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Synthetic studies of lignanes: Attempted synthesis of arctigenin and enterolactone

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Abstract: A synthetic approach to bioactive lignans was devised, which relies on photocatalyzed [2+2] cycloaddition of cinnamyl cinnamates, followed by catalytic hydrogenolysis of C–C bond between two benzylic positions in the strained cycloadduct. However, while feasible in the model study, this approach could not be applied to the synthesis of bioactive derivatives arctigenin and enterolactone. Calculations were performed in order to rationalize the surprising lack of reactivity of substituted cinnamates and cyclobutane-fused γ -butyrolactone.

Keywords: photocycloaddition; photocatalysis; butanolide; hydrogenolysis; natural products.

INTRODUCTION

Lignans are an important class of plant natural products, composed of phenylpropanoid fragments.^{1,2} They have important physiological roles in planta, notably as defense chemicals – hence their antioxidant, allelopathic, antifungal, antimicrobial, insecticidal, nematocidal, antifeedant and (occasionally) poisonous properties. In addition, oligomerization and deposition of lignans has a structural role. Apart from plants, these compounds have an important role in human nutrition, health protection and disease treatment.³ The spectrum of their activities encompasses protection against onset of breast and prostate cancer, antitumor properties, hepatotoxic preventive effect, antiviral properties, anti-inflammatory, antiasthmatic and antidepressant effects, *inter alia*. In this context, three compounds originating from *Arctium lappa* L. – a well-known medicinal plant in the traditional Chinese, as well as in Kampo medicine – are particularly noteworthy: arctigenin

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1, its glycoside arctiin **2** and its metabolite enterolactone **3** (Fig. 1). In addition to the abovementioned biological properties of lignanes, some specific features of these three lactones are noteworthy. Thus, arctigenin exhibits antiinflammatory, antioxidant, immunomodulatory, multiple sclerosis fighting, antitumor and anti-leukemia properties.^{4,5} Remarkably, arctigenin possesses preferential cytotoxicity, targeting selectively nutrient-deprived cells, thus allowing for the antiausterity treatment – a possible therapeutic approach against, *e.g.*, pancreatic cancer, which is resistant to chemotherapy.^{6–8} Comprehensive reviews of molecular mechanisms of anticancer activity of arctigenin⁹ and the corresponding glycoside – arctiin – are available.¹⁰ Enterolactone, which is the first lignane to be found in the mammalian gut, inhibits tumour growth and metastasis in ovarian cancer allograft mouse model. This effect is especially efficient in combination of enterolactone with 3TSR, which act synergistically, mostly by inhibiting tumour angiogenesis.¹¹ It was also found to act as phytoestrogen, and is reported to protect against breast cancer and cardiovascular disease.¹²

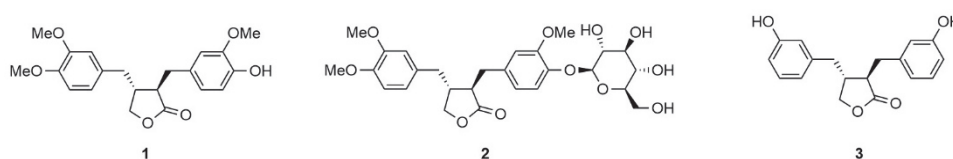
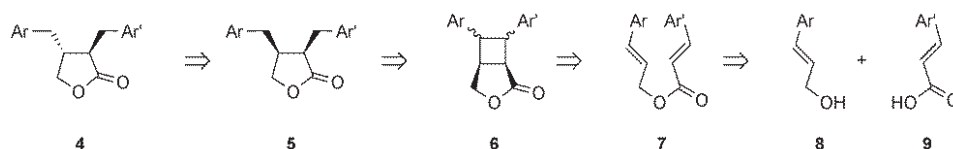


Fig. 1. Structures of arctigenin **1**, arctiin **2** and enterolactone **3**.

Due to a wide range of important biological activities, arctigenin and enterolactone have attracted considerable attention from the synthetic community. Pharmacology and total synthesis of arctigenin have been recently reviewed,¹³ as well as biological properties and total synthesis of enterolactone.¹⁴ Several total syntheses of arctigenin have been reported, based on radical carboxyarylation,¹⁵ intermolecular radical addition,¹⁶ α -alkylation of dihydrocinnamic acid chiral oxazolidinone¹⁷ and intramolecular carbene insertion.¹⁸ An interesting isomerization of 3,5- into 4,5-dibenzylated butanolides was successfully applied in synthesis of arctigenin;¹⁹ this approach also provided the nonnatural *cis*-isomer.²⁰ Enterolactone was also an inspiring challenge for synthetic chemists, whose efforts encompassed alkylation of succinic acid-derived Evans oxazolidinones,²¹ enantioselective radical addition to fumarate-derived oxazolidinone,¹⁶ alkylation of dihydrocinnamic oxazolidinones,²² chiron-based synthesis starting from malic acid,²³ catalytic asymmetric intramolecular carbene insertion (for the formation of the optically enriched butanolide structural unit),²⁴ alkylation of π -allylpalladium complex,²⁵ allylic nucleophilic substitution with organocuprates,²⁶ organocatalyzed aldolization,²⁷ enzymatic resolution,²⁸ Michael addition of a benzylic dithiane to an optically pure butanolide,²⁹ as well as semisynthesis from the readily available natural lignan 7-hydroxymatairesinol.³⁰

RESULTS AND DISCUSSION

Naturally occurring lignanes are of *trans*-stereochemistry, and virtually all syntheses reported so far target *trans*-lignanes. However, structure–bioactivity relationship studies have shown that *cis*-configured products also possess bioactivity;²⁰ thus, *cis*-arctigenin was shown to be a more potent inhibitor of cyclic AMP phosphodiesterase than the *trans*-isomer.³¹ Therefore, we set out to develop a method that would allow for synthesis of both *cis*- and *trans*-stereoisomers, and which would allow for a modular synthesis of a small library of lignanes suitable for structure–activity relationship (SAR) study. Our approach, blueprinted in Scheme 1, relies on [2+2] cycloaddition of cinnamyl cinnamates (**7** → **6**) as a pivotal step for the construction of the bicyclic framework **6**, from which the non-natural, *i.e.*, *cis*-configured butanolides of type **5** should be obtained by hydrogenolysis. Base-catalyzed isomerization of *cis*-derivatives (**5**) into thermodynamically more stable *trans*-ones should then provide lignans **4** from the natural series. The cyclization substrates **7** are readily available cinnamyl cinnamates, which makes the whole sequence suitable for the creation of a library of arctigenine analogues for SAR studies.

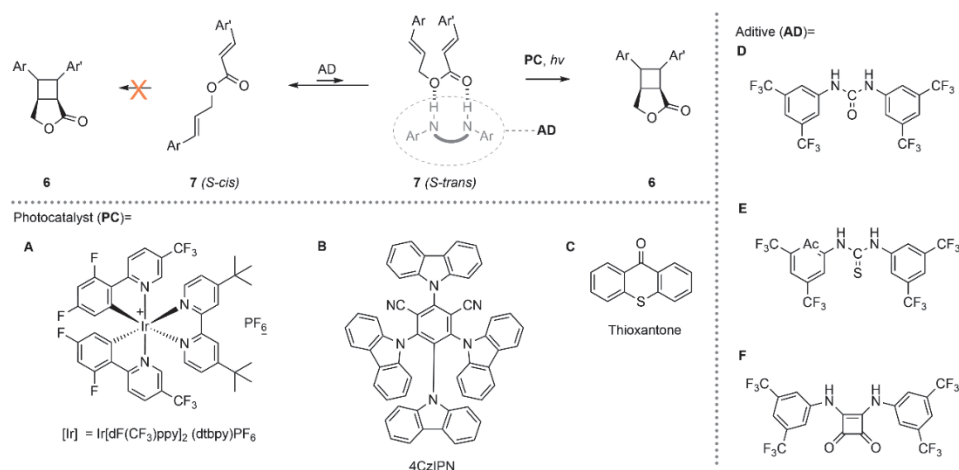


Scheme 1. Synthetic approach to *cis*- and *trans*-configured arctigenine and analogues thereof.

The feasibility of this approach was tested on the parent cinnamyl cinnamate **7a** (Ar, Ar' = Ph) as a model substrate. Exposure of this compound to UV-light (254 nm) gave no formation of product **6a**, as only decomposition of the starting material was observed. We then resorted to photoredox conditions:³² exposure of **7a** to blue LED light (440 nm) in the presence of Ir-catalyst (**A**) provided cycloadduct **6a** in 55 % yield. Performing the reaction at 55 °C compared favorably with the literature (29 % at 80 °C). The moderate yield may be attributed to the known propensity of esters to adopt in solution the *S-cis*-conformation, which is unfavorable for the cyclization. Therefore, to surmount *S-cis*/*S-trans* energy barrier and facilitate conformational interconversion, we postulated that additives which may engage into hydrogen bonding with both ester oxygen atoms may invert the conformational equilibrium and promote cyclization (Scheme 2).

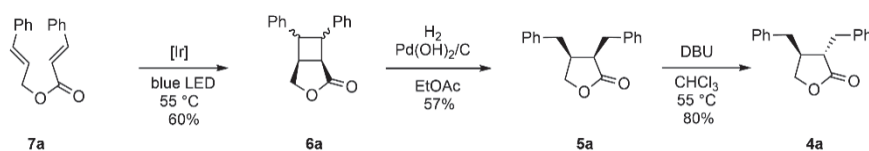
To this end, several urea- and squaramide-based catalysts were tried (**D**, **E** and **F**, Scheme 2), unfortunately to no avail: urea **D** and thiourea **E** actually slowed the reaction and lowered the yield, whereas squaramide **F** catalyst induced only modest yield improvement (from 55 to 60 %). We also tested few organic dyes as

photocatalysts 4-CzIPN **B** and thioxantone **C** (Scheme 2), with medium to poor outcome (38 and 14 %, respectively).



Scheme 2. Postulated organocatalyst-induced inversion of *S-cis/S-trans* conformational equilibrium of esters.

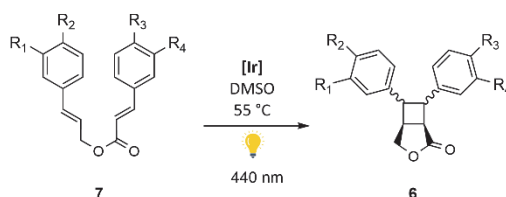
The cycloadduct **6a** was obtained as a mixture of isomers with the ratio 3.6:1 (regarding the relative positions of Ph groups with respect to each other), which was inconsequential for the synthesis, as the stereogenic centers at Ph-bearing carbon atoms are to be destroyed in the next step. Our plan called for the cyclobutane ring scission, where the bond between two benzylic positions was expected to cleave selectively. Surprisingly, to the best of our knowledge, only two such precedents exist in the literature: 1) palladium hydroxide-catalyzed hydrogenolysis³³ and 2) reductive cleavage under the conditions of Birch reduction.³⁴ To our pleasure, upon exposure to hydrogen atmosphere in the presence of palladium hydroxide on charcoal (10–20 %), bicyclic lactone **6a** was converted into *cis*-configured butanolide **5a** in 57 % yield. Finally, treatment of **5a** with a stoichiometric amount of DBU in chloroform, at 55 °C afforded the *trans*-isomer **4a** in 80 % yield (Scheme 3).



Scheme 3. Synthesis of *cis*- and *trans*-1,2-dibenzylbutanolides **5a** and **4a**.

The successful accomplishment of the model-study encouraged us to apply this protocol in the synthesis of substituted, natural targets. However, and much to

our surprise, out of eleven structurally different cinnamic esters, only three underwent cyclization, in modest yields (Scheme 4, Table I, entries **6b**, **6c** and **6e**). The reasons for this lack of reactivity are not clear, as the catalyzed reaction was expected to proceed *via* energy transfer, where the presence of electron-donating substituents at the aromatic rings should not be a handicap; in fact, two such cyclizations occurred, albeit in modest yields (Table I, entries **6b** and **6e**).



Scheme 4. General scheme for the photocycloaddition reaction.

TABLE I. Attempts to cyclize cinnamyl cinnamates

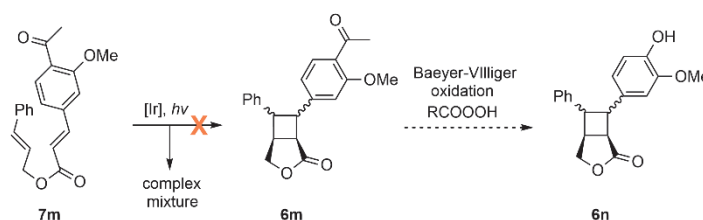
Entry	R ₁	R ₂	R ₃	R ₄	Yield, %
6a	–H	–H	–H	–H	55
6b	–OBn	–H	–H	–OBn	38
6c	–Br	–H	–H	–Br	25
6d	–OMe	–H	–H	–OMe	0 ^a
6e	–OMe	–OMe	–H	–H	25
6f	–OMe	–OMe	–OBn	–OMe	0 ^b
6g	–OMe	–OMe	–Ac	–OMe	0 ^b
6h	–H	–H	–Ac	–OMe	0 ^b
6i	–H	–H	–H	–NO ₂	0 ^a
6j	–OBn	–H	–H	–NO ₂	0 ^a
6k	–NO ₂	–H	–H	–H	0 ^a
6l	–NO ₂	–H	–H	–OBn	0 ^a

^aMixture of starting material isomers; ^bcomplex mixture

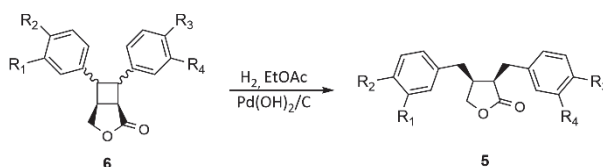
Suspecting that electron-donating substituents at the aromatic ring may be responsible for the diminished reactivity, we prepared 4-acetyl cinnamic derivative **7m**, which should be electron deficient enough to cyclize to product **6m**, from which the desired compound **6n** should be obtained by Baeyer–Villiger reaction (Scheme 5). However, upon exposure to photocatalytic conditions **7m** gave a complex mixture of products.

Photocatalytic [2+2] cycloaddition was not the only obstacle hampering the extension of the model study to natural products and structural analogues, as cycloadducts (Table I, entries **6b**, **6c** and **6e**) proved resilient to hydrogenolytic cyclobutane ring opening with Pd(OH)₂ (Scheme 6). Attempts to force the bond cleavage using other catalysts, such as Pd/C or PtO₂/C as catalysts, or by increasing pressure and/or temperature resulted in partial or total reduction of benzene rings

(Table II, entries **5a** and **5e**, in addition to the expected debenzilylation in the case of **5b**), as well as debromination (in the case of **5c**, Table II).



Scheme 5. An attempt towards indirect preparation of 4-hydroxy-substituted cycloadduct **6n**.



Scheme 6. General reaction scheme for hydrogenolytic cyclobutane ring opening.

TABLE II. Attempts of hydrogenolytic cyclobutane ring opening

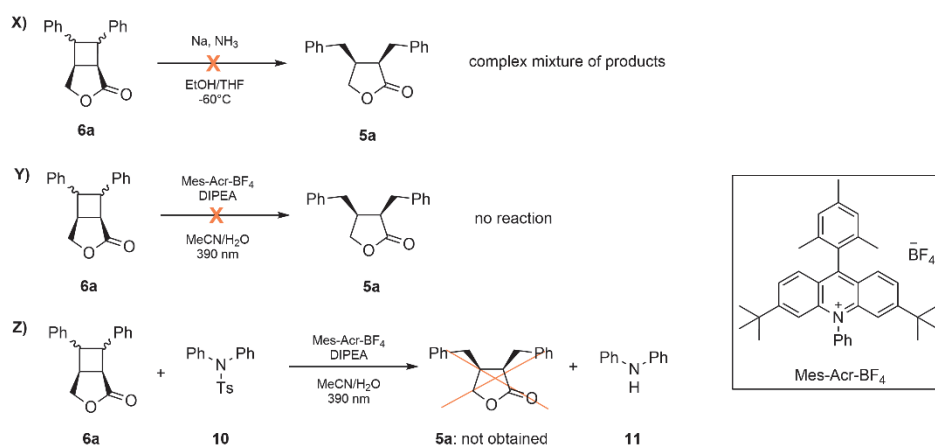
Entry	R ₁	R ₂	R ₃	R ₄	Yield, %
5a	-H	-H	-H	-H	57 ^a
5b	-OBn	-H	-H	-OBn	0
5c	-Br	-H	-H	-Br	0
5e	-OMe	-OMe	-H	-H	0
5o	-OAc	H	H	-OAc	0

^aDetermined by ¹H-NMR spectroscopy using TMB as an internal standard

Other reductive attempts to effect hydrogenolytic scission included Birch reduction (Scheme 7, **X**)³³ and photoredox-catalysis (Scheme 7, **Y**),³⁵ both to no avail as, in the case of Birch reduction only decomposition of the starting material was observed, whereas **6a** didn't react under photoredox conditions, with the recovery of the starting material. As for the latter reaction – we performed a control experiment (Scheme 7, **Z**), with competitive reduction of compound **10** (described in the literature)³⁵ and our substrate **6a** in the same reaction vessel. Whereas tosylamine **10** underwent reductive detosylation, as described, and provided diphenylamine **11**, substrate **6a** remained unchanged, thus proving that selective reduction of **6a** into **5a** under these photoredox conditions is not possible.

To explain the lack of reactivity of substituted analogues, we performed density functional theory (DFT) calculations. The [2+2] cycloaddition reaction is presumed to occur *via* the energy-transfer mechanism, where the energy of the excited photocatalyst in the triplet state should equal or exceed the corresponding triplet energy gap of cinnamyl cinnamate. We hypothesized that if substituents on the

aromatic ring significantly increased this energy gap (*i.e.*, $S_0 \rightarrow T_1$), it could result in the inability of the catalyst to excite the substrate, and subsequent lack of reactivity. To test this, vertical triplet excitation energies were calculated *via* time-dependent DFT (TD-DFT) at the wB97x-D4rev/def2-TZVP level of theory for derivatives bearing $-H$ (**7a**), $-OBn$ (**7b**) and $-OMe$ (**7d**) substituents at the meta-positions. The calculations yielded vertical excitation values of 2.75, 2.71 and 2.73 eV for the **7a**, **7b** and **7d** derivatives, respectively. Although range-separated hybrids typically exhibit a systematic blueshift, leading to slightly higher absolute vertical excitation energies (on the order of 0.2–0.4 eV), the relative differences within the series remain negligible ($\Delta\Delta E \leq 0.04$ eV). Thus, substituents do not perturb the triplet energy in a way that could account for the observed reactivity differences. Instead, other factors, such as alternative quenching pathways, steric hindrance, or further nuances of the reaction mechanism, may be at play.



Scheme 7. Other attempts of reductive cyclobutane ring opening.

We also performed calculations to clarify the lack of reactivity of substituted cyclobutane-fused γ -butyrolactones towards hydrogenolytic cleavage, which contrasted with the smooth reaction of the parent compound **6a**. Although counter-intuitive, our initial hypothesis was that substituents may increase the strength of the C–C bond between the two benzylic positions. To evaluate this, we calculated the corresponding C–C bond dissociation energy comparing the parent system **6a** to the substituted analogue **6e**. The Gibbs energy difference between the closed cyclobutane form and the homolytically cleaved open-shell singlet form was calculated at the wB97x-D4rev/def2-TZVP level. We obtained values of 169.03 kJ/mol for the H-substituted system and 166.52 kJ/mol for the OMe-substituted system. This marginal difference suggests that the aromatic substituents do not inherently strengthen the C–C bond. Consequently, the lack of reactivity in these

cases might be attributed to kinetic barriers or catalyst-surface interactions that a simple thermodynamic bond-strength model does not capture. Additional studies are required to elucidate this mechanistic divergence fully.

EXPERIMENTAL

General experimental

All chromatographic separations were performed on Silica gel 60 (0.063–0.2 mm), Merck. Standard techniques were used for the purification of reagents and solvents.³⁶ NMR spectra were recorded on Varian/Agilent 400 (¹H-NMR at 400 MHz, ¹³C-NMR at 100 MHz) and on Bruker Avance III 500 (¹H-NMR at 500 MHz, ¹³C-NMR at 125 MHz), in deuterated chloroform, if not otherwise stated. Chemical shifts are expressed in ppm (δ) using tetramethylsilane as internal standard, coupling constants (*J*) are in Hz. IR spectra were recorded on Thermo Scientific Nicolet Summit FT-IR instrument and are expressed in cm⁻¹. Mass spectra were obtained on Orbitrap Exploris 240 spectrometer. Blue light irradiation was performed using blue LED KessilPR160L 440 nm lamp. Additional synthetic procedures are listed in the Supplementary material to this paper.

Experimental procedures

Synthesis of 6,7-diphenyl-3-oxabicyclo[3.2.0]heptan-2-one (6a).³² The solution of compound **7a** (43.8 mg, 0.166 mmol, 1 eq.) and [Ir(dtbbpy)(dF(CF₃)ppy)₂](PF₆)₂ (4.0 mg, 0.0035 mmol, 0.02 eq.) in DMSO (4.0 mL) was sparged with Ar for 10 min and stirred at 55 °C for 2 h with a blue LED setup (see Supplementary material for details). After completion, the reaction mixture extracted with diethyl ether, organic phase was washed with brine and dried over anhydrous MgSO₄ and concentrated on rotovap. The residue was purified by dry-flash chromatography (petroleum ether/ethyl acetate = 9:1), to afford 26.5 mg (55 %) of compound **6a** as a mixture of isomers in a relative ratio 3:1, as a colorless oil. Spectral data (Supplementary material) are in accordance with the literature.

Synthesis of cis-(3R,4R)-dihydro-3,4-bis(phenylmethyl)-2(3H)-furanone (5a).³⁷ A suspension of compound **2** (18 mg; 0.068 mmol, 1 eq.) and Pd(OH)₂/C (10–20 %, 64 mg, 0.068 mmol, 1eq.) in ethyl acetate (1.2 mL) was stirred under hydrogen atmosphere (2 bar) for 48 h at room temperature. The reaction mixture was then filtered through the Celite. The product yield (57 %) was determined via ¹H-NMR spectroscopy with iodoform as internal standard. Spectroscopic data are in accordance with the literature.

Synthesis of trans-(3R,4R)-dihydro-3,4-bis(phenylmethyl)-2(3H)-furanone (4a).³⁷ To a solution of compound **5a** (12 mg, 0.045 mmol, 1 eq.) in 0.5 mL (CDCl₃) in NMR tube was added DBU (6.8 μ L, 0.045 mmol, 1 eq.) and stirred at 55 °C for 96 h. Upon completion, monitored by ¹H-NMR spectroscopy, the reaction mixture was purified by dry-flash chromatography (petroleum ether/ethyl acetate = 9:1), to afford 8 mg (80 %) of the product **4a**, as a colorless oil. Spectroscopic data in accordance with the literature.

Synthesis of 6,7-bis(3-(benzyloxy)phenyl)-3-oxabicyclo[3.2.0]heptan-2-one (6b). The solution of compound **7b** (380.0 mg, 0.79 mmol, 1 eq.) and [Ir(dtbbpy)(dF(CF₃)ppy)₂](PF₆)₂ (18.0 mg, 0.016 mmol, 0.02 eq.) in DMSO (16 mL) was sparged with Ar for 10 min and stirred at 55 °C for 6.5 h with a blue LED setup. After completion, the reaction mixture extracted with diethyl ether, organic phase was washed with brine and dried over anhydrous MgSO₄ and concentrated on rotovap. The residue was purified by dry-flash chromatography (benzene/ethyl acetate = 99:1), to afford 145.3 mg (38 %) of compound **6b** as a mixture of isomers in a relative ratio 3:1, as a colorless oil.

Synthesis of 6,7-bis(3-bromophenyl)-3-oxabicyclo[3.2.0]heptan-2-one (**6c**). The solution of compound **7c** (100.0 mg, 0.24 mmol, 1 eq.) and [Ir(dtbppy)(dF(CF₃)ppy)₂]PF₆ (5.3 mg, 0.005 mmol, 0.02 eq.) in DMSO (4.7 mL) was sparged with Ar for 10 min and stirred at 55 °C for 4 h with a blue LED setup. After completion, the reaction mixture was extracted with diethyl ether, organic phase washed with brine and dried over anhydrous MgSO₄ and concentrated on rotovap. The residue was purified by dry-flash chromatography (benzene/ethyl acetate = 925:75), to afford 25 mg (25 %) as a mixture of isomers of compound **6c** in a relative ratio 5.8:1, as a colorless oil.

Synthesis of 6-(3,4-dimethoxyphenyl)-7-phenyl-3-oxabicyclo[3.2.0]heptan-2-one (**6e**). The solution of compound **7e** (27.2 mg, 0.08 mmol, 1 eq.) and [Ir(dtbppy)(dF(CF₃)ppy)₂]PF₆ (2.0 mg, 0.0017 mmol, 0.02 eq.) in DMSO (1.6 mL) was sparged with Ar for 10 min and stirred at 55 °C for 4 h with a blue LED setup. After completion, the reaction mixture was extracted with diethyl ether, organic phase washed with brine and dried over anhydrous MgSO₄ and concentrated on rotovap. The residue was purified by dry-flash chromatography (benzene/ethyl acetate = 9:1), to afford 9.3 mg (25 %) of compound **6e**, as a mixture of isomers of compound **6e** in a relative ratio 1:1, as a colorless oil.

Synthesis of (2-oxo-3-oxabicyclo[3.2.0]heptane-6,7-diyl)bis(3,1-phenylene) diacetate (**6o**). To compound **13** (see Supplementary material) (12.0 mg, 0.04 mmol, 1 eq.) were added acetic anhydride (31 µL) and pyridine (2 µL) and reaction mixture was stirred at 120 °C for 15 min. After completion, the reaction mixture was purified by column chromatography (petroleum ether/ethyl acetate = 1:1), to afford 13.9 mg (91 %) of compound **6o** as a mixture of isomers in a relative ratio 2.8:1, as a colorless oil.

Spectral data of compounds **6b**, **6c**, **6e** and **6o** are given in the Supplementary material.

Computational details

All electronic structure calculations were conducted using the ORCA 6.1.1 software package.³⁸ Initial conformational ensembles for **6a**, **7a** and their substituted derivatives were generated using the global geometry optimization and ensemble generator (GOAT) module³⁹ at the GFN2-xTB level.³⁹ These ensembles were refined using the B97-3c composite method,⁴⁰ followed by final geometry optimizations and frequency analysis with wB97x-D4rev⁴¹ range-separated hybrid functional and def2-TZVP basis set. The nature of the final stationary points was confirmed as true minima by the absence of imaginary frequencies. Vertical singlet–triplet (S₀→T₁) energy gaps were evaluated *via* TD-DFT vertical excitations at the wB97x-D4rev/def2-TZVP level of theory. Bond dissociation energies were determined as the Gibbs energy difference between the closed-shell bicyclic lactone and the open-shell singlet biradical resulting from homolytic cleavage of the C–C bond between the two benzylic positions.

CONCLUSIONS

To summarize, a synthetic approach to both *cis*- and *trans*-configured lignane-type butanolides was devised, which works well on the parent compound, but fails with substituted substrates and structurally more complex targets. Further studies are required to clarify the reasons behind this lack of reactivity and to expand the method scope to the naturally occurring lignanes and the analogues thereof.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/14007>, or from the corresponding author on request.

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The authors acknowledge the contribution of Djordje Stefanovic, an undergraduate student, who performed the synthesis and characterization of compounds **7b**, **7c** and **7e**.

ИЗВОД

СИНТЕТИЧКЕ СТУДИЈЕ ЛИГНАНА: ПОКУШАЈ СИНТЕЗЕ АРКТИГЕНИНА И ЕНТЕРОЛАКТОНА

МИЛЕНА ТРМЧИЋ¹, БОЈАН ВУЛОВИЋ², МАТИЈА ЗЛАТАР³ И РАДОМИР Н. САИЧИЋ^{2,4}

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Осмишљен је синтетички приступ биолошки активним лигнанима, који се заснива на фотокатализованој [2+2] циклоадицији цинамил-цинамата, праћеној хидрогенолизом везе угљеник–угљеник између два бензилна положаја у угаоно-напетом циклоадукту. Међутим, иако је ова реакциона секвенца успешно реализована на модел-једињењу, није могла бити примењена у синтези биоактивних деривата арктигенина и ентеролактона, јер присуство супституената на ароматичном језгру инхибира жељене реакције. Изненађујући изостанак реактивности супституисаних цинамата (према [2+2] циклоадицији) и циклобутанобутиролактона (према хидрогенолизи) анализиран је рачунарским методама.

(Примљено 15. јуна, ревидирано 30. јуна, прихваћено 10. јула 2026)

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SUPPLEMENTARY MATERIAL TO
**Synthetic studies of lignanes: Attempted synthesis of arctigenin
and enterolactone**

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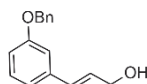
General experimental	S174
(<i>E</i>)-3-(3-(benzyloxy)phenyl)prop-2-en-1-ol (12)	S175
(<i>E</i>)-3-(3-(benzyloxy)phenyl)allyl (<i>E</i>)-3-(3-(benzyloxy)phenyl)acrylate (7b)	S175
(<i>E</i>)-3-(3-bromophenyl)allyl (<i>E</i>)-3-(3-bromophenyl)acrylate (7c)	S175
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GENERAL EXPERIMENTAL

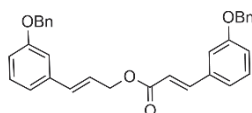
All chromatographic separations¹ were performed on Silica gel 60 (0.063–0.2 mm), Merck. Standard techniques were used for the purification of reagents and solvents.² NMR spectra were recorded on Varian/Agilent 400 (¹H NMR at 400 MHz, ¹³C NMR at 100 MHz) and on Bruker Avance III 500 (¹H NMR at 500 MHz, ¹³C NMR at 125 MHz), in deuterated chloroform, if not otherwise stated. Chemical shifts are expressed in ppm (δ) using tetramethylsilane as internal standard, coupling constants (*J*) are in Hz. IR spectra were recorded on Thermo Scientific Nicolet Summit FT-IR instrument, and are expressed in cm^{−1}. Mass spectra were obtained on Orbitrap Exploris 240 spectrometer. Blue light irradiation was performed using KessilPR160L 440 nm lamp (www.kessil.com).

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(E)-3-(3-(benzyloxy)phenyl)prop-2-en-1-ol (**12**)

Ethyl chloroformate (0.54 mL, 5.64 mmol, 1 Eq) was added dropwise to a cold ($-7\text{ }^{\circ}\text{C}$) solution of *(E)*-3-(3-(benzyloxy)phenyl)acrylic acid³ (1.43 g, 5.64 mmol, 1 Eq) and triethylamine (1.57 mL, 11.27 mmol, 2 Eq) in dry THF (15 mL), under an argon atmosphere. After stirring for 30 minutes, reaction mixture was filtered and washed with additional THF (3 mL). NaBH_4 (0.64 g, 16.9 mmol, 3 Eq) was then added to the reaction mixture as one portion at $10\text{ }^{\circ}\text{C}$ and MeOH (8.4 mL) was added dropwise. The suspension was stirred at $10\text{ }^{\circ}\text{C}$ for 1 hour before quenched with water and extracted with DCM. The organic extract was dried over anhydrous MgSO_4 and concentrated on rotovap. The residue was purified by dry-flash chromatography (petroleum ether/ethyl acetate = 1:1), to afford 1.22 g (89%) of compound **12**, as a colorless oil. Spectroscopic data in accordance with the literature.⁴

(E)-3-(3-(benzyloxy)phenyl)allyl *(E)*-3-(3-(benzyloxy)phenyl)acrylate (**7b**)

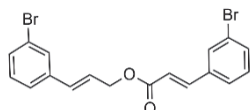
(E)-3-(3-(benzyloxy)phenyl)acrylic acid³ (129.7 mg, 0.52 mmol, 1 Eq), compound **12** (147.0 mg, 0.61 mmol, 1.2 Eq), DCC (126.0 mg, 0.61, 1.2 Eq) and DMAP (12.0 mg, 0.01, 0.2 Eq) were suspended in DCM (5.4 mL) and stirred for 30 minutes. The reaction mixture was filtered through Celite and purified by dry-flash chromatography (benzene/ethyl acetate = 99:1), to afford 243.0 g (100%) of pure **7b**, as a colorless oil.

¹H NMR (500 MHz, CDCl_3) δ 7.72 (d, $J=16.0$, 1H), 7.49–7.24 (m, 12H), 7.18–7.14 (m, 2H), 7.08–7.00 (m, 3H), 6.92 (ddd, $J=8.2, 2.6, 1.0$ Hz, 1H), 6.73–6.66 (m, 1H), 6.48 (dd, $J=16.0, 0.7$ Hz, 1H), 6.37 (dt, $J=15.9, 6.4$ Hz, 1H), 5.09 (d, $J=3.6$ Hz, 4H), 4.89 (dd, $J=6.4, 1.3$ Hz, 2H).

¹³C NMR (126 MHz, CDCl_3) δ 166.7, 159.2, 159.1, 145.1, 137.8, 137.0, 136.7, 135.9, 134.2, 130.1, 129.7, 128.8, 128.7, 128.2, 128.1, 127.6, 123.7, 121.2, 119.7, 118.3, 117.2, 114.7, 114.1, 113.0, 70.2, 70.1, 65.2.

IR (ATR): $\nu=3064, 3033, 2934, 2873, 1713, 1638, 1597, 1580, 1490, 1445, 1381, 1309, 1293, 1260, 1162, 1110, 1081, 1027, 979, 910\text{ cm}^{-1}$.

$R_f=0.8$ (PhH/EtOAc=95:5).

(E)-3-(3-bromophenyl)allyl *(E)*-3-(3-bromophenyl)acrylate (**7c**)

(E)-3-(3-bromophenyl)acrylic acid⁵ (100.0 mg, 0.44 mmol, 1 Eq), *(E)*-3-(3-bromophenyl)prop-2-en-1-ol⁶ (112.5 mg, 0.53 mmol, 1.2 Eq), DCC (109.0 mg, 0.53, 1.2 Eq) and DMAP (11.0 mg, 0.09, 0.2 Eq) were dissolved in DCM (4.6 mL) and was stirred for 30 minutes. The reaction mixture was filtered through Celite and purified by dry-flash

chromatography (benzene/ethyl acetate = 99:1), to afford 168.0 g (90.4%) of pure **7c**, as a colorless oil.

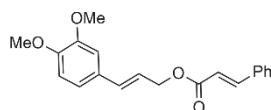
¹H NMR (500 MHz, CDCl₃) δ 7.69–7.61 (m, 2H), 7.57–7.54 (m, 1H), 7.51 (d, J = 8 Hz, 1H), 7.44 (d, J = 7.7 Hz, 1H), 7.39 (d, J = 8 Hz, 1H), 7.31 (d, J = 8 Hz, 1H), 7.29–7.23 (m, 1H), 7.19 (t, J = 7.8 Hz, 4H), 6.63 (d, J = 15.9 Hz, 3H), 6.47 (d, J = 16.0 Hz, 4H), 6.35 (dt, J = 15.9, 6.2 Hz, 4H), 4.87 (dd, J = 6.3, 1.4 Hz, 7H).

¹³C NMR (126 MHz, CDCl₃) δ 166.2, 143.6, 138.4, 136.5, 133.3, 132.6, 131.0, 130.9, 130.5, 130.2, 129.6, 126.8, 125.4, 124.9, 123.2, 122.9, 119.3, 119.3, 65.0.

IR (ATR): ν = 3060, 2925, 2854, 1714, 1639, 1591, 1473, 1424, 1374, 1312, 1268, 1198, 1166, 1111, 1089, 1072, 995 cm⁻¹.

R_f = 0.9 (PhH/EtOAc = 95:5).

(*E*)-3-(3,4-dimethoxyphenyl)allyl cinnamate (**7e**)



Cinnamic acid (32.0 mg, 0.21 mmol, 1 Eq), (*E*)-3-(3,4-dimethoxyphenyl)prop-2-en-1-ol (50.0 mg, 0.26 mmol, 1.2 Eq), DCC (53.0 mg, 0.26, 1.2 Eq) and DMAP (5.0 mg, 0.04, 0.2 Eq) were dissolved in DCM (2.5 mL) and was stirred for 30 minutes. The reaction mixture was filtered through Celite and purified by dry-flash chromatography (benzene/ethyl acetate = 9:1), to afford 31.6 g (45%) of pure **7e**, as colorless oil.

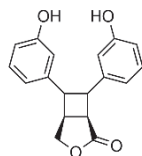
¹H NMR (400 MHz, CDCl₃) δ 7.73 (d, J = 16.0 Hz, 1H), 7.54 – 7.49 (m, 2H), 7.40 – 7.37 (m, 3H), 6.99 – 6.93 (m, 2H), 6.83 – 6.79 (m, 1H), 6.68 – 6.62 (m, 1H), 6.48 (dd, J = 16.0, 0.6 Hz, 1H), 6.24 (dt, J = 15.8, 6.6 Hz, 1H), 4.86 (dd, J = 6.6, 1.3 Hz, 2H), 3.88 (dd, J = 8.8, 1.4 Hz, 6H).

¹³C NMR (101 MHz, CDCl₃) δ 166.8, 149.3, 149.1, 145.1, 134.4, 134.4, 130.4, 129.3, 129.0, 128.1, 121.3, 120.1, 118.0, 111.1, 108.9, 65.4, 55.9, 55.9.

IR (ATR): ν = 3060, 3001, 2936, 2836, 1712, 1637, 1602, 1582, 1515, 1464, 1451, 1419, 1376, 1328, 1310, 1202, 1165, 1141, 1073, 1027, 970 cm⁻¹.

R_f = 0.6 (PhH/EtOAc = 9:1).

6,7-bis(3-hydroxyphenyl)-3-oxabicyclo[3.2.0]heptan-2-one (**13**)



To a solution of **6b** (30.7 mg, 0.064 mmol, 1 Eq) in EtOAc (1.2 mL) was added Pd(OH)₂ on carbon (12 mg, 0.013 mmol, 0.2 Eq) and stirred overnight under H₂ atmosphere (1 bar). After reaction was completed, reaction mixture was filtered through pad of Celite and purified by dry-flash chromatography (petroleum ether/ethyl acetate = 1:1), to afford 13.2 g (70%) of **13** as a mixture of isomers in a ratio 2.7:1, as a colorless oil.

Major isomer: **¹H NMR** (500 MHz, CDCl₃) δ 6.95 (t, J = 7.8 Hz, 1H), 6.89 (t, J = 7.8 Hz, 1H), 6.56 (d, J = 7.5 Hz, 1H), 6.53 – 6.48 (m, 2H), 6.47 – 6.42 (m, 2H), 6.42 – 6.39 (m, 1H), 4.58 (dd, J = 9.5, 6.3 Hz, 1H), 4.42 (dd, J = 9.5, 1.4 Hz, 1H), 4.03 – 3.97 (m, 1H), 3.95 (dd, J = 9.5, 3.2 Hz, 1H), 3.70 (q, J = 7.1 Hz, 1H), 3.39 (ddd, J = 7.9, 3.2, 1.1 Hz, 1H).

^{13}C NMR (126 MHz, CDCl_3) δ 182.4, 158.1, 158.0, 141.9, 141.5, 130.0, 129.8, 120.4, 120.2, 116.1, 115.8, 114.4, 114.2, 74.7, 42.3, 39.9.

IR (ATR): ν = 3370, 2968, 2927, 2854, 1740, 1588, 1493, 1454, 1376, 1313, 1257, 1228, 1172, 1086, 1047 cm^{-1} .

HRMS (ESI-Orbitrap) m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{18}\text{H}_{16}\text{O}_4$: 319.0941, found: 319.0940.

R_f =0.5 (PE /EtOAc=6:4).

Experimental setup for photocatalyzed [2+2] cycloaddition

Borosilicate culture tube (8 mL, round bottom, screw cap finish 13-415; height 100 mm, diameter 13 mm) was used as the reaction vessel. The distance between reaction vessel and light source was 3 cm. The lamp was cooled using Sunon 92x92x25 12VDC (1.68 W) fan. With this experimental setup, temperature reaches 55 °C, as measured using thermometer placed next to the reaction vessel.



Figure S2 Blue LED setup

6,7-Diphenyl-3-oxabicyclo[3.2.0]heptan-2-one (6a)

¹H NMR (400 MHz, CDCl₃) δ 7.42–7.27, 7.22–7.01, 7.00–6.95, 6.88–6.84 (4 x m, 10H major and 10H minor), 4.56 (dd, J = 9.7, 6.1 Hz, 1H), 4.48–4.37 (m, 1H major and 1H minor), 4.27 (dd, J = 10.2, 4.9 Hz, 1H minor), 4.20–4.07 (m, 2H major and 2H minor), 3.71 (q, J = 6.7 Hz, 1H major), 3.60–3.50 (m, 1H minor), 3.47–3.41 (m, 1H major), 3.35–3.28 (m, 1H minor). Major and minor: **¹³C NMR** (101 MHz, CDCl₃) δ 179.5, 178.4, 141.7, 138.7, 138.5, 138.1, 129.0, 128.9, 128.2, 128.1, 128.0, 127.7, 127.3, 127.2, 127.1, 126.7, 126.6, 126.4, 48.2, 47.7, 46.7, 45.9, 42.6, 40.6, 39.0, 35.4.

Cis-(3R,4R)-dihydro-3,4-bis(phenylmethyl)-2(3H)-furanone (5a)

¹H NMR (400 MHz, CDCl₃) δ 7.40–7.19 (m, 8H), 7.05–7.01 (m, 2H), 4.07–3.98 (m, 2H), 3.35 (dd, J = 15.0, 4.7 Hz, 1H), 3.13 (ddd, J = 11.4, 7.1, 4.6 Hz, 1H), 3.00 (dd, J = 13.6, 3.9 Hz, 1H), 2.85 (dd, J = 14.9, 10.9 Hz, 1H), 2.73–2.63 (m, 1H), 2.44–2.34 (m, 1H). **¹³C NMR** (101 MHz, CDCl₃) δ 178.1, 138.7, 138.6, 129.1, 129.0, 128.9, 128.5, 126.9, 126.8, 69.5, 45.4, 40.0, 33.0, 31.0.

Trans-(3R,4R)-dihydro-3,4-bis(phenylmethyl)-2(3H)-furanone (4a)

¹H NMR (400 MHz, CDCl₃) δ 7.34–7.14 (m, 8H), 7.02–6.97 (m, 2H), 4.11–4.05 (m, 1H), 3.89–3.83 (m, 1H), 3.12–3.06 (m, 1H), 2.96 (dd, J = 14.0, 7.1 Hz, 1H), 2.70–2.57 (m, 2H), 2.56–2.46 (m, 2H). **¹³C NMR** (101 MHz, CDCl₃) δ 178.6, 138.1, 137.8, 129.4, 129.1, 128.9, 128.7, 128.5, 127.0, 126.9, 77.5, 77.4, 77.2, 76.8, 71.3, 46.6, 46.4, 41.4, 41.3, 38.6, 35.2, 35.1.

6,7-Bis(3-(benzyloxy)phenyl)-3-oxabicyclo[3.2.0]heptan-2-one (6b)

¹H NMR (400 MHz, CDCl₃) δ 7.46–7.28, 7.11–7.02, 6.98–6.86, 6.80–6.68, 6.64–6.59, 6.51–6.46 (6 x m, 18H major and 18H minor), 5.06, 4.90, 4.89, 4.86 (4 x bs, 4H major and 4H minor), 4.51 (dd, J = 9.7, 6.1 Hz, 1H major), 4.40 (d, J = 9.7 Hz, 1H major), 4.48–4.32 (m, 1H minor), 4.25 (dd, J = 10.3, 5.0 Hz, 1H minor), 4.17–4.02 (m, 2H major and 2H minor), 3.60 (q, J = 6.9 Hz, 1H), 3.52–3.44 (m, 1H minor), 3.39 (dd, J = 8.0, 2.6 Hz, 1H), 3.28 (t, J = 7.2 Hz, 1H minor). Major and minor: **¹³C NMR** (101 MHz, CDCl₃) δ 179.4, 178.2, 159.3, 159.2, 158.8, 158.7, 143.3, 140.4, 140.2, 139.9, 137.0, 130.1, 130.0, 129.3, 129.1, 128.7, 128.2, 128.1, 127.7, 127.6, 127.5, 120.7, 120.5, 119.6, 119.0, 115.0, 114.6, 114.1, 113.3, 113.2, 72.7, 70.1, 70.1, 69.1, 48.1, 47.6, 46.7, 45.7, 42.5, 40.6, 39.2, 35.3. **IR** (ATR): ν = 3372, 3063, 3032, 2907, 2871, 1765, 1602, 1583, 1491, 1452, 1378, 1316, 1293, 1263, 1162, 1081, 1045, 1027, 915 cm⁻¹. **HRMS** (ESI-Orbitrap) m/z : [M+K]⁺ calcd. for C₃₂H₂₈O₄K: 515.1619, found: 515.1622. R_f = 0.5 (PE/EtOAc = 6:4).

6,7-Bis(3-bromophenyl)-3-oxabicyclo[3.2.0]heptan-2-one (6c)

¹H NMR (400 MHz, CDCl₃) δ 7.46–7.39, 7.31–7.28, 7.26–7.19, 7.18–7.14, 7.13–7.09, 7.05–6.95, 6.89–6.84, 6.77–6.72 (8 x m, 8H major and 8H minor), 4.56 (dd, J = 9.8, 6.1 Hz, 1H), 4.45–4.37 (m, 1H major and 1H minor), 4.22 (dd, J = 10.4, 4.7 Hz, 1H minor), 4.15–4.03 (m, 2H major and 2H minor), 3.73–3.63 (m, 1H major), 3.54 (m, 1H minor), 3.40 (dd, J = 8.0, 1.7 Hz, 1H major), 3.29 (dd, J = 7.9, 6.5 Hz, 1H minor). Major and minor: **¹³C NMR** (101 MHz, CDCl₃) δ 178.7, 143.5, 140.4, 140.0, 130.9, 130.8, 130.7, 130.1, 130.0, 129.9, 129.8, 129.3, 126.7, 126.4, 125.2, 122.6, 122.5, 72.6, 68.9, 47.7, 47.3, 46.1, 45.4, 42.4, 40.3, 38.7, 35.3. **IR** (ATR): ν = 3373, 3061, 2967, 2906, 1764, 1594, 1566, 1476, 1428, 1372, 1332, 1251, 1165, 1094, 1047, 997 cm⁻¹. **HRMS** (ESI-Orbitrap) m/z : [M+H]⁺ calcd. for C₁₈H₁₄Br₂O₂: 420.9433, found: 420.9433. R_f = 0.4 (PE/EtOAc = 7:3).

6-(3,4-Dimethoxyphenyl)-7-phenyl-3-oxabicyclo[3.2.0]heptan-2-one (6e)

¹H NMR (400 MHz, CDCl₃) δ 7.38–7.31 (m, 5H), 7.19–7.14 (m, 2H), 7.13–7.10 (m, 1H), 7.02–6.98 (m, 2H), 6.89–6.86 (m, 1H), 6.77 (dd, J = 8.3, 2.1 Hz, 1H), 6.66 (d, J = 2.1 Hz, 1H), 6.63 (d, J = 8.2 Hz, 1H), 6.50 (dd, J = 8.4, 2.1 Hz, 1H), 6.16 (d, J = 2.1 Hz, 1H), 4.53 (dd, J = 9.6, 6.0 Hz, 1H), 4.44–4.39 (m, 1H), 4.39–4.36 (m, 1H), 4.29 (dd, J = 10.2, 4.7 Hz, 1H), 4.12–4.09 (m, 3H), 4.02 (t, J = 8.5 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.77 (s, 3H), 3.63–3.57 (m, 1H), 3.56 (s, 3H), 3.53–3.44 (m, 2H), 3.34–3.29 (m, 1H). **¹³C NMR** (101 MHz, CDCl₃) δ 179.6, 178.6, 149.5, 148.6, 148.4, 147.9, 141.9, 138.6, 131.1, 130.9, 128.9, 128.7, 128.4, 128.0, 127.3, 126.8, 126.4, 120.1, 119.9, 119.4, 111.6, 111.4, 110.6, 110.5, 72.7, 69.1, 66.0, 56.1, 55.9, 55.8, 48.0, 47.8, 47.0, 46.1, 42.4, 40.3, 40.1, 35.6, 15.4, 0.1. **IR** (ATR): ν = 3060, 2959, 2935, 2908, 2836, 1763, 1670, 1641, 1590, 1517, 1464, 1419, 1372, 1333, 1258, 1163, 1080, 1027, 912 cm⁻¹. **HRMS** (ESI-Orbitrap) m/z : [M+K]⁺ calcd. for C₂₀H₂₀O₄K: 363.0993, found: 363.0997. **R_f** = 0.6 (PhH/EtOAc = 8:2).

(2-Oxo-3-oxabicyclo[3.2.0]heptane-6,7-diyl)bis(3,1-phenylene) diacetate (6o)

¹H NMR (400 MHz, CDCl₃) δ 7.42–7.32, 7.20–7.25, 7.15–7.07, 7.07–6.87, 6.85–6.74, 6.73–6.68, 6.65–6.63 (7 x m, 8H major and 8H minor), 4.55–4.48, 4.43–4.37, 4.28–4.23, 4.19–4.06 (4 x m, 4H major and 4H minor), 3.70–3.62 (m, 1H major), 3.56–3.48 (m, 1H minor), 3.44–3.38 (m, 1H major), 3.34–3.28 (m, 1H minor), 2.31–2.27 (m, 6H minor), 2.26–2.22 (m, 6H major). Major and minor: **¹³C NMR** (101 MHz, CDCl₃) δ 179.0, 178.0, 169.4, 169.3, 151.3, 151.2, 150.8, 150.7, 143.2, 140.2, 139.9, 139.5, 130.2, 130.0, 129.4, 129.2, 125.6, 125.3, 124.5, 123.9, 121.1, 121.0, 120.7, 120.4, 120.0, 119.5, 72.6, 69.0, 47.9, 47.4, 46.3, 45.3, 42.4, 40.4, 39.1, 35.4, 21.3, 21.2. **IR** (ATR): ν = 3060, 2927, 2853, 1765, 1609, 1587, 1444, 1370, 1208, 1154, 1088, 1046, 1015, 921 cm⁻¹. **HRMS** (ESI-Orbitrap) m/z : [M+H]⁺ calcd. for C₂₂H₂₀O₆: 381.1332, found: 381.1326. **R_f** = 0.4 (PE /EtOAc = 1:1).

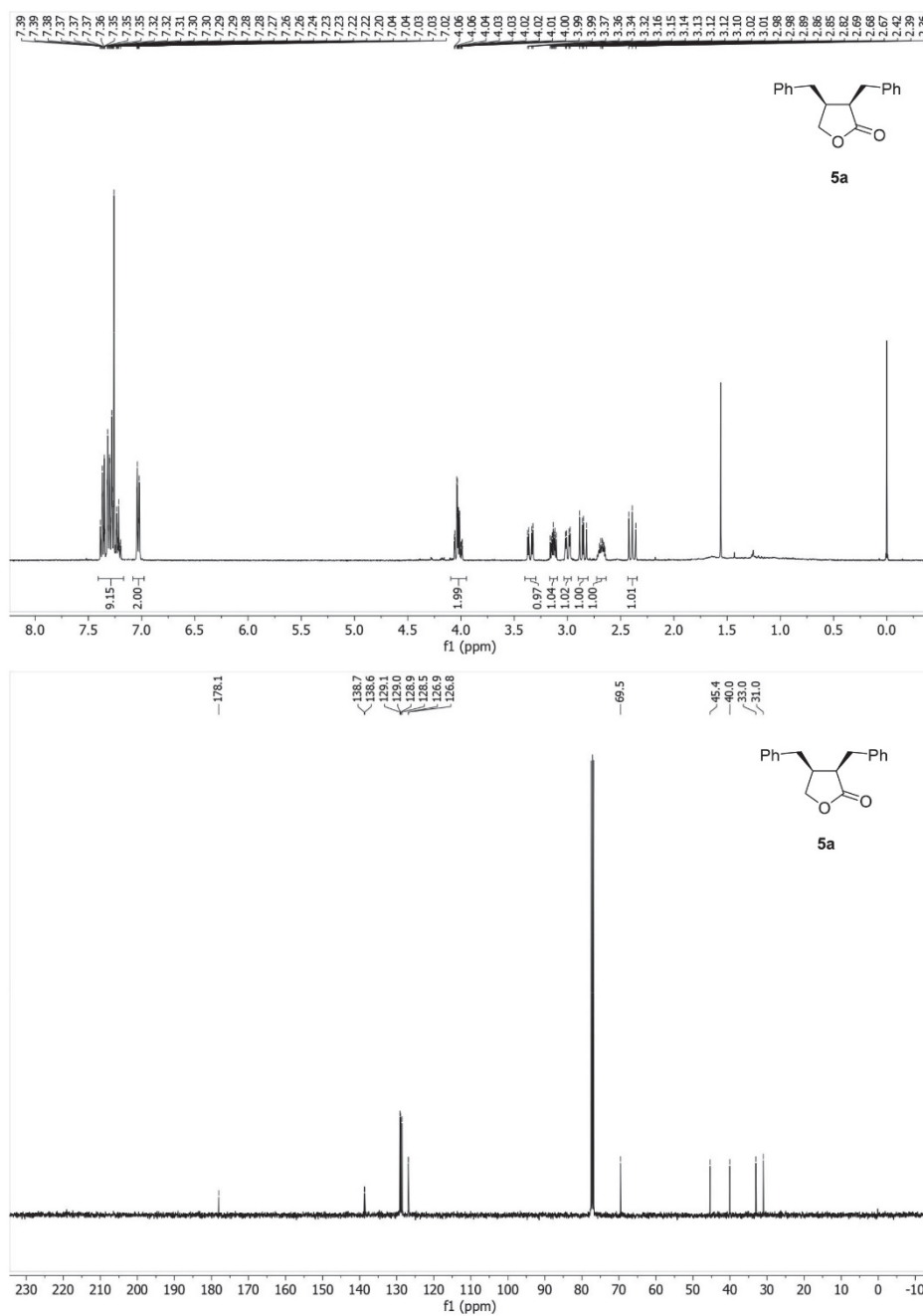
The figure displays the ^1H and ^{13}C NMR spectra of compound **6a**, which is 2,2-diphenyl-3-oxobicyclo[3.1.0]hex-6-ene. The chemical structure is shown above the spectra.

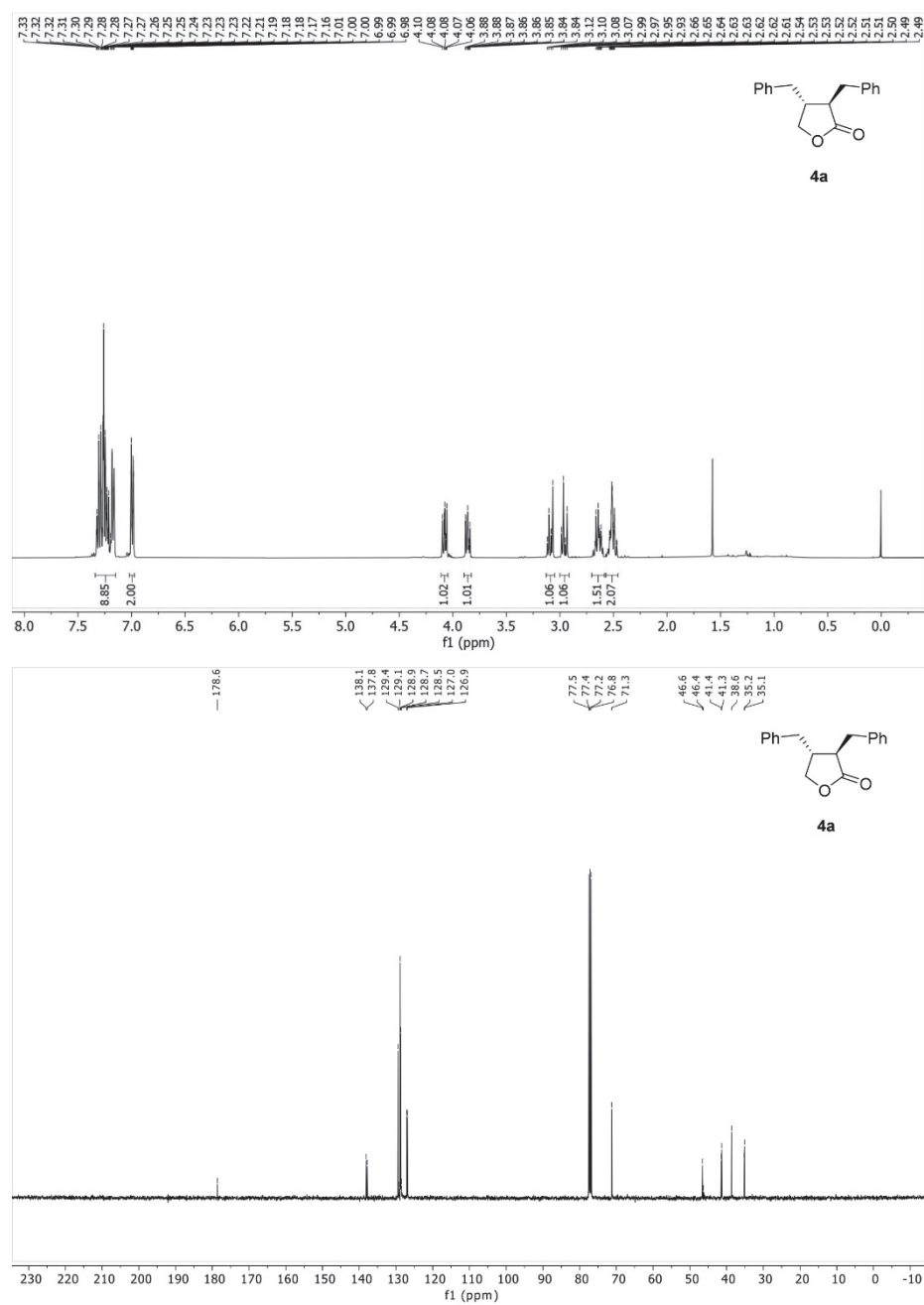
^1H NMR Spectrum (Top): The spectrum is recorded in CDCl_3 and shows peaks from 0.0 to 8.0 ppm. Integration values are provided below the baseline. The chemical shift values (ppm) are listed at the top of the spectrum.

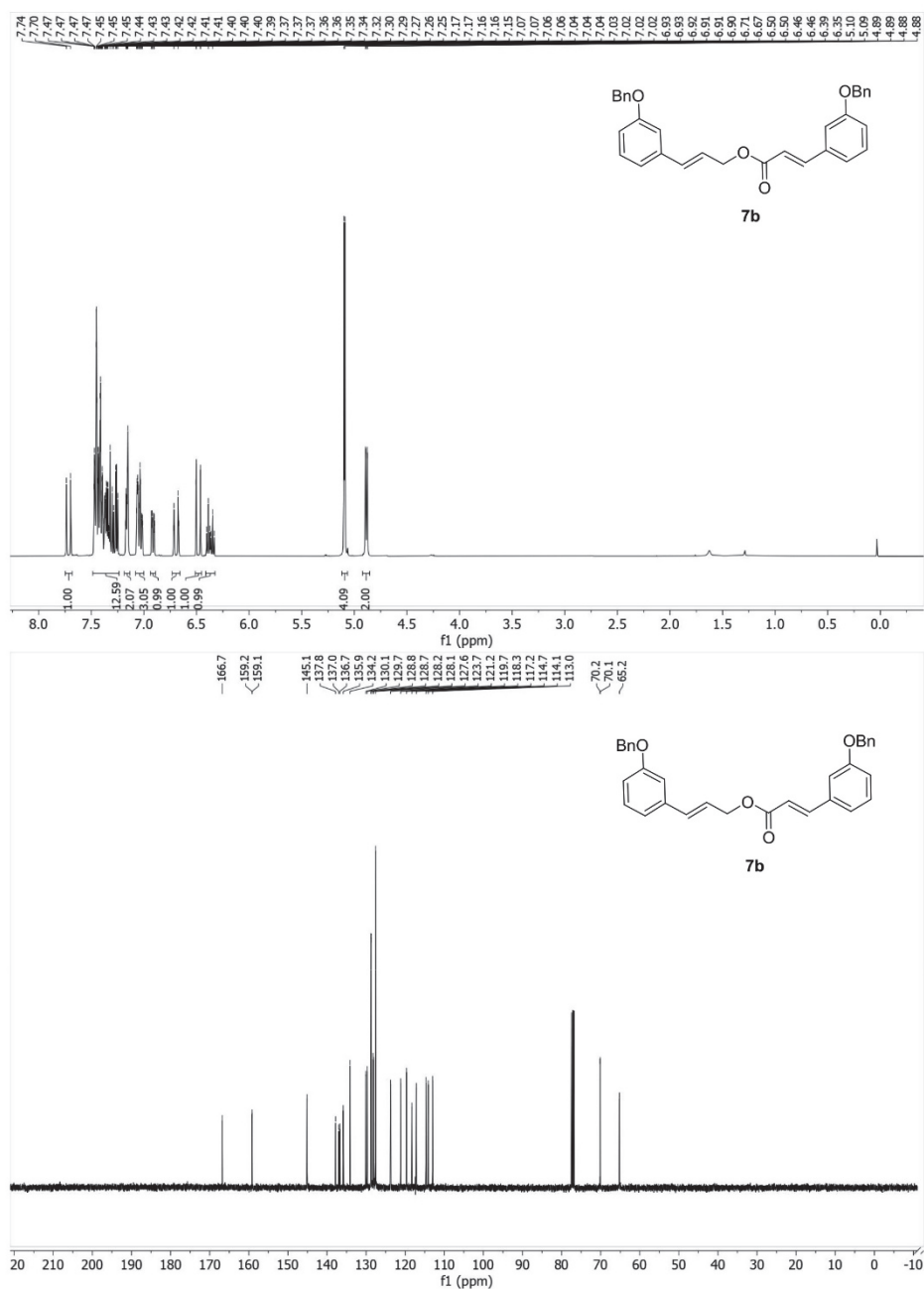
Chemical Shift (ppm)	Integration
7.46, 7.38, 7.36, 7.35, 7.33, 7.32, 7.30, 7.29, 7.28, 7.27, 7.26	3.36, 1.28, 2.02, 2.02
7.20, 7.18, 7.15, 7.13, 7.11, 7.10, 7.09, 7.08, 7.07, 7.06, 7.05, 7.03, 7.03, 6.99, 6.99, 6.97, 6.86, 6.84, 6.84, 4.58, 4.57, 4.56, 4.54, 4.45, 4.43, 4.41, 4.41, 4.38, 4.29, 4.28, 4.27, 4.25, 4.15, 4.17, 4.15, 4.13, 4.13, 4.11, 4.09, 3.71, 3.70, 3.68, 3.54, 3.46, 3.46, 3.44, 3.43, 3.34, 3.32	1.04, 1.39, 0.29, 2.59, 1.07, 0.34, 1.00, 0.29

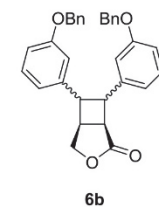
^{13}C NMR Spectrum (Bottom): The spectrum is recorded in CDCl_3 and shows peaks from 35.4 to 198.5 ppm. The chemical shift values (ppm) are listed below the baseline.

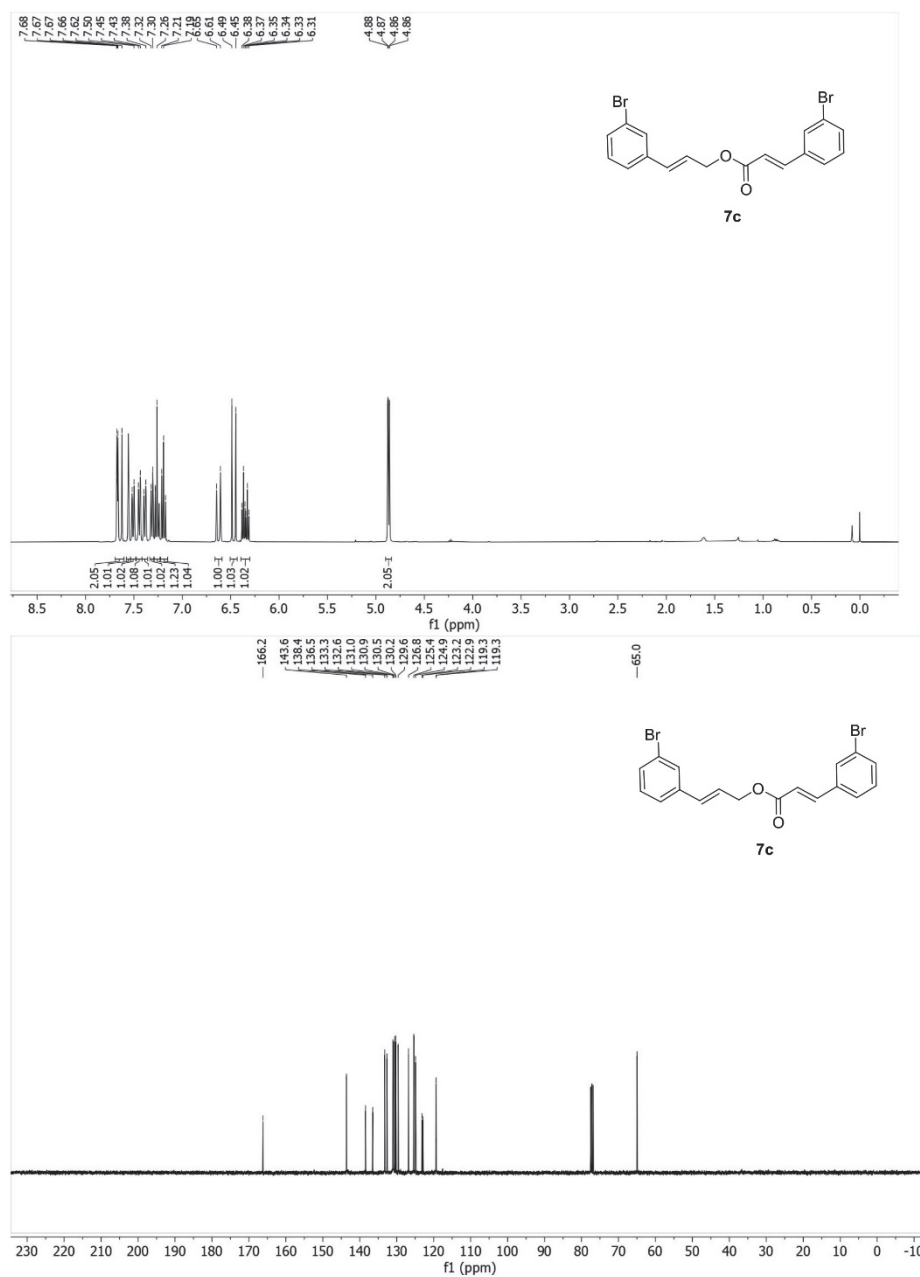
Chemical Shift (ppm)
198.5, 178.4, 141.7, 138.7, 138.5, 138.1, 138.0, 129.0, 128.8, 128.2, 128.1, 128.0, 127.7, 127.3, 127.1, 126.7, 126.6, 126.4, 48.2, 47.7, 46.7, 45.9, 42.6, 40.6, 38.0, 35.4

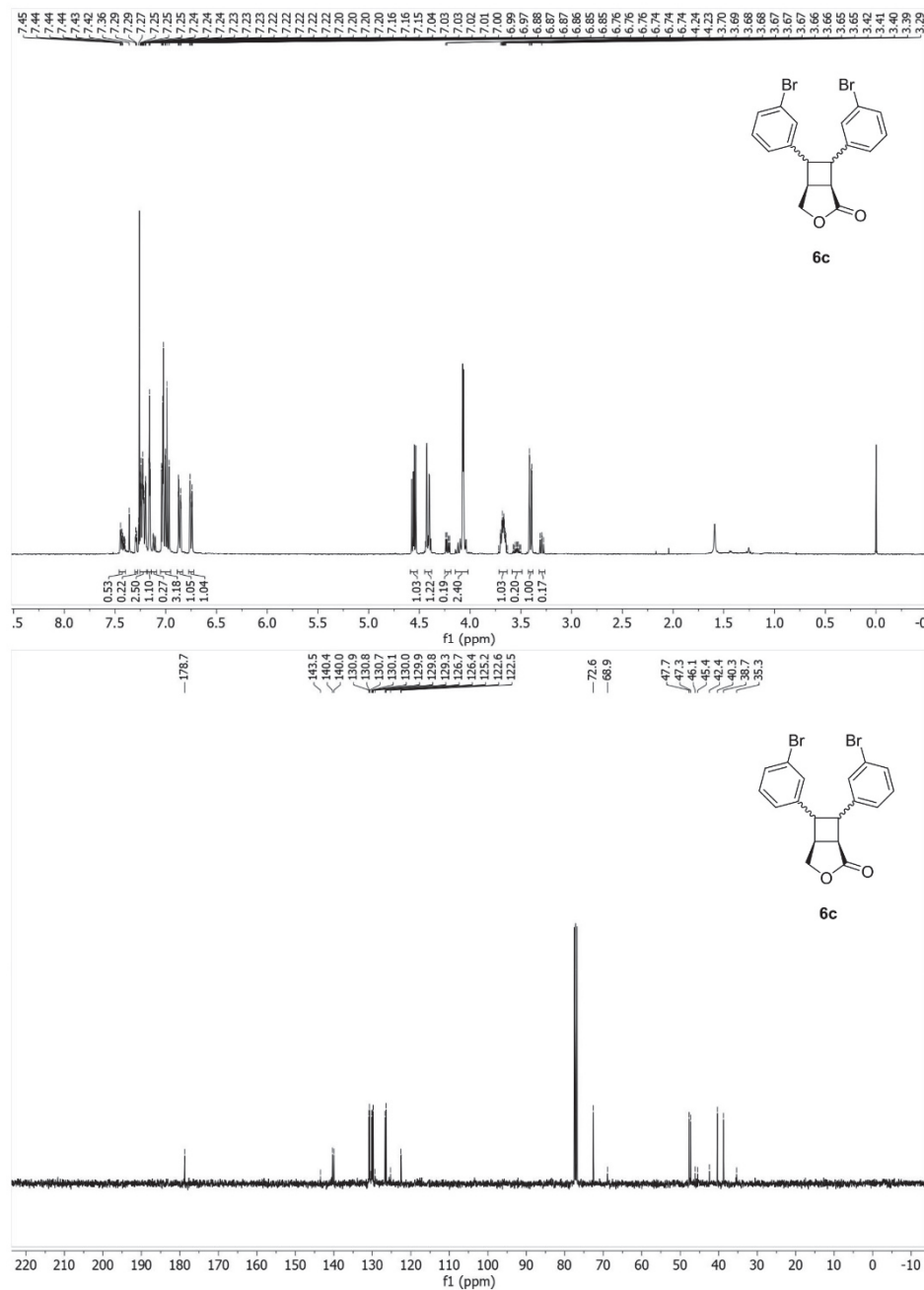


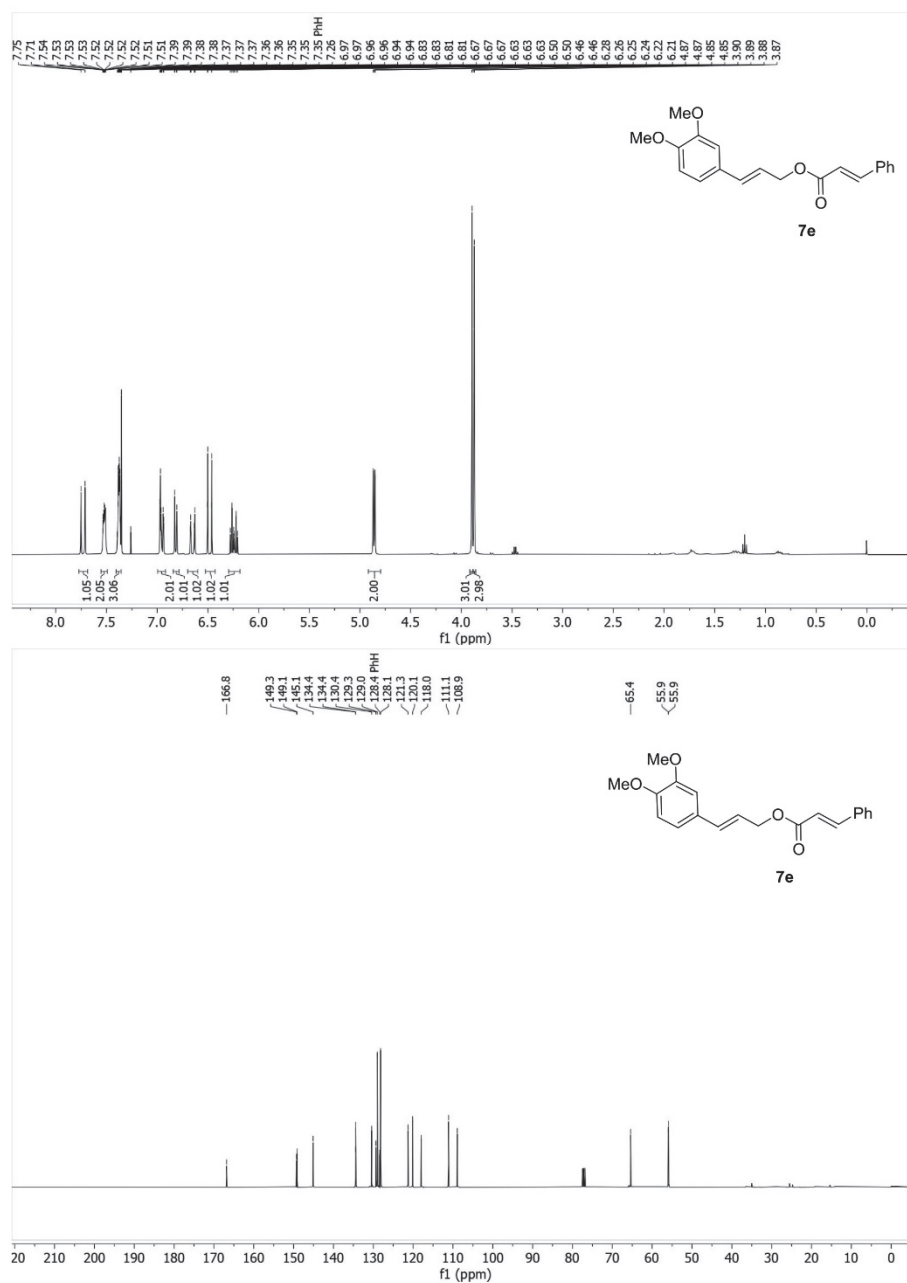


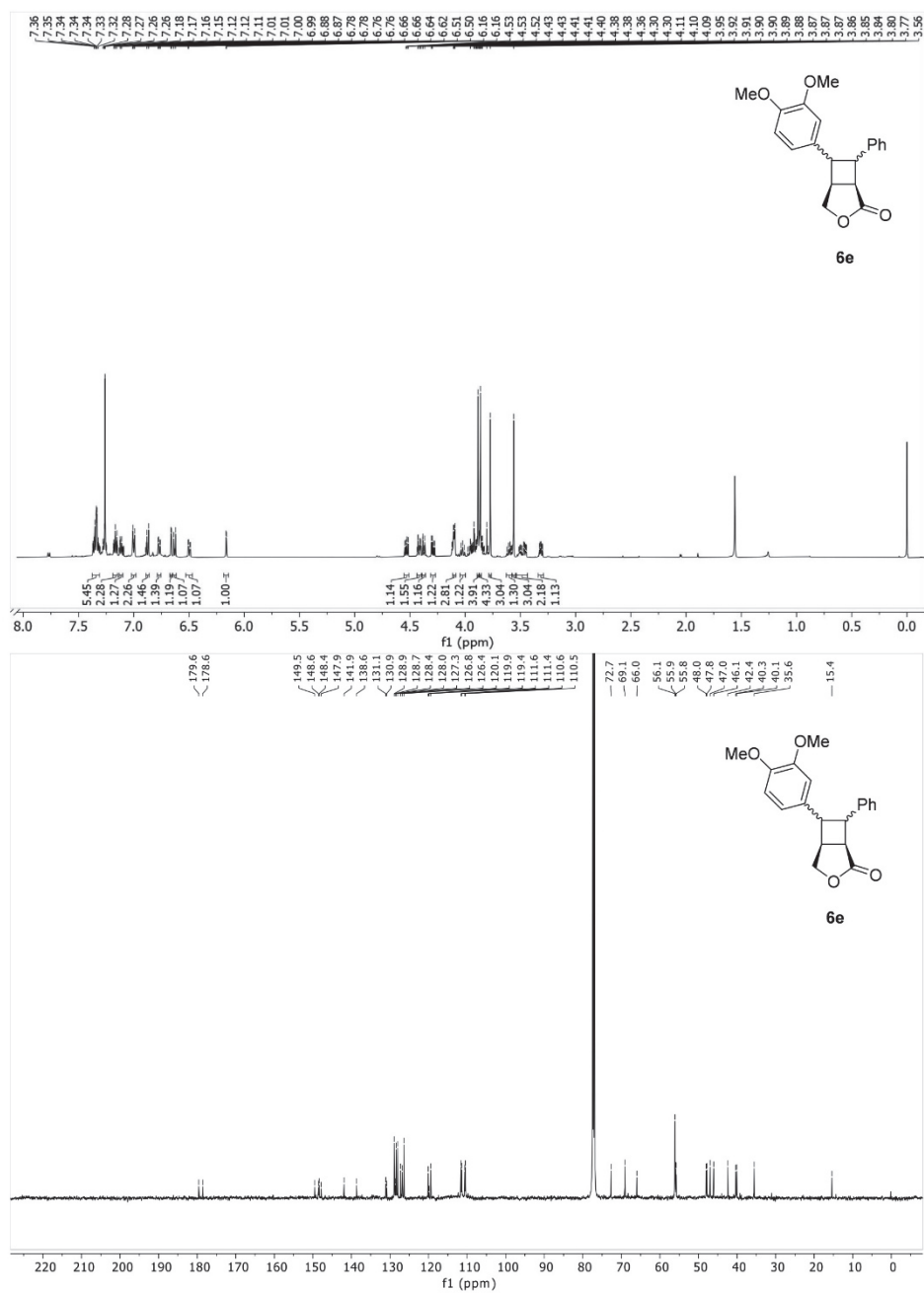


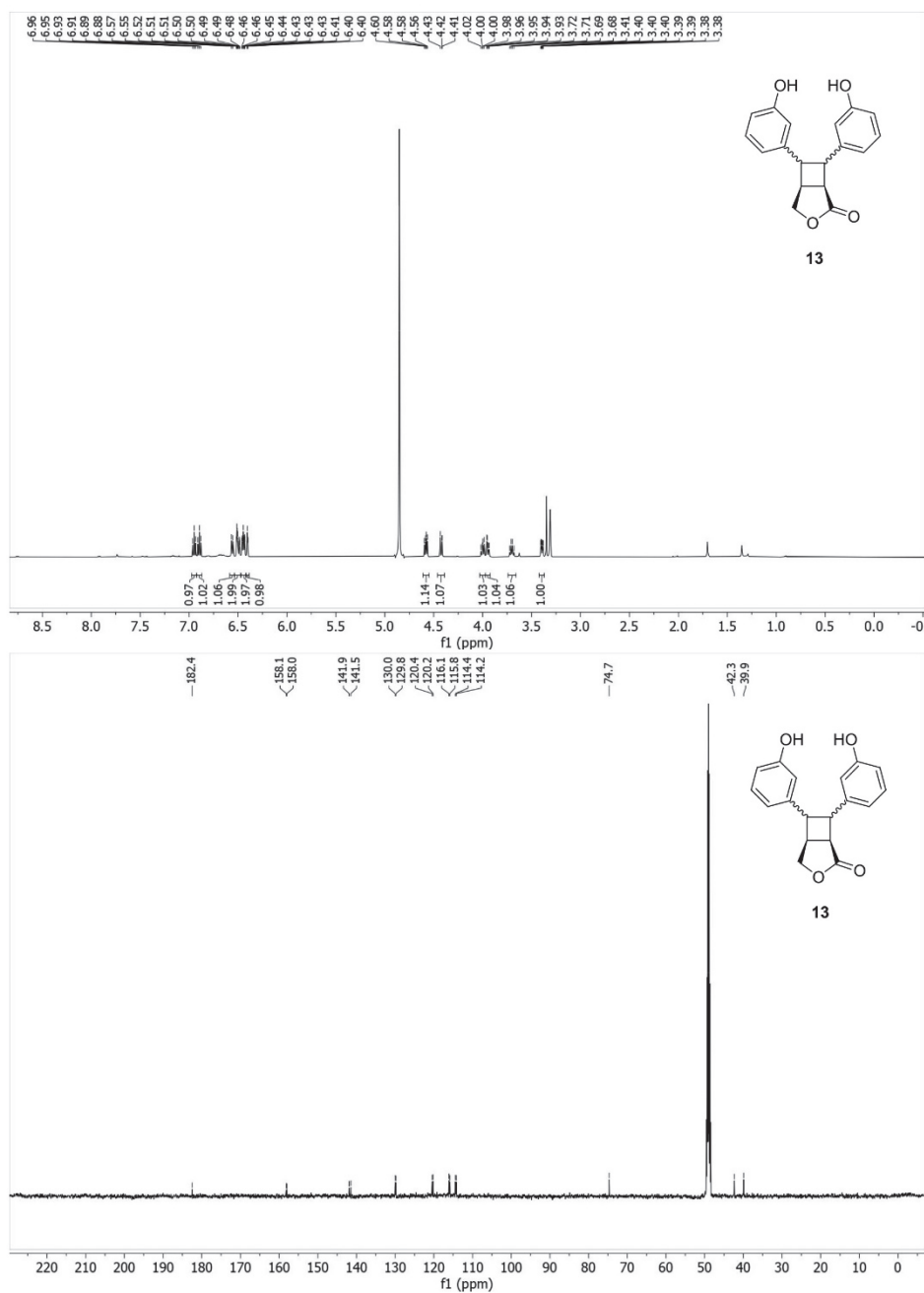


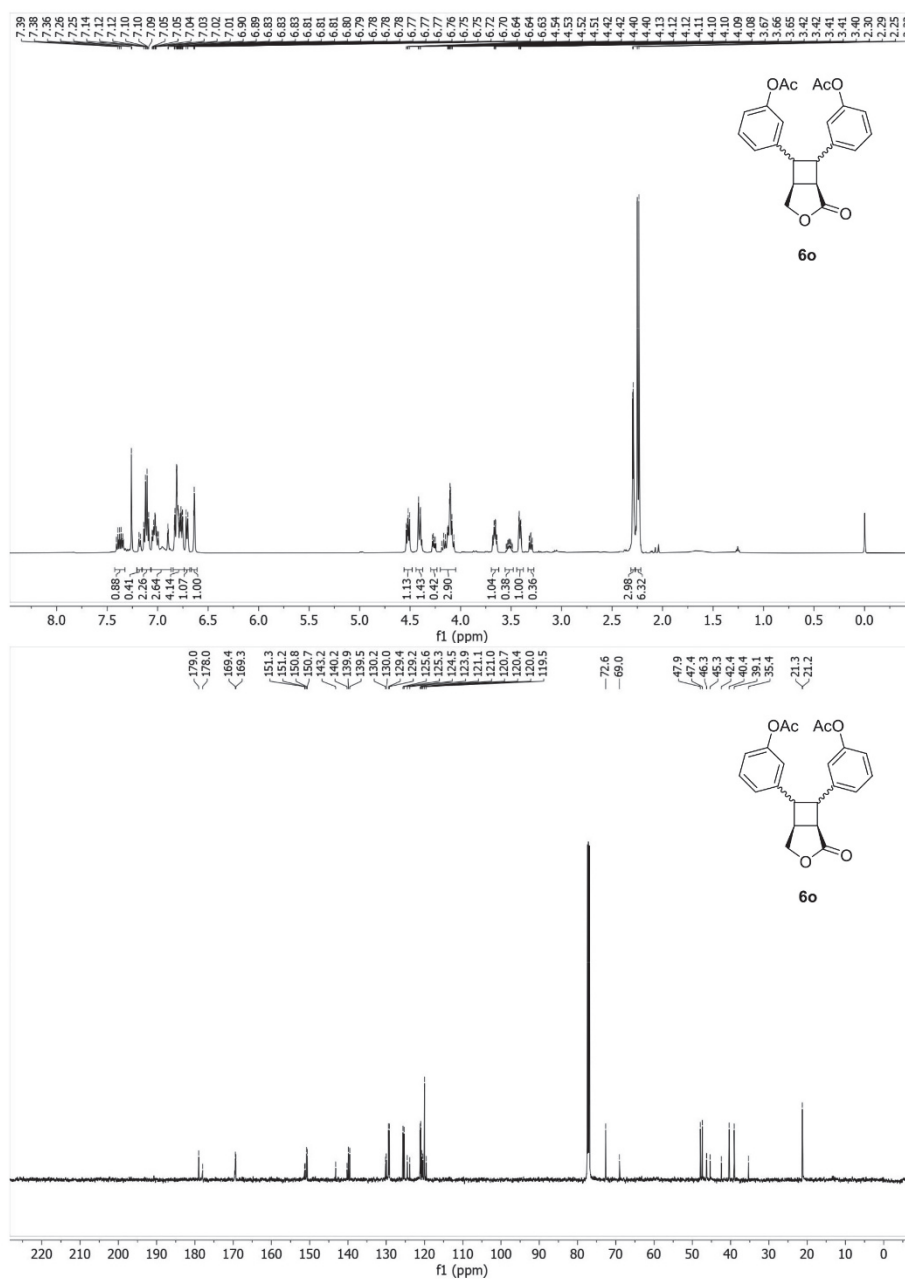












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JSCS–5518

Metal complexes with Schiff base and benzimidazolyphenol ligands with *tert*-butyl and methoxy groups: Structural characterization and biological evaluation

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Abstract: Two chelating ligands bearing *tert*-butyl and methoxy groups, an ONO type Schiff base (**H₂L¹**) and a benzimidazolyphenol derivative (**HL²**), NO type ligand, and their complexes with CoCl₂, NiCl₂, CuCl₂, ZnCl₂ and K₂PdCl₄ (**1a–e** and **2a–e**, respectively) were synthesized and characterized. Various physico-chemical and spectroscopic techniques were used to characterize the ligands. In addition, the crystal structure of **H₂L¹** was determined by X-ray diffraction spectroscopy. Keto–enol tautomerism is observed in **H₂L¹** according to spectral data. Elemental analysis, molar conductivity, magnetic moment and thermogravimetric analysis, as well as FT-IR, fluorescence, UV–Vis and NMR (for diamagnetic complexes) spectroscopic techniques, were utilized to characterize the complexes. Then, antibacterial, antifungal and antiviral activities of the compound were investigated. Antimicrobial activities of the compounds were tested toward six bacteria and three fungi. Some of the complexes exhibited higher activity towards some microorganisms than the ligands. **HL²** and its complexes were observed to show higher activity compared to **H₂L¹** and its complexes, especially against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli* bacteria, and the fungus *Candida tropicalis*. It was found that **2a** has a potent activity against *S. aureus* and *E. coli*, **2b** against both Gram-positive bacteria (*S. aureus* and *S. epidermidis*) and **2c** against *S. aureus*, *S. epidermidis* and *E. coli* compared to other complexes. Antiviral activity of the compounds was tested against Parainfluenza type-2 virus. In terms of antiviral effect, **H₂L¹** showed

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higher activity than **HL**² and the Co(II) and Cu(II) complexes of **HL**² had the highest activity among the compounds, with rates of 62 and 64 %, respectively.

Keywords: chelating ligand; imine; transition metal complex; antimicrobial; antiviral.

INTRODUCTION

Imine compounds derived from *o*-aminophenols and salicylaldehydes are known as ONO type Schiff bases. Such ligands constitute a class of compounds of considerable interest because of their ability to form chelate structures, as for variety of applications and many interesting properties.¹⁻⁴ They form strong chelate complexes due to tridentate characteristics.⁵ ONO type Schiff bases are known to exhibit a wide range of biological effects such as antibacterial,^{6,7} antifungal,^{8,9} anticancer¹⁰⁻¹³ and antidiabetic¹⁴ activities. Due to these versatile characteristics, they have significant applications in both industry and biology.

A benzimidazole derivative containing a phenol group is called benzimidazolylphenol. Current and widespread research is being conducted on benzimidazolylphenols, and the results of these studies might be applicable in many areas. In addition, since these compounds have strong fluorescent properties, many studies were published examining photophysical properties along with their complexes.¹⁵⁻¹⁷ We have also conducted various studies on many benzimidazolylphenol derivatives and some of their metal complexes, and we reported that these compounds have antimicrobial effects on some bacteria and fungi.¹⁸⁻²¹ For example, it was determined that 2-(5-bromo-1*H*-benzimidazol-2-yl)phenol compounds, especially those containing second and even third hydroxyl groups in the phenol ring, have both antioxidant and antibacterial effects considerably higher than others.²² Cl, Br and NO₂ groups in some 5-methoxy-2-(5-substituted-1*H*-benzimidazol-2-yl)phenols were observed to increase the antimicrobial activity toward *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans*.²³

As is known, Cu(II) and Zn(II) ions are essential microelements having important roles in the human body such as enzyme modulators, catalytic and regulatory, oxidative-reductive, *etc.* Cobalt(II), which is a part of vitamin B12, is also an essential metal ion. Various biological activities of some Ni(II) and Pd(II) complexes were also reported.^{24,25}

The aim of this study is to prepare a salicylic-based Schiff base and benzimidazolylphenol ligands (**H₂L**¹ and **HL**², Fig. 1) containing *tert*-butyl and methoxy groups and their metal complexes with CoCl₂, NiCl₂, CuCl₂, ZnCl₂ and K₂PdCl₄ and to investigate their structural properties and some biological effects. In the context of biological activity, the antibacterial, antifungal and antiviral activities of all compounds were investigated. **H₂L**¹ and its various complexes were reported.²⁶⁻³⁰ 3d/4f Coordination clusters as cooperative catalysts for diastereoselective Michael addition reactions of Zn₂Y₂ complexes were investigated by Griffiths

*et al.*²⁶ Ke *et al.* studied structures and magnetic properties of heterometallic Zn_2Dy_2 tetranuclear clusters.^{27,28} Antimicrobial activities of Ni(II) and Ni(II)+Zn(II) complexes of H_2L^1 were investigated by Zhao *et al.*²⁹ Crystallographic insights and biological potency against malaria and oxidative stress of organotin complexes of H_2L^1 were considered.³⁰ HL^2 derivative of benzimidazolyphenol is commercially available but is being published here for the first time.³¹

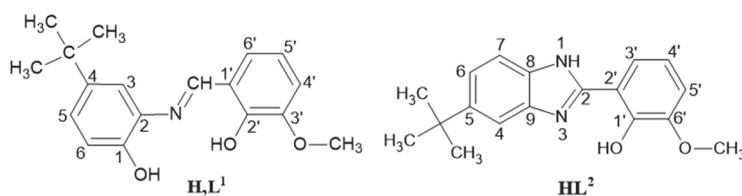


Fig. 1. Chemical structures of the ligands in the study.

EXPERIMENTAL

Chemistry and apparatus

All chemicals and solvents were of reagent grade and they were used without further purification.

The devices and techniques used are as follows. Elemental analysis: LECO combustion analyzer CHNS-932. Melting points: Buchi M-560 melting-point apparatus. Molar conductivity: WTW Cond315i conductivity meter (in DMF at 25 °C). Magnetic measurements: MK1 Sherwood Scientific apparatus (at room temperature by Gouy's method. Diamagnetic corrections were calculated using Pascal's constants). NMR spectroscopy: Varian Unity Inova 500 NMR spectrometer. FT-IR spectroscopy: Perkin Elmer Spectrum Two spectrometer with attenuated total reflection (ATR) techniques, between 400 and 4000 cm^{-1} . The electron spray ionization-mass spectroscopy (ESI-MS positive ion mode): Thermo Finnigan LCQ Advantage MAX LC/MS/MS (in MeOH). Thermogravimetric studies: TG-60WS Shimadzu, between 40 and 700 °C, with a heating rate of 10 °C/min and air flowing at the rate of 50 mL/min. UV-Vis spectra were performed on Shimadzu UV-1800 spectrophotometer in EtOH. Fluorescence spectra: Shimadzu RF-5301 PC spectrofluorophotometer ($c \approx 1 \times 10^{-4}$ mol/L in EtOH).

Synthesis of the ligands

4-tert-Butyl-2-[(E)-(2-hydroxy-3-methoxyphenyl)methylidene]amino}phenol (H_2L^1). 3-methoxysalicylaldehyde (0.78 g, 5 mmol) and 2-amino-4-tert-butylphenol (0.825 g, 5 mmol) were dissolved in EtOH (25 mL) and refluxed for 3 h. The crystals formed in the solution that was filtered and kept at room temperature. Red solid. Yield: 77 %.

2-(5-tert-Butyl-1H-benzimidazol-2-yl)-6-methoxyphenol (HL^2). a modified method developed by us utilizing two different methods available in the literature was applied for synthesis of HL^2 .³²⁻³⁴ 3-Methoxysalicylaldehyde (0.78 g, 5 mmol), 4-tert-butylbenzene-1,2-diamine (0.82 g, 5 mmol) and 250 mg H_3BO_3 as catalyst were dissolved in DMF (25 mL) and then this mixture was refluxed for 3 h. After cooling to the room temperature, the reaction mixture was poured into water (250 mL) and then a precipitate was formed. It was filtered, dried and crystallized from ethanol after. Light yellow solid. Yield: 81 %.

Preparation of the complexes

Appropriate metal salt solutions (1 mmol of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{ZnCl}_2 \cdot 6\text{H}_2\text{O}$ in 10 mL of ethylacetate; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in 10 mL methanol, K_2PdCl_4 (was obtained by dissolving 1 mmol PdCl_2 (0.177 g) and 2 mmol KCl (0.15 g) in 10 mL $\text{MeOH} + \text{H}_2\text{O}$ mixture (6:4 volume ratio))) were added gradually to the solutions of the ligands (1 mmol, *i.e.*, ~300 mg of H_2L^1) in appropriate solvent (10 mL) and heated for 3 h under reflux. Cu(II) and Pd(II) salts gave immediately precipitates with the ligands. The other complex solutions were kept at room temperature; precipitates formed within a few days. The precipitates were filtered off, washed with distilled water and kept at room temperature to dry.

Analytical and spectral data of the synthesized ligands, and complexes **1a–e** and **2a–e** compounds are given in the Supplementary material to this paper.

Determination of antibacterial and antifungal activity

Antimicrobial activities of the compounds were investigated against standard ATCC strains of bacteria and fungi by the microbroth dilutions way according to the Clinical and Laboratory Standards Institute (CLSI) guidance.^{35,36} The antimicrobial activity procedure and the microorganisms used are the same as in our previous studies.^{8,9} The experiments were carried out in duplicate.

Antiviral activity assay

For Parainfluenza Type-2 virus (PIV-2), African green monkey kidney cell line (VERO, ATCC) was cultured in Dulbecco's modified Eagle's medium (Wisent, Multicell) supplemented with 10 % fetal bovine serum (FBS; Sigma), 100 U/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin. The PIV-2 and cells were maintained at 37 °C under 5 % CO_2 atmosphere. An experiment was conducted based on the plaque formation test for PIV-2 strain that grown on VERO cell culture.³⁷ VERO cells were infected with PIV-2 at 100 PFU per well, one of the wells was used as a control and the antiviral activities of the samples were tested in the other wells. The virus-infected control (ribavirin) well was accepted as 100 %, and the efficacy results were calculated as a percentage by comparing this control value. The concentration of the compounds is 10 mg/mL.

X-ray single crystal diffraction analysis

H_2L^1 was crystallized from EtOH. The red colored single crystals were obtained. Suitable crystals were selected for data collection which was performed on a Bruker-D8 QUEST diffractometer equipped with a graphite-monochromatic Mo-K_α radiation at 296 K. The structure of H_2L^1 was solved by direct methods using SHELXS-2013³⁸ and refined by full-matrix least-squares methods on F^2 using SHELXL-2013.³⁹ All non-hydrogen atoms were refined with anisotropic parameters. The H atoms of C atoms were located from different maps and then treated as riding atoms with C–H distance of 0.93–0.97 Å. The other H atoms were located in a difference map refined freely. The following procedures were implemented in our analysis. Data collection: Bruker APEX2;⁴⁰ program used for molecular graphics were as follow: Mercury programs;⁴¹ software used to prepare material for publication: WinGX.⁴² Details of data collection and crystal structure determinations are given in Table S-I of the Supplementary material. Crystallographic data for the structural analysis are deposited with the Cambridge Crystallographic Data Centre, CCDC No. 2298000.

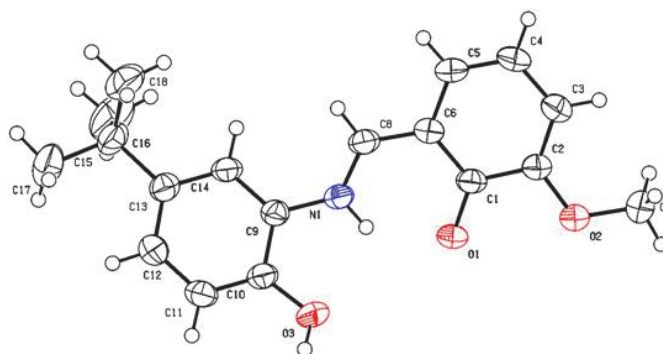
RESULTS AND DISCUSSION

Crystal structure of $\mathbf{H_2L^1}$

Crystal data and structure refinement and hydrogen bond parameters related $\mathbf{H_2L^1}$ are given in Tables S-I and I, respectively. Selected bond distances and angles are given in Table S-II of the Supplementary material. Molecular structure of $\mathbf{H_2L^1}$ is shown in Fig. 2.

TABLE I. Hydrogen bonding parameters for $\mathbf{H_2L^1}$; symmetry code: (i) $-x+1/2, y+1/2, z$

D-H...A	D-H, Å	H...A, Å	D...A, Å	D-H...A, °
N1-H1...O1	0.88(2)	1.88(2)	2.598(2)	138(2)
O3-H3A...O1 ⁱ	0.83(2)	1.83(2)	2.657(2)	178(3)

Fig. 2. Molecular structure of $\mathbf{H_2L^1}$ showing the atom numbering scheme.

From the X-ray data, it appears that the keto structure is valid for $\mathbf{H_2L^1}$ in solid state as stated in our previous studies.^{2,9} In the crystal structure of $\mathbf{H_2L^1}$, the C–O bond length in the 2-hydroxy-3-methoxyphenyl part is shorter than the other (4-*tert*-butyl-2-hydroxyphenylimino part, 1.289(2) and 1.353(3) Å, respectively). This difference between the C–O bond lengths is due to the keto structure being more dominant than the enol structure.

General properties

$\mathbf{H_2L^1}$, an ONO type imine compound, is a dibasic and tridentate chelating ligand whereas $\mathbf{HL^2}$ is a monobasic bidentate chelating NO type ligand. ESI-MS data showing formation of the ligands are given in the experimental section, and the spectra are given in the Supplementary material (Figs. S-1 and S-2). Both ligands appear to tend to form 1:1 complexes, which is probably due to the steric effect of the substituents. It was also expected that $\mathbf{HL^2}$, which is a bidentate ligand, would give a 1:2 complex, but the steric effect of the substituents and the

character of the metal salt (chloride salts) can be considered factors that favor the 1:1 formation.

The magnetic moment values of the Ni(II) complexes were found to be 2.04 and 2.05 BM for **H₂L¹** and **HL²** complexes, respectively. These values are lower than the 2.83 BM expected for octahedral or tetrahedral geometries, which are paramagnetic structures that the d⁸ ion with two unpaired electrons may have. Such low values are generally thought to be due to the distorted geometry between the tetrahedral and square planar systems, called quasi-tetrahedral.^{43–45} Co(II) complex of **H₂L¹** (**1a**) has a magnetic moment of 4.06 BM according to the measurements. It was reported that geometry of Co(II) complexes, having magnetic moment values below 4.70 BM, is trigonal bipyramide or square pyramidal (penta-coordinated).⁴⁶ According to this finding, the geometry of **1a** may be regarded as square pyramidal in a high-spin structure (three unpaired electrons). The magnetic moment value of Cu(II) complex of **H₂L¹** (**1c**) at room temperature was found to be 1.72 BM, indicating formation of a monomeric Cu(II) complex. This value is very close to the theoretical value of 1.71 BM for the d⁹ electron configuration. The Cu(II) complex of **HL²** (**2c**) was found to be 1.01 BM, which indicates a Cu(II)–Cu(II) interaction. It suggests that a dimeric structure with a chloride bridge is formed in this complex and thus the Cu(II)–Cu(II) interaction occurs.

The magnetic moment value of the Co(II) complex of **HL²** (**2a**) was measured as 2.17 BM. This value indicates a structure with 1 and 2 unpaired electrons, which may result from a chloride-bridged structure or five-coordination geometry showing M–M interaction for the Co(II) ion with the d⁷ structure.⁴⁷

The molar conductivity values of the complexes, except for the Pd(II) complex of **H₂L¹** (**1e**) in DMF, were measured to be below 50 Ω^{–1} cm² mol^{–1}. According to Geary, compounds with a molar conductivity below 50 Ω^{–1} cm² mol^{–1} are considered non-ionic or non-electrolyte.⁴⁸ It can be concluded that the Pd(II) complex of **H₂L¹**, which has a molar conductivity value of 83.2 Ω^{–1} cm² mol^{–1}, is 1:1 electrolyte. This result, which is also supported by the elemental analysis values, shows that the K[Pd(L¹)Cl] structure is possible.⁸

NMR spectra

NMR spectral data and their assignments of the ligands and the diamagnetic complexes are given in the Supplementary material. ¹H- and ¹³C-NMR spectra of the compounds are given in Figs. S-3–S-14.

There is significant information for structural characterization of Schiff base **H₂L¹** having two hydroxyl groups (salicylic and phenolic OHs) in the ¹H-NMR. The signals at 9.57 and 9.00 ppm should belong to phenolic OH and N=CH protons, respectively.¹⁰ Salicylic OH proton, on the other hand, was dissociated due to its high acidic character and its signal could not be detected by the device. As a result, it can be asserted that the keto state of **H₂L¹** is dominant in the solid state

while the enol state is dominant in the solution (Fig. 3). According to the crystal structure analysis, **H₂L¹** has a keto structure in the solid state.

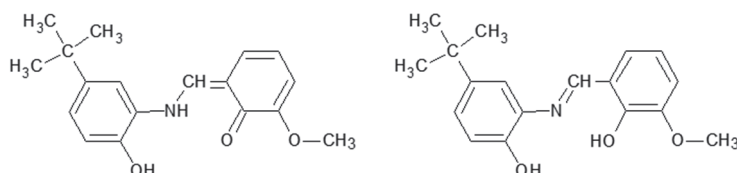


Fig. 3. Structure of **H₂L¹** in solid state (keto form, left) and solution with polar solvents (enol form, right).

Phenolic protons of the ligands are not observed in the ¹H-NMR spectra of the Pd(II) and Zn(II) complexes, since they removed along with the complex formation. In addition, the azomethine proton (-N=CH-) of the Schiff base ligand shifted considerably to the lower frequency (upfield) on complexation. For example, N=CH proton shows shifting with 0.36 ppm (from 9.00 to 8.64 ppm) in the Pd(II) complex of **H₂L¹**. It appeared at 8.86 ppm in the Zn(II) complex.

The signal at 161.6 ppm in the ¹³C-NMR spectra of **H₂L¹** should be due to azomethine carbon atom in enol form of the imine compound. The signals of the carbon atoms bonded to the oxygen atom (C1, C2' and C3') of **H₂L¹** are detected at the 148.6–152.5 ppm range. In the Pd(II) complex, the azomethine carbon shifted to 174.3 ppm, while the carbon atoms to which the oxygen atoms are bonded were found between 161.5 and 146.9 ppm. In the Zn(II) complex, the N=CH carbon was detected at 164.8 ppm, while the carbon atoms bonded to oxygen were detected between 161.3 and 144.8 ppm.

In **HL²**, the NH and OH protons gave a single broad band at 13.12 ppm (integral value = 2H). The broad signals corresponding to a single proton seen at 13.64 and 13.22 ppm in the Zn(II) and Pd(II) complexes, respectively, should belong to the NH proton. The disappearance of the signal belonging to the OH proton indicates that this proton was removed with the complexation of the phenolic oxygen. Sufficient data could not be obtained from ¹³C-NMR analyses of complexes of **HL²**.

IR spectra

The major IR spectral data are presented in the Supplementary material. FTIR spectra of the compounds are given as supplementary material (Figs. S-15–S-26). The IR spectra of the Schiff base (**H₂L¹**) and its complexes show medium intensity absorption bands at 1630–1647 cm⁻¹ assigned to the ν(C=N). The C=N stretching mode of the benzimidazolyphenol derivative (**HL²**) and its complexes are observed at the 1602–1619 cm⁻¹ range. The medium bands seen between 1576 and 1619 cm⁻¹ can be attributed to the C=C stretching mode of the ring systems, which is expected to appear around 1600 cm⁻¹. On complexation, considerable changes

such as a shift to a higher wavenumber and a decrease in the intensity of absorptions were observed in the C=N band characteristic. These changes may support the argument of the imine nitrogen atom coordination in the complexes of both Schiff base and benzimidazolyphenol ligands.

The strong bands at the 1182–1258 cm^{-1} range are attributed to the C–O stretching vibrations of the ligands and the complexes.^{49,50}

In the spectra of all the compounds, generally weak bands appearing between 3054 and 3080 cm^{-1} can be attributed to stretching vibration of aromatic CH; strong or medium intensity bands detected in the range of 812–831 cm^{-1} can be interpreted as originating from the bending modes of aromatic CH groups.⁵¹

As a difference from the ligands, the new broad medium or weak bands between 667 and 688 cm^{-1} in the IR spectra of the complexes, can be attributed to $\nu(\text{M} \leftarrow \text{N})$ coordination band (Fig. 4).²²

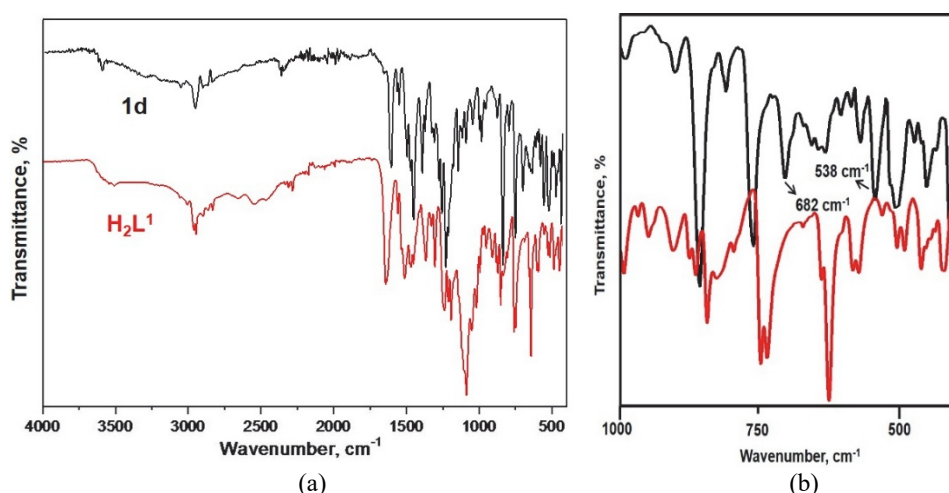


Fig. 4. Infrared spectra of H_2L^1 (a) and its Zn(II) complex (b), **1d**.

New medium bands at lower frequency at the 536–545 cm^{-1} range in the complexes may be assigned to $\nu(\text{M} \leftarrow \text{OC})$ vibration frequencies and this finding can be considered as supporting information for the phenolic oxygen atom coordination (Fig. 4).⁵²

UV–Vis spectra

The UV–Vis absorption spectra were obtained in dimethylsulfoxide (DMSO) at room temperature. Experimental maximum absorption wavelength data of the compounds are presented in the Supplementary material. UV–Vis spectra of the compounds are given in the Supplementary material (Figs. S-27–S-38). The bands between 300 and 350 nm involve $\pi \rightarrow \pi^*$ transitions whereas the bands at the range

of 350–400 nm can be attributed to $n \rightarrow \pi^*$ transitions. The band at 456 nm in **H₂L¹** is associated with intramolecular charge transfer transitions.⁵³

The absorptions at 451 and 458 nm in the Pd(II) complexes of **H₂L¹** and **HL²**, respectively, can be assigned to d–d transitions and indicate square planar stereochemistry in solution and can be assigned to $^1A_{1g} \rightarrow ^1A_{2g}$ transition.⁹ The weak bands appearing in the visible region can be attributed to d–d transitions. For example, the bands in the range of 433–478 nm in **1b**, **1c**, **2b** and **2c** (Ni(II) and Cu(II) complexes), can be associated with the $^4A_2 \rightarrow ^4T_1(P)$ (v_3) transition arising from a tetrahedral geometric structure.⁴² The 464 and 473 nm transitions observed in Co(II) complexes, for **1a** and **2a**, respectively, can be attributed to the $^3B(F) \rightarrow ^3E(P)$ transitions in the square pyramidal structure in solution environment.^{54,55} Since there are no allowed d–d transitions in Zn(II) complexes (**1d** and **2d**), the bands at the 350–465 nm range probably originate from the intra-ligand and metal-to-ligand charge transfer transitions.

Fluorescence spectra

Fluorescence spectroscopy measurements of **HL²**, a benzimidazolyphenol derivative, and its complexes were carried out. Fluorescence spectral data of the compounds, obtained at a concentration of approximately 1×10^{-4} mol/L in ethanol at room temperature, are given in experimental section (excitation wavelength: 354 nm). The fluorescence spectra of **HL²** and its complexes are given in Fig. 5.

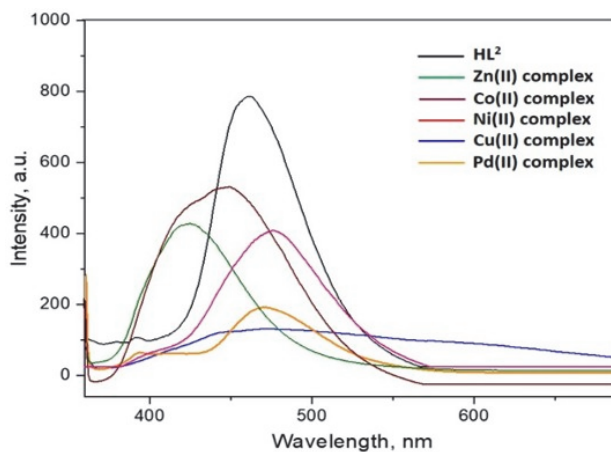


Fig. 5. Comparative fluorescence spectra of **HL²** and its complexes.

HL² emit at 463 nm strongly. In the complexes, the emission values vary between 425 and 478 nm and the intensity decreases. In Co(II), Cu(II) and Zn(II) complexes, emission is observed at a lower wavelength (blue shift) relative to the ligand, while in Ni(II) and Pd(II) complexes, it is observed at a higher wavelength

(red shift). The common feature of the last two metal ions is that they both have the d^8 electronic configuration system. The fluorescence intensity decreases significantly in Pd(II) and Cu(II) complexes. This effect is particularly pronounced in the Cu(II) complex. This indicates that the Cu(II) ion quenches the fluorescence property of the ligand (quenching effect).

Thermogravimetric analysis

The weight loss data obtained from thermal analysis studies are outlined in the Supplementary material. The samples were heated from room temperature up to 700 °C in air atmosphere. Thermogravimetric analysis (TGA) curves of complexes provide important clues for explaining the thermal behavior of molecules and especially the state of water molecules. It is known that lattice H_2O molecules remove from the complex below 100 °C and coordinate H_2O molecules up to 200 °C. Approximately 9.5 and 12.3 % mass losses observed in TGA curves of Co(II) and Ni(II) complexes of H_2L^1 (**1a** and **b**, respectively), below 200 °C can be considered as an indication of presence of two moles of coordinated water molecules (Fig. 6). This valuation is reasonable, considering that two moles of H_2O in the complexes correspond to theoretical masses of 8.8 and 9.2 %, respectively. The mass losses of 3.6 to 4.5 % observed in compounds **1a**, **1d**, **1e**, **2b** and **2d** below 100 °C indicate the presence of one mole of lattice water in these compounds (the theoretical values for these complexes are in the 3.6–4.5 % range). TGA curves of **2a–e** are given in the Supplementary material as Figs. S-39–S-43.

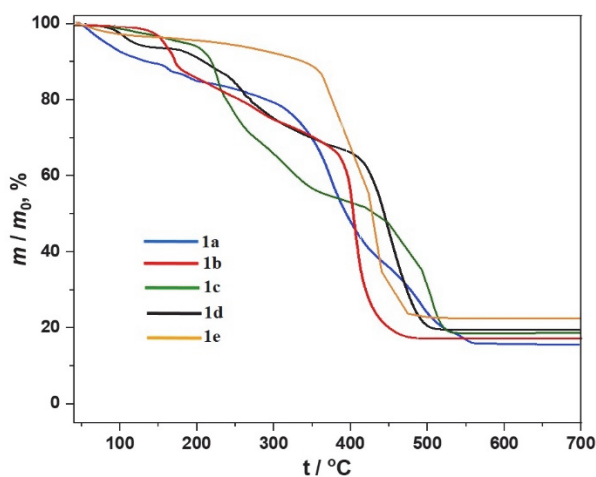


Fig. 6. TGA curves of the complexes **1a–e**.

It was observed that the complexes began to decompose above 300 °C and are completely decomposed above 500 °C, forming metal oxide forms. The mass

ratios remaining from the complexes after complete decomposition are consistent with the theoretical mass ratios calculated for metal oxides.

Various complexes of **H₂L¹** were reported in the literature. Griffiths *et al.* reported the synthesis and crystal structure of heterobimetallic 3d/4f coordination cluster formulated as $[\text{Zn}_2\text{Y}_2(\text{L}^1)_4(\text{NO}_3)_2(\text{DMF})_2]$ and study of its catalytic properties toward the Michael addition of nitrostyrenes with barbituric acid derivatives.²⁶ Effect of alcoholic solvent and acetate anion coordination mode variations on structures and magnetic properties of heterometallic Zn_2Dy_2 tetranuclear clusters was studied.^{27,28} Zhao *et al.* reported tetranuclear complexes of **H₂L¹**, $[\text{Ni}_4(\text{L}^1)_4(\text{CH}_3\text{OH})_4]$ and $[\text{Ni}_2\text{Zn}_2\text{Cl}_2(\text{L}^1)_2(\mu_{1,1}\text{-N}_3)_2]\cdot\text{H}_2\text{O}$, and investigated their antimicrobial activities.²⁹ All of these complexes have a tetranuclear structure. Some mononuclear diorganotin(V) complexes of **H₂L¹**, R_2SnL^1 (R = Me, Et, Bu, Ph), were obtained and their antimalarial and antioxidant activities were investigated by Taxak *et al.*³⁰

Factors such as the crystallization method, the kind of metal salt and the solvent used, are significant in complex formation. In our study, we used chloride salts of metals and attempted to obtain single crystals using various solvents, but we were unsuccessful. Therefore, we do not have evidence to suggest whether our complexes are mononuclear or polynuclear. It is also possible that some of our complexes are polynuclear as in the literature. Consequently, in the light of the analytical and spectroscopic data obtained, the tentatively proposed structures in Fig. 7 can be suggested for the complexes in the study.

Antimicrobial activity

In vitro antimicrobial activity test results of the ligands (**H₂L¹** and **HL²**) and their metal complexes in terms of MIC are presented in Table III in comparison with the values of the antibiotics and antifungal agents.

Table III shows that the benzimidazole derivative (**HL²**) exhibits a higher antimicrobial effect compared to the Schiff base (**H₂L¹**), particularly against *Staphylococcus epidermidis* (a Gram-positive bacteria) and *Candida tropicalis* (a fungi). On the other hand, it appears that the Co(II) complex of **HL²** (**2a**) has a high activity against *Staphylococcus aureus* and *Escherichia coli*, the Ni(II) complex (**2b**) against both Gram-positive bacteria (*S. aureus* and *S. epidermidis*), and the Cu(II) complex (**2c**) against *S. aureus*, *S. epidermidis* and *E. coli* compared to other complexes. Another noteworthy finding of the study is that **1a**, **1c**, **1d** and **1e** showed significantly higher activity against *C. tropicalis* compared to the ligand.

In the literature, the antimicrobial activities of **H₂L¹** and its tetranuclear Ni(II) complex were investigated by Zhao *et al.* against *S. aureus* and *E. coli* bacterial strains, which are also included in the bacterial groups that we studied.²⁹ They reported antimicrobial activities for **H₂L¹** of 75 μM (*S. aureus*) and 37.5 μM (*E. coli*), which differ greatly from our results (625 $\mu\text{g/mL}$ for both bacterial strains).

There could be several reasons for such significantly different results in the studies. First, there is a difference in methods: the literature uses a colorimetric method using dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) published in 2000.⁵⁶ Our method is a relatively new published by CLSI in 2021.³⁶ In addition, factors such as user errors and the chemicals used (DMSO, culture medium, *etc.*) may also be influential. The reproducibility of the experiment is also important. Our experiments were repeated twice. The number of repetitions in the literature is not specified. Therefore, the difference in results can be considered reasonable.

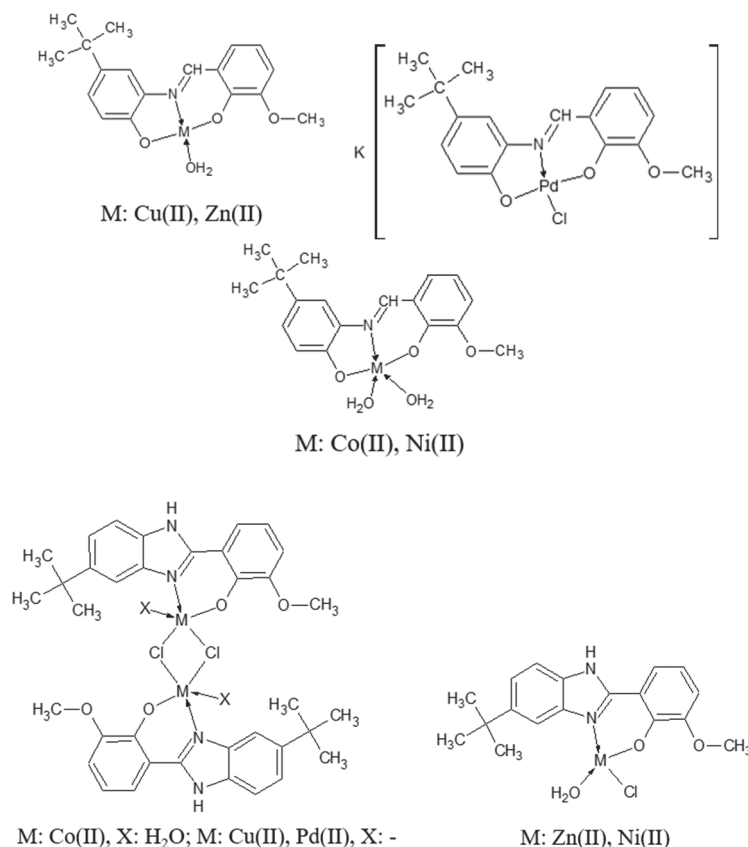


Fig. 7. Tentatively proposed structures for the complexes in the study.

Antiviral activity

Antiviral activity of the compounds was tested against Parainfluenza type-2 virus and the results are given at Table IV as percent inhibition. It was found that while **H₂L¹** inhibited viruses by 51 %, **HL²** was found to be effective with a rate

of 26 %. Accordingly, it appears that Schiff base, **H₂L¹**, has the antiviral effect approximately twice higher than the benzimidazole compound, *i.e.*, **HL²**.

TABLE III. *In vitro* antimicrobial activity of the compounds (*MIC* / $\mu\text{g mL}^{-1}$); *Sa* – *Staphylococcus aureus* ATCC 6538; *Se* – *Staphylococcus epidermidis* ATCC 12228; *Ec* – *Escherichia coli* ATCC 8739; *Kp* – *Klebsiella pneumoniae* ATCC 4352; *Pa* – *Pseudomonas aeruginosa* ATCC 1539; *Pm* – *Proteus mirabilis* ATCC 14153; *Ca* – *Candida albicans* ATCC 10231; *Cp* – *Candida parapsilosis* ATCC 22019; *Ct* – *Candida tropicalis* ATCC 750; ^aGram-positive; ^bGram-negative; –: no antimicrobial effect at 5000 $\mu\text{g/mL}$ and lower dilutions; ciprofloxacin and amphotericin B were used as references for bacteria and fungi, respectively

Compound	Microorganism								
	<i>Sa</i>	<i>Se</i> ^a	<i>Ec</i> ^b	<i>Kp</i> ^b	<i>Pa</i> ^b	<i>Pm</i> ^b	<i>Ca</i>	<i>Cp</i>	<i>Ct</i>
H₂L¹	625	625	625	625	312	625	312	156	625
1a	312	312	625	625	625	625	312	–	156
1b	625	625	1250	625	–	625	1250	1250	312
1c	625	625	1250	1250	625	1250	625	625	156
1d	1250	156	1250	312	1250	625	1250	625	156
1e	312	625	312	625	625	625	312	156	156
HL²	625	156	312	–	625	625	–	1250	156
2a	78	156	78	156	312	312	312	625	156
2b	19.5	39	156	625	625	–	–	625	–
2c	78	19.5	78	312	625	–	625	625	312
2d	312	312	156	626	625	312	312	625	312
2e	312	312	312	312	625	–	–	312	–
Reference	0.25	0.25	0.007	0.007	0.125	0.007	0.5	1.0	1.0

In all complexes of **HL²**, the antiviral effect significantly increased compared to the ligand. It is particularly interesting that **2a** and **2c** showed virus inhibition effect at the rates 62 and 64 %, respectively.

TABLE IV. Antiviral activity of the compounds; ribavirin: reference antiviral agent

Compound	Virus Inhibition, %
H₂L¹	51
1a	37
1b	29
1c	34
1d	33
1e	42
HL²	26
2a	62
2b	52
2c	64
2d	43
2e	35
Ribavirin	100

This study is limited to one Schiff base containing *tert*-butyl and methoxy groups, one benzimidazolylphenol ligand and five complexes of each. More comprehensive studies can be conducted using a larger number of similar ligands and different complexes. For example, it might be interesting to investigate how would the activities of compounds, obtained by substituting the methoxy groups (*e.g.*, 4- or 5-substituted), change. Changes in activity can be observed by forming complexes with different metal ions such as Mn(II), Fe(III), Ag(I) and VO(II). One of the advantages of our study is that the activity against a wide range of bacteria and fungi has been investigated. Another advantage is that the antiviral effect has been investigated, and studies on the antiviral activity of such compounds are quite limited.

CONCLUSION

This study deals with two chelating ligands containing *tert*-butyl and methoxy groups in their structures, an ONO-type Schiff base (**H₂L¹**) and benzimidazolylphenol (**HL²**), an NO-type ligand, and their various transition metal complexes (**1a–e**, **2a–e**) and their various biological properties. A study group consisting of a total of 12 compound samples was formed by obtaining Co(II), Ni(II), Cu(II), Zn(II) and Pd(II) complexes of both ligands.

The structure of **H₂L¹** was investigated using single crystal X-ray diffraction spectroscopy. There is keto-enol tautomerism in **H₂L¹** according to spectral data. All the compounds were characterized using physicochemical and spectroscopic methods such as elemental analysis, FT-IR, UV–Vis and NMR spectroscopic techniques. Molar conductivity and magnetic moment measurements and thermogravimetric analysis were applied the complexes also. Fluorescence spectroscopy was performed on **HL²** and its complexes due to their fluorescence properties.

In terms of biological applications, antibacterial, antifungal, and antiviral tests were performed on all compounds toward six bacteria, three fungi and Parainfluenza type-2 virus. Generally, **HL²** and its complexes showed higher activity compared to **H₂L¹** and its complexes, especially on *S. aureus*, *S. epidermidis* and *E. coli* bacteria, and the fungus *C. tropicalis*. Some of the complexes, such as **1a** and **b**, and all complexes of **HL²**, exhibited higher activity than the ligands against many bacteria and *C. tropicalis*. In the comparison between the complexes, it was observed that **2a**, **b** and **c** showed higher antibacterial activity, especially against *S. aureus*, *S. epidermidis* and *E. coli*, compared to the others. Regarding antiviral activity, **H₂L¹** showed higher activity than **HL²** on Parainfluenza type-2 virus. However, Co(II) and Cu(II) complexes of **HL²** (**2a** and **c**) were observed to have the highest activity among the compounds, with rates of 62 and 64 %, respectively.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13789>, or from the corresponding author on request.

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ИЗВОД

КОМПЛЕКСИ МЕТАЛА СА ШИФОВИМ БАЗАМА И БЕНЗИМИДАЗОЛИЛФЕНОЛНИМ
ЛИГАНДИМА СА ТЕРЦ-БУТИЛ И МЕТОКСИ ГРУПАМА: СТРУКТУРНА
КАРАКТЕРИЗАЦИЈА И БИОЛОШКО ИСПИТИВАЊЕ

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Синтетисана су и окарактерисана два хелатна лиганда са *терц*-бутил и метокси групама, Шифова база ОНО типа (H_2L^1) и бензимидазолилфенолни дериват (HL^2), лиганд NO типа, као и њихови комплекси у реакцијама са CoCl_2 , NiCl_2 , CuCl_2 , ZnCl_2 и K_2PdCl_4 (**1a–e**, **2a–e**). За карактеризацију лиганда коришћене су различите физичкохемијске и спектроскопске технике. Поред тога, кристална структура H_2L^1 лиганда одређена је рендгенском структурном анализом. На основу спектроскопских података за H_2L^1 примећена је појава кето–енолне таутомерије. За карактеризацију комплекса коришћене су елементална анализа, моларна проводљивост, магнетна мерења, термогравиметријска анализа, FT-IR, флуоресцентна, UV–Vis и NMR спектроскопија (за дијамагнетне комплексе). Испитиване су антибактеријска, антигљивична и антивирусна активност синтетисаних једињења. Антимикробна активност једињења је испитивана према шест бактеријских и три гљивичне врсте. Неки од комплекса су показали већу активност према појединим микроорганизмима у односу на лиганде. Уочено је да HL^2 лиганд и његови комплекси показују већу активност у поређењу са H_2L^1 и његовим комплексима, нарочито према бактеријама *Staphylococcus aureus*, *Staphylococcus epidermidis* и *Escherichia coli*, као и према гљивици *Candida tropicalis*. Утврђено је да **2a** показује изражену активност према *S. aureus* и *E. coli*, **2b** према обе Грам-позитивне бактерије (*S. aureus* и *S. epidermidis*), а **2c** према *S. aureus*, *S. epidermidis* и *E. coli* у поређењу са осталим комплексима. Антивирусна активност једињења испитивана је према вирусу параинфлуенце тип-2. У погледу антивирусног ефекта, H_2L^1 је показао већу активност од HL^2 , док су кобалт(II) и бакар(II) комплекси HL^2 лиганда показали највећу активност међу свим једињењима, са процентима инхибиције од 62 и 64 %, редом.

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SUPPLEMENTARY MATERIAL TO
**Metal complexes with Schiff base and benzimidazolyphenol
ligands with *tert*-butyl and methoxy groups: Structural
characterization and biological evaluation**

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4-*tert*-Butyl-2-[(*E*)-(2-hydroxy-3-methoxyphenyl)methylidene]amino}phenol (**H₂L¹**). Decomposition point (Dec. p.): 138 °C. (+)ESI-MS (*m/z*): calculated for [C₁₈H₂₁NO₃]⁺ 299.4, observed 297.3 (90.6 %) ([C₁₈H₂₁NO₃ - 2H]⁺ and 300.4 for ([C₁₈H₂₁NO₃ + H]⁺ (64.1 %). Combustion analysis for C₁₈H₂₁NO₃: Calculated C 72.22, H 7.07, N 4.68; found C 72.41, H 7.11, N 4.60. FT-IR (ATR, cm⁻¹): 3509m,br ν(NH), 3054w ν(CH)_{ar}, 2958m ν(CH)_{al}, 1630m ν(C=N), 1619m ν(C=C), 1498m, 1455m, 1351m, 1289m, 1225m ν(C–O), 1204m ν(C–O), 1070s, 831m δ(CH)_{ar}, 740m, 623m, 572m, 465m, 425m. ¹H NMR (500 MHz, DMSO-*d*₆, δ / ppm): 9.57 (*s*, 1H, OH1), 9.00 (*s*, 1H, N=CH), 7.36 (*d*, 1H, *J* = 2.4, H3), 7.21 (*dd*, 1H, *J*₁ = 7.9, *J*₂ = 1.4, H6'), 7.14 (*dd*, 1H, *J*₁ = 8.5, *J*₂ = 2.4, H5), 7.06 (*dd*, 1H, *J*₁ = 8.0, *J*₂ = 1.4, H4'), 6.89 (*d*, 1H, *J* = 8.5, H6), 6.84 (*t*, 1H, *J*₁ = 7.9, *J*₂ = 7.9, H5'), 3.81 (*s*, 3H, OCH₃), 1.28 (*s*, 9H, –C(CH₃)₃). ¹³C NMR (125 MHz, DMSO-*d*₆, δ / ppm): 161.6 (N=CH), 152.5 (C–OH(2')), 149.0 (C–OCH₃), 148.6 (C–OH(1')), 142.5 (C–C(CH₃)), 134.0 (C2), 125.2 (C5), 124.3 (C6'), 119.7 (C3), 118.2 (C6), 116.7 (C1'), 116.5 (C5'), 115.5 (C4'), 56.3 (OCH₃), 34.4 (–C(CH₃)₃). 31.8 (–C(CH₃)₃). UV-vis spectra (DMSO, λ_{max} / nm): 315m,br, 348m,br, 456w,br.

2-(5-*tert*-Butyl-1*H*-benzimidazol-2-yl)-6-methoxyphenol (**HL²**): Dec.p.: 219 °C. (+)ESI-MS (*m/z*): calculated for [C₁₈H₂₀N₂O₂]⁺ 296.4, observed 297.3 (100 %) ([C₁₈H₂₀N₂O₂ - H]⁺ and 298.3 for ([C₁₈H₂₀N₂O₂ + 2H]⁺ (20.7 %). Combustion analysis for C₁₈H₂₀N₂O₂: Calculated C 72.95, H 6.80, N 9.45; found C 73.07, H 6.91, N 9.30. FT-IR (ATR, cm⁻¹): 3351m ν(NH+OH), 3072w ν(CH)_{ar}, 2960m ν(CH)_{al}, 1619m ν(C=N), 1604m ν(C=C), 1576m, 1462m, 1364m, 1254s ν(C–O), 1202m ν(C–O), 1059m, 972m, 808m δ(CH)_{ar}, 785m, 732m, 655m, 557m, 511m, 457m, 425m. ¹H NMR (500 MHz, DMSO-*d*₆, δ / ppm): 13.12 (*s*,br, 2H, NH+OH),

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7.59 (*dd*, 1H, $J_1 = 8.0$, $J_2 = 1.3$, H6), 7.55 (*s,br*, 2H, H4+H6'), 7.36 (*dd*, 1H, $J_1 = 8.5$, $J_2 = 1.5$ Hz, H4'), 7.06 (*dd*, 1H, $J_1 = 8.0$, $J_2 = 1.2$, H7), 6.93 (*t*, 1H, $J_1 = 8.0$, $J_2 = 8.0$, H5'), 3.81 (*s*, 3H, OCH₃), 1.35 (*s*, 9H, -C(CH₃)₃). ¹³C NMR (125 MHz, DMSO-d₆, δ / ppm): 161.4 (C2), 152.7 (C-OCH₃), 149.6 (C-OH), 145.6 (C-C(CH₃)), 132.2 (C9), 131.3 (C8), 122.0 (C3'), 121.1 (C4'), 120.7 (C6), 120.1 (C2'), 117.1 (C7), 113.9 (C4), 109.3 (C5'), 56.2 (OCH₃), 34.5 (-C(CH₃)₃), 31.7 (-C(CH₃)₃). UV-vis spectra (DMSO, $\lambda_{\max}$ / nm): 304m, 316m, 330m,br, 382m,br. Fluorescence spectra (EtOH, $c = 1 \times 10^{-4}$ mol L⁻¹, $\lambda_{\max}$ / nm): 463m.

[Co(L^I)(H₂O)₂·H₂O (**1a**): Brown solid. Yield: 67 %. Dec. p.: 167 °C. Combustion analysis for C₁₈H₂₅NO₆Co (FW: 410.33): Calculated C 52.69, H 6.14, N 3.41; found C 52.40, H 5.84, N 3.28. Magnetic moment, μ_{eff} : 4.06 μ_B . A_M (DMF, 25 °C, S m² mol⁻¹): 21.4. FT-IR (ATR, cm⁻¹): 3223m,br ν (OH), 3062w,br ν (CH)_{ar}, 2957m ν (CH)_{al}, 1646m ν (C=N), 1611m ν (C=C), 1565m, 1490m, 1440m, 1362m, 1257s ν (C-O), 1225m, 1096m, 1050m, 957m, 822m δ (CH)_{ar}, 723m, 684m ν (N→M), 541m ν (O→M), 495m, 452m. TGA (t / °C: weight loss, %): 50: 0.3; 75: 1.5; 100: 4.6 (1 mole of H₂O); 150: 7.8; 200: 9.5 (1 mole of H₂O); 250: 12.3; 300: 18.3; 350: 36.5; 400: 47.8; 450: 58.0; 500: 67.5; 550: 80.1; 600: 80.1; 650: 80.1; 700: 80.2 (Theoretical value of CoO: 18.3 %). UV-vis spectra (DMSO, $\lambda_{\max}$ / nm): 227w, 253m, 278m,br, 354m, 464m,br.

[Ni(L^I)(H₂O)₂] (**1b**): Greenish brown solid. Yield: 70 %. Dec. p.: 186 °C. Combustion analysis for C₁₈H₂₃NO₅Ni (FW: 392.07): Calculated C 55.14, H 5.91, N 3.57; found C 55.33, H 6.06, N 3.51. Magnetic moment, μ_{eff} : 2.04 μ_B . A_M (DMF, 25 °C, S m² mol⁻¹): 21.4. FT-IR (ATR, cm⁻¹): 3065w,br ν (CH)_{ar}, 2957m ν (CH)_{al}, 1633sh ν (C=N), 1615m ν (C=C), 1506m, 1440m, 1362m, 1245m ν (C-O), 1222s, 1129m, 1078m, 975m, 820m δ (CH)_{ar}, 732s, 682m ν (N→M), 587m, 536m ν (O→M), 496m, 454m, 425m. TGA (t / °C: weight loss, %): 50: 0.2; 75: 0.3; 100: 0.4; 150: 3.1; 200: 12.3 (2 mole of H₂O); 250: 15.5; 300: 22.1; 350: 27.2; 400: 60.1; 450: 78.9; 500: 80.0; 550: 80.0; 600: 80.0; 650: 80.0; 700: 80.0 (Theoretical value of NiO: 19.1 %). UV-vis spectra (DMSO, $\lambda_{\max}$ / nm): 232w, 255m, 300m,br, 343m,br, 356sh, 440m,br.

[Cu(L^I)(H₂O)] (**1c**): Brown solid. Yield: 73 %. Dec. p.: 295 °C. Combustion analysis for C₁₈H₂₁NO₄Cu (FW: 378.9): Calculated C 57.69, H 4.36, N 2.64; found C 57.43, H 4.21, N 2.49. Magnetic moment, μ_{eff} : 1.72 μ_B . A_M (DMF, 25 °C, S m² mol⁻¹): 32.0. FT-IR (ATR, cm⁻¹): 3346m,br ν (OH), 3080w,br ν (CH)_{ar}, 2962m ν (CH)_{al}, 1647m ν (C=N), 1612m ν (C=C), 1588m, 1506m, 1463m, 1351m, 1258m ν (C-O), 1093m, 1027m, 821m δ (CH)_{ar}, 781m, 731m, 688m ν (N→M), 584m, 537m ν (O→M), 477m, 422m. TGA (t / °C: weight loss, %): 50: 0.1; 75: 0.4; 100: 0.6; 150: 4.4; 200: 5.0 (1 mole of H₂O); 250: 10.5; 300: 29.8; 350: 45.1; 400: 47.0; 450: 59.7; 500: 68.0; 550: 79.2; 600: 79.4; 650: 79.9; 700: 79.9 (Theoretical value of CuO: 21.0 %). UV-vis spectra (DMSO, $\lambda_{\max}$ / nm): 242w, 254m, 272m,br, 307sh, 363m,br, 478w,br.

[Zn(L^I)(H₂O)]·H₂O (**1d**): Dark yellow solid. Yield: 65 %. Dec. p.: 173 °C. Combustion analysis for C₁₈H₂₃NO₅Zn (FW: 398.8): Calculated C 49.79, H 5.34, N 3.23; found C 49.90, H 5.20, N 3.11. A_M (DMF, 25 °C, S m² mol⁻¹): 23.4. FT-IR (ATR, cm⁻¹): 3336m,br ν (OH), 3211m,br, 3061w,br ν (CH)_{ar}, 2957m ν (CH)_{al}, 1637m ν (C=N), 1615m ν (C=C), 1506m, 1435m, 1363m, 1285m, 1234m ν (C-O), 1195m ν (C-O), 1022m, 875m, 819m δ (CH)_{ar}, 734m, 684m ν (N→M), 586m, 537m ν (O→M), 497m, 415m. ¹H NMR (500 MHz, DMSO-d₆, δ / ppm): 8.86 (*s*, 1H, N=CH), 7.50 (*s,br*, 1H, $J = 2.4$, H3), 7.19 (*d*, 1H, $J = 7.1$, H6'), 7.03 (*s,br*, 1H, H5), 6.88 (*s,br*, 1H, H4'), 6.78 (*d,br*, 1H, $J = 6.8$, H6), 6.38 (*t*, 1H, $J_1 = 7.7$, $J_2 = 7.8$, H5'), 3.75 (*s*, 3H, OCH₃), 1.27 (*s*, 9H, -C(CH₃)₃). ¹³C NMR (125 MHz, DMSO-d₆, δ / ppm): 164.8 (N=CH), 161.3 (C-OH₂), 152.7 (C-OCH₃), 144.8 (C-OH1'), 142.4 (C-C(CH₃)), 130.1 (C2), 127.3 (C5), 123.8 (C6'), 119.4 (C3), 117.1 (C6), 114.3 (C1'), 111.5 (C5'), 107.6 (C4'), 55.0 (OCH₃), 32.2 (-C(CH₃)₃), 29.5 (-C(CH₃)₃). TGA (t / °C: weight loss, %): 50: 1.1; 75: 2.5; 100: 4.6 (1 mole of

H₂O); 150: 4.4; 200: 9.5 (1 mole of H₂O); 250: 12.2; 300: 23.1; 350: 29.5; 400: 33.0; 450: 63.0; 500: 78.6; 550: 78.8; 600: 78.9; 650: 79.0; 700: 79.0 (Theoretical value of ZnO: 20.4%). UV-vis spectra (DMSO, λ_{max} / nm): 228 w, 253 w, 279 m, 354 m, br, 464 m, br.

K[Pd(L¹)Cl]·H₂O (**1e**): Light brown solid. Yield: 77 %. Dec. p.: 265 °C. Combustion analysis for C₁₈H₂₁ClNO₄KPd (FW: 496.3): Calculated C 43.56, H 4.26, N 2.82; found C 43.70, H 4.11, 2.68. A_M (DMF, 25 °C, S m² mol⁻¹): 83.2. FT-IR (ATR, cm⁻¹): 3036m, br $\nu(\text{CH})_{\text{ar}}$, 2958m $\nu(\text{CH})_{\text{al}}$, 1646m $\nu(\text{C}=\text{N})$, 1611m $\nu(\text{C}=\text{C})$, 1506m, 1456m, 1351m, 1289m, 1257s $\nu(\text{C}-\text{O})$, 1204m $\nu(\text{C}-\text{O})$, 1097m, 1024m, 965m, 912m, 826m $\delta(\text{CH})_{\text{ar}}$, 730m, 673m $\nu(\text{N} \rightarrow \text{M})$, 564m, 540m $\nu(\text{O} \rightarrow \text{M})$, 500m, 462m. ¹H-NMR (500 MHz, DMSO-d₆, δ / ppm): 8.64 (s, 1H, N=CH), 7.94 (d, 1H, J=3.0, H3), 7.43 (d, 1H, J=8.1 H6'), 7.30 (d, 1H, J=8.5, H5), 7.09 (d, 1H, J=8.2, H4'), 7.02 (d, 1H, J=8.6, H6), 6.72 (t, 1H, J₁=8.7, J₂=8.5, H5'), 3.72 (s, 3H, OCH₃), 1.30 (s, 9H, -C(CH₃)₃). ¹³C NMR (125 MHz, DMSO-d₆, δ / ppm): 174.3 (N=CH), 161.5 (C-OH2), 148.4 (C-OCH₃), 146.9 (C-OH1'), 139.5 (C-C(CH₃)₃), 134.3 (C2), 126.3 (C5), 125.3 (C6'), 118.4 (C3), 118.3 (C6), 115.2 (C1'), 113.6 (C5'), 112.5 (C4'), 55.9 (OCH₃), 35.7 (-C(CH₃)₃), 32.1 (-C(CH₃)₃). TGA (t / °C: weight loss, %): 50: 0.7; 75: 2.0; 100: 3.7 (1 mole of H₂O); 150: 3.9; 200: 4.1; 250: 4.3; 300: 6.0; 350: 7.5; 400: 20.0; 450: 67.0; 500: 74.7; 550: 75.3; 600: 75.6; 650: 75.9; 700: 76.0 (Theoretical value of PdO: 24.6 %). UV-vis spectra (DMSO, λ_{max} / nm): 233w, 255m, 309sh, 318m, br, 337sh, 430m, br, 451sh.

[Co(L²)Cl(H₂O)] (**2a**): Dark brown solid. Yield: 72 %. Dec. p.: 201 °C. Combustion analysis for C₁₈H₂₁ClN₂O₃Co (FW: 407.8): Calculated C 53.02, H 5.19, N 6.87; found C 54.10, H 5.00, N 6.72. Magnetic moment, μ_{eff} : 2.17 μ_B . A_M (DMF, 25 °C, S m² mol⁻¹): 35.4. FT-IR (ATR, cm⁻¹): 3198m, br $\nu(\text{OH})$, 3088m, br $\nu(\text{CH})_{\text{ar}}$, 2960m $\nu(\text{CH})_{\text{al}}$, 1606m $\nu(\text{C}=\text{N})$, 1573m $\nu(\text{C}=\text{C})$, 1463m, 1435m, 1361m, 1244s $\nu(\text{C}-\text{O})$, 1195m, 1060m, 981m, 813m $\delta(\text{CH})_{\text{ar}}$, 786m, 732s, 66 m $\nu(\text{N} \rightarrow \text{M})$, 601m, 574m, 545m $\nu(\text{O} \rightarrow \text{M})$, 501m, 470m, 420m. TGA (t / °C: weight loss, %): 50: 0.1; 75: 0.2; 100: 0.4; 150: 3.1; 200: 5.2 (1 mole of H₂O); 250: 9.9; 300: 29.0; 350: 66.0; 400: 67.1; 450: 68.2; 500: 69.4; 550: 82.4; 600: 82.5; 650: 82.6; 700: 82.6 (Theoretical value of CoO: 18.4 %). UV-vis spectra (DMSO, λ_{max} / nm): 240w, 253w, 307m, br, 320m, br, 334sh, 473w, br. Fluorescence spectra (EtOH, $c = 1 \times 10^{-4}$ mol L⁻¹, λ_{max} / nm): 425sh, 451m, br.

[Ni(L²)Cl(H₂O)]·H₂O (**2b**): Light brown solid. Yield: 70 %. Dec. p.: 198 °C. Combustion analysis for C₁₈H₂₃ClN₂O₄Ni (FW: 425.5): Calculated C 50.81, H 5.45, N 6.58; found C 50.90, H 5.51, N 6.43. Magnetic moment, μ_{eff} : 2.05 μ_B . A_M (DMF, 25 °C, S m² mol⁻¹): 38.8. FT-IR (ATR, cm⁻¹): 3373m, br $\nu(\text{OH})$, 3228m, br, 3070m, br $\nu(\text{CH})_{\text{ar}}$, 2962m $\nu(\text{CH})_{\text{al}}$, 2872m, 1611m $\nu(\text{C}=\text{N})$, 1598m $\nu(\text{C}=\text{C})$, 1545m, 1440m, 1362m, 1249m $\nu(\text{C}-\text{O})$, 1198m $\nu(\text{C}-\text{O})$, 1015m, 983m, 863m, 814m $\delta(\text{CH})_{\text{ar}}$, 731m, 671m $\nu(\text{N} \rightarrow \text{M})$, 581m, 540m $\nu(\text{O} \rightarrow \text{M})$, 457m, 420m. TGA (t / °C: weight loss, %): 50: 0.3; 75: 2.2; 100: 4.6 (1 mole of H₂O); 150: 7.8; 200: 9.0 (1 mole of H₂O); 250: 19.3; 300: 22.8; 350: 24.4; 400: 36.9; 450: 53.0; 500: 65.1; 550: 72.1; 600: 75.3; 650: 82.6; 700: 83.0 (Theoretical value of NiO: 17.5 %). UV-vis spectra (DMSO, λ_{max} / nm): 241w, 253w, 309m, br, 357m, br, 373m, br, 461m, br. Fluorescence spectra (EtOH, $c = 1 \times 10^{-4}$ mol L⁻¹, λ_{max} / nm): 399w, 478m.

[Cu(L²)Cl] (**2c**): Brown solid. Yield: 76 %. Dec. p.: 235 °C. Combustion analysis for C₁₈H₁₉ClN₂O₂Cu (FW: 394.4): Calculated C 54.82, H 4.86, N 7.10; found C 55.90, H 5.05, N 6.92. Magnetic moment, μ_{eff} : 1.01 μ_B . A_M (DMF, 25 °C, S m² mol⁻¹): 48.8. FT-IR (ATR, cm⁻¹): 3215m, br $\nu(\text{OH})$, 3057w, br $\nu(\text{CH})_{\text{ar}}$, 2962m $\nu(\text{CH})_{\text{al}}$, 1608m $\nu(\text{C}=\text{N})$, 1589m $\nu(\text{C}=\text{C})$, 1543m, 1485m, 1384m, 1242s $\nu(\text{C}-\text{O})$, 1199m $\nu(\text{C}-\text{O})$, 1062m, 981m, 815m $\delta(\text{CH})_{\text{ar}}$, 786m, 732s, 667m $\nu(\text{N} \rightarrow \text{M})$, 576m, 543m $\nu(\text{O} \rightarrow \text{M})$, 470m, 418m. TGA (t / °C: weight loss, %): 50: 0.2; 75: 0.3; 100: 0.4; 150: 0.5; 200: 1.6; 250: 4.2; 300: 6.4; 350: 13.4; 400: 26.5; 450: 43.0; 500: 65.0; 550: 76.0; 600: 78.1; 650: 80.0; 700: 81.0 (Theoretical value of CuO: 20.2 %). UV-

vis spectra (DMSO, $\lambda_{\max}$ / nm): 243w, 255m, 297sh, 307m, 321m, 335m,br, 371sh, 433m,br. Fluorescence spectra (EtOH, $c = 1 \times 10^{-4}$ mol L⁻¹, $\lambda_{\max}$ / nm): 404w,br, 461sh.

[Zn(L²)Cl(H₂O)]·H₂O (**2d**): Dark yellow solid. Yield: 65 %, Dec. p.: 229 °C. Combustion analysis for C₁₈H₂₃ClN₂O₄Zn (FW: 432.3): Calculated C 50.02, H 5.36, N 6.48; found C 50.30, H 5.44, N 6.34. A_M (DMF, 25 °C, S m² mol⁻¹): 38.6. FT-IR (ATR, cm⁻¹): 3406m,br ν (OH), 3215m,br ν (NH), 3059w,br ν (CH)_{ar}, 2962m ν (CH)_{al}, 1614m ν (C=N), 1591m ν (C=C), 1554m, 1467m, 1386m, 1249s ν (C–O), 1199m ν (C–O), 1109m, 1078m, 975m, 852m, 825m δ (CH)_{ar}, 732s, 669m ν (N→M), 601m, 543m ν (O→M), 439m, 410m. ¹H NMR (500 MHz, DMSO-d₆, δ / ppm): 13.64 (*s,br*, 1H, NH), 7.65 (*dd,br*, 1H, $J_1 = 8.6$, $J_2 = 1.7$, H6), 7.37 (*d,br*, 2H, $J = 8.9$, H4+H6'), 7.05 (*s,br*, 1H, H4'), 6.95 (*d,br*, 1H, $J = 7.9$, H7), 6.89 (*t,br*, 1H, $J_1 = 8.0$, $J_2 = 8.1$, H5'), 3.82 (*s*, 3H, OCH₃), 1.36 (*s*, 9H, –C(CH₃)₃). ¹³C NMR (125 MHz, DMSO-d₆, δ / ppm): 167.9 (C2), 159.0 (C–OCH₃), 131.9, 104.7, 54.2 (OCH₃), 32.8 (–C(CH₃)₃). TGA (t / °C: weight loss, %): 50: 1.9; 75: 4.1; 100: 4.3 (1 mole of H₂O); 150: 8.4; 200: 9.0 (1 mole of H₂O); 250: 21.7; 300: 34.3; 350: 75.6; 400: 80.9; 450: 81.1; 500: 81.5; 550: 81.6; 600: 81.8; 650: 82.0; 700: 82.1 (Theoretical value of ZnO: 18.8 %). UV-vis spectra (DMSO, $\lambda_{\max}$ / nm): 237w, 254w, 296sh, 304m, 332sh, 354sh. Fluorescence spectra (EtOH, $c = 1 \times 10^{-4}$ mol L⁻¹, $\lambda_{\max}$ / nm): 424m,br.

[Pd(L²)Cl(H₂O)] (**2e**): Light brown solid. Yield: 77%, Dec. p.: 265. Combustion analysis for C₁₈H₂₁ClN₂O₃Pd (FW: 455.2): Calculated C 47.49, H 4.65, N 6.15; found C 47.12, H 4.63, N 5.90. A_M (DMF, 25 °C, S m² mol⁻¹): 49.6. FT-IR (ATR, cm⁻¹): 3293m,br ν (OH), 3197m ν (NH), 3058w,br ν (CH)_{ar}, 2958m ν (CH)_{al}, 2866m, 1602m ν (C=N), 1592m ν (C=C), 1561m, 1492m, 1436m, 1364m, 1242s ν (C–O), 1182m ν (C–O), 1075m, 981m, 812m δ (CH)_{ar}, 733m, 669m ν (N→M), 599m, 543m ν (O→M), 465m, 441m. ¹H NMR (500 MHz, DMSO-d₆, δ / ppm): 13.22 (*s,br*, 1H, NH), 8.02 (*d*, 1H, $J = 9.1$, H6), 7.61 (*d,br*, 1H, $J = 2.8$, H4), 7.39 (*d,br*, 1H, $J = 8.8$, H6'), 7.30 (*dd*, 1H, $J_1 = 8.4$, $J_2 = 1.8$, H4'), 7.23 (*dd*, 1H, $J_1 = 8.6$, $J_2 = 1.6$, H7), 6.96 (*t*, 1H, $J_1 = 8.3$, $J_2 = 7.9$, H5'), 3.84 (*s*, 3H, OCH₃), 1.39 (*s*, 9H, –C(CH₃)₃). ¹³C NMR (125 MHz, DMSO-d₆, δ / ppm): 170.8 (C2), 166.2 (C–OCH₃), 159.8 (C–OH), 158.0 (C–C(CH₃)₃), 140.4, 139.3, 132.5, 119.0, 116.1, 56.2 (OCH₃), 33.2 (–C(CH₃)₃). TGA (t / °C: weight loss, %): 50: 0.2; 75: 0.5; 100: 0.6; 150: 3.3; 200: 5.1 (1 mole of H₂O); 250: 7.5; 300: 15.0; 350: 23.0; 400: 28.5; 450: 32.5; 500: 35.2; 550: 44.2; 600: 68.2; 650: 74.1; 700: 74.2 (Theoretical value of PdO: 26.8 %). UV-vis spectra (DMSO, $\lambda_{\max}$ / nm): 233m, 255m, 308m,br, 321m,br, 335m,br, 458m,br. Fluorescence spectra (EtOH, $c = 1 \times 10^{-4}$ mol L⁻¹, $\lambda_{\max}$ / nm): 393w, 472m,br.

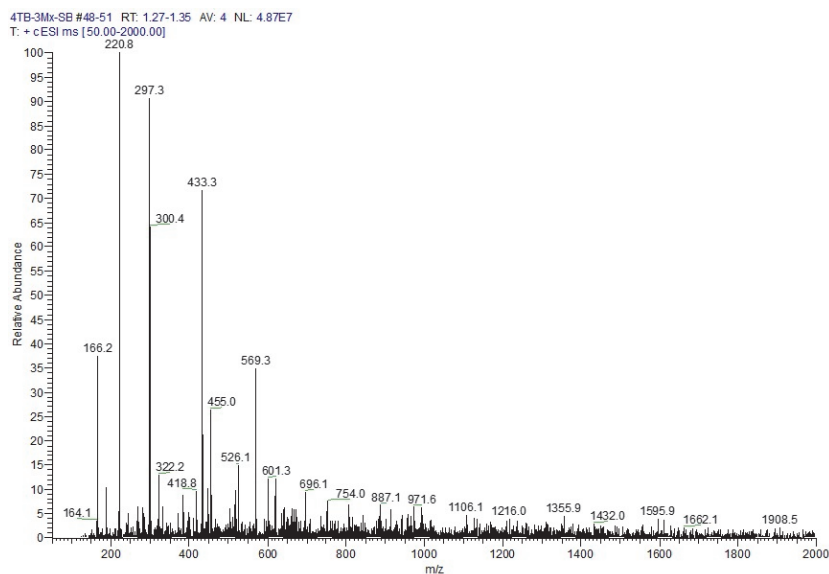
TABLE S-I. Crystal data and structure refinement parameters for H₂L¹

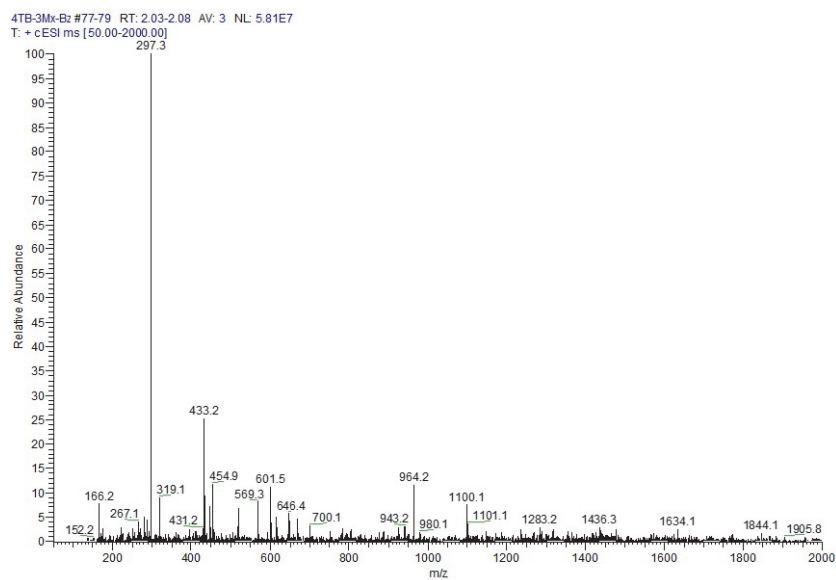
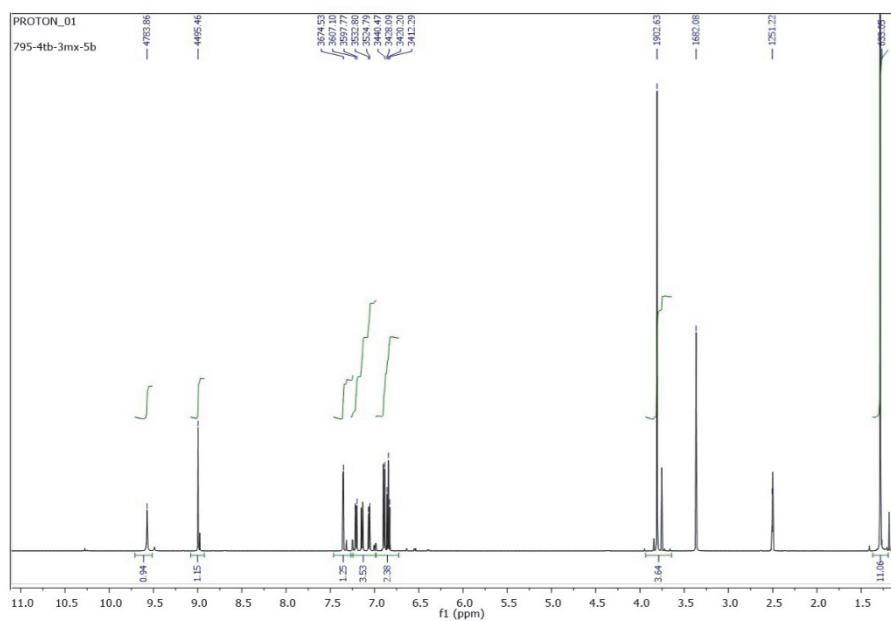
Empirical formula	C ₁₈ H ₂₁ NO ₃
Formula weight	299.36
Crystal system	Orthorhombic
Space group	<i>Pbca</i>
<i>a</i> (Å)	14.2349 (10)
<i>b</i> (Å)	9.5415 (5)
<i>c</i> (Å)	24.1061 (16)
α, β, γ (°)	90
<i>V</i> (Å ³)	3274.1(4)
<i>Z</i>	8
<i>D_c</i> (g cm ⁻³)	1.215
μ (mm ⁻¹)	0.082
θ range (°)	2.5–25.2

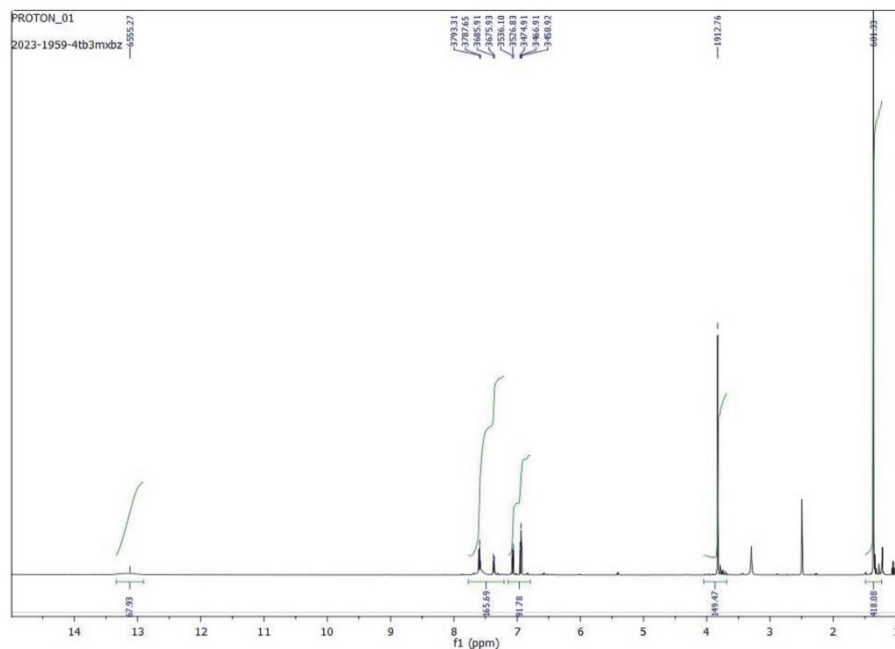
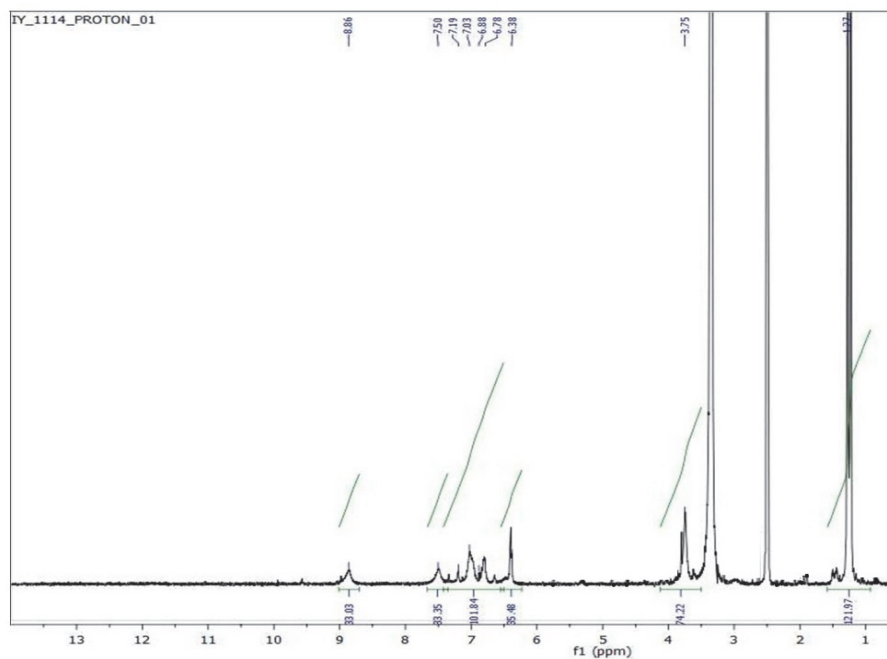
Measured refls.	46454
Independent refls.	3898
R_{int}	0.062
S	1.04
R1/wR2	0.059/0.154
$\Delta\rho_{\text{max}}/\Delta\rho_{\text{min}}$ (eÅ ⁻³)	0.19/-0.21
CCDC	2298000

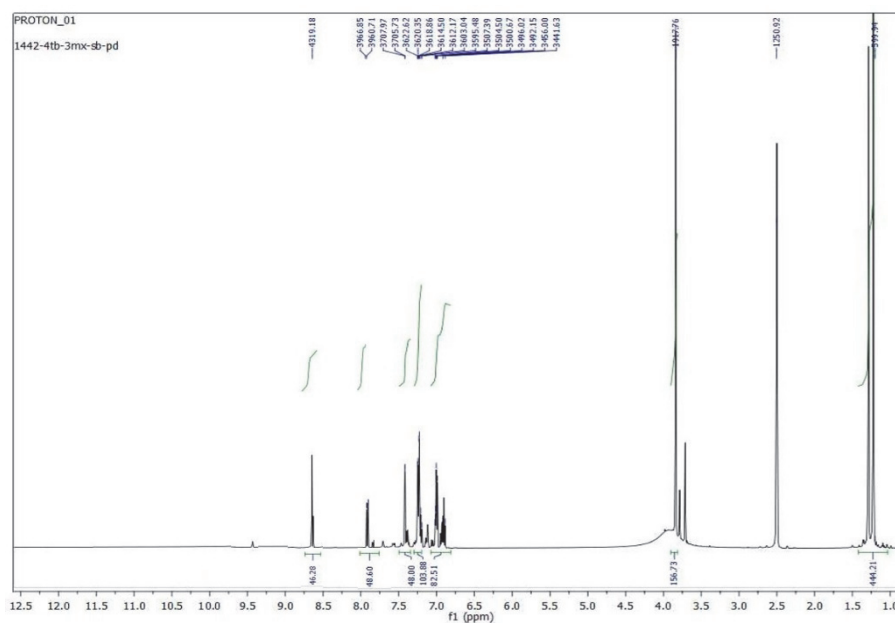
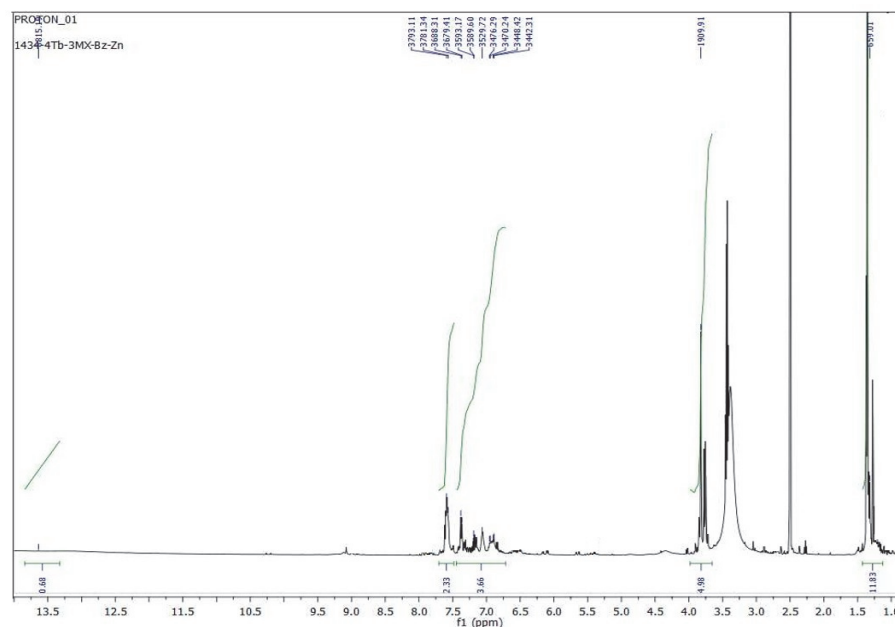
TABLE S-II. Selected bond distances and angles for **H₂L¹** (Å, °)

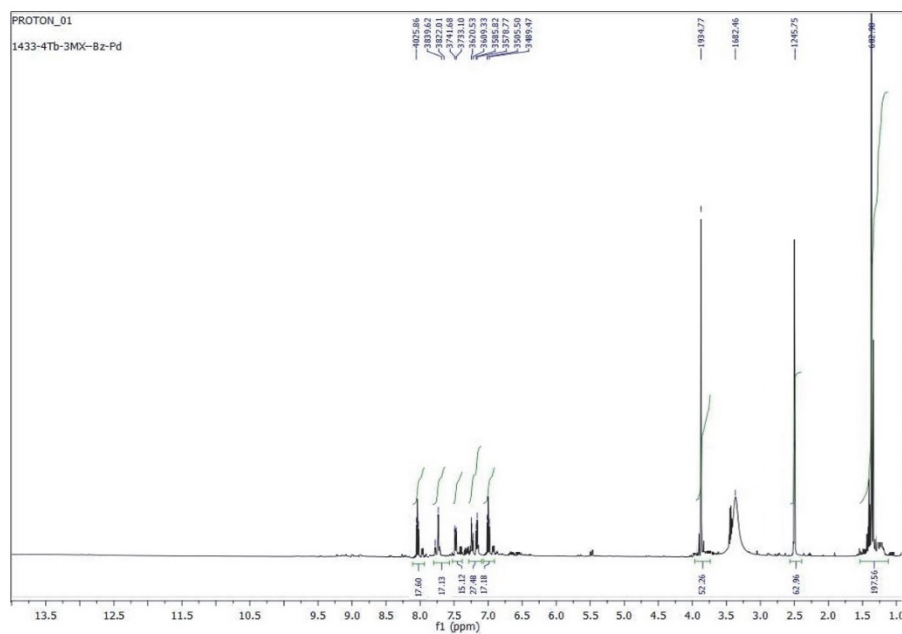
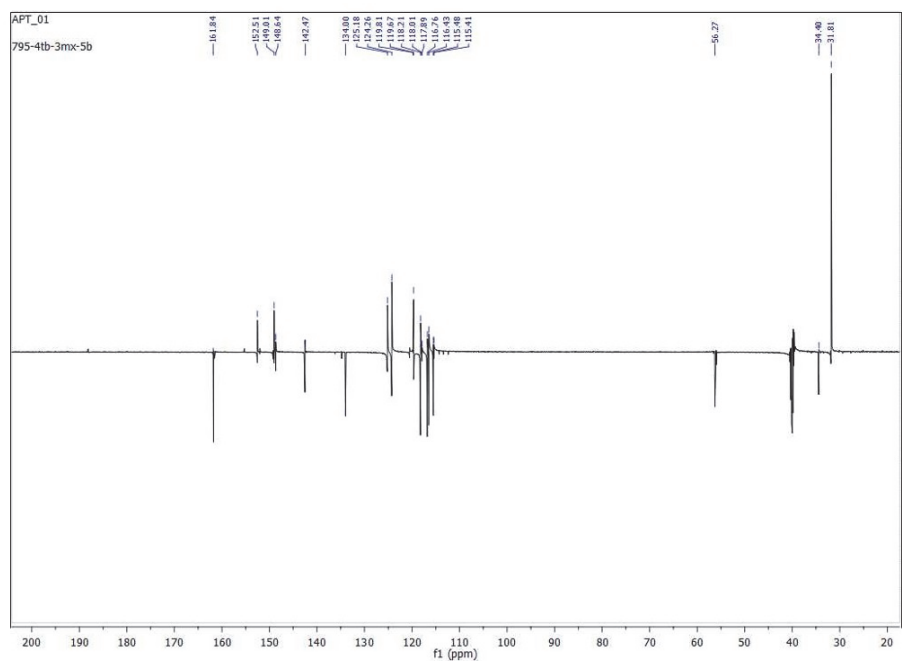
C8—N1	1.305(3)	C3—C2—O2	125.58(19)
C1—O1	1.289(2)	C13—C15—C18	110.40(19)
C2—O2	1.364(2)	C13—C15—C17	111.9(2)
C6—C8	1.407(3)	C16—C15—C13	108.3(2)
C7—O2	1.420(3)	O3—C10—C11	125.82(19)
C9—C14	1.384(3)	O3—C10—C9	116.1(2)
C9—C10	1.396(3)	C14—C9—N1	123.33(18)
C9—N1	1.414(3)	C10—C9—N1	115.90(18)
C10—O3	1.353(3)	C8—N1—C9	127.13(19)
O1—C1—C6	122.17 (19)	N1—C8—C6	123.8(2)
O1—C1—C2	121.55 (18)	C2—O2—C7	117.37(17)
O2—C2—C1	113.32 (17)		

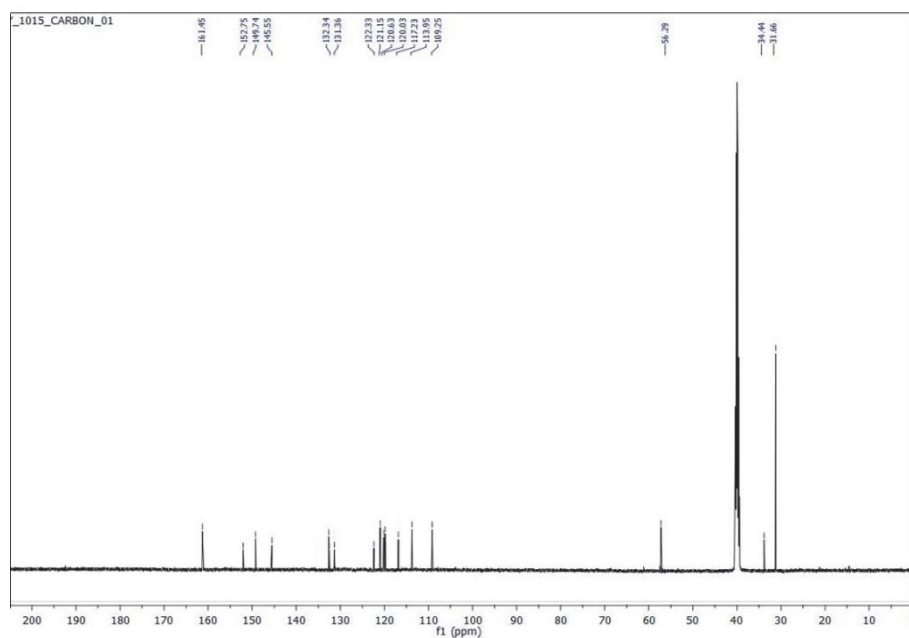
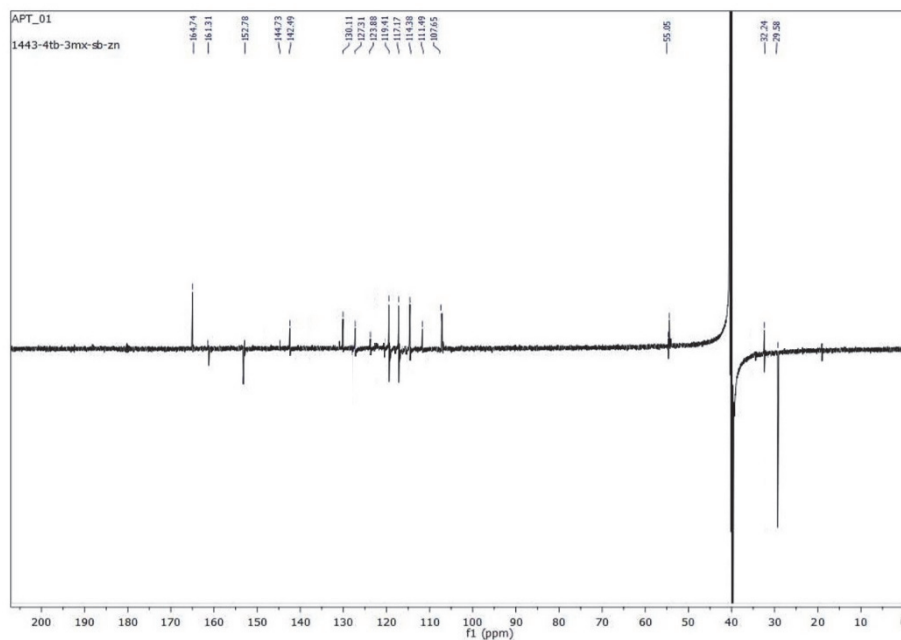
Fig. S1. ESI-MS spectrum of **H₂L¹**

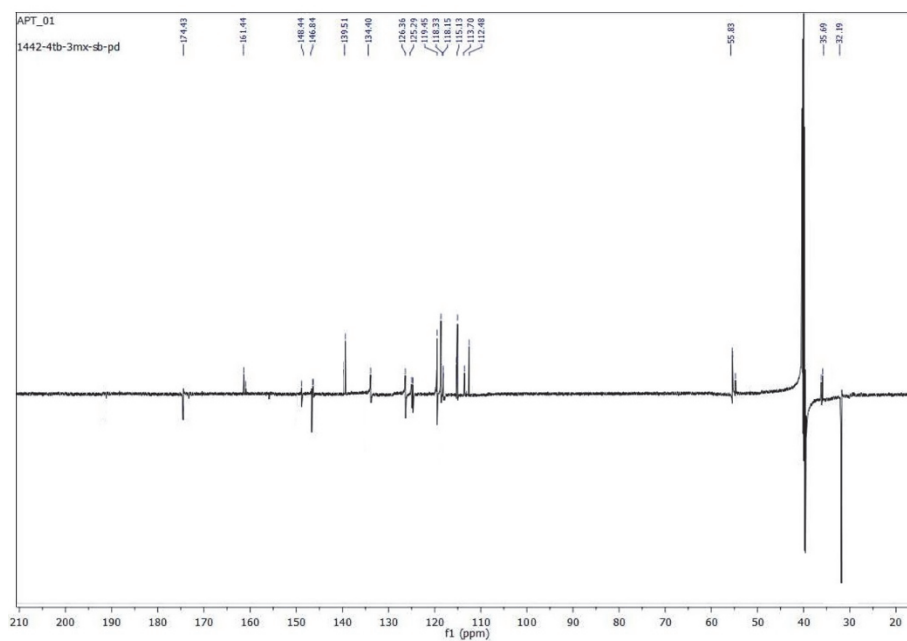
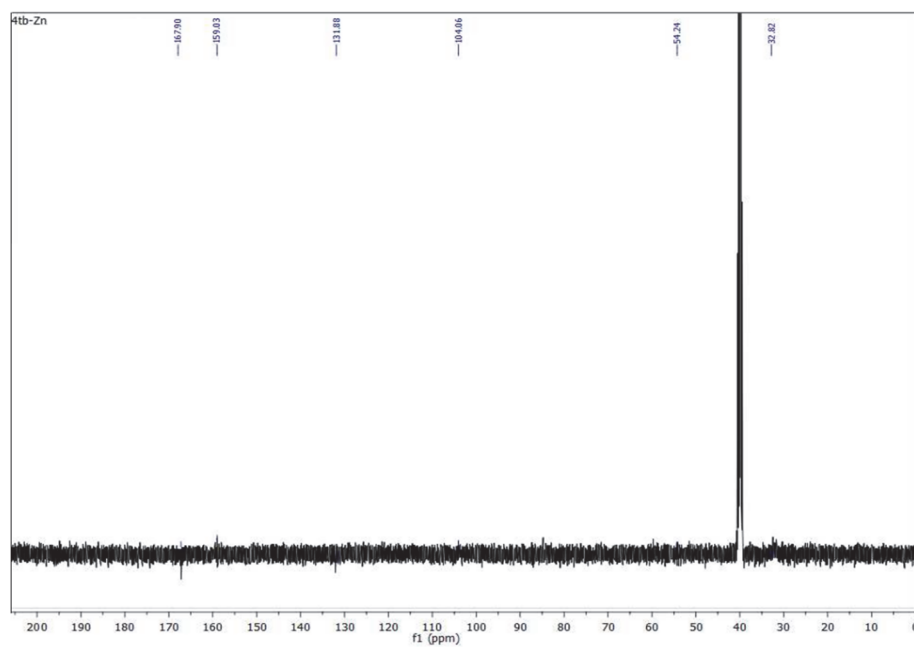
Fig. S2. ESI-MS spectrum of HL^2 Fig. S3. ^1H -NMR spectrum of H_2L^1

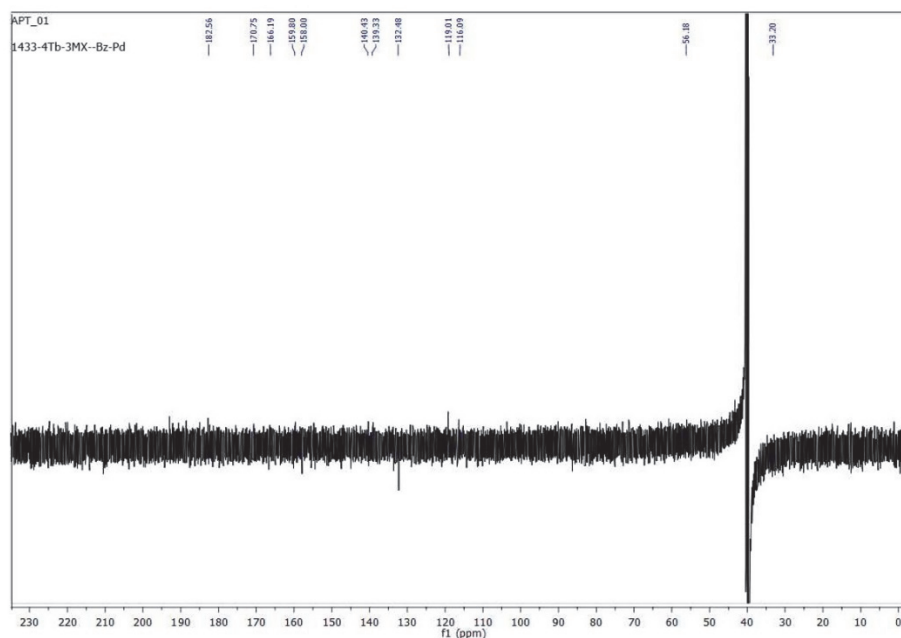
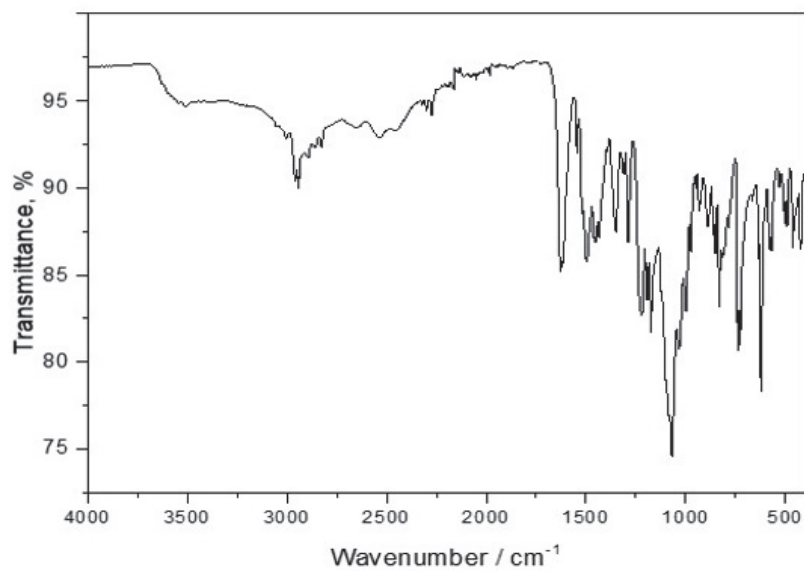
Fig. S4. ^1H -NMR spectrum of HL^2 Fig. S5. ^1H -NMR spectrum of 1d (Zn(II) complex of H_2L^1)

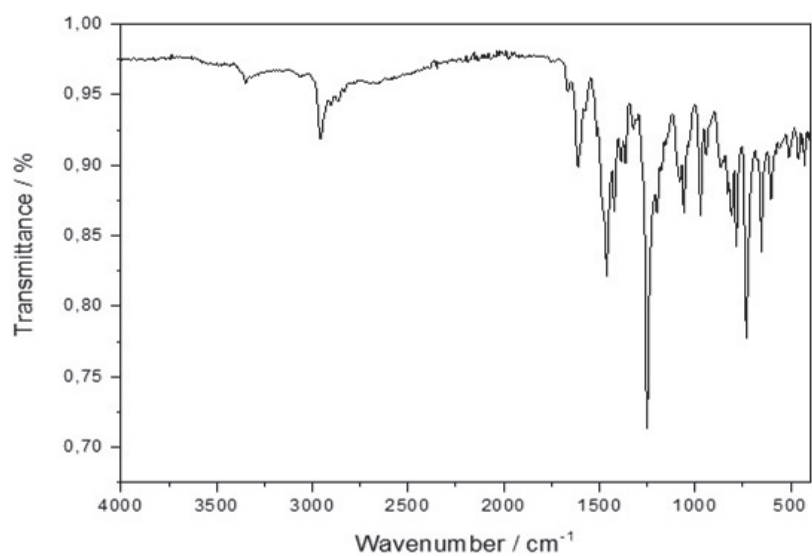
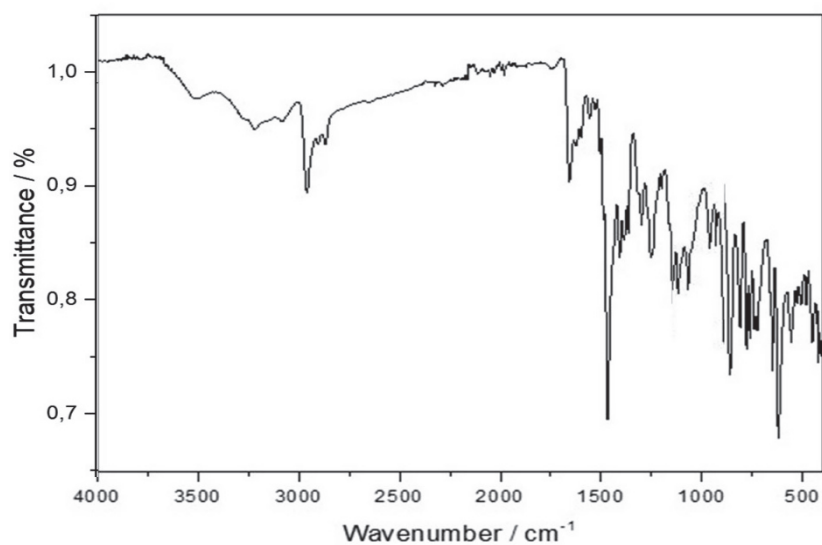
Fig. S6. ^1H -NMR spectrum of **1e** (Pd(II) complex of H_2L^1)Fig. S7. ^1H -NMR spectrum of **2d** (Zn(II) complex of HL^2)

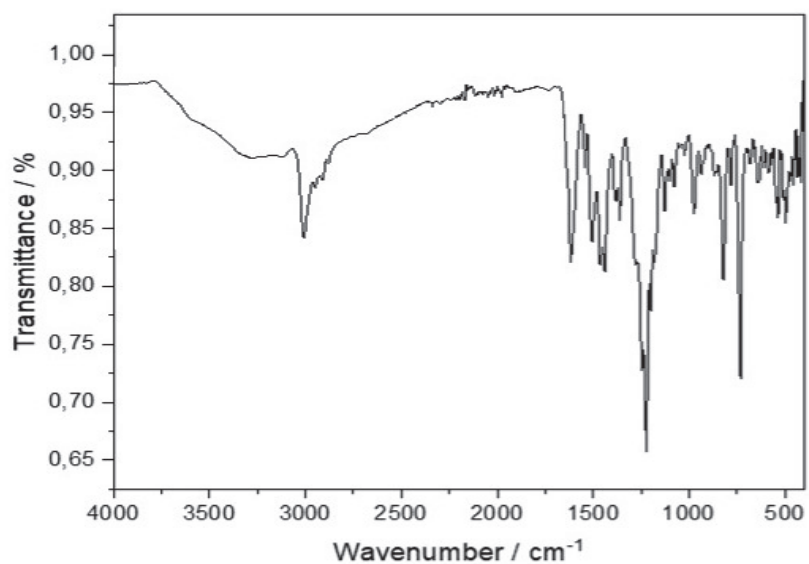
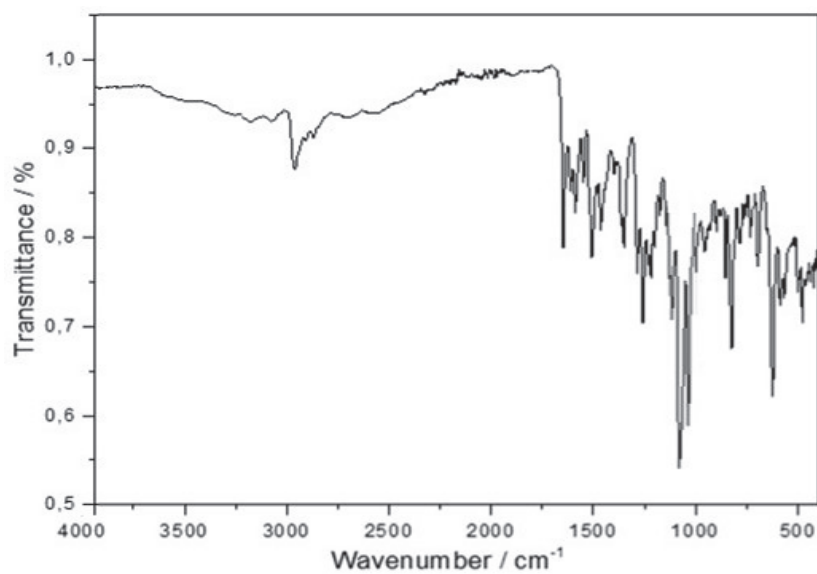
Fig. S8. ^1H -NMR spectrum of **2e** (Pd(II) complex of **HL**²)Fig. S9. ^{13}C -NMR spectrum of **H**₂**L**¹

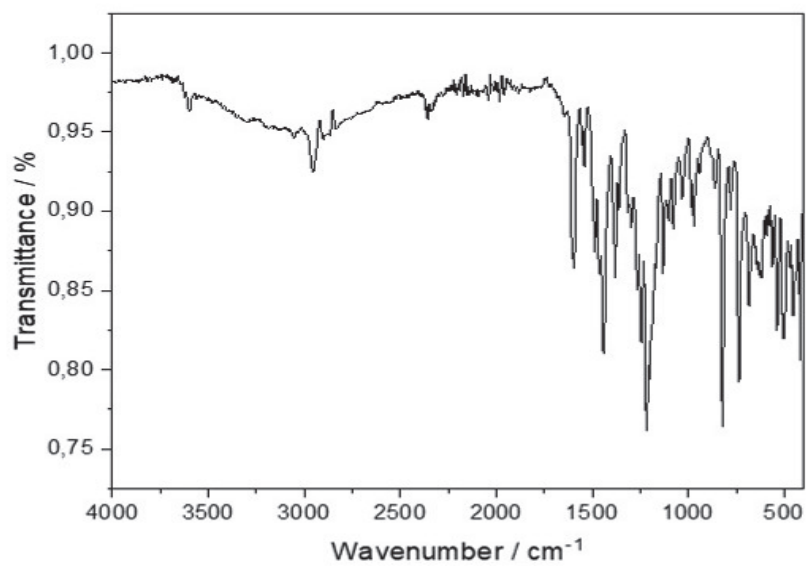
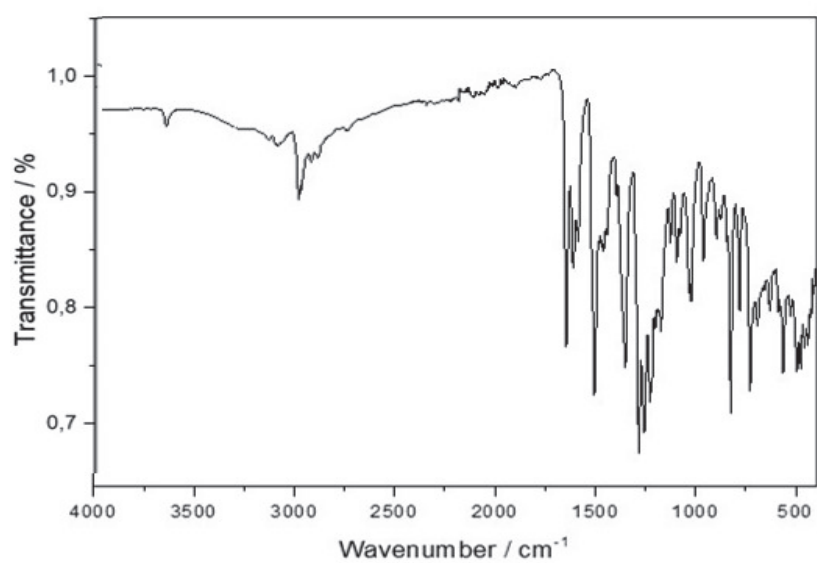
Fig. S10. ¹³C-NMR spectrum of **HL**²Fig. S11. ¹³C-NMR spectrum of **1d** (Zn(II) complex of **H**₂**L**¹)

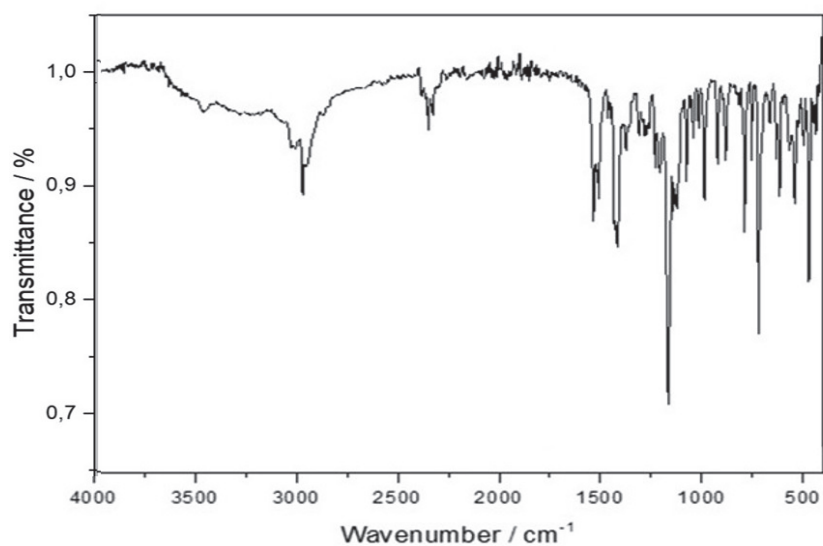
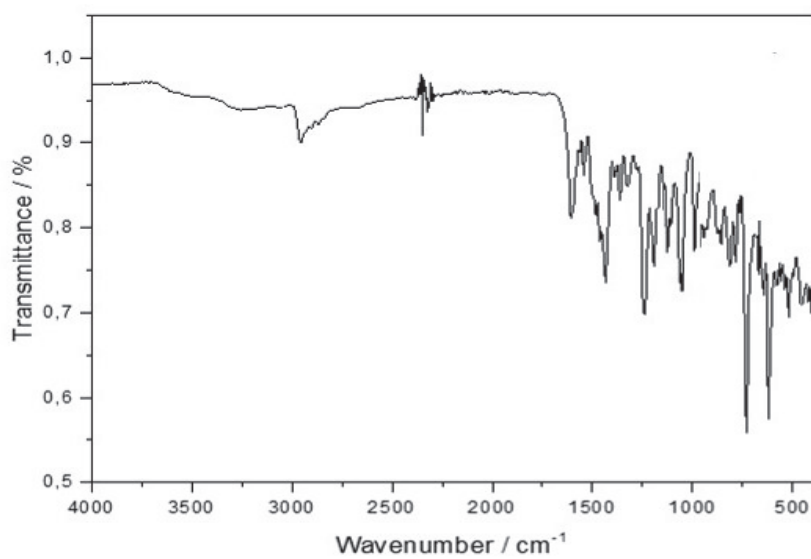
Fig. S12. ^{13}C -NMR spectrum of **1e** (Pd(II) complex of H_2L^1)Fig. S13. ^{13}C -NMR spectrum of **2d** (Zn(II) complex of HL^2)

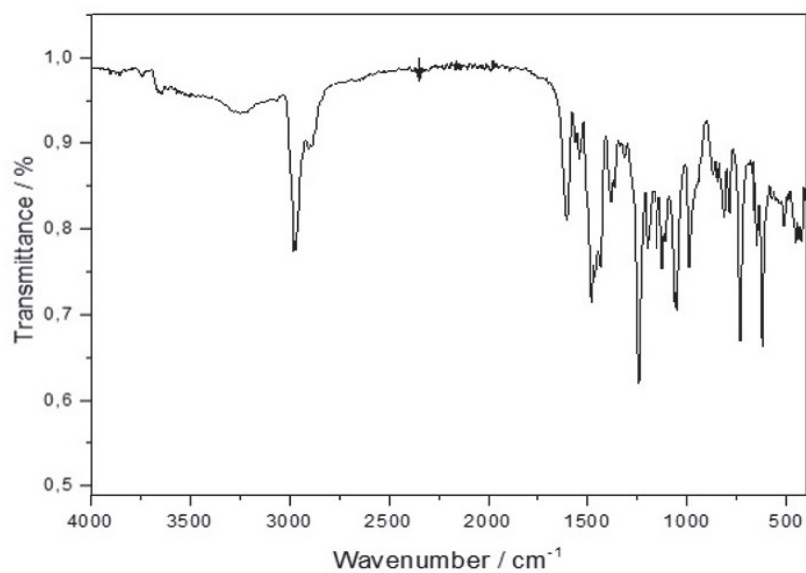
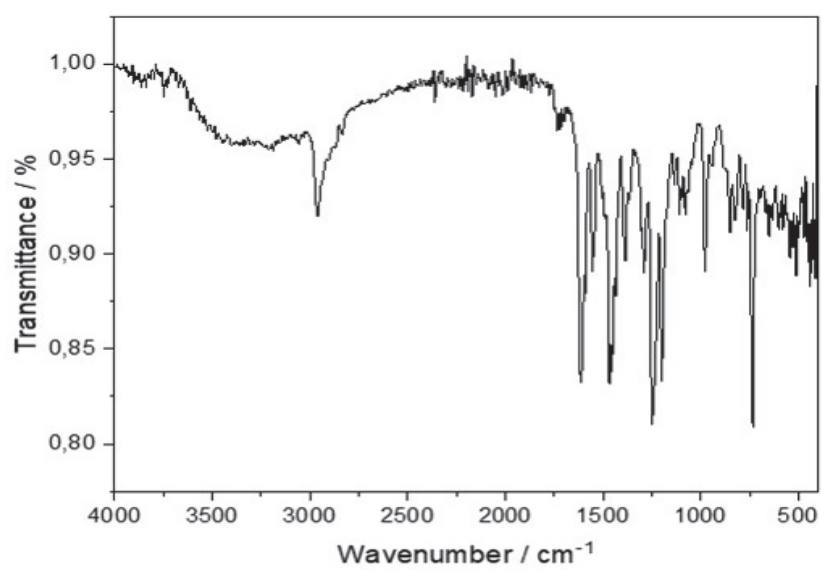
Fig. S14. ^{13}C -NMR spectrum of **2e** (Pd(II) complex of **HL**²)Fig. S15. FTIR spectrum of **H₂L**¹

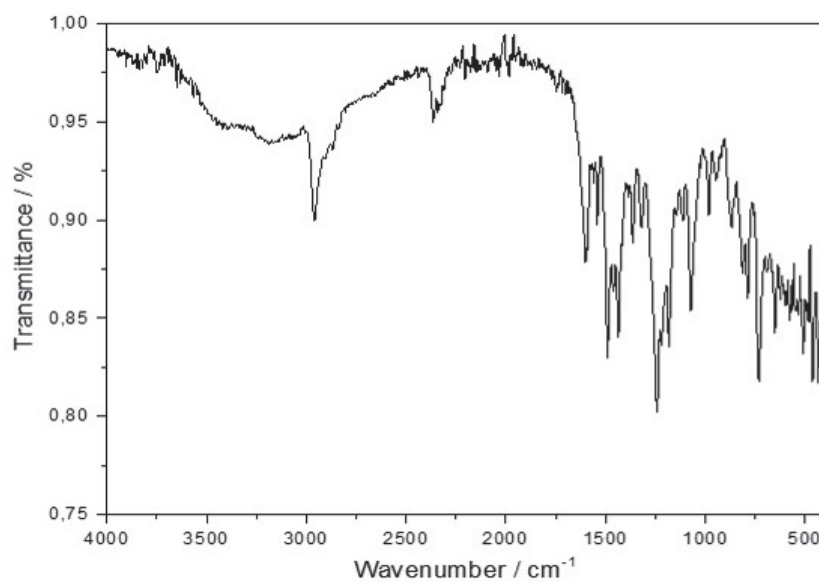
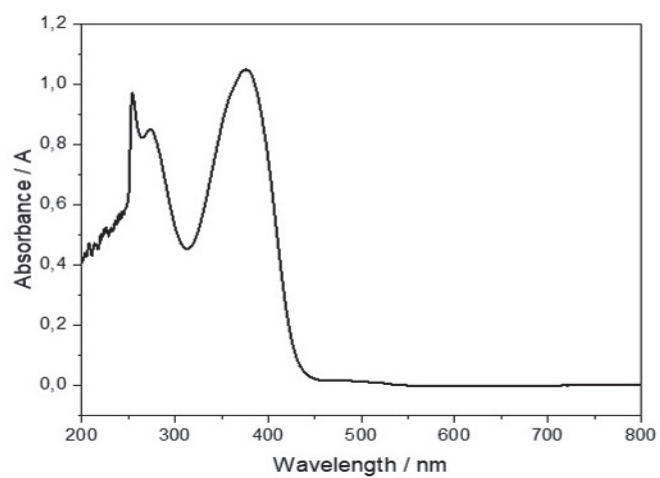
Fig. S16. FTIR spectrum of **HL**²Fig. S17. FTIR spectrum of **1a**

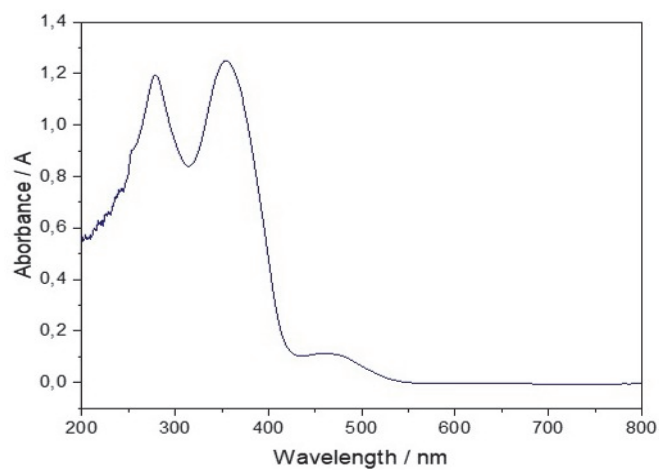
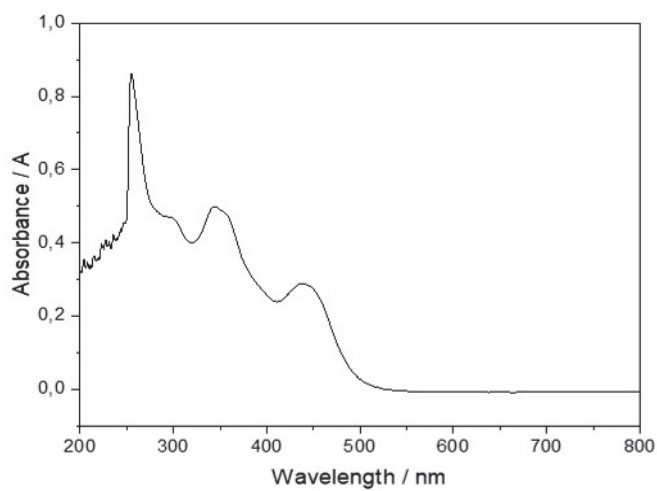
Fig. S18. FTIR spectrum of **1b**Fig. S19. FTIR spectrum of **1c**

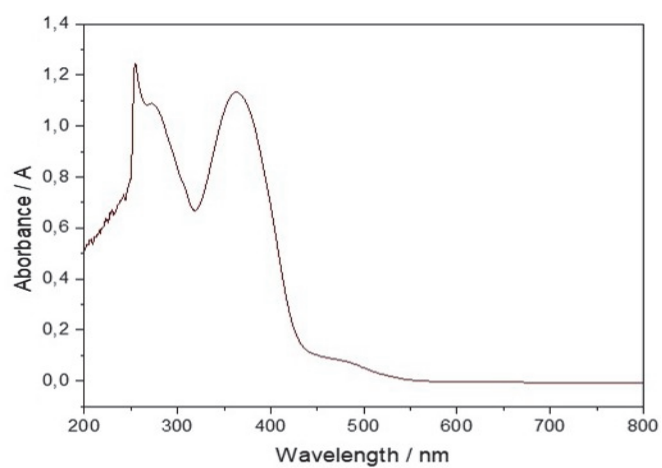
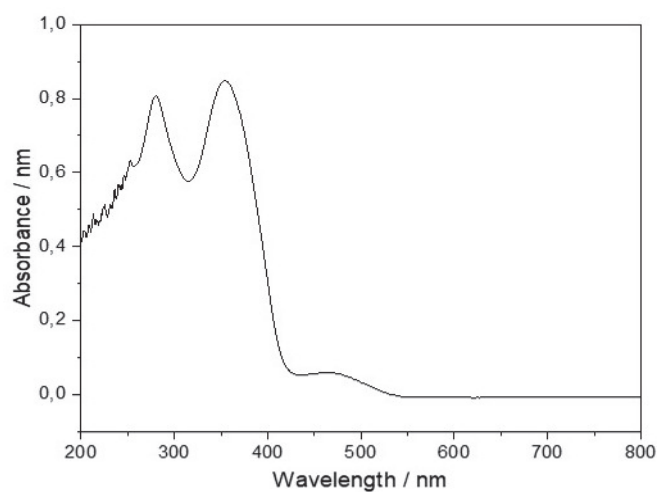
Fig. S20. FTIR spectrum of **1d**Fig. S21. FTIR spectrum of **1e**

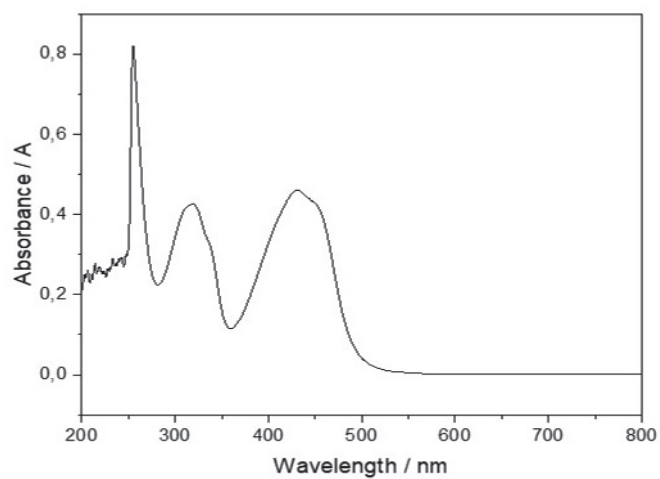
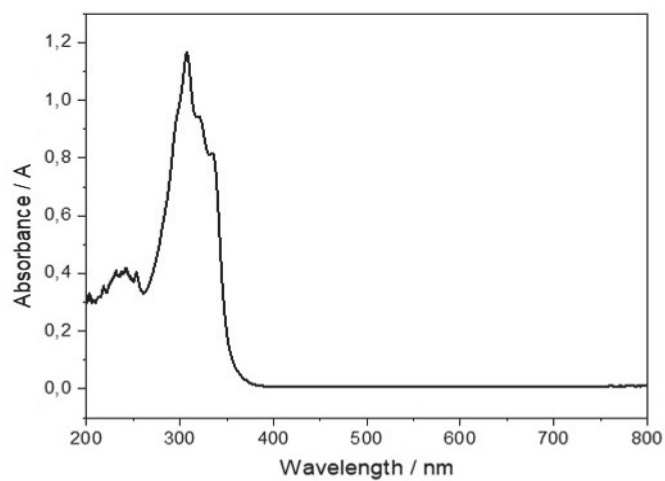
Fig. S22. FTIR spectrum of **2a**Fig. S23. FTIR spectrum of **2b**

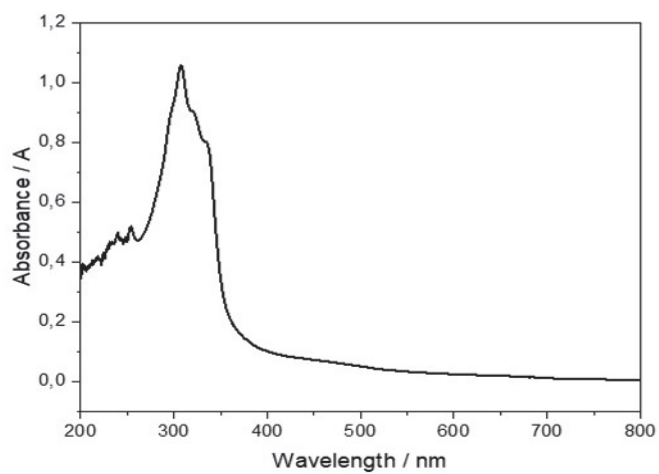
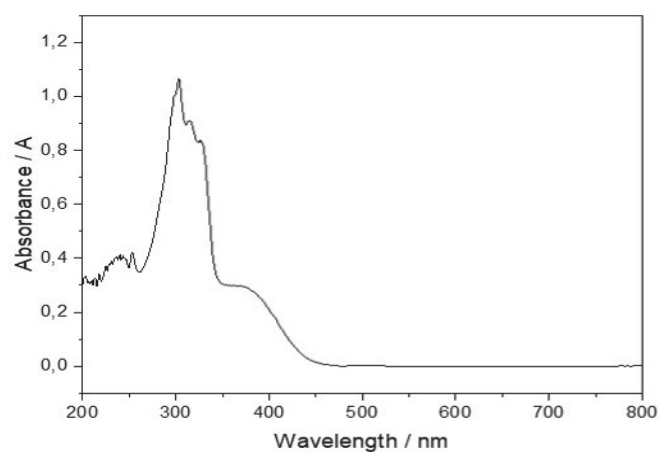
Fig. S24. FTIR spectrum of **2c**Fig. S25. FTIR spectrum of **2d**

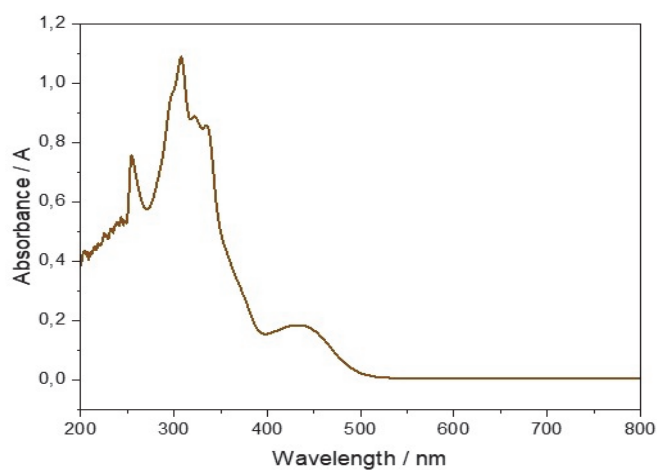
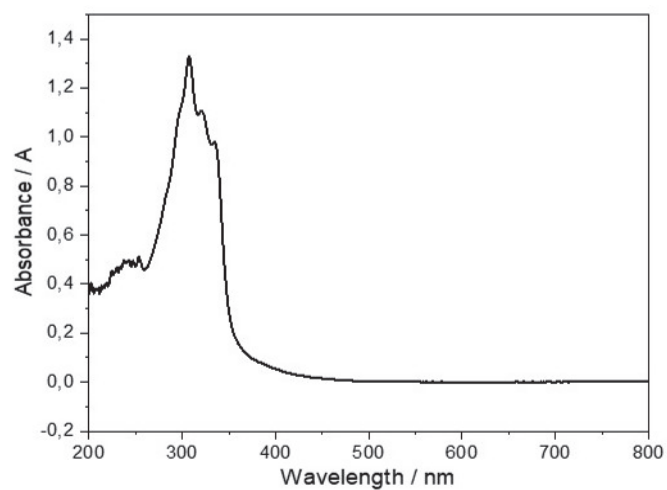
Fig. S26. FTIR spectrum of **2e**Fig. S27. UV-visible spectrum of **H₂L¹**

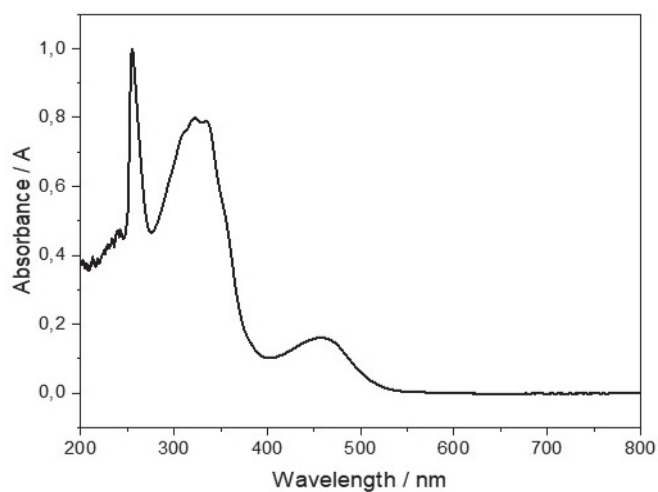
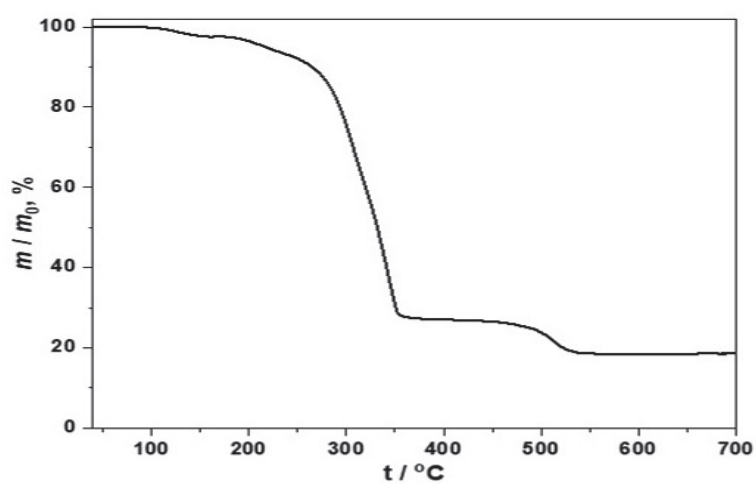
Fig. S28. UV-visible spectrum of **1a**Fig. S29. UV-visible spectrum of **1b**

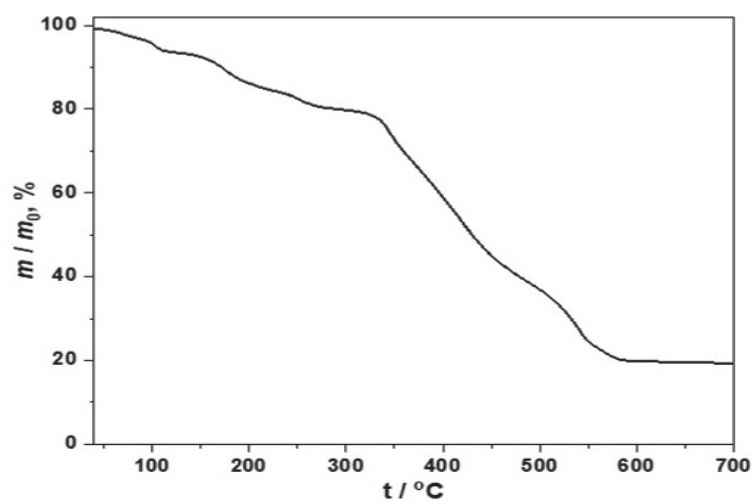
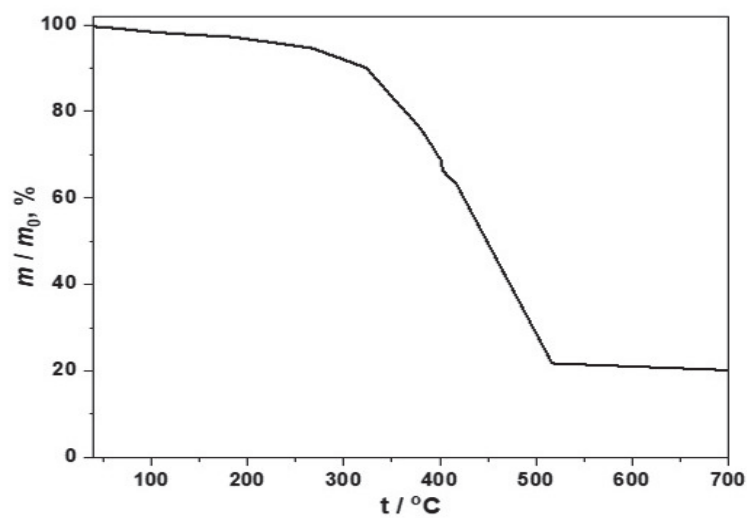
Fig. S30. UV-visible spectrum of **1c**Fig. S31. UV-visible spectrum of **1d**

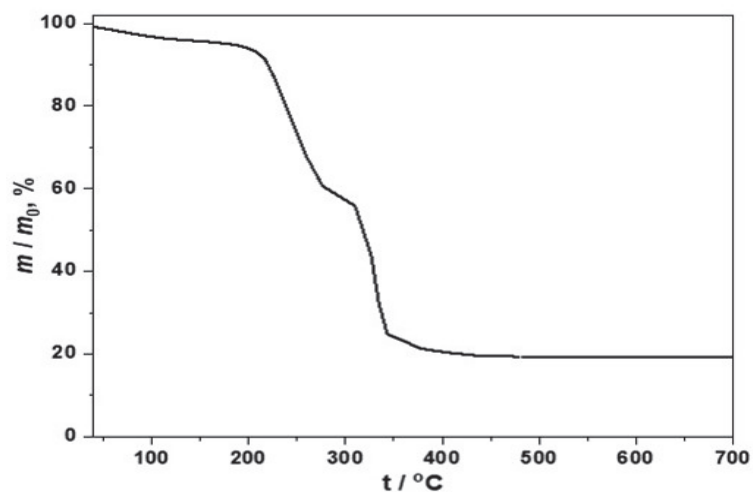
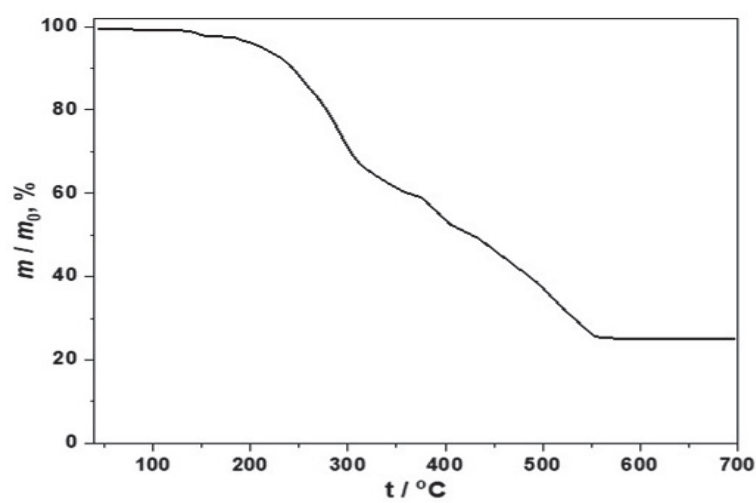
Fig. S32. UV-visible spectrum of **1e**Fig. S33. UV-visible spectrum of **HL²**

Fig. S34. UV-visible spectrum of **2a**Fig. S35. UV-visible spectrum of **2b**

Fig. S36. UV-visible spectrum of **2c**Fig. S37. UV-visible spectrum of **2d**

Fig. S38. UV-visible spectrum of **2e**Fig. S39. TGA curve of **2a**

Fig. S40. TGA curve of **2b**Fig. S41. TGA curve of **2c**

Fig. S42. TGA curve of **2d**Fig. S43. TGA curve of **2e**.



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Redox pathways in the carnosine–manganese system mediated by semiquinone radicals

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Abstract: The interaction of carnosine histidine-containing dipeptide with the semiquinone radical of 3,4-dihydroxybenzoic dianion in dimethyl sulfoxide solution under aerobic conditions was investigated in the present study. The interaction of dipeptide–manganese system with the same semiquinone was also studied under the identical conditions. The metal-binding and redox chemistry of these systems were investigated using electronic, ESR, ¹H-NMR spectroscopies and cyclic voltammetry. The *in situ* formal semiquinone radicals (extensively studied in previous works) were significantly modified upon addition of carnosine, as evidenced by the redistribution of the visible absorption bands and the change of the redox couples of the catecholic product. Subsequent influence of Mn ions induced further spectral and electrochemical changes, consistent with metal centered oxidation and formation of ligand-stabilized manganese species. Paramagnetic broadening in the ¹H-NMR spectra supported the presence of higher-valent Mn intermediates in solution. The combined data support a cooperative redox pathway in which semiquinone species mediate both peptide oxidation and Mn centered electron transfer. These results provide chemical insight into the understanding of multicomponent systems usually present in nature.

Keywords: carnosine; manganese; semiquinone radicals; catechol oxidation; ESR spectroscopy; cyclic voltammetry.

INTRODUCTION

L-Carnosine (β -alanyl-L-histidine) is a naturally occurring histidine-containing dipeptide widely distributed in excitable tissues such as skeletal muscle and brain. It has attracted considerable attention because of its buffering capacity,

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antioxidant activity, carbonyl scavenging properties and ability to coordinate transition-metal ions through the imidazole and carboxylate donor groups.^{1,2} These physicochemical characteristics make carnosine an important model compound for studying peptide-mediated redox and coordination processes in solution.

Catechol derivatives such as dihydroxybenzoic acids are well known to undergo oxidation in the presence of dioxygen, generating semiquinone radicals and quinonoid products through sequential one-electron transfer processes.^{3–5} Such intermediates are of particular interest because they often display characteristic visible absorption bands, persistent ESR signals and rich electrochemical behavior. In addition, semiquinone species can participate in coupled reactions with nucleophiles, peptides and transition-metal ions, thereby modifying both the redox profile and coordination chemistry of multicomponent systems.⁶

Manganese is especially relevant in this context because it can access multiple oxidation states under mild conditions, most commonly Mn(II) and Mn(III), and frequently forms kinetically labile yet spectroscopically informative complexes with O- and N-donor ligands.^{7–10} Redox-active manganese species are central to many chemical transformations, while ligand environment strongly influences their stability, electron-transfer behavior and reactivity toward oxygen-derived intermediates.^{11,12} Accordingly, mixed systems containing manganese ions, catechol-derived radicals and chemically relevant ligands provide useful models for investigating cooperative electron-transfer pathways.

Despite the well-established metal-binding and antioxidant properties of carnosine, its behavior in radical-mediated manganese redox systems remains insufficiently understood, particularly under non-enzymatic conditions. In our previous studies we have characterized the spectroscopic and electrochemical properties of catechol-derived semiquinone systems and their redox transformations in solution.^{13,14} However, the interplay between such radical intermediates, peptide ligands and manganese ions has not been systematically investigated.

In systems where catechol derivatives generate semiquinone species, complex reaction pathways may arise involving simultaneous radical transformations, peptide modification and metal-centered electron transfer. The present study addresses this gap by examining the interactions among L-carnosine, Mn(II) ions and semiquinone radicals derived from 3,4-dihydroxybenzoic acid in a controlled chemical environment. By combining spectroscopic (UV–Vis, ESR, ¹H-NMR) and electrochemical techniques, we aim to elucidate the redox pathways governing this multicomponent system and to define the role of semiquinone intermediates in mediating manganese oxidation and peptide transformation.

It should be emphasized that the present study focuses on a simplified chemical model system and does not attempt to directly reproduce enzymatic or biological conditions.

EXPERIMENTAL

Materials and instrumentation

All chemicals were used as received: L-carnosine (99 % crystalline, Sigma–Aldrich), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (Merck), manganese(II) acetate tetrahydrate (Merck), tetrabutylammonium hexafluorophosphate (98 %, Fluka Chemical), tetrabutylammonium hydroxide 30-hydrate (Sigma–Aldrich), 2,3-dihydroxybenzoic acid (2,3-DHBA, Sigma–Aldrich), 3,4-dihydroxybenzoic acid (3,4-DHBA, Sigma–Aldrich), EtOH (min. 99.8 %, Carlo Erba), hydrogen peroxide (30 %, Merck), D_2O (99.90 % D, Eurisotop) and high-purity Argon (99.99 %).

UV–Vis spectra were recorded on Hitachi U-2000 and Varian Cary 3E spectrophotometers. IR spectra were recorded on a Perkin–Elmer 883 infrared spectrophotometer. ^1H - and ^{13}C -NMR spectra were recorded on a Varian Unity Plus 300 MHz instrument ($\pm 0.1^\circ\text{C}$). X-Band EPR (ESR) spectra were recorded at NCSR “Demokritos” on a Bruker ER-200D-SCR instrument equipped with an Oxford ESR 900 cryostat. Cyclic voltammetry (CV) was performed on a Pine Research Instrumentation Bipotentiostat (model AFCBP1). Elemental analysis (C, H, N) was carried out on a Perkin–Elmer 2400 analyzer. Raman spectra were obtained using a Renishaw inVia reflex Raman microscope (532 and 785 nm excitation).

Experimental procedures

For UV–Vis spectroscopy, a solution was prepared by dissolving 10 mg (0.065 mmol) of 3,4-DHBA in 25 mL of DMSO, followed by the addition of 0.100 g (0.125 mmol) of tetrabutylammonium hydroxide (TBAH). The UV–Vis spectra were recorded before and after the addition of 0.0147 g (0.065 mmol) of carnosine. Subsequently, 5 mL of a $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ solution (0.0129 g, 0.065 mmol) in DMSO was added. For comparison, the spectrum of a solution containing equimolar isolated carnosine–manganese complex was also recorded.

For EPR (ESR) spectroscopy, the spectrum of the 3,4-DHBA dianion ($c = 0.0065\text{ M}$) in DMSO was recorded at $T = 4.2\text{ K}$. Measurements were also performed at 4.2 and 13 K following the addition of an equimolar amount of carnosine (0.0147 g) to 10 mL of the initial solution.

Cyclic voltammetry (CV) measurements involved adding 3 mg (0.020 mmol) of 3,4-DHBA to 20 mL of 0.1 M tetrabutylammonium chloride (TBAC) in DMSO, followed by 32 mg (0.040 mmol) of TBAH. Voltammograms were obtained for the initial solution, after the addition of 4.5 mg (0.020 mmol) of carnosine, and after adding 4 mg (0.02 mmol) of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$. The electrochemical behavior of the isolated carnosine–Mn complex was also studied using a three-electrode setup: graphite (working), platinum/gold (auxiliary) and Ag/AgCl (reference).

^1H -NMR spectra were obtained by adding carnosine ($c = 0.0065\text{ mmol L}^{-1}$) and $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ($c = 0.0065\text{ mmol L}^{-1}$) gradually to a solution of 3,4-DHBA/TBAH in $\text{DMSO}-d_6$. Spectra were collected at each titration step. Finally, a spectrum was recorded for a solution containing the isolated carnosine–Mn complex (Mn/carnosine 1:1) in a 1:2 mixture of 3,4-DHBA/TBAH in $\text{DMSO}-d_6$.

Spectra were processed uniformly for baseline correction and visualization purposes without altering the analytical content.

RESULTS AND DISCUSSION

UV–Vis and ESR spectroscopy

The dianionic form of 3,4-DHBA prepared in DMSO under aerobic conditions exhibits broad visible absorption bands centered at 672, 676 and 706 nm (Fig. S-1 of the Supplementary material to this paper). These features are consistent with the

formation of oxygen-stabilized semiquinone radicals arising from catechol oxidation, where overlapping mono- and di-ionized forms contribute to the observed spectral envelope.^{3–5} Additional shoulders in the 320–400 nm region and a strong transition near 300 nm can be attributed to $\pi \rightarrow \pi^*$ excitations associated with partial oxidation of the aromatic framework.^{4,15}

Addition of equimolar carnosine produces an immediate color change to deep purple and generates new absorption bands at approximately 500 and 590 nm (Fig. S-2 of the Supplementary material). The redistribution of visible absorption bands, accompanied by increased molar absorptivity, indicates the formation of a new redox-active species involving chemical interaction between semiquinone intermediates and carnosine. The persistence of semiquinone- and quinonoid-type features suggests that the process involves redox coupling rather than simple radical quenching.⁶

Introduction of Mn^{2+} results in further spectral evolution, producing a brown–yellow solution with weak extended visible absorption (Fig. 1). These features are consistent with the formation of Mn(III) species, likely stabilized through coordination with oxygen- and nitrogen-donor ligands present in the system. Comparable spectral behavior observed after addition of the isolated carnosine–manganese complex (solid 24, Fig. S-3 of the Supplementary material)²⁵ further supports the formation of electronically coupled Mn-containing intermediates.

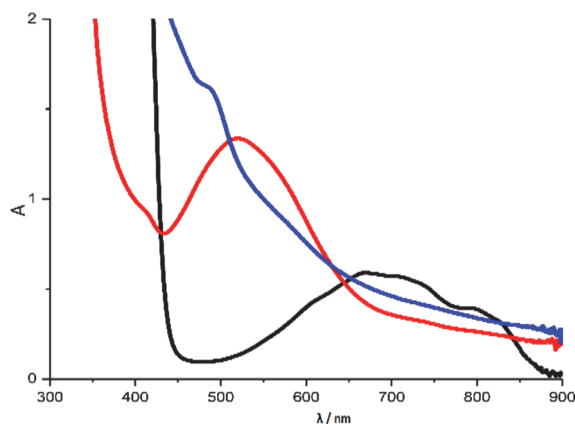


Fig. 1. Electronic spectra of 3,4-DHBA dianion in DMSO (black line), upon addition of carnosine (1:1 ratio, red line) and subsequent addition of equimolar $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (blue line).

A solution of 3,4-DHBA dianion in DMSO under aerobic conditions exhibits a strong ESR signal characteristic of a radical species (Fig. 2). The signal remains largely unchanged upon increasing the temperature to 15 or 30 K, indicating appreciable persistence of the paramagnetic intermediate under the experimental conditions.

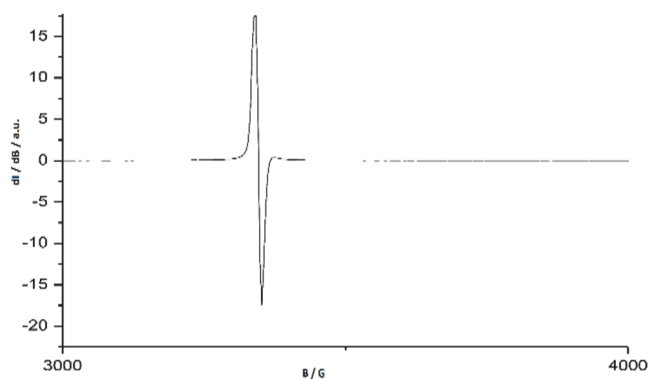


Fig. 2. ESR spectrum of a cyan solution of the 3,4-DHBA dianion ($c = 0.0065$ M) in DMSO under aerobic conditions, recorded at $T = 4.2$ K. Microwave power 0.3 mW, modulation amplitude 1.0 G, receiver gain 26 dB.

The addition of carnosine to the radical-containing solution in a 1:1 mole ratio leads to measurable changes in the ESR spectrum (Fig. 3), indicating interaction between the semiquinone species and the dipeptide. These observations indicate chemical transformation of the radical species upon interaction with carnosine, supporting the involvement of semiquinone intermediates in subsequent redox processes.

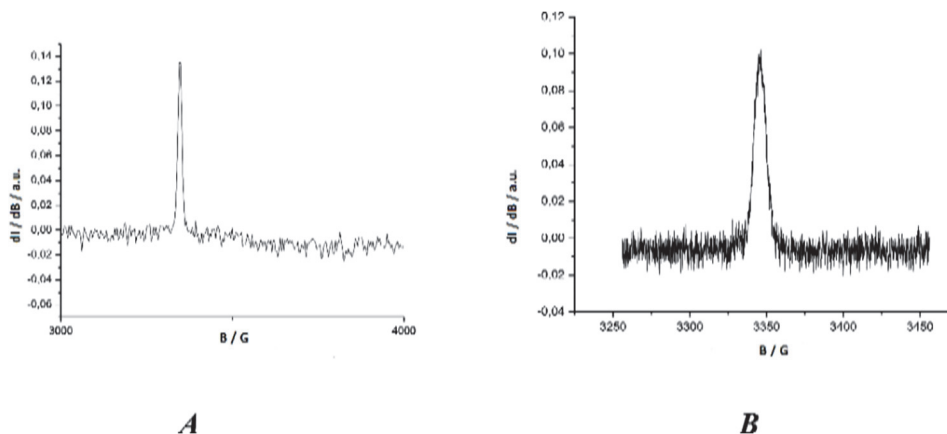
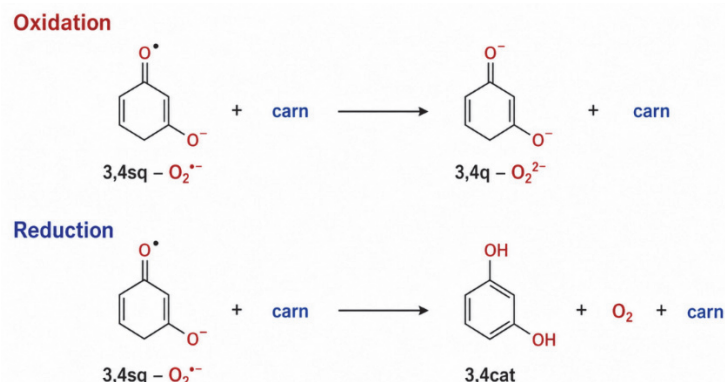


Fig. 3. ESR spectra of a green DMSO solution containing the 3,4-DHBA dianion ($c = 0.0065$ M) and carnosine ($c = 0.0065$ M) recorded at: A) 4.2 and B) 13 K. Microwave power 0.3 mW, modulation amplitude 1.0 G, receiver gain 26 dB.

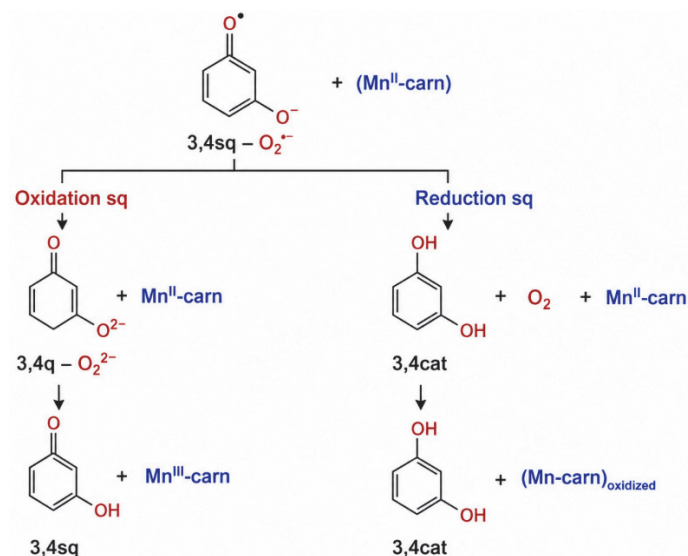
If the reaction between the radical and the dipeptide involves reduction of the semiquinone species (*e.g.*, in a superoxide-associated form, $3,4\text{-SQ}^{\bullet}\text{-O}_2^-$), Scheme 1, regeneration of the catechol form of DHBA would be expected. In such a case, partial decolorization of the solution might occur, as catechol derivatives

typically exhibit weak absorption in the visible region, while the released molecular oxygen would not be expected to react rapidly with carnosine under the present conditions.



Scheme 1. Proposed reaction pathways for the interaction between 3,4-DHBA semiquinone radical and carnosine, illustrating the competitive oxidative and reductive routes.

Conversely, if the process involves oxidation of the semiquinone, Scheme 2, formation of the corresponding quinone species and hydrogen peroxide is plausible. Both species are known to participate in oxidative reactions with nucleophilic sites of peptides, including the imidazole moiety of carnosine. Under these conditions, the development of brown–green coloration is expected, consistent with the experimental observations.



Scheme 2. Proposed mechanism for the metal-assisted electron transfer between 3,4-DHBA semiquinone and the Mn(II)–carnosine complex.

The oxidative pathway likely involves the formation of a semiquinone–carnosine intermediate, which may facilitate interaction with dioxygen-derived species and contribute to charge-transfer stabilization within the system.

Electrochemical behavior

Cyclic voltammetry (CV) of the 3,4-DHBA dianion reveals two well-defined quasi-reversible redox couples (Fig. S-4 of the Supplementary material), consistent with sequential one-electron catechol/semiquinone/quinone transformations.¹ The peak separation suggests moderately fast electron transfer with partial chemical follow-up reactions, likely involving oxygen coordination.

Upon addition of equimolar carnosine, a positive shift in the first redox couple is observed, accompanied by an irreversible oxidation wave at 0.68 V (Fig. 4). This behavior indicates that carnosine perturbs the redox equilibrium through chemical coupling rather than simple hydrogen bonding. The irreversible feature can be attributed to oxidative modification of the imidazole-containing dipeptide or formation of electrochemically unstable quinonoid adducts.

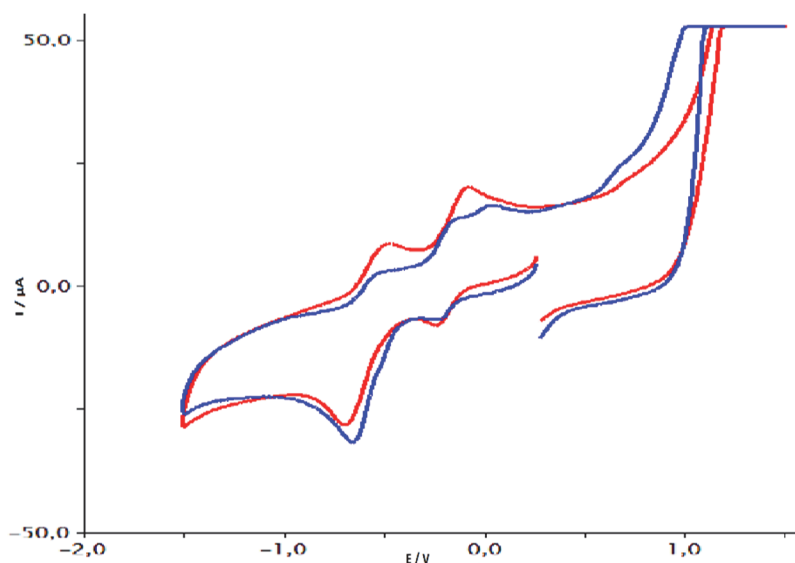


Fig. 4. Cyclic voltammograms of 3,4-DHBA dianion in DMSO before (red line) and after addition of carnosine (blue line). Scan rate: 100 mV s⁻¹.

Introduction of Mn²⁺ dramatically alters the voltammetric profile (Fig. 5). The attenuation of the semiquinone oxidation signal and the emergence of broad irreversible processes between 0.30–0.80 V are consistent with manganese-centered oxidation.

These signatures are consistent with Mn²⁺ → Mn³⁺ conversion followed by rapid ligand coordination. The disappearance of the semiquinone oxidation signal

and persistence of a multi-electron reduction wave indicate kinetically slow Mn(II) oxidation to Mn(III), likely occurring within a DHBA-associated coordination environment.

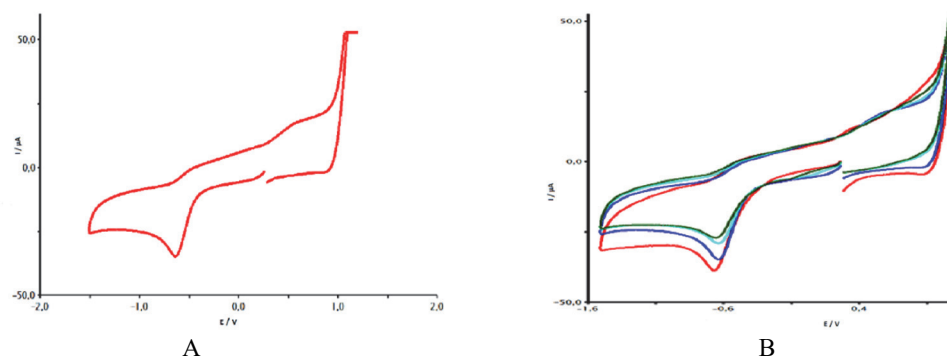


Fig. 5. Cyclic voltammograms of the 3,4-DHBA dianion in DMSO with carnosine: A) immediately after Mn^{2+} addition; B) time-dependent evolution: 0 min (red), 2 min (blue), 4 min (green), 6 min (pink) and 8 min (black). Scan rate: 100 mV s^{-1} .

Comparable irreversible behavior observed for the isolated carnosine–manganese complex (solid 27, Fig. S-5 of the Supplementary material)²⁵ reinforces the assignment of ligand-stabilized Mn(III) intermediates. Overall, the electrochemical data support a mechanism in which semiquinone radicals act as transient electron shuttles, likely promoting manganese oxidation while undergoing peptide-mediated trapping.

A comparative summary of the electrochemical parameters for all investigated systems is provided in Table I.

TABLE I. Summary of redox potentials for the investigated systems in DMSO; E_{pa} : anodic peak potential; E_{pc} : cathodic peak potential; ΔE_{p} : peak-to-peak separation

System	E_{pa} V	E_{pc} V	ΔE_{p} V	Electrochemical behavior
3,4-DHBA dianion	~0.45	~0.30	~0.15	Quasi-reversible catechol/semiquinone redox couple
3,4-DHBA + carnosine (1:1)	~0.50	~0.32	~0.18	Positive shift; appearance of irreversible oxidation at ~0.68 V
3,4-DHBA + carnosine + Mn^{2+}	—	—	—	Broad irreversible processes (0.30–0.80 V); Mn-centered oxidation
Carnosine–Mn complex	—	—	—	Predominantly irreversible behavior; ligand-stabilized Mn(III) formation

The stability of Mn(III) species in solution is strongly dependent on ligand environment. In the absence of stabilizing ligands, Mn(III) is typically prone to disproportionation. However, coordination to oxygen- and nitrogen-donor ligands,

such as carnosine and catechol-derived species, can provide both kinetic and thermodynamic stabilization. In the present system, the persistence of electrochemical and spectroscopic signatures attributed to Mn(III) suggests that ligand coordination effectively stabilizes the higher oxidation state, at least on the experimental timescale.

NMR analysis

^1H -NMR spectroscopy provides complementary evidence for interaction between carnosine and oxidized DHBA species (Fig. 6). Pronounced upfield shifts of the imidazole resonances indicate changes in the electronic environment, consistent with possible coordination or interaction with electron-deficient quinonoid structures. Such perturbations are consistent with partial redistribution of electron density within the heteroaromatic ring following oxidative coupling.

Following Mn^{2+} addition, the spectrum exhibits substantial line broadening and reduced resolution (Fig. 7), behavior typical of paramagnetic Mn(III) centers.^{22,23}

The appearance of a broad resonance near 9.03 ppm is consistent with the formation of metal-associated species undergoing rapid exchange on the NMR timescale. Addition of isolated carnosine-manganese complex to the DHBA dianion generates an even more intricate spectral pattern (Fig. 8), suggesting the presence of multiple equilibrating coordination environments rather than a single dominant complex. This observation aligns with the electrochemical evidence for dynamic metal–ligand speciation.

Taken together, the NMR data support a model in which carnosine participates both as a redox-active partner and as a ligand scaffold capable of stabilizing higher oxidation states of manganese. The combined observations are consistent with a mechanism involving initial semiquinone formation, followed by peptide-mediated oxidative trapping and subsequent electron transfer to Mn(II), yielding ligand-stabilized Mn(III) intermediates.²⁴

Proposed redox sequence

The spectroscopic and electrochemical results support a cooperative redox sequence in the 3,4-DHBA–carnosine– Mn^{2+} system.

1. *Semiquinone formation.* Aerobic oxidation of 3,4-dihydroxybenzoic acid leads to the formation of oxygen-stabilized semiquinone radicals,¹⁶ as evidenced by characteristic ESR signals and visible absorption bands.

2. *Interaction with carnosine.* Carnosine interacts with these intermediates, likely through an imidazole-mediated pathway,^{16,17} leading to attenuation of the ESR signal, redistribution of visible absorption bands and perturbation of the catechol redox couple, consistent with the formation of quinonoid or peptide-associated species.

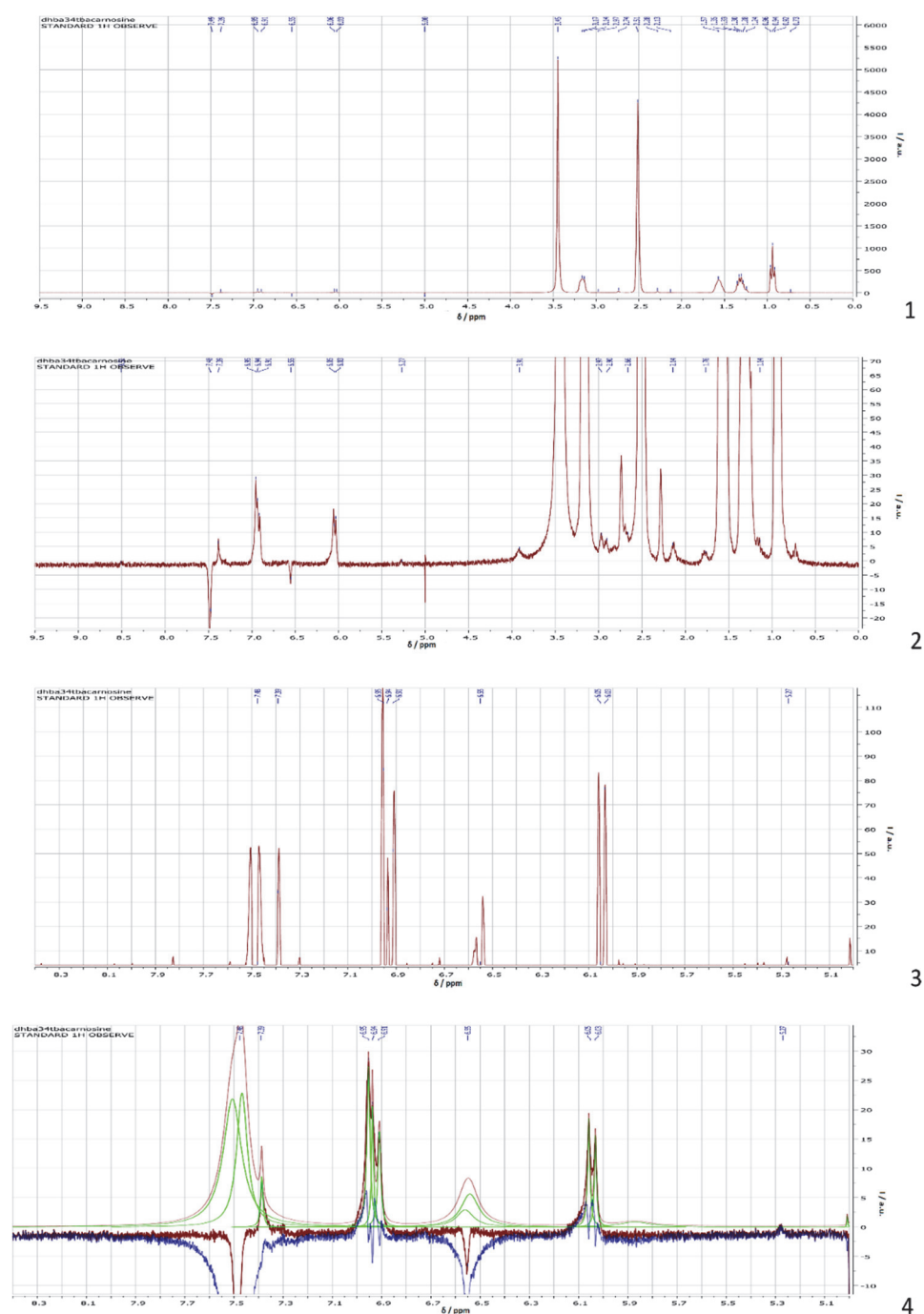


Fig. 6. ^1H -NMR spectrum of the 3,4-DHBA/carnosine in $\text{DMSO}-d_6$; panels 3 and 4 show the 5–10 ppm region processed with resolution booster and global spectral deconvolution (GSD).

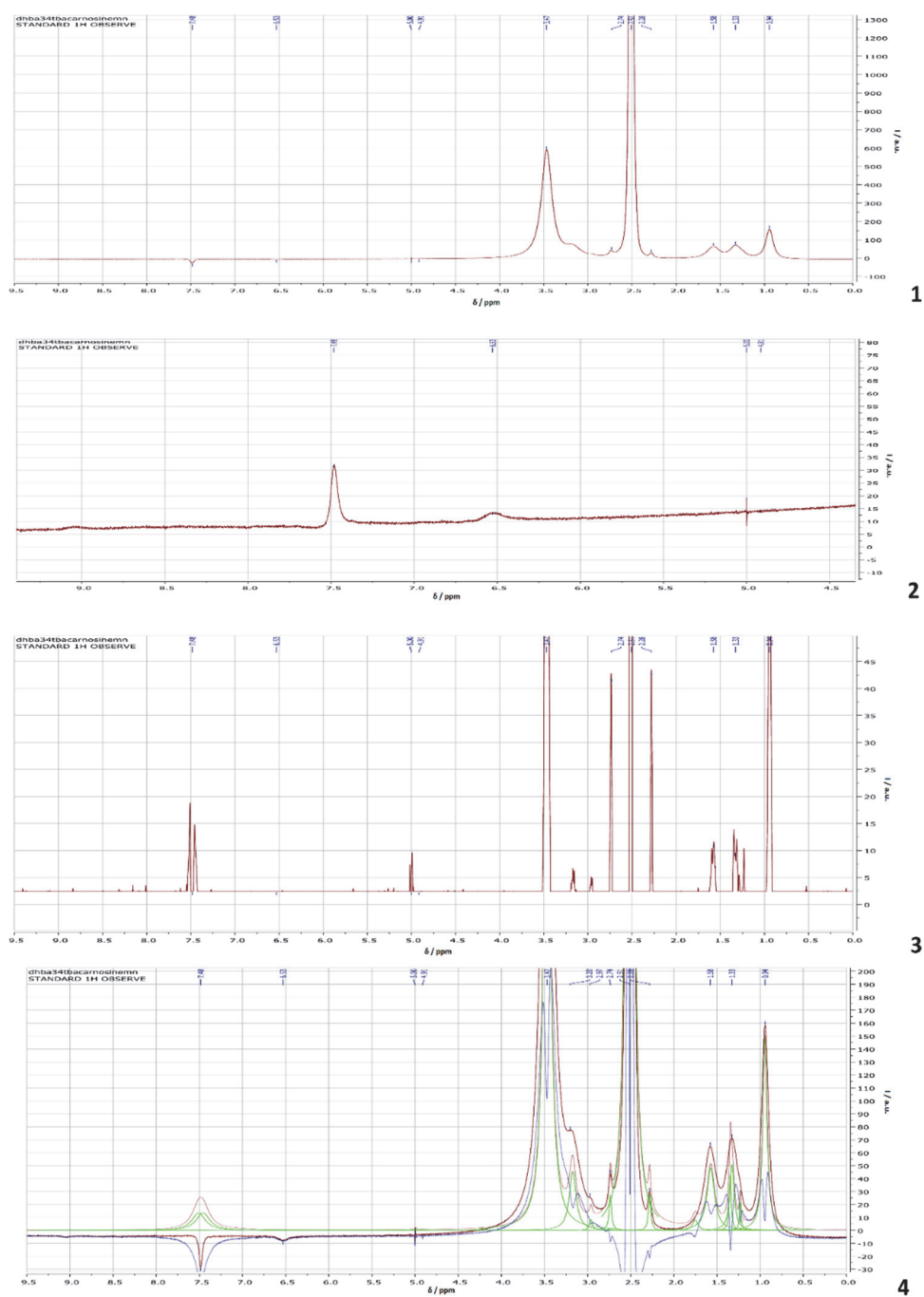


Fig. 7. ^1H -NMR spectrum of the 3,4-DHBA/carnosine/ $\text{Mn}^{2+}(\text{aq})$ mixture in $\text{DMSO}-d_6$; panels 3 and 4 represent resolution booster and GSD analyses.

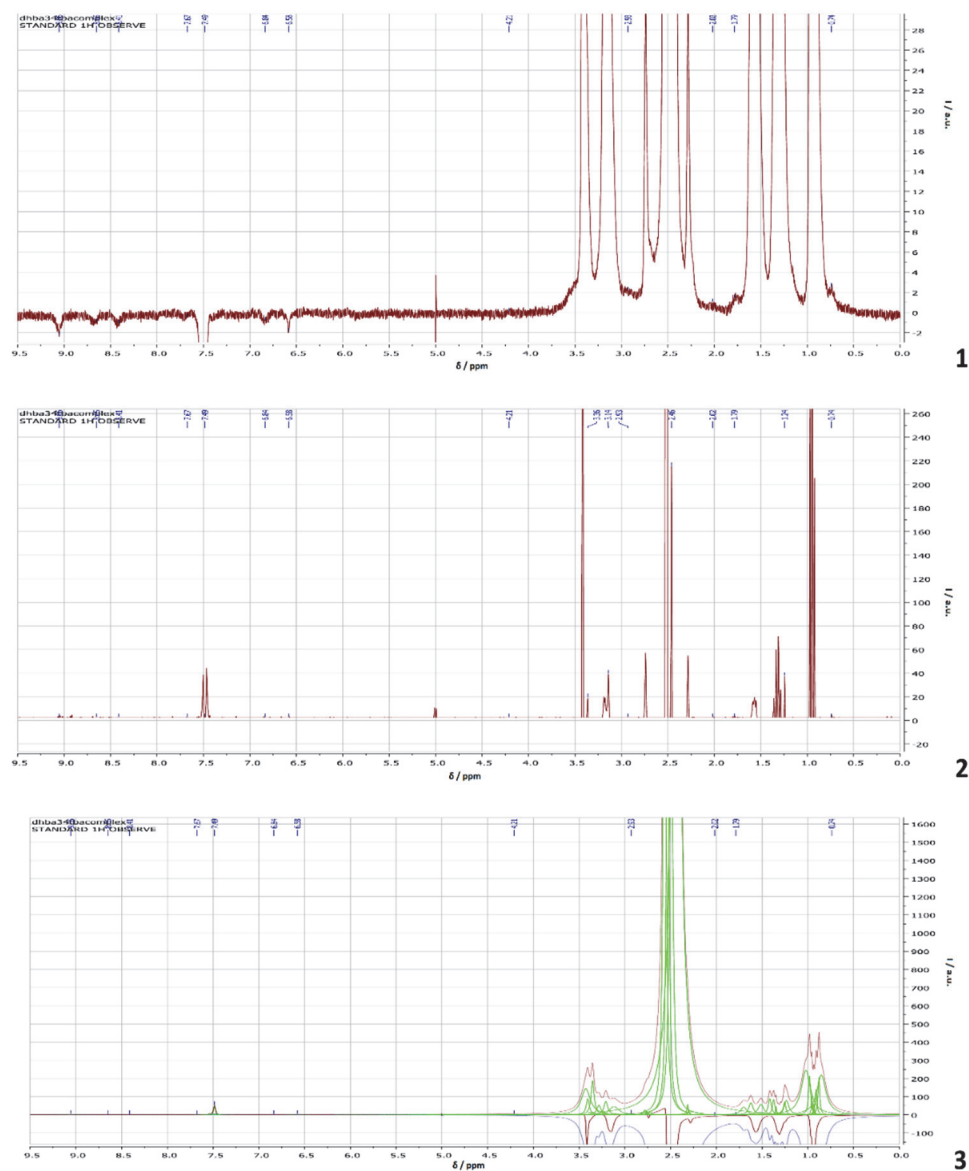


Fig. 8. ¹H-NMR spectrum of the 3,4-DHBA/ isolated carnosine-manganese complex (solid 24)²⁵ in DMSO-*d*₆; panels 2 and 3 show GSD and resolution booster analyses.

3. *Semiquinone-mediated Mn oxidation.* In the presence of Mn²⁺, semiquinone intermediates can act as electron-transfer mediators, promoting oxidation of the metal center to higher oxidation states.¹⁸ Attenuation of semiquinone electrochemical signals and the appearance of irreversible anodic processes are consistent with coupled metal-centered redox processes.

4. *Stabilization of Mn(III)*. The resulting Mn(III) species are likely stabilized through coordination to oxygen-donor groups and the imidazole functionality of carnosine, forming dynamic solution structures.^{18–21} Paramagnetic ¹H-NMR broadening and irreversible voltammetric behavior in the 0.30–0.80 V region support the presence of ligand-stabilized high-spin Mn(III) species.^{9,18,21}

The proposed sequence is consistent with the observed ESR attenuation, irreversible voltammetry, and paramagnetic NMR effects, as summarized schematically in Fig. 9.

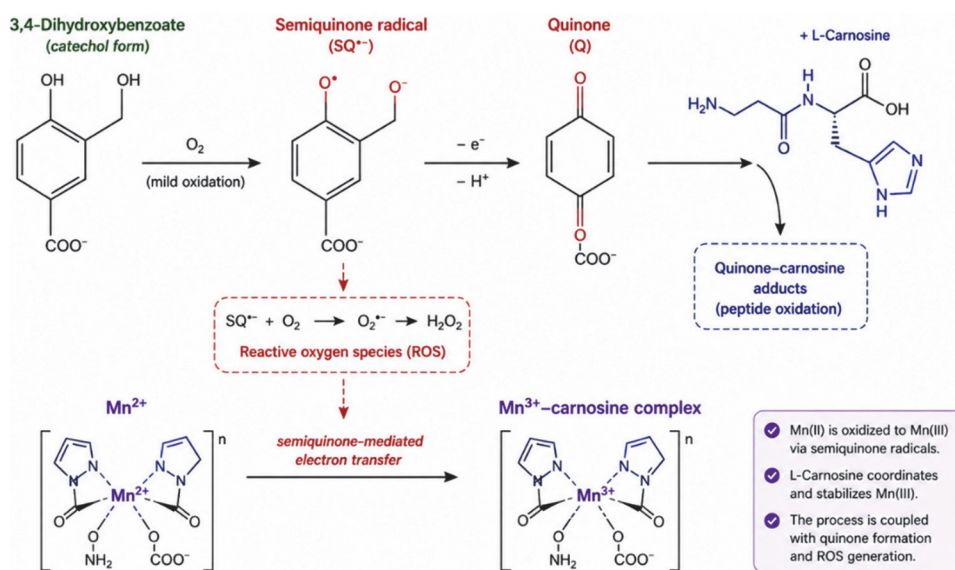


Fig. 9. Schematic representation of the proposed redox sequence in the 3,4-DHBA–carnosine–Mn²⁺ system illustrating semiquinone-mediated electron transfer leading to oxidation of Mn(II) to Mn(III) and stabilization of the resulting metal complex by L-carnosine.

CONCLUSION

This study establishes a coherent redox framework for the interactions between L-carnosine, Mn²⁺ and semiquinone radicals derived from 3,4-dihydroxybenzoic acid under non-enzymatic conditions. Combined UV–Vis, ESR, ¹H-NMR and cyclic voltammetry measurements demonstrate that semiquinone intermediates participate in both peptide oxidation and manganese-centered electron-transfer processes.

Carnosine acts as both a redox-active component and a coordinating ligand, interacting with semiquinone species and contributing to the stabilization of higher-valent manganese intermediates. The formation of ligand-stabilized Mn(III) species is supported by spectroscopic and electrochemical evidence, including paramagnetic NMR behavior and irreversible voltammetric features.

Overall, the results support a cooperative redox pathway in which semiquinone radicals function as transient electron-transfer mediators within a multicomponent system. These findings provide insight into redox interactions between manganese ions, catechol-derived species and peptide ligands in solution, offering a chemically consistent basis for understanding such processes in complex environments.

NOMENCLATURE

3,4-DHBA: 3,4-dihydroxybenzoic acid
DMSO: dimethyl sulfoxide
ESR: electron spin resonance
CV: cyclic voltammetry
NMR: nuclear magnetic resonance
UV–Vis: ultraviolet–visible spectroscopy
E_{pa}: anodic peak potential
E_{pc}: cathodic peak potential
 ΔE_p : peak-to-peak separation
g: g-factor (ESR spectroscopy)
 δ : chemical shift (NMR spectroscopy)

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13801>, or from the corresponding author on request.

ИЗВОД

РЕДОКС РЕАКЦИЈЕ У КАРНОЗИН–МАНГАН СИСТЕМУ У ПРИСУСТВУ СЕМИХИНОНСКИХ РАДИКАЛА

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У овом раду испитивана је интеракција карнозина, дипептида који садржи хистидин, са семихинонским радикалом дианјона 3,4-дихидроксибензојеве киселине у раствору диметил-сулфоксида у аеробним условима. Поред тога, испитивана је интеракција система дипептид–манган са истим семихинонским радикалом при истим условима. Координација за метал и редокс хемија ових система испитивани су применом електронске, ESR и ¹H-NMR спектроскопије, као и цикличне волтаметрије. *In-situ* формирану семихинонски радикали (детаљно проучавани у претходним радовима) били су значајно модификовани након додавања карнозина, што је потврђено прерасподелом апсорпционих трака и променом редокс парова катехолног производа. Утицај јона мангана изазвао је додатне спектралне и електрохемијске промене, у складу са оксидацијом усмереном на јон метала и формирањем комплексних врста мангана стабилованих лигандом. Параметар магнетно ширење сигнала у ¹H-NMR спектрима указало је на присуство интермедијера

мангана виших оксидационих стања у раствору. Комбиновани подаци указују на кооперативни редокс механизам у којем семихинонске врсте посредују и у оксидацији пептида и у преносу електрона усмереном на јон мангана. Ови резултати пружају увид у разумевање вишекомпонентних система који су често присутни у природи.

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SUPPLEMENTARY MATERIAL TO
**Redox pathways in the carnosine–manganese system mediated
by semiquinone radicals**

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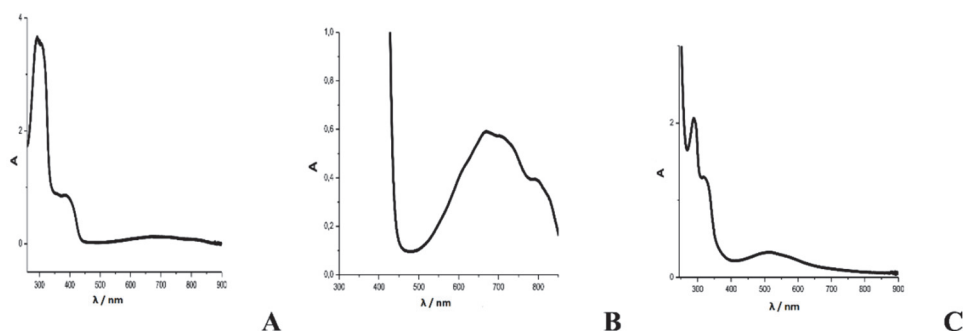


Fig. S-1. Electronic spectrum of 3,4-DHBA dianion ($c = 2.6 \times 10^{-3}$ M) in DMSO under aerobic conditions: (A, B) UV and Vis regions for the cyan form and (C) for the green form.

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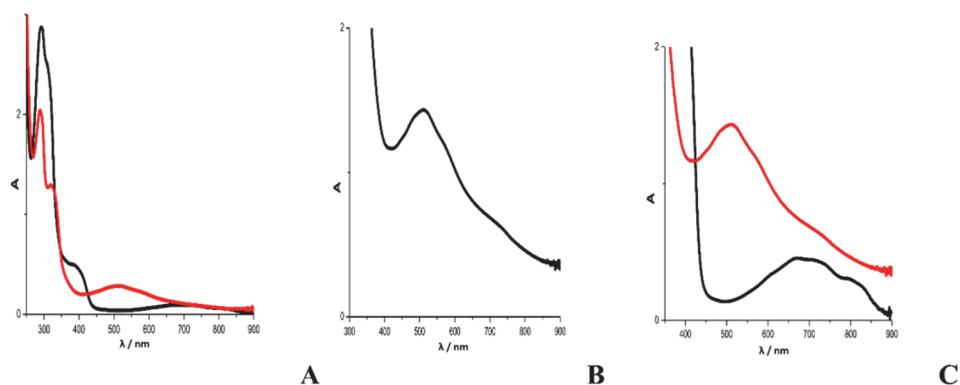


Fig. S-2. Electronic spectra of a 1:1 mixture of 3,4-DHBA dianion and carnosine in DMSO under aerobic conditions: A) comparison between 3,4-DHBA dianion (black line) and its mixture with carnosine (red line); B) visible region showing the blue form; C) comparison of the visible region.

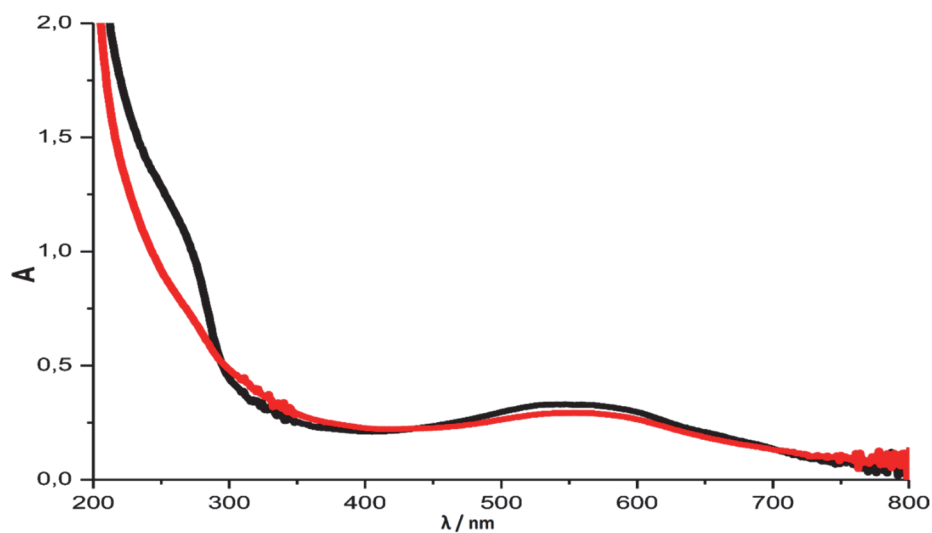


Fig. S-3. Electronic spectrum of the 3,4-DHBA dianion mixture in DMSO (black trace) compared with the spectrum after addition of an equimolar solution of isolated carnosine-manganese complex (solid 24)²⁵ (red trace).

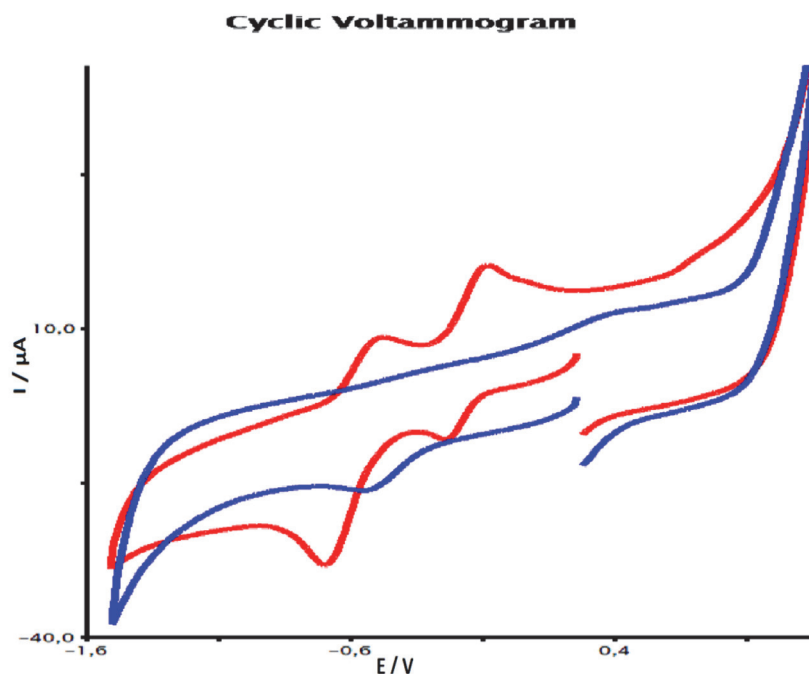


Fig. S-4. Cyclic voltammograms of 3,4-DHBA dianion in DMSO before (red line) and after 25 min of electrolysis (blue line). Potential range: -1500 to 1500 mV; scan rate: 100 mV s^{-1} .

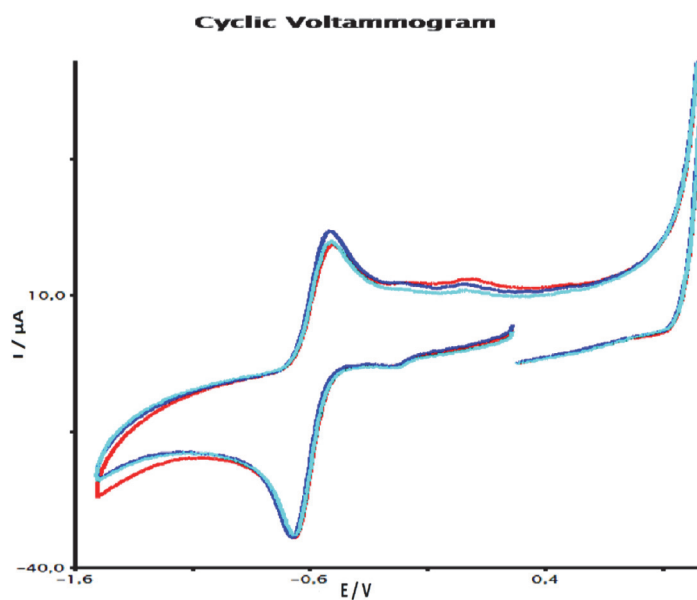


Fig. S-5. Cyclic voltammograms of isolated carnosine-manganese complex (solid 27)²⁵ ($c = 10^{-3} \text{ M}$) in a DMSO solution of 3,4-DHBA dianion at $t = 0$ min (red), 10 min (blue), and 25 min (green). Scan rate: 100 mV s^{-1} .



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The features of the intrinsic photoconductivity spectra of silver-doped zinc sulphide

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Abstract: The intrinsic photoconductivity of silver-doped zinc sulphide (ZnS:Ag) was investigated. It was shown that doping with silver leads to significant modifications in the electronic structure and optical properties of ZnS due to the formation of localized energy levels within the band gap. Mechanical polishing of the sample surface resulted in the appearance of an additional photoconductivity maximum near the fundamental absorption edge, which gradually disappears during sample ageing. These effects are attributed to the compensation of donor and acceptor states, as well as to changes in charge carrier recombination mechanisms. The dependence of the photoconductivity spectrum on ageing time, the applied electric field, and the position of illumination relative to the contacts was established. The results are explained by variations in the lifetime of non-equilibrium charge carriers with depth. A qualitative model describing the photoconductivity spectra is proposed, taking into account the influence of silver compensation on the energy structure of ZnS. The proposed model provides insight into the photoconductive mechanisms and supports the development of ZnS-based optoelectronic materials.

Keywords: photoconductivity spectra; absorption coefficient; optoelectronic material.

INTRODUCTION

The great importance of modern research on photoconductivity is reflected in the recent rapid growth of interest in all areas of solid-state physics and chemistry.^{1–5} Silver alloying in single crystals leads to significant compensation and results in high sensitivity to radiation.^{6–10}

The main interest in ZnS:Ag is due to the fact that its photoconductivity excitation spectrum coincides with the emission spectrum of serial GaAs-based LEDs,

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which allows ZnS to be considered a promising material for the manufacture of high-performance resistor opt couplers.^{11–18} The reasons for the high photosensitivity of ZnS:Ag and CdS:Ag samples are not fully understood, but there is evidence of the presence of two levels in the forbidden band gap of these semiconductor sulphides, spaced 0.3 eV apart^{12–17} and located above the top of the valence band.^{19–21} The authors explored the idea of a possible relation between the indicated levels and the localization features of silver in the ZnS lattice to explain the appearance of an additional photoconductivity maximum in the region of the fundamental absorption edge.^{22–24} It is assumed that silver impurities exhibit similar behavior in the investigated semiconductor sulphides.^{25–30}

EXPERIMENTAL

For silver alloying, zinc sulphide grown by the Czochralski method with an electron concentration of $9.2 \times 10^{14} \text{ cm}^{-3}$ was used. Diffuse saturation of silver was carried out using a silver film applied to both surfaces in vacuum-sealed quartz ampoules evacuated to 10^{-5} Torr at temperatures of 800–900 °C. The diffusion time 1–10 h. After annealing, the ampoules were quenched in cold water and the samples were ground with abrasive powders and polished with diamond paste. Hall effect measurements of the free charge carrier concentration in annealed samples indicated that alloying enables the formation of both high-resistivity ($n = 2.4 \times 10^8 - 1 \times 10^9 \text{ cm}^{-3}$) and low-resistivity ($n = 1.4 \times 10^{15} \text{ cm}^{-3}$) materials. Contacts made of liquid Zn, Cd eutectic were applied to the polished surface of the samples in the form of two parallel strips, 2.2–3 mm long, with a gap between them. The photoconductivity signal was measured in the constant field mode using a modulation technique with synchronous detection at a modulation frequency of 8–400 Hz. For the study, high-quality samples were used in order to achieve a photocurrent that significantly exceeds the dark current when illuminated.

RESULTS AND DISCUSSION

Immediately after mechanical polishing of the sample surface, the photoconductivity spectrum exhibits two distinct maxima in the region of the fundamental absorption edge (Fig. 1). The short-wavelength maximum gradually disappears during storage under ambient conditions and is no longer observed after approximately two days. A similar effect is observed after thermal annealing, indicating that the additional peak is associated with surface-related states.

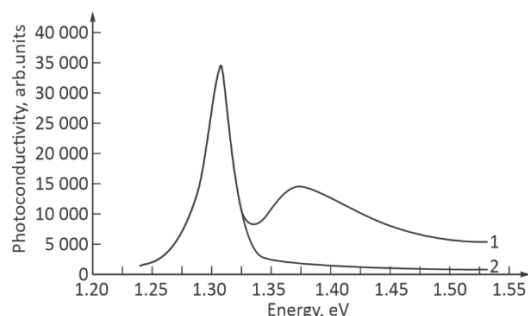


Fig. 1. Photoconductivity spectrum of ZnS:Ag immediately after polishing and after two days. Experimental data; 1 – after polishing at 250 V; 2 – after 50 h at a voltage of 250 V.

Fig. 2 shows the spectral dependence of the photoconductivity of Ag-doped ZnS single crystals as a function of photon energy in the region close to the fundamental absorption edge. Fig. 2 shows the photoconductivity spectra for 60 and 450 V.

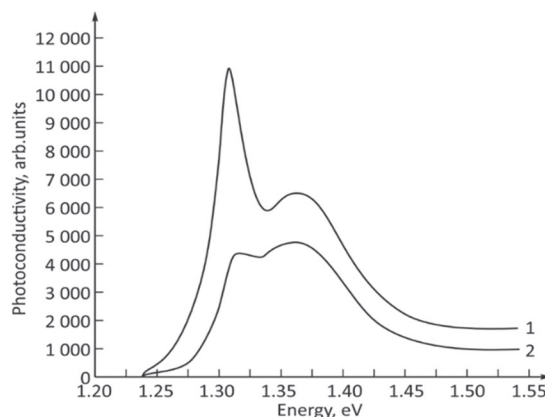


Fig. 2. Photoconductivity spectrum of a polished ZnS:Ag sample at different voltages. Experimental data at: 1 – 60 V; 2 – 450 V.

As seen from the spectra, the photoconductivity begins to increase rapidly when the photon energy approaches the fundamental absorption edge of ZnS. This behavior is associated with the generation of electron–hole pairs due to inter-band optical transitions. A pronounced maximum is observed in the short-wavelength region near approximately 1.30–1.32 eV. This peak can be attributed to intrinsic photoconductivity related to electronic transitions involving defect levels or localized states in the band gap introduced by silver doping. Two curves are presented in Fig. 2, corresponding to different surface conditions of the sample. Curve 1 exhibits a significantly higher photoconductivity signal compared to curve 2. This difference indicates that surface treatment strongly affects the photoconductive response of the crystal. Mechanical polishing modifies the surface defect structure and may create additional surface states that participate in charge carrier generation or recombination processes. After the main maximum, the photoconductivity gradually decreases with increasing photon energy. This behavior can be explained by changes in the efficiency of carrier generation, recombination processes, and carrier transport in the crystal. Overall, the observed spectral features demonstrate that both bulk defect states and surface-related states contribute to the formation of the intrinsic photoconductivity spectra in Ag-doped ZnS crystals. The results confirm that surface conditions play an important role in determining the magnitude and shape of the photoconductivity response.

It was noted that when the beam was focused near the negative contact, the short-wave peak predominated, whereas focused near the positive contact, the

long-wave peak predominated (Fig. 3). The interval between the start of the two experiments was no more than 10 minutes.

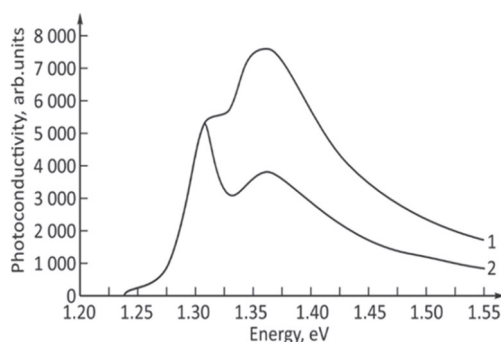


Fig. 3. Photoconductivity spectrum of a ZnS:Ag sample immediately after polishing with the focusing beam near the positive and negative contacts at a voltage of 200 V. Experimental data for field focusing 1 – near the negative contact, 2 – near the positive contact.

Previously, the observation of additional extrema beyond the fundamental absorption band edge in the ZnS:Ag photoconductivity spectra has been explained.²

Let us consider a sample model consisting of several layers. We assume that in ZnS:Ag the quantum yield η is constant in each layer, and the change in mobility from layer to layer is small.² Then the photoconductivity, $\Delta\sigma$, of the entire sample can be described by the following equation:

$$\begin{aligned} \Delta\sigma(h\nu) = & Q_1(1 - \exp(-\alpha t_1)) + Q_2 \exp(-\alpha t_1)(1 - \exp(-\alpha t_2)) + \\ & + Q_3 \exp(-\alpha t_1) \exp(-\alpha t_2)(1 - \exp(-\alpha t_3)) + \\ & + Q_4 \exp(-\alpha t_1) \exp(-\alpha t_2) \exp(-\alpha t_3)(1 - \exp(\alpha(d - t_1 - t_2 - t_3))) \end{aligned} \quad (1)$$

where Q_i is a coefficient proportional to the lifetime for each layer; $\alpha = f(h\nu)$ is a performance dependence of the absorption coefficient of ZnS; t_1 is the layer thickness; d is the sample thickness. Q_1 characterizes the contribution of fast recombination processes occurring in the near-surface layer of the crystal. Q_2 describes the trapping and recombination of charge carriers through localized states associated with surface defects. Q_3 represents the contribution of recombination processes occurring in the bulk region of the crystal. Q_4 characterizes the transport of photogenerated charge carriers into deeper layers of the crystal and delayed recombination processes. These parameters are used to fit the shape of the photoconductivity spectra and make it possible to evaluate the relative influence of different recombination mechanisms.

The absorption coefficient α of zinc sulphide was measured independently and the result presented in Fig. 4 is in good agreement with previously reported data.³ Regions of weak and strong absorption were approximated. In the region of the

fundamental absorption, as the energy of light quanta increases, the absorption coefficient increases significantly, which leads to a decrease in the penetration depth of light. For the energy range from 1.20 to 1.50 eV, the sample can be conditionally divided into 4 layers.

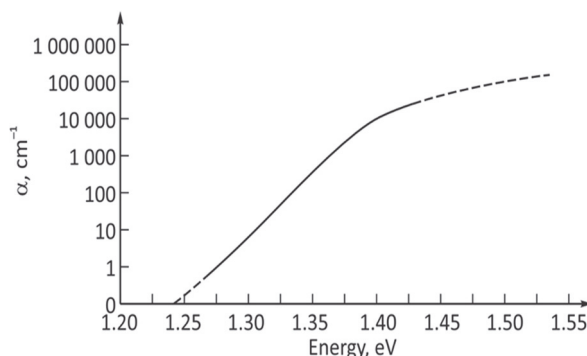


Fig. 4. Spectral dependence of the absorption coefficient of zinc sulphide.

Eq. (1) describes the photoconductivity spectra after polishing for ageing times from 0 to 50 h. The standard deviation did not exceed 4.285×10^{-3} . The values of the obtained parameters of Eq. (1) are given in Table I.

TABLE I Measurement data for the Q -parameters and thickness values t_1 – t_4

t_1 – t_4	Q_1	Q_2	Q_3	Q_4	$t_1 / \mu\text{m}$	$t_2 / \mu\text{m}$	$t_3 / \mu\text{m}$	$t_4 / \mu\text{m}$	$d / \mu\text{m}$
0	4720	14845	2788	50820	0.1843	20.05	205.1	1508.7	1675
1	3790	11440	2885	51840	0.1948	21.06	210.8	1539.8	1675
2	2880	10032	2886	51841	0.1946	21.07	210.7	1542.2	1675
3	2390	8195	2877	51846	0.1947	21.09	210.6	1543.1	1675
4	1880	6545	2881	51849	0.1942	21.02	210.5	1543.4	1675
5	1539	5895	2882	51852	0.1949	21.05	210.3	1544.5	1675
6	1440	5506	2875	51853	0.1943	21.04	210.2	1543.6	1675
7	1180	5356	2873	51854	0.1944	21.06	210.7	1542.2	1675
24	580	3107	2871	51855	0.1946	21.07	210.9	1542.9	1675
47	280	1608	2882	51857	0.1942	21.08	210.4	1543.4	1675

When approximating the photoconductivity spectra at different voltages using Eq. (1) (Fig. 2), it was noted that with an increase in voltage from 60 to 450 V, the parameter Q_1 increased by a factor of 1.7, Q_2 and Q_3 by a factor of 1.3, and αQ_4 by a factor of 3. This means that with an increase in the electric field strength, the lifetime of carriers in the deeper regions of the sample increases most significantly. The thickness of the first layer increased by a factor of 2 compared to the data in Table I, however, the thicknesses of the remaining layers did not change significantly. Table II shows the values of the parameters of Eq. (1) for approximating the

photoconductivity spectra when focusing the beam near the negative (–) and positive (+) contacts (Fig. 3).

TABLE II. Comparison of positive and negative focusing on Q -values and layer thicknesses

Focusing the beam near the contact	Q_1	Q_2	Q_3	Q_4	$t_1 / \mu\text{m}$	$t_2 / \mu\text{m}$	$t_3 / \mu\text{m}$	$t_4 / \mu\text{m}$	$d / \mu\text{m}$
+	941	4034	1804	0.3938	0.3839	41.15	208.7	1524.6	1675
–	1850	7884	3803	5997	0.2852	61.05	210.8	1503.8	1675

The spectral characteristics of the intrinsic photoconductivity of silver-doped (ZnS:Ag) have been studied. It has been established that the presence of deep defect levels formed within the forbidden band of the crystal plays an important role. The compensation effect influences both the spectral sensitivity and the intensity of photoconductivity.

The photoconductivity mechanism in Ag-compensated ZnS crystals has a complex nature and is determined by the interaction between the band structure and defect-related states. The observed changes in the spectral response are associated with recombination processes involving deep levels. Similar effects have been reported in previous studies of ZnS-based materials, where defect states and surface conditions strongly influence carrier recombination and transport processes. Silver-doped ZnS materials are widely used in electroluminescent panels, CRT phosphors and UV detectors because of their favorable optical and electrical characteristics.

One of the main results of the present study is the appearance of an additional short-wavelength photoconductivity maximum near the fundamental absorption edge after mechanical polishing of the crystal surface. This observation indicates that surface treatment modifies the defect structure in the near-surface region and introduces additional localized states within the band gap. The gradual decrease in the intensity of this maximum with ageing time suggests relaxation or recombination of the surface-related defect states.

The experimental results also show that increasing the applied electric field enhances the photoconductivity signal due to more effective separation and transport of photogenerated charge carriers. In addition, the photoconductivity spectra depend on the position of illumination relative to the electrical contacts, indicating the contribution of carrier injection processes. To explain the experimental observations, a multilayer phenomenological model was used, in which the crystal is considered a system of layers with different recombination properties and carrier lifetimes. This approach makes it possible to describe the depth-dependent behavior of photogenerated carriers in the crystal.

CONCLUSION

The intrinsic photoconductivity spectra of Ag-compensated ZnS crystals exhibit characteristic features associated with electronic transitions near the fundamental absorption edge. In particular, an additional short-wavelength photoconductivity maximum appears after mechanical polishing of the sample surface. Similar surface-related effects have been reported in previous studies on ZnS-based materials, where surface treatment was shown to introduce localized states within the band gap and modify recombination processes.

The observed decrease in the intensity of this maximum with increasing ageing time indicates relaxation of surface defect states. This behavior is in agreement with the literature data, where the evolution of surface states and space charge regions has been shown to influence photoconductivity in II–VI semiconductors.

The experimental results also demonstrate that the photoconductivity depends on the applied electric field and the position of illumination relative to the contacts. The increase in photoconductivity with electric field strength can be attributed to enhanced separation of photogenerated carriers, which is consistent with previously reported results for ZnS and related compounds. In addition, the influence of carrier injection from contacts, leading to recharging of impurity centers, has also been discussed in earlier works.

To interpret the experimental observations, a multilayer phenomenological model was applied, in which the crystal is considered a system of layers with different carrier lifetimes. Such an approach allows one to take into account the depth-dependent behavior of non-equilibrium carriers and the variation of the space charge region near the surface. Similar modelling approaches have been successfully used in the analysis of photoconductivity in compensated semiconductors.

The appearance of the short-wavelength maximum can be attributed to mechanical stresses induced by surface polishing, as well as to the formation of a space charge region influenced by interaction with the ambient atmosphere. The variation of photoconductivity maxima with electric field strength and illumination geometry indicates that the lifetime of non-equilibrium carriers changes with depth, which is consistent with the proposed model.

ИЗВОД

КАРАКТЕРИСТИКЕ СПЕКТАРА СОПСТВЕНЕ ФОТОПРОВОДЉИВОСТИ
ЦИНК-СУЛФИДА ДОПИРАНОГ СРЕБРОМ

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Испитивана је сопствена фотопроводљивост цинк-сулфида допираног сребром (ZnS:Ag). Показано је да допирање сребром доводи до значајних модификација у елек-

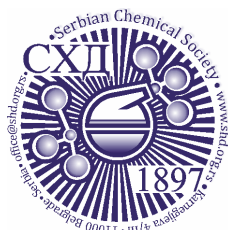
тронској структури и оптичким својствима ZnS због формирања локализованих енергетских нивоа унутар забрањене зоне. Механичко полирање површине узорка резултира појавом додатног максимума фотопроводљивости близу фундаменталне ивице апсорпције, који постепено нестаје током старења узорка. Ови ефекти се приписују компензацији донорског и акцепторског стања, као и променама у механизмима рекомбинације носилаца наелектрисања. Утврђена је зависност спектра фотопроводљивости од времена старења, примењеног електричног поља и положаја осветљења у односу на контакте. Резултати се објашњавају варијацијама у животном веку неравнотежних носилаца наелектрисања са дубином. Предложен је квалитативни модел који описује спектре фотопроводљивости, узимајући у обзир утицај компензације сребра на енергетску структуру ZnS. Предложени модел пружа увид у фотопроводљиве механизме и подржава развој оптоелектронских материјала на бази ZnS.

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The effect of catalyst composition and surface properties on the selective catalytic dehydrogenation of bioethanol

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Abstract: The article provides a comparative analysis of bioethanol dehydrogenation using Cr–Cu–O and Ce–Cu–O catalyst systems. For the Cr–Cu–O catalysts, bioethanol is converted into a range of products including acetaldehyde, acetone and ethylene. Advanced characterization revealed that these catalysts are structurally complex, containing not only individual copper and chromium oxides but also a distinct compound, Cu₂CrO₄. A key finding is the clear structure–property relationship: the catalyst’s crystallinity, which varied widely from 41.9 (mostly amorphous) to 73.8 % (highly crystalline), has a major effect on its catalytic activity. The Ce–Cu–O system converted bioethanol into a diverse mixture of products such as acetaldehyde, acetone and ethyl acetate, indicating the occurrence of multiple simultaneous reactions, including dehydrogenation and condensation. Most compositions resulted in separate CeO₂ and CuO phases; however, a specific Ce:Cu ratio of 1:9 is unique. At this ratio, the material is reduced to form CeO₂ with elemental copper, rather than CuO, and its crystallinity increased from 52.7 to 71.2 %. The research showed that structural order is crucial for steering the reaction pathway, as only catalysts with low crystallinity produce ethyl acetate. Furthermore, while the specific surface area of a catalyst (5.0 to 17.0 m²/g in this study) is a key factor in surface-driven reactions such as dehydrogenation, it does not affect all products equally.

Keywords: acetaldehyde; binary Ce–Cu–O catalysts; binary Cr–Cu–O catalysts; specific surface area; X-ray diffraction.

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INTRODUCTION

Bioethanol^{1–4} is produced from renewable biomass resources, including sugarcane, corn, lignocellulosic feedstocks and even micro- and macro-algae.^{5,6} Beyond its widespread use as a biofuel, bioethanol serves as a versatile chemical platform molecule, acting as a pivotal precursor for the synthesis of numerous industrially important chemicals, fuels and value-added materials.⁷ Its renewable origin, coupled with its inherent chemical reactivity and compatibility with existing conversion technologies, makes bioethanol an essential building block in sustainable chemical manufacturing pathways and an important driver for the transition toward circular and low-carbon bio-based industries. Bioethanol can undergo multiple catalytic transformations, including dehydrogenation, dehydration, oxidation, esterification, amination, *etc.*^{8–10} Consequently, the design and development of advanced catalysts are a primary focus of ongoing research aimed at optimising this transformative technology. The bioethanol dehydrogenation reaction is a typical example of a heterogeneous catalytic process. This means that the reaction takes place not within a uniform solution, but on the surface of a solid catalyst.^{11–13} The physical and chemical properties of this solid surface – such as its acidity, the arrangement of its atoms and its pore structure – are critical in controlling the reaction's speed, selectivity (which products are formed) and overall efficiency.^{14,15} The catalytic process is highly dependent on the physical makeup of the catalyst's surface. Its structural and textural characteristics, particularly the specific surface area (m^2/g), are vital as they dictate how many active sites are available for the reaction to occur. This availability is the primary factor controlling the catalyst's activity, selectivity and operational efficiency. Essentially, a larger internal surface area gives the chemical reaction more room to occur, which improves performance.

Bioethanol dehydrogenation is a well-studied and industrially vital process, primarily for producing acetaldehyde – a key intermediate in synthesizing acetic acid, acetone and other chemicals.¹⁶ Through this reaction, other valuable products, such as ethyl acetate, acetone and diethyl ether can also be obtained.

Copper-based catalysts are highly selective for this reaction, but their practical use is limited by stability issues.^{24,25} To address this, modification with secondary metal oxides is common. In recent years, binary metal oxide systems, particularly chromium–copper (Cr–Cu–O) and cerium–copper (Ce–Cu–O) oxides, have gained significant attention for their enhanced catalytic activity, selectivity and thermal stability. While copper oxide (CuO) alone is active, the incorporation of Cr_2O_3 or CeO_2 creates synergistic effects that improve catalyst stability, enhance redox properties and favourably modify the surface structure. For instance, in Cr–Cu–O systems, a mixed copper chromite (CuCr_2O_4) phase can form, which is highly active. In Ce–Cu–O systems, the presence of CeO_2 , CuO and metallic Cu phases depends on the synthesis conditions, and these structural variations strongly influence catalytic behaviour.^{26,27}

A primary factor governing catalytic performance is the specific surface area. A higher surface area generally corresponds to more exposed active sites per unit mass, which can enhance both the reaction rate and selectivity towards desired products like acetaldehyde and ethyl acetate. In Cr–Cu–O catalysts, the chromium oxide promotes the dispersion of the copper phase, thereby increasing the surface area.^{20–23} In Ce–Cu–O systems, the redox properties of ceria facilitate the activation of copper sites. Consequently, optimizing surface properties is a critical strategy for improving catalytic performance.

This study aims to systematically investigate the role of specific surface area in bioethanol dehydrogenation over Cr–Cu–O and Ce–Cu–O catalysts with varying atomic ratios. The catalysts were synthesised and characterised for their structural, textural and surface properties. Their activity, product selectivity and conversion efficiency were evaluated to establish a correlation between surface area and performance. This approach is expected to contribute to both fundamental knowledge in heterogeneous catalysis and the practical development of more efficient catalysts for industrial bioethanol transformation.

EXPERIMENTAL

*Catalyst preparation*²⁵

Binary chromium–copper oxide catalysts with varying atomic ratios were synthesized using a co-precipitation method. The synthesis involved mixing aqueous solutions of chromium(III) nitrate and copper(II) nitrate, followed by multistage thermal processing. This included evaporation at 368–373 K, drying at 373–393 K, thermal decomposition at 523–573 K to stop the evolution of nitrogen oxides and a final calcination at 823 K for 10 h. The resulting catalysts covered a broad compositional range, with Cr:Cu atomic ratios extending from 9:1 to 1:9.

The Ce–Cr–O catalysts were prepared using a procedure similar to that established for the Cr–Cu–O system. In this approach, aqueous solutions of cerium(III) nitrate and chromium(III) nitrate were co-precipitated under equivalent conditions, then subjected to an identical series of thermal steps involving evaporation, drying, thermal decomposition and final calcination.

Catalytic test

The catalytic activity of synthesized chromium–copper oxide and cerium–copper oxide catalysts was assessed for bioethanol dehydrogenation. The investigation was carried out in a lab-scale flow reactor. A fixed bed of 5 ml of catalyst (with a particle size of 1.0–2.0 mm) was subjected to a feed of bioethanol at a volume rate of 1200 h^{–1} under nitrogen carrier gas. The reaction was studied over a temperature range of 423 to 723 K to determine its effect on catalyst performance. Subsequently, the conversion of bioethanol and the yields of the dehydrogenation products were quantified using chromatographic analysis.

The composition and yields of reaction products were determined by gas chromatography using detector and column configurations optimized for different classes of compounds.

Condensable organic products (acetaldehyde, acetone, ethyl acetate) were analyzed using an Agilent 7820A gas chromatograph equipped with a flame ionization detector (FID). Separation was achieved on a 3 m packed column filled with Polysorb-1 sorbent. Nitrogen served as the carrier gas at a constant flow rate of 30 mL·min^{–1}. The injector and detector temperatures

were maintained at 473 and 523 K, respectively. The column oven was operated under isothermal conditions, with the temperature selected to ensure efficient separation of oxygenated compounds.

Carbon monoxide was analyzed using a dedicated gas chromatographic system equipped with a packed column (2 m, activated carbon). Nitrogen was used as the carrier gas at a flow rate of 25 mL·min⁻¹. The column temperature was maintained under isothermal conditions optimized for CO separation, while the detector temperature was set at 423 K.

Gaseous products, including butene-1, *cis*-butene-2 and carbon dioxide, were analyzed using a gas chromatograph equipped with a thermal conductivity detector (TCD). Separation was performed on a packed column under isothermal conditions, with nitrogen as the carrier gas at a flow rate of 30 mL·min⁻¹. The TCD temperature was maintained at 473 K to ensure stable detection of permanent gases and light hydrocarbons.

All analyses were conducted under steady-state reaction conditions. Quantification was carried out using external calibration with standard gas and liquid mixtures of known composition. The carbon balance was maintained within ±5 %, confirming the reliability and accuracy of the analytical procedure.

Catalyst characterization

The textural properties of the catalysts were characterized by low-temperature nitrogen adsorption–desorption measurements conducted at 77 K using a SORBI-MS surface area and porosity analyzer (ZAO META, Russia). Prior to analysis, all samples were degassed under vacuum at 473 K for 3–4 h to remove physically adsorbed moisture and other volatile contaminants. The specific surface area (S_{BET}) was determined by applying the multipoint Brunauer–Emmett–Teller (BET) method within the relative pressure (P/P_0) range of 0.05–0.30. The total pore volume (V_p) was derived from the nitrogen uptake at a relative pressure of approximately 0.99. Pore size distributions and average pore diameters were calculated using the Barrett–Joyner–Halenda (BJH) method applied to the desorption branch of the isotherms.

The crystalline structures of the chromium–copper oxide (Cr–Cu–O) and cerium–copper oxide (Ce–Cu–O) catalysts were investigated by X-ray diffraction (XRD) using a Bruker D2 Phaser diffractometer equipped with CuK α radiation ($\lambda = 1.5406 \text{ \AA}$) and a nickel filter. Diffraction patterns were collected over a 2θ range of 3–80° with an appropriate step size and scan rate to ensure reliable phase identification. The analysis aimed to determine the phase composition and structural characteristics of these binary catalyst systems.

Phase identification was performed by comparison with standard reference patterns from the PDF database. For the Cr–Cu–O catalysts, diffraction peaks corresponding to CuO and Cr₂O₃ phases were observed, along with the formation of mixed oxide phases such as Cu₂CrO₄, depending on the catalyst composition. In the Ce–Cu–O system, characteristic reflections of fluorite-structured CeO₂ and monoclinic CuO were detected. For specific compositions, particularly those with high copper content, partial reduction of copper species to metallic Cu was also identified.

The average crystallite size (D) of the oxide phases was estimated using the Scherrer equation:

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (1)$$

where: K is the shape factor (taken as 0.9), λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum of the selected diffraction peak (in radians) and θ is the Bragg angle (in rad).

The relative degree of crystallinity (X_c) of the catalysts was evaluated by comparing the integrated intensity of the crystalline diffraction peaks with the total scattered intensity, including the amorphous background. The crystallinity percentage was calculated according to:

$$X_c = 100 \frac{A_{\text{crystalline}}}{A_{\text{total}}} \quad (2)$$

$A_{\text{crystalline}}$ is the cumulative area of all resolved diffraction peaks and A_{total} represents the total area under the diffraction pattern after baseline correction.

Peak deconvolution and background subtraction were performed to separate overlapping reflections and the amorphous contribution. The crystallinity values of the catalysts were found to vary over a broad range (approximately 40–75 %), indicating significant differences in structural order depending on composition.

A pronounced correlation between crystallinity and catalytic performance was observed. Catalysts with higher crystallinity exhibited well-defined oxide phases and enhanced structural stability, whereas samples with lower crystallinity (*i.e.*, higher amorphous content) provided a greater density of defect sites. These defect-rich structures are likely responsible for promoting parallel reaction pathways, including condensation reactions that yield products such as ethyl acetate. Thus, the degree of crystallinity plays a critical role in modulating both activity and selectivity in bioethanol conversion.

RESULT AND DISCUSSION

The catalytic performance of the Cr–Cu (1:9) catalyst in the bioethanol dehydrogenation reaction is presented in Fig. 1. The onset of the dehydrogenation reaction is observed at 373 K. Upon increasing the temperature to 423 K, acetaldehyde is formed with an initial yield of 23 %. Further elevation of the reaction temperature leads to a progressive increase in acetaldehyde yield, reaching a maximum of 34.5 %. The yield of acetaldehyde passes through a maximum at 573 K (46.2 %). A further increase in temperature leads to the formation of carbon dioxide, acetone, ethylene and ethyl acetate. Fig. 1 shows that bioethanol conversion on the studied catalyst reaches (93.5 %) at 723 K.

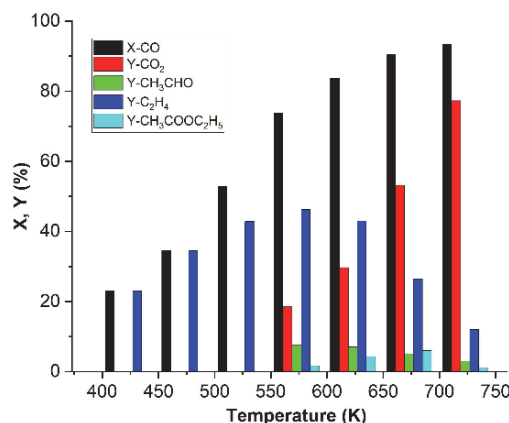


Fig. 1. Investigation of bioethanol dehydrogenation over bimetallic Cr–Cu (1:9) catalyst.

The data in Fig. 2 show that bioethanol dehydrogenation begins at 423 K, producing only acetaldehyde with an initial yield of 9.9 %. As the temperature increases, other products begin to form. The yield of acetaldehyde itself peaks at 573 K, reaching a maximum of 82.5 % with a remarkably high selectivity of 97.6 %, before declining at higher temperatures due to the formation of competing by-products.

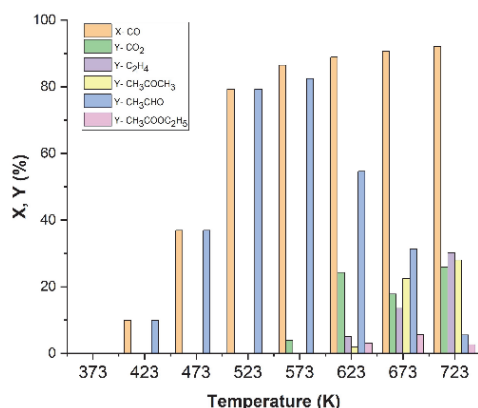


Fig. 2. Investigation of bioethanol dehydrogenation over bimetallic Cr–Cu (6:4) catalyst.

Product yields are highly dependent on temperature. While ethyl acetate production peaks at 673 K, its maximum yield is modest at only 5.6 %. The major products are carbon dioxide, ethylene and acetone, which all reach their highest concentrations at 723 K, with significant yields of 25.8, 30.2 and 28 %, respectively. This shift towards these by-products is linked to the catalyst's efficiency, which drives bioethanol conversion to 92.1 % at the same temperature.

Research into chromium–copper oxide catalysts demonstrates that temperature critically controls the outcome of bioethanol dehydrogenation. Low temperatures favor the high-yield production of acetaldehyde and its subsequent conversion to ethyl acetate. In contrast, high temperatures (above 573 K) trigger different reactions, leading predominantly to a mixture of light olefins like ethylene and the complete breakdown of bioethanol into waste products like carbon monoxide and carbon dioxide.

Beyond identifying the reaction pathway, the present study also identifies the specific outputs of bioethanol dehydrogenation catalyzed by cerium–copper oxides. The process generates a suite of chemicals, primarily acetaldehyde, acetone, carbon dioxide, ethylene and ethyl acetate, alongside smaller quantities of other organic fragments derived from the breakdown of bioethanol. As a key example of this catalytic activity, the performance data for the Ce–Cu catalyst with a 1:9 atomic ratio are explicitly provided in Fig. 3, offering a clear view of its effectiveness in facilitating the reaction.

Over bimetallic cerium–copper oxide catalysts the products of bioethanol dehydrogenation include acetaldehyde, ethyl acetate, acetone, ethylene and carbon monoxide. Fig. 3 illustrates the temperature dependence of the activity of the catalyst Ce–Cu = 1:9 in the dehydrogenation reaction of bioethanol. As can be seen from Fig. 3, the yields of acetaldehyde, acetone, ethyl acetate and ethylene reach their maximum values before declining with increasing reaction temperature, while the yield of carbon dioxide increases slightly and reach 12.5 % at 723 K. As the temperature rises, the conversion of bioethanol increases to 78.1 % at 573 K, after which it remains virtually unchanged.

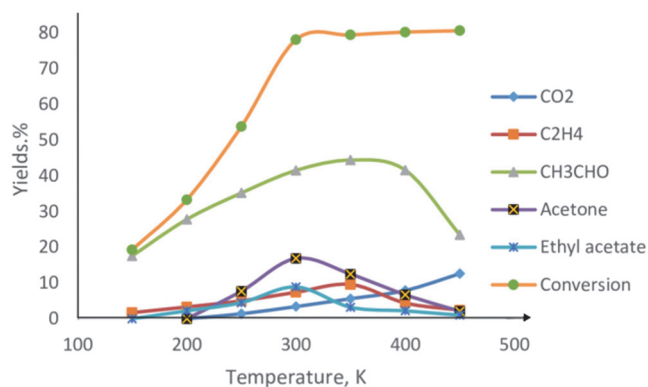


Fig. 3. The influence of temperature on the yield of the products of the dehydrogenation reaction of bioethanol over a bimetallic catalyst Ce–Cu (1:9).

The catalytic activity for bioethanol dehydrogenation over the Ce–Cu catalyst with a 1:9 ratio is presented in Fig. 3. The process becomes active at 423 K, generating an initial acetaldehyde yield of 15.2 %. The yield then climbs progressively with temperature, reaching a maximum yield of 44.8 % at 573 K. Following this peak, a decrease in yield is observed, suggesting the onset of secondary reactions or catalyst deactivation at higher temperatures.

The studies demonstrate that the products of bioethanol dehydrogenation over bimetallic cerium–copper oxide catalysts are acetaldehyde, acetone, carbon dioxide, ethylene, ethyl acetate and a small amount of organic decomposition products of the initial alcohol. As with the reaction at 523 K, at 673 K the yields of acetone and ethyl acetate also illustrate a bimodal distribution depending on the composition of the cerium–copper oxide catalysts. The results of the bioethanol dehydrogenation reaction at different temperatures over the Ce–Cu (2:8) catalyst are shown in Table I.

The bioethanol dehydrogenation process is highly sensitive to temperature, as detailed in Table I. Beginning at 423 K, a 6.8 % yield of acetaldehyde is observed. The acetaldehyde yield rises with increasing temperature, achieving a maximum

of 34.7 % at 573 K, but then plummets to 9.5 % at 723 K. In parallel, ethylene formation follows a similar trend, reaching a peak yield of 14.4 % at 623 K. Ethyl acetate generation is activated at 473 K and optimizes at 523 K with a 26.1 % yield. Notably, this peak temperature for ethyl acetate also serves as the starting point for acetone production. Beyond 523 K, acetone becomes the dominant product, with its yield escalating to a maximum of 34.7 % at 573 K. Carbon dioxide formation begins at 523 K and increases throughout the studied temperature range, reaching its maximum yield of 41.3 % at 723 K. According to Table I, bioethanol conversion reaches 78 % at 723 K over this catalyst.

TABLE I. Bioethanol dehydrogenation over the Ce–Cu (2:8) catalyst

T / K	Yield, %					Conversion, %
	$\text{CO} + \text{CO}_2$	C_2H_4	CH_3CHO	CH_3COCH_3	$\text{CH}_3\text{COOC}_2\text{H}_5$	
373	0	–	–	0	–	0
423	–	–	6.8	–	0	6.8
473	0	–	12.4	–	19.8	32.2
523	2.2	–	26.6	0	26.1	54.9
573	4.1	0	34.7	10.5	23.5	72.5
623	5.7	7.2	27.2	18.2	17.4	75.7
673	7.6	14.4	18.2	23.7	12.9	76.8
723	41.3	2.4	9.5	14.4	10.5	78

The data on the activity of the Ce–Cu (3:7) catalyst in the bioethanol dehydrogenation reaction are given in Table II. The reaction starts at 423 K, as in the previous samples, producing 2 % ethylene and 1.3 % acetaldehyde. With increasing temperature, acetaldehyde yield reaches a maximum of 33.8 % at 573 K. Ethylene yield also passes through a maximum, reaching 13.5 % at 673 K.

TABLE II. Bioethanol dehydrogenation over the Ce–Cu (3:7) catalyst

T / K	Yield, %					Conversion, %
	$\text{CO} + \text{CO}_2$	C_2H_4	CH_3CHO	CH_3COCH_3	$\text{CH}_3\text{COOC}_2\text{H}_5$	
273	0	–	–	0	–	0
423	0	2	1.3	0	0	3.3
473	0	3.2	9.3	1.2	7.6	21.3
523	1.7	4.4	23.8	6.5	19.8	56.2
573	6.8	6.4	33.8	16.2	13.7	76.9
623	13.4	10.6	23.6	31.7	5.4	84.7
673	18.2	13.5	14.5	42.2	1.1	89.5
723	24.4	4.8	9.6	47	0	91.6

The thermal decomposition process leads to the formation of oxygenated by-products, including acetone and ethyl acetate, which are first detected at a reaction temperature of 473 K. The maximum attainable yields for these key compounds

are 47.0 % for acetone and 19.8 % for ethyl acetate, indicating that acetone is the predominant oxygenated product under these conditions. Concurrently, the conversion of bioethanol, the likely feedstock, is also enhanced at higher temperatures. Furthermore, the table indicates that carbon dioxide production follows a continuous upward trajectory across the entire experimental range, culminating in a maximum yield of 24.4 % at the highest temperature studied, 723 K, suggesting progressive decarbonylation or combustion reactions at more severe conditions.

The results show that bioethanol conversion over this catalyst reaches 91.6 % at 723 K. Thus, based on the investigation of bioethanol dehydrogenation over binary copper-containing oxide catalysts, the following conclusions can be drawn: up to 573 K, bioethanol is mainly converted to ethyl acetate and acetaldehyde over cerium–copper oxide catalysts. At higher temperatures, the main products become acetone and acetaldehyde.

Both catalysts (Fig. 4) exhibit high ethanol conversion, with Ce–Cu–O (75.7 %) slightly outperforming Cr–Cu–O (73.8 %). However, selectivity patterns differ substantially, reflecting distinct active site chemistries. Cr–Cu–O yields significantly more acetaldehyde (46.2 %) than Ce–Cu–O (34.7 %), indicating greater selectivity toward direct dehydrogenation. This is attributed to well-dispersed CuO/Cu sites and the stabilizing effect of the CuCr₂O₄ mixed oxide phase. Ce–Cu–O produces markedly higher yields of ethyl acetate (24.9 % vs. 1.6 %) and acetone (11.2 % vs. trace), consistent with its bifunctional CeO₂ phase and higher density of defect-rich interfacial sites that promote C–C coupling *via* esterification and ketonization pathways. Cr–Cu–O shows increased formation of ethylene (7.5 % vs. 1.5 %) and CO_x (18.5 % vs. 3.4 %), suggesting the presence of acidic Cr₂O₃ sites and enhanced thermal stability that facilitate dehydration and deep oxidation pathways.

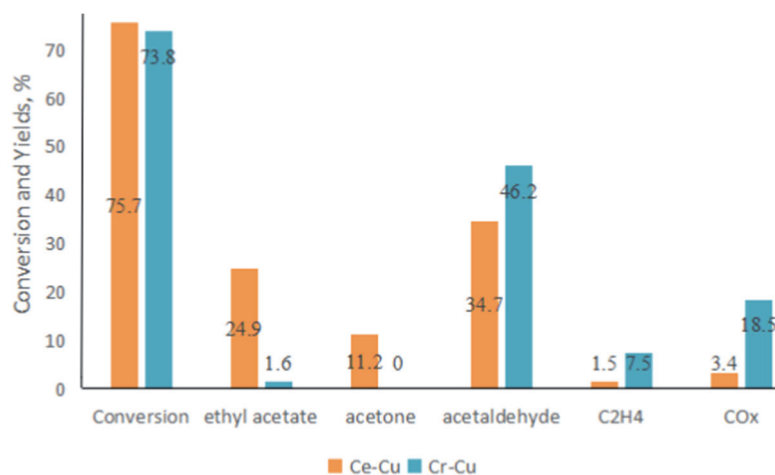


Fig. 4. Effect of catalyst composition on product selectivity in bioethanol dehydrogenation (Cr–Cu–O and Ce–Cu–O, 1:9, 573 K).

These results clearly demonstrate that, even under identical reaction conditions, the catalytic behavior is strongly phase-dependent: Cr–Cu–O favors selective dehydrogenation, whereas Ce–Cu–O promotes a broader reaction network involving condensation and secondary transformations.

The provided Fig. 5 displays the combined X-ray diffraction (XRD) patterns for the nine synthesized samples with varying chromium-to-copper (Cr:Cu) atomic ratios. For reference, the standard diffractograms for pure Cr_2O_3 and CuO are positioned at the top and bottom, respectively. Analysis of these patterns confirms that all samples within the Cr–Cu–O system consist of a mixture of three distinct phases: chromium oxide (Cr_2O_3), copper oxide (CuO) and the composite spinel compound, copper chromite (CuCr_2O_4). The systematic variation in the intensity of the characteristic diffraction peaks across the samples provides strong evidence that the proportional relationships between these phases are maintained consistently. X-ray diffraction analysis on all figures showed that all Cr–Cu–O samples contain three phases: Cr_2O_3 , CuO and CuCr_2O_4 .

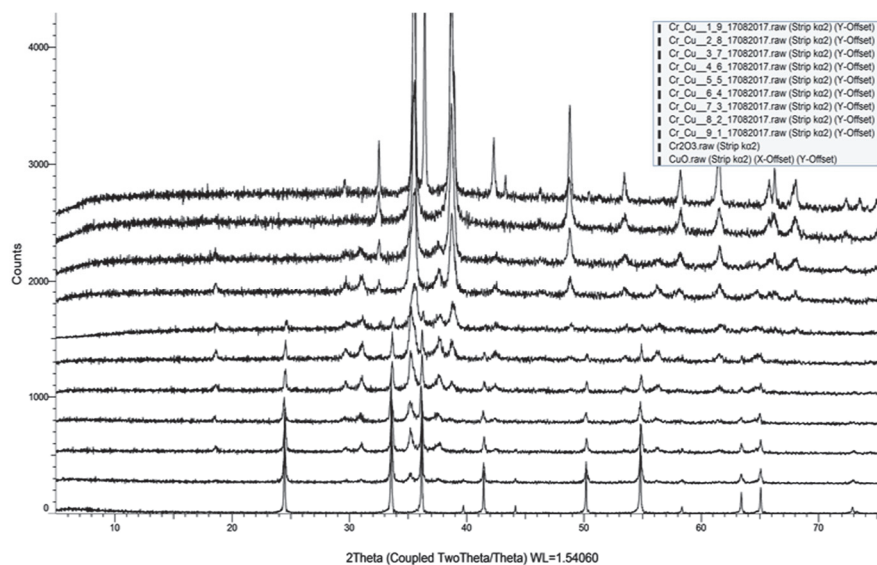


Fig. 5. X-Ray diffraction patterns of chromium and copper oxides and all nine samples of the Cr–Cu–O catalytic system.

Fig. 6 shows the diffraction patterns for all nine Ce:Cu ratios. CeO_2 and CuO phases were observed in all samples, while metallic Cu appeared in the Ce–Cu (1:9) composition.

Fig. 6 presents the combined diffraction patterns for all Ce:Cu ratios. For comparison, CeO_2 and CuO reference diffractograms are included at the start and end. The consistent variation in peak intensities across samples indicates that the

phase ratios remain stable. The Ce–Cu–O system forms CeO₂, CuO and, in some compositions, metallic Cu phases (Table III).

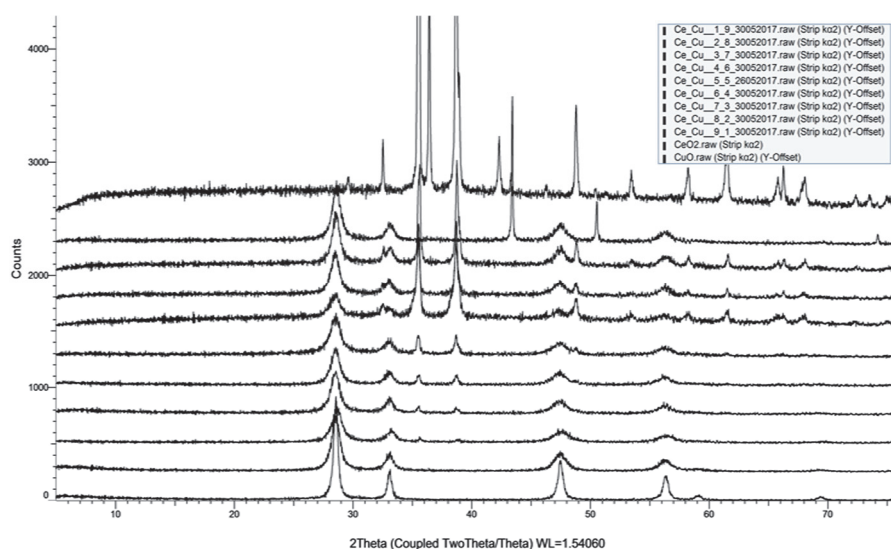


Fig. 6. X-Ray diffraction patterns of CeO₂ and CuO and nine Ce–Cu–O catalyst samples.

TABLE III. Phases formed in the Ce–Cu–O system

Ce:Cu Ratio	Formed phases
1 Ce – 9Cu	CeO ₂ and Cu
2 Ce – 8Cu	CeO ₂ and CuO
3 Ce – 7Cu	
4 Ce – 6Cu	
5 Ce – 5Cu	
6 Ce – 4Cu	
7 Ce – 3Cu	
8 Ce – 2Cu	
9 Ce – 1Cu	

Crystallinity in Cr–Cu–O increases from 41.6 to 73.3 %. In Ce–Cu–O, it increases slightly from 66.7 to 71.2 % with increasing cerium content (Table IV).

In Cr–Cu–O, the specific surface area ranges from 9.1 to 14.1 m²/g. The maximum value is observed for Cr–Cu for the ratio of 8:2. For comparison, Cr₂O₃ and CuO have specific surface areas of 2.4 and 0.6 m²/g, respectively. The specific surface area of Ce–Cu–O catalysts varies between 5.0 and 17.0 m²/g (Table V).

Fig. 7 illustrates how the specific surface area influences product yields in the bioethanol dehydrogenation reaction over Cr–Cu–O catalysts. As the surface area increases, acetaldehyde yield and bioethanol conversion rise, while ethyl acetate

yield decreases. The selectivity towards acetaldehyde also improves with increasing surface area.

TABLE IV. Degree of crystallinity in the Cr–Cu–O and Ce–Cu–O systems

Cr–Cu–O		Ce–Cu–O	
Cr:Cu Ratio	Crystallinity, %	Ce:Cu Ratio	Crystallinity / %
1:9	41.6	1:9	66.7
2:8	41.9	2:8	59.1
3:7	50.2	3:7	63.0
4:6	57.3	4:6	52.7
5:5	64.4	5:5	62.5
6:4	67.1	6:4	70.4
7:3	69.7	7:3	67.9
8:2	73.3	8:2	64.6
9:1	71.2	9:1	71.2

TABLE V. Specific surface area of Cr–Cu–O catalytic system samples (m²/g)

Cr–Cu										
Cr ₂ O ₃	Atomic ratio								CuO	
	1–9	2–8	3–7	4–6	5–5	6–4	7–3	8–2		9–1
2.4	12.5	9.1	9.9	11.4	12.5	12.5	13.7	14.1	13.3	0.6
Ce–Cu										
CeO	Atomic ratio								CuO	
	1–9	2–8	3–7	4–6	5–5	6–4	7–3	8–2		9–1
6.2	7.1	6.1	5.6	5.6	5.0	8.0	6.7	10.5	17.0	0.6

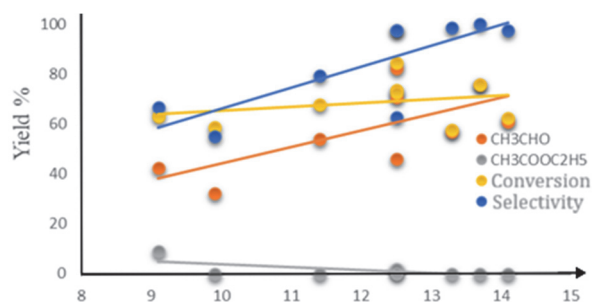


Fig. 7. Effect of specific surface area on the activity of Cr–Cu–O catalysts in bioethanol dehydrogenation ($T = 573$ K); CH_3CHO , $\text{CH}_3\text{COOC}_2\text{H}_5$, conversion.

Fig. 8 illustrates how the specific surface area of Ce–Cu–O catalysts governs the resulting product yields. A non-monotonic relationship is observed for acetaldehyde and acetone, whose yields pass through a distinct maximum. In contrast, the formation of ethyl acetate and the complete oxidation product CO_2 is disfavoured on catalysts with higher surface areas. Increasing surface area in Cr–Cu–O catalysts leads to higher acetaldehyde yield and bioethanol conversion. In Ce–Cu–O

catalysts, acetaldehyde and acetone yields pass through maxima with increasing surface area, while ethyl acetate and CO₂ yields decrease. Bioethanol conversion is only slightly affected by surface area in Ce–Cu–O.

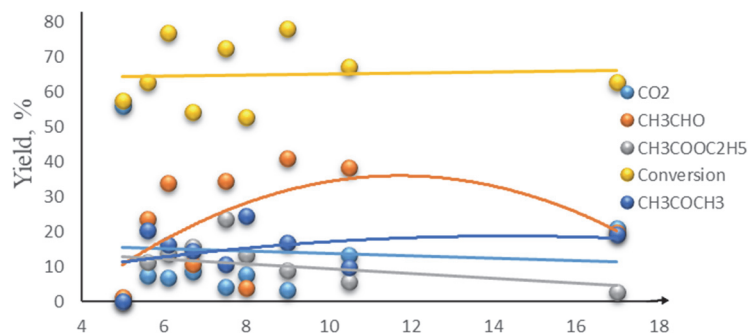


Fig. 8. Effect of specific surface area on the activity of Ce–Cu–O catalysts in bioethanol dehydrogenation ($t = 300\text{ }^{\circ}\text{C}$).

To rationalize the influence of specific surface area on product distribution, it is essential to distinguish between total surface area and catalytically active sites. In the Cr–Cu–O and Ce–Cu–O systems, the surface is not uniformly active; rather, catalytic performance is governed by specific active centers associated with particular phases and interfacial structures. XRD analysis (Figs. 5 and 6) confirms that the catalysts are multiphase systems. In the Cr–Cu–O system, CuO, Cr₂O₃ and CuCr₂O₄ are present; in the Ce–Cu–O system, CeO₂ and CuO are observed, with metallic Cu appearing in selected compositions. Each phase contributes distinctively to catalytic behavior:

- CuO and Cu serve as primary sites for ethanol dehydrogenation, favoring acetaldehyde formation;
- mixed oxide phases (*e.g.*, CuCr₂O₄) and phase boundaries facilitate C–C coupling and condensation reactions, promoting ethyl acetate formation;
- CeO₂ acts as a promoter, enhancing oxygen mobility and stabilizing dispersed copper species, thereby modulating both activity and selectivity.

Thus, the dependence of product yields on specific surface area (Figs. 7 and 8) cannot be interpreted solely in terms of the total available surface area. Instead, the surface area reflects the dispersion, accessibility, and relative abundance of distinct active sites.

Importantly, surface area and crystallinity are interrelated. Catalysts with lower crystallinity (higher amorphous content) exhibit a greater density of structural defects and interfacial sites, which promote secondary reactions such as esterification and condensation, leading to increased ethyl acetate yields. Conversely, highly crystalline catalysts possess well-defined oxide phases with fewer defect sites, favoring selective dehydrogenation to acetaldehyde.

Although direct quantification of individual phase activity is challenging in such multicomponent systems, correlative analysis combining compositional variation, XRD phase identification, crystallinity (Table IV), and catalytic data (Tables I and II, Figs. 7 and 8) provides strong evidence that:

- Acetaldehyde formation is primarily associated with Cu-based active sites;
- ethyl acetate formation is linked to defect-rich and interfacial sites;
- total conversion depends on the combined contribution of all accessible active centers.

Consequently, the influence of specific surface area on catalytic performance is phase-dependent and site-specific, explaining why different reaction products respond differently to changes in surface properties.

CONCLUSION

A comparative investigation was conducted into the catalytic dehydrogenation of bioethanol using two distinct binary oxide systems: chromium–copper (Cr–Cu–O) and cerium–copper (Ce–Cu–O). The study revealed a significant divergence in product selectivity between the two catalysts. The Cr–Cu–O system primarily facilitated the formation of acetaldehyde as the major dehydrogenation product, alongside valuable oxygenates such as acetone and ethyl acetate, as well as the dehydration product ethylene and fully oxidized carbon dioxide. In contrast, the Ce–Cu–O catalysts produced a similar suite of compounds, including acetaldehyde, acetone, ethyl acetate and ethylene, but also demonstrated a propensity for further decomposition, evidenced by the generation of carbon monoxide in addition to carbon dioxide.

It was shown that binary chromium–copper oxide catalysts consist not only of chromium and copper oxide phases but also of the Cu_2CrO_4 compound phase, with crystallinity varying between 41.9 and 73.8 %. In bioethanol dehydrogenation over these catalysts, an increase in crystallinity leads to the yield of acetaldehyde and the selectivity of bioethanol dehydrogenation to acetaldehyde passing through a minimum. The study of binary cerium–copper oxide systems showed that, except for the Ce:Cu ratio of 1:9, CeO_2 and CuO phases are formed in all compositions. At Ce:Cu of 1:9, however, CeO_2 and elemental Cu phases are observed, and their crystallinity increases from 52.7 to 71.2 %. It was determined that in bioethanol dehydrogenation over cerium–copper oxide catalysts, acetaldehyde formation is predominant in samples with medium crystallinity, whereas ethyl acetate is mainly produced in poorly crystalline samples. The specific surface area of binary chromium–copper oxide catalysts varies between 9.1–14.1 m^2/g , while for cerium–copper oxide catalysts it varies between 5.0–17.0 m^2/g , depending on the composition. It was established that changes in the specific surface area influence the yields of reaction products differently.

When bioethanol is dehydrogenated using chromium–copper oxide catalysts, it produces a range of products including acetaldehyde, ethyl acetate, acetone, ethylene, carbon monoxide and carbon dioxide. These catalysts are composed of a mixture of separate chromium and copper oxide phases, along with a distinct compound known as copper chromite (CuCr_2O_4).

Their degree of crystallinity ranges from 42.6 to 73.8 %. In bioethanol dehydrogenation over binary Cr–Cu oxide catalysts, increasing crystallinity enhances acetaldehyde yield, bioethanol conversion and the selectivity of the reaction toward acetaldehyde. The effect of specific surface area on the activity of the binary chromium–copper oxide catalytic system was also studied. It was established that an increase in surface area increases acetaldehyde yield and bioethanol conversion to acetaldehyde. For the Cr–Cu (6:4) catalyst, the highest acetaldehyde yield was 82.5 % with a selectivity of 97.6 %.

ИЗВОД

УТИЦАЈ САСТАВА И ПОВРШИНСКИХ СВОЈСТАВА КАТАЛИЗАТОРА НА СЕЛЕКТИВНУ КАТАЛИТИЧКУ ДЕХИДРОГЕНИЗАЦИЈУ БИОЕТАНОЛА

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Представљена је упоредна анализа дехидрогенизације етанола коришћењем Cr–Cu–O и Ce–Cu–O катализаторских система. У случају Cr–Cu–O катализатора, биоетанол се преводи у низ производа укључујући ацеталдехид, ацетон и етилен. Напредна карактеризација је показала да су испитивани катализатори структурно сложени, јер садрже не само појединачне оксиде бакара и хрома, већ и једињење Cu_2CrO_4 . Кључни резултат је јасна веза између структуре и својстава: кристалиничност катализатора, која се кретала у широком распону од 41,9 % (углавном аморфни) до 73,8 % (високо кристалинични), има велики утицај на његову каталитичку активност. Ce–Cu–O систем је преводио биоетанол у разноврсну смешу производа као што су ацеталдехид, ацетон и етил-ацетат, што указује на истовремено одвијање више реакција, укључујући дехидрогенизацију и кондензацију. Различити састави најчешће резултирају одвојеним фазама CeO_2 и CuO , осим при односу Ce:Cu од 1:9. При овом односу, материјал се редукује тако да, поред CeO_2 , настаје и елементарни бакар, а не CuO , а његова кристалиничност се повећава са 52,7 на 71,2 %.

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Development of a sensitive extraction–spectrophotometric method for Cu(II) based on 1-(*o*-tolylamino)- and 1-(phenylamino)propane-2-thiol complexes

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Abstract: The reactions of copper(II) with 1-(*o*-tolylamino)-propane-2-thiol (**L**¹, **TPT**) and 1-(phenylamino)-propane-2-thiol (**L**², **PPT**) was studied by the extraction-photometric method. The ligands were characterized with IR and NMR spectroscopy. The influence of aqueous phase pH on the formation of CuL₂ complexes and thermal decomposition were studied. Chloroform was chosen as the best extractant. A single extraction with chloroform extracted 98.6–97.9 % of Cu(II). The yield of CuL₂ was maximum at $c_L = 9 \times 10^{-3}$ M and did not decompose for 48 h or more than 45 days after extraction. Phase volume ratios of 5:5–140:5 did not affect the recovery. The optimum acidity is in the range of pH_{opt} 3.1–5.3 (pH_{form} 1.0–7.1). The molar absorption coefficients are, $\epsilon_{450-470}$, $(3.4-3.8) \times 10^4$. Compliance with the fundamental law of light absorption is achieved at $c_{Cu(II)} = 0.08 - 19 \mu g mL^{-1}$. Thermal decomposition of CuL₂ complexes ($[C_{20}H_{28}N_2S_2Cu]$ and $[C_{18}H_{24}N_2S_2Cu]$; Cu²⁺:L = 1:2) occurs stepwise due to the different stability of the Cu–S and Cu–N bonds. In the range of 65–135 °C, a mass loss of 2.78–2.84 % is observed, associated with the removal of weakly coordinated water. At 345–400 °C, profound destruction of the ligand occurs with the rupture of coordination bonds and the destruction of the chelate cycle (mass loss of 87.06–87.35 %). The proposed methods were successfully applied to determine copper in various complex samples.

Keywords: Cu(II) determination; extraction–spectrophotometry; aminothiols; liquid–liquid extraction; trace analysis; spectrophotometric method.

INTRODUCTION

Environmental contamination with heavy metals represents one of the most acute global ecological problems of our time, as it is accompanied by pronounced

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toxic and mutagenic effects on living organisms, including genetic alterations. Consequently, in recent years there has been a significant increase in interest in the development of effective methods for monitoring and removing such pollutants. At the same time, the advancement of high technologies and accelerated industrialization has led to substantially higher requirements for material purity, thereby increasing the importance of determining trace amounts of elements that exert a noticeable influence on metabolic processes in the human body.¹

Among trace elements, copper occupies a special position, ranking third in abundance in the human body. In terms of copper content, the liver is second only to the brain, which is associated with the crucial role of copper ions in numerous biological functions, including neuromodulation, antioxidant defense and cellular responses to hypoxia.¹ The redox-active nature of copper determines its participation in a number of essential physiological processes and explains the widespread use of Cu^{2+} as auxiliary component in enzymatically catalyzed reactions due to its high coordination ability.²

At the same time, disruption of copper homeostasis in the body leads to the development of severe pathologies. In terms of toxicity in drinking water, Cu^{2+} is second only to mercury compounds.³ Excessive intake of copper from contaminated water promotes its accumulation in humans and animals, posing a threat to ecosystems and increasing the risk of neurodegenerative diseases.⁴ In addition, Cu^{2+} are common contaminants in aquaculture systems and exhibit pronounced toxic effects on aquatic organisms.⁵

Significant interest in copper is also due to its extensive industrial use.⁶ Owing to its high electrical and thermal conductivity, ductility and corrosion resistance, more than half of the extracted copper is used in the electrical industry for the production of wires, cables and current-carrying components.⁷ A considerable portion of copper is utilized in the form of alloys, in heat-exchange equipment, radio electronics, artistic products, as well as in the form of various compounds in medicine, catalysis, electroplating, agriculture and pigment production. At the same time, all soluble copper salts are toxic, causing irritation of mucous membranes, damage to the gastrointestinal tract and liver, and, under inhalation exposure, can lead to chronic poisoning.

In this context, one of the urgent tasks of modern coordination chemistry is the investigation of complexation reactions between transition metal ions and polydentate organic ligands.⁷ Coordination compounds that combine the properties of organic and inorganic components represent a promising basis for the development of functional materials and for modeling chemical–biological processes.

A special place in this field is occupied by aminothiols and their metal complexes, which, due to the presence of nitrogen and sulfur donor atoms in their molecules, exhibit pronounced N,S-coordination properties and are capable of forming stable chelate structures with transition metal ions.^{8–12} The biological significance

of aminated thiols is confirmed by the key role of coenzyme A, containing an amino-ethanethiol fragment, in metabolic processes. This determines the interest in the synthesis of new aminated thiols and their metal complexes as potential physiologically active compounds with tunable properties.¹¹

Metal complexes of aminated thiols find applications not only in bioorganic and medical coordination chemistry but also in materials science, where they are used as stabilizing additives for polymer systems, antioxidants for lubricants, and exhibit antimicrobial activity and can be applied in agrotechnology. In analytical coordination chemistry, aminated thiols acquire particular importance as selective ligands for Cu(II), Ni(II) and Co(II) ions, enabling the development of highly sensitive extraction–spectrophotometric methods for metal determination with low detection limits and high selectivity.¹³

A wide range of analytical reagents has been proposed for the spectrophotometric determination of copper, including naphthazarin,¹⁴ various hydrazones, thiosemicarbazones and azo compounds,^{15–21} halogenated hydroxythiophenols,^{22,23} and alkylthiophenols.²⁴ However, most of these reagents suffer from significant drawbacks, such as long color development times, the need for heating, narrow linearity ranges and substantial interference from accompanying ions. Previously, we synthesized and studied copper(II) complexes with related aminated thiol derivatives using extraction–photometric analysis.²⁴

Recent studies (2020–2025) have emphasized the growing importance of environmentally safer and highly sensitive analytical methods for trace metal determination.¹⁰

In the present work, the complexation of copper(II) ions with aminated thiol ligands (**HL**, **L**) 1-(*o*-tolylamino)-propane-2-thiol (**L**¹, **TPT**) and 1-(phenylamino)-propane-2-thiol (**L**², **PPT**) was investigated. This study is aimed at the further development of this approach and the evaluation of the analytical potential of new ligand systems.

EXPERIMENTAL

Reagents and solutions

Copper(II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\geq 99.0\%$), potassium nitrate (KNO_3 , $\geq 99.0\%$), hydrochloric acid (HCl , 37 %), nitric acid (HNO_3 , 65 %), sulfuric acid (H_2SO_4 , 98 %) and hydrogen peroxide (H_2O_2 , 30 %) were purchased from Merck (Germany) and Sigma–Aldrich and used without further purification. Organic solvents including CHCl_3 ($\geq 99.5\%$, Merck), $\text{C}_2\text{H}_4\text{Cl}_2$ ($\geq 99\%$, Sigma–Aldrich), CCl_4 ($\geq 99\%$, Carlo Erba), C_6H_6 ($\geq 99.5\%$, Merck), $\text{C}_6\text{H}_5\text{Cl}$ ($\geq 99\%$, Merck), $\text{C}_6\text{H}_5\text{CH}_3$ ($\geq 99.5\%$, Sigma–Aldrich), xylene ($\geq 99\%$, Merck), isobutanol ($\geq 99\%$, Fluka), amyl alcohol ($\geq 99\%$, Merck) and benzyl alcohol ($\geq 99\%$, Sigma–Aldrich) were used as received. Double-distilled water was used throughout the experiments.

A stock solution of copper(II) (1.0 mg mL^{-1}) was prepared by dissolving 3.9296 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in double-distilled water with the addition of 2–3 drops of concentrated H_2SO_4 , and diluting to 1 L in a volumetric flask. The working solutions were obtained by appropriate

dilution of the stock solution.²⁵ The copper content was standardized iodometrically according to the reported procedure.²⁶

A 0.01 M solution of ligands (**HL**¹ and **HL**²) was prepared in chloroform and used for extraction. Chloroform was selected as the main extractant, while other organic solvents were tested to evaluate the extraction efficiency of CuL₂ complexes.

The ionic strength of the aqueous phase ($\mu = 0.1$) was adjusted using KNO₃. The pH of the solutions was controlled by addition of 0.1 M HCl.

Apparatus

Spectrophotometric measurements were performed using a KPK-2 photoelectric colorimeter (Russia, 400–700 nm) and an SP-26 UV–Vis spectrophotometer (Russia, 190–1100 nm). Quartz cuvettes with optical path lengths of 0.5 and 1.0 cm were used. Solution pH was measured using an I-120.2 pH meter (Russia) equipped with a combined glass electrode, calibrated with standard buffer solutions.

IR spectra of the compounds in thin films were recorded on a Specord 75 IR spectrometer (Germany). The ¹H- and ¹³C-NMR spectra of the substances in CDCl₃ were recorded on a Bruker SP-300 instrument (Bruker, Germany), at 300.13 Hz for ¹H, 75 MHz for ¹³C, with HMDS used as the internal standard. Elemental analysis for C, H, N and S atoms was performed on a Carlo Erba 1106 elemental analyzer (Italy). Chromatographic separation was carried out on a glass column (*l* = 20 cm, *d* = 2 cm) filled with silica gel of grade L 100/160. The eluent was a hexane–ethyl acetate mixture in a 9:1 ratio.

Thermogravimetric (TG) and derivative thermogravimetric (DTG) analyses were carried out using a simultaneous thermal analyzer (Netzsch STA 449 or equivalent, Germany) under controlled heating conditions.

General procedure for the synthesis of ligands HL¹ and HL²

The 0.74 g (0.01 mol) of 1,2-epithiopropene and 2.14 g (0.02 mol) of ortho-toluidine (1.86 g (0.02 mol) of aniline) are placed in a 10 mL ampoule. After sealing the ampoule, the reaction mixture is heated in a water bath at 95–100 °C for 10 h. After the specified time, the ampoule is cooled and opened, and the contents are subjected to vacuum distillation to purify the reaction product from *o*-toluidine (aniline).

Analytical and spectral data of the synthesized ligands are given in the Supplementary material to this paper.

General procedure for measuring light absorption

An aliquot of the Cu(II)-containing solution (0.1–20 µg mL⁻¹) was transferred to a separatory funnel. The pH of the aqueous phase was adjusted to 3.5–5.0 with 1 M HCl. Then 1 mL of a 0.01 M ligand solution (**L**¹ or **L**²) in chloroform was added. The mixture was shaken for 5–6 min to ensure complete complex formation and extraction. After phase separation, the organic layer was collected, and its absorbance was measured at 470 nm (for **L**¹) or 450 nm (for **L**²). The Cu(II) concentration was determined using a calibration curve.

Determination of Cu(II) in pharmaceutical samples

Boiling with 10 mL of aqua regia dissolves 0.5 g of a pharmaceutical sample. The solution is evaporated to dryness, the residue is dissolved in 10 mL of 1 M HCl, filtered if necessary, and the resulting solution is diluted to 100 mL with double-distilled water. The working solution is prepared by appropriate dilution of the stock solution. From an aliquot of this solution, 1 mL is analyzed for Cu(II) using the procedure described earlier.

Determination of copper in standard alloy samples

Weighed samples, 0.1–0.5 g of alloy (A 95-4 (0.180 Al; 0.025 Fe; 0.025 Pb; 0.010 Cd; 0.010 Cu; 0.010 Sb; 0.007 % Sn; rest Zn), A 95-5 (0.230 Al; 0.040 Fe; 0.040 Pb; 0.015 Cu; 0.015 % Sn; rest Zn), A-195-3 (11.3 Si; 0.3 Mn; 0.23 Zn; 0.14 Cu; 0.08 Ti; 0.17 % Mg; rest Al), A-195-4 (12.3 Si; 0.13 Mn; 0.9 Fe; 0.3 Zn; 0.11 Cu; 0.2 Ti; 0.12 % Mg; rest Al), A-195-5 (13.2 Si; 0.08 Mn; 1.1 Fe; 0.38 Zn; 0.04 Cu; 0.4 Ti; 0.09 % Mg; rest Al)) were dissolved in 10 mL of a mixture of an aqueous solution of 5 mL HCl (1:1) + 1–2 mL HNO₃ (1:1) at 50 °C. Silicon does not dissolve, so the solution was filtered and the filtrate was transferred to a 50 mL flask and diluted with water to the mark. A 20 mL aliquot of this solution was analyzed for Cu(II) content according to the extraction–spectrophotometric procedure described in the *General procedure for measuring light absorption* section.

Determination of copper in gelatin

Gelatin (5 g) in a porcelain dish was soaked in 50 mL of distilled water for 2–3 h. 25 mL of HNO₃ (1:1) was added to the swollen gelatin and heated in a boiling water bath for 2 h. The solution was filtered and neutralized with NaOH (1:1). A 20 mL aliquot of this solution was analyzed for Cu(II) content following the general spectrophotometric procedure described in *General procedure for measuring light absorption* section.

Determination of copper in wheat bran

A 5 g sample of wheat bran was dried in a drying oven in porcelain dishes at 105 °C until air-dry. The cup was then placed on an asbestos plate, and the contents were burned over an open flame. The charred residue, along with the cup, was transferred to a muffle furnace and calcined at 800 °C. The mineralized residue was dissolved in 0.1 M HNO₃ and filtered through a medium-density filter into a 100 mL flask. A 20 mL aliquot of this solution was analyzed for Cu(II) content following the general spectrophotometric procedure described in *General procedure for measuring light absorption* section.

Determination of copper in beans

A weighed sample of beans (≈10 g) was ground and dried in a porcelain dish, first at 60–70 °C, then at 105 °C. The dry residue was calcined in a muffle furnace at 500 °C. The ash was dissolved in dilute (1:1) HNO₃ and evaporated to form wet salts, which were then dissolved in double-distilled water and filtered into a 100 mL volumetric flask. A 20 mL aliquot of this solution was analyzed for Cu(II) content following the general spectrophotometric procedure described in *General procedure for measuring light absorption* section.

Determination of copper in ram kidneys

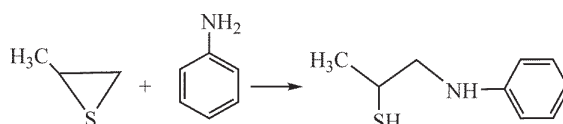
A sample (3 g) of kidneys was dried in crucible porcelain until moisture was removed and ached, first at low temperature, then at higher temperatures. After vapor evolution ceased, better air flow to the substance being ached was ensured. Heat was simultaneously increased until the crucible walls and lid were cleared of volatile combustion products. The lid was removed and ashing was continued in a muffle furnace heated to a deep red glow, corresponding to ~500 °C. To accelerate ashing, the crucibles were removed from the muffle furnace, cooled and the crucible contents were moistened with a 3 % H₂O₂ solution. They were dried in a water bath and a drying oven and then calcined again to obtain a homogeneous brown mass. The mass was dissolved in hot water acidified with 2 M HCl. The mixture was filtered into a 100 mL volumetric flask. A 20 mL aliquot of this solution was analyzed for Cu(II) content following the general spectrophotometric procedure described in *General procedure for measuring light absorption* section.

Determination of copper in water

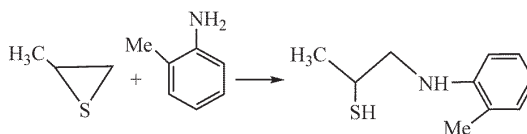
The natural sample, surface water (Kura River), was pre-cleaned by filtration through a fine-pored glass Schott filter to remove suspended matter or soil. Ammonium persulfate was added, and the mixture was boiled for 20 min. A 20 mL aliquot was added to the extraction vessel, and an $n \times 10^3$ -fold excess of PO_4^{3-} was added to mask the Fe^{3+} . The copper content was determined using the calibration curve. The validity of the proposed method was verified using the standard addition method (method of standard additions)

RESULTS AND DISCUSSION

The reaction Schemes 1 and 2 for the synthesis of **HL**¹ and **HL**² are given below.



Scheme 1. Synthesis scheme and chemical structures of 1-(phenylamino)-propane-2-thiol studied in this work.



Scheme 2. Synthesis scheme and chemical structures of 1-(*o*-tolylamino)-propane-2-thiol studied in this work.

The reaction schemes for the synthesis of **HL**¹ and **HL**² are shown below (Schemes 1 and 2). We first investigated the reaction of aniline and *o*-toluidine with 1,2-epithiopropene. This reaction proceeds smoothly upon mixing equimolar amounts of the starting compounds in methanol at room temperature, leading to the formation of the target aminothiols **HL**¹ and **HL**² in yields of up to 80 %. The structures of the aminothiols were confirmed by spectral techniques and elemental analysis.

The IR spectra of the resulting ligands display characteristic absorption bands corresponding to the --NH stretching vibrations at $3300\text{--}3400\text{ cm}^{-1}$ and the --SH stretching vibrations at $2556\text{--}2560\text{ cm}^{-1}$. In the $^1\text{H-NMR}$ spectra, the NH protons appear as broad singlets at $4.5\text{--}5.5\text{ ppm}$, while the --SH protons resonate as broad singlets in the region of $3.0\text{--}3.5\text{ ppm}$. These spectral features are consistent with the successful formation of the desired aminothiols.

Determination of the charge sign of the complexes and selection of extractant

Colored $\text{Cu}(\text{L}^{1,2})_2$ complexes are highly soluble in chloroform and dichloroethane. To determine the charge of the complexes by electromigration, experiments were carried out in a U-shaped tube at R 200–220 V and I 0.4–0.8 mA for

4.0 h. During the electrolysis of the solutions of the complexes, no movement of charge was observed either to the anode or to the cathode, *i.e.*, it is electrically neutral. Extractability of complexes was estimated in coefficient of distribution (D) and extent of extraction (R , %):²⁷

$$D = \frac{c(\text{Cu})_{\text{org}}}{c(\text{Cu})_{\text{aq}}} \quad (1)$$

$$D = \frac{100D}{D + \frac{V_{\text{aq}}}{V_{\text{org}}}} \quad (2)$$

Rapid phase separation and maximum molar absorption coefficients were achieved during the extraction of complexes with CHCl_3 , $\text{C}_2\text{H}_4\text{Cl}_2$ and CCl_4 . Single-step extraction of Cu(II) with chloroform resulted in recovery rates of 97.9 and 98.6 % for complexes $\text{Cu(II)(L}^1\text{)}_2$ (**1**) and $\text{Cu(II)(L}^2\text{)}_2$ (**2**), respectively. Further research were conducted with chloroform.

Although chloroform is not considered an environmentally friendly solvent, it was selected due to its superior extraction efficiency, rapid phase separation and higher sensitivity compared to other tested solvents. Therefore, its use is justified in this study from an analytical performance perspective.

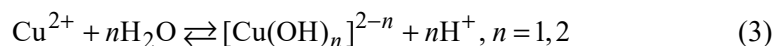
The concentration of copper in the organic phase was determined with sodium dithiocarbamate²⁸ by photometric measurements after back extraction, while in the aqueous phase it was determined by the difference. Photometric measurements after back extraction with sodium dithiocarbamate were used to measure the copper concentration in the organic phase. Experiments have shown that for 98.6 % (for **2** 99.3 %) re extraction of copper it is necessary to shake the organic phase with an equal volume of 0.2 M HCl and perhydrol solutions in a ratio of 1:1. The high extraction efficiency, stability of the complexes in the organic phase and favorable spectrophotometric characteristics demonstrate that the proposed extraction system is well suited for the extractive–spectrophotometric determination of trace amounts of Cu(II) in environmental and technological samples.

Influence of aqueous phase pH

The formation of Cu(II)–aminothiol complexes is strongly dependent on the pH of the aqueous phase, reflecting the influence of protonation–deprotonation equilibria of both the ligand and the metal ion. Systematic studies reveal that the efficiency of complex formation and subsequent extraction is highly sensitive to the solution's acidity.

The optimal acidity range for the complexes **1** and **2** is at pH_{opt} 3.6–5.3 (pH_{form} 2.4–7.1) and pH_{opt} 3.1–4.0 (pH_{form} 1.0–6.2), respectively (Fig. 1). Within these pH ranges, the ligands exist predominantly in their deprotonated or partially

deprotonated forms, which facilitates effective coordination to Cu(II) ions, leading to stable chelate formation and high extraction efficiency. At solution pH > 7.1, extraction of complexes is not observed. This is attributed to the hydrolysis of Cu(II) ions, which predominates under alkaline conditions:



Here, the formation of hydroxo-species reduces the availability of free Cu(II) ions for complexation with the aminothiols ligands, thereby inhibiting the extraction process. These observations underscore the critical role of solution pH in modulating the speciation of both the metal ion and the ligand and consequently, the efficiency and selectivity of Cu(II) complex formation. The pH of the aqueous phase was adjusted using 0.1 M HCl. Although no buffer system was employed, the pH stability during extraction was ensured due to the short equilibration time (5–6 min) and the relatively low ionic strength of the solutions. Under these conditions, no significant drift in pH was observed during the complex formation and extraction process. The difference between formal and optimal pH values is attributed to changes in the deprotonation equilibria of the ligand and metal hydrolysis processes under varying acidity conditions.

Absorption spectra

The absorbance of complexes **1** and **2** at their absorption maxima (λ_{max}) is practically the same. However, there is a small difference in the position of these λ_{max} maxima: 470 (**1**) and 450 nm (**2**), Fig. 2. In contrast, the ligands **HL**¹ and **HL**² have maximum absorption at 270 and 255 nm, respectively. Bathochromic shifts are calculated using the Eq. (4) and are 200 nm for **1** and 195 nm for **2**. The molar absorption coefficients are $\varepsilon_{470} = 3.8 \times 10^4$ for **1** and $\varepsilon_{450} = 3.4 \times 10^4$ for **2**.

$$\Delta = \lambda_{\text{Cu(II)-L}} - \lambda_{\text{L}} \quad (4)$$

Effect of phase volume ratio, ligand concentration and holding time

The influences of the aqueous-to-organic phase volume ratio, ligand concentration, and equilibration time on the extraction efficiency and spectrophotometric determination of Cu(II) ions were systematically investigated. The term “holding time” refers to the time interval between phase separation and absorbance measurement, during which the stability of the extracted complex in the organic phase was evaluated. It was established that variations in the phase volume ratio within the range 5:(5–140):5 do not exert a statistically significant effect on the extraction recovery of the Cu(**L**^{1,2})₂ complex, indicating a high extraction capacity and robustness of the proposed system. This behavior enables the simultaneous implementation of preconcentration and extractive–spectrophotometric determination of copper(II) without loss of analytical performance.

An increase in the aqueous phase volume up to a 28-fold excess relative to the organic phase did not adversely affect the extraction completeness, which reflects a favorable distribution coefficient and strong affinity of the $\text{Cu}(\text{L}^{1,2})_2$ complex toward the organic solvent. This characteristic is particularly advantageous for trace-level analysis, where large sample volumes are typically required.

The maximum extraction efficiency of the $\text{Cu}(\text{L}^{1,2})_2$ complex was achieved at ligand concentrations in the c_L range $(8\text{--}9)\times 10^{-3} \text{ mol L}^{-1}$. Compliance with the fundamental law of light absorption is achieved within the $c_{\text{Cu(II)}}$ concentration range $0.08\text{--}19 \mu\text{g mL}^{-1}$.

Under these optimized conditions, the extracted complex exhibited pronounced thermodynamic and kinetic stability, with no detectable changes in absorbance over at least 48 h in solution and remaining stable for more than 45 days after extraction. This stability confirms the absence of decomposition, hydrolytic degradation, or secondary complexation processes. Kinetic studies revealed that the maximum absorbance was reached within 6 min, demonstrating rapid complex formation and phase transfer. Consequently, the kinetics of complexation and extraction of copper with ligands **HL**¹ and **HL**² can be classified as fast and nearly quantitative, rendering the proposed methodology suitable for rapid and reliable analytical applications.

The limit of detection (*LOD*) and limit of quantification (*LOQ*) were calculated using the following equations:

$$LOD = \frac{3\sigma}{S} \quad (5)$$

$$LOQ = \frac{10\sigma}{S} \quad (6)$$

where σ is the standard deviation of the analytical response and S is the slope of the calibration curve.

The calibration equations are calculated as: $y = 0.291x + 0.026$ ($LOD = 3 \times 4.328/0.291 = 47 \text{ ng mL}^{-1}$ and $LOQ = 10 \times 4.328/0.291 = 149 \text{ ng mL}^{-1}$) for complex **1** and $y = 0.295x + 0.033$ ($LOD = 3 \times 4.9153/0.295 = 50 \text{ ng mL}^{-1}$ and $LOQ = 10 \times 4.9153/0.295 = 167 \text{ ng mL}^{-1}$) for complex **2** (Table I).

The Sandell sensitivity (*SS*) values were calculated from the slopes of the calibration curves using the relation $SS = 0.001/S$. Accordingly, the Sandell sensitivity was found to be $0.00344 \mu\text{g cm}^{-2}$ for the $\text{Cu(II)}\text{--TPT}$ complex (complex **1**) and $0.00339 \mu\text{g cm}^{-2}$ for complex **2**, indicating high analytical sensitivity of the proposed spectrophotometric method.

The Sandell sensitivity values for the $\text{Cu(II)}\text{--aminothiol}$ complexes varied within the range of $0.0095\text{--}0.025 \mu\text{g Cu cm}^{-2}$, demonstrating the high analytical sensitivity of the developed spectrophotometric methods, with the $\text{Cu(II)}\text{--TPT}$ complex exhibiting the most pronounced response.

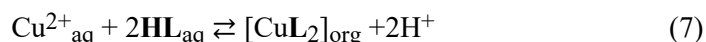
TABLE I. Analytical characteristics of Cu(II)(L^{1,2})₂ complexes

Parameter		1	2
pH	Formation	2.4–7.1	1.0–6.2
	Optimal	3.6–5.3	3.1–4.0
λ / nm		470	450
$\varepsilon \times 10^{-4}$		3.8	3.4
Log K_{eq}		12.27	11.68
Log K_{ex}		16.31	15.48
log β		9.7	8.4
Beer's law execution interval / $\mu\text{g mL}^{-1}$		0.08–19	0.1–18
$y = ax + b$		$y = 0.291x + 0.026$	$y = 0.295x + 0.033$
LOD / ng mL ⁻¹		47	149
LOQ / ng mL ⁻¹		50	167

Some analytical characteristics of the Cu(L^{1,2})₂ complexes are presented in Table I.

Equilibrium, extraction and stability constants

The complexation reaction can be predicted as follows:



Equilibrium constant (K_{eq}) of the reaction:

$$K_{eq} = \frac{[\text{Cu}(\text{L}^{1,2})_2]_{\text{org}} [\text{H}^+]_{\text{eq}}^2}{[\text{Cu}^{2+}]_{\text{aq}} [\text{HL}]_{\text{eq}}^2} = \frac{D \times [\text{H}^+]^2}{[\text{HL}]^2} \quad (8)$$

$$D = \frac{[\text{Cu}]_{\text{org}}}{[\text{Cu}^{2+}]_{\text{aq}}} = \log \frac{A_x}{A_0 - A_x} \quad (9)$$

where, A_x – is the optical density for a given experiment; A_0 is the optical density for complete binding of the copper ion into a complex.

Taking the logarithm of Eq (8), we obtain:

$$\log K_{eq} = \log D - 2\text{pH} - 2\log [\text{HL}] \quad (10)$$

The process of extraction of the complex can be represented as:



The value of the extraction constant (K_{ex}) can be calculated as:

$$K_{ex} = \frac{[\text{Cu}(\text{L}^{1,2})_2]_{\text{org}}}{[\text{Cu}^{2+}]_{\text{aq}} [\text{L}^{-}]_{\text{eq}}^2} = \frac{D}{[\text{HL}]^2} \quad (12)$$

Taking the logarithm of expression (12), we obtain:

$$\log K_{ex} = \log D - 2\log [\text{HL}] \quad (13)$$

The stability constants, calculated as:

$$\log \beta_{\text{S.C.}} = \frac{c_{\text{C}}}{(c_{\text{Cu}} - c_{\text{C}})(c_{\text{L}^{1,2}} - nc_{\text{C}})^n} \quad (14)$$

are 9.7 for **1** and 8.4 for **2**, where c_{Cu} , $c_{\text{L}^{1,2}}$ and c_{C} are the concentrations of copper ion, aminothiols and complexes, respectively.

The values of K_{eq} , K_{ex} and $\beta_{\text{S.C.}}$, calculated using formulas (10), (13) and (14) respectively, are given in Table I.

Composition of the complexes

In the formation of the investigated complexes, the copper(II) ion acts as the coordination center. Experimental evidence indicates that, during complexation, one proton is displaced from each **HL**^{1,2} ligand molecule, demonstrating that the ligands coordinate to Cu(II) in their deprotonated form (**L**[−]).

The degree of aggregation (γ) of the extracted complexes was calculated from the slope of the linear graph: $\log A = f(\log c_{\text{Cu(II)}})$, where $c_{\text{Cu(II)}}$ is the copper ion concentration, and A is the optical density of the extract. Evaluation of the aggregation degree, calculated from extraction–spectrophotometric data, confirms that the $\text{Cu}(\text{L}^{1,2})_2$ complexes do not undergo polymerization in the organic phase. The calculated γ values are close to unity ($\gamma = 1.05$ for complex **1** and $\gamma = 1.08$ for complex **2**), indicating that the complexes exist predominantly as monomeric species. Minor deviations from the ideal value of $\gamma = 1$ can be attributed to experimental uncertainty and weak intermolecular interactions within the extraction medium.

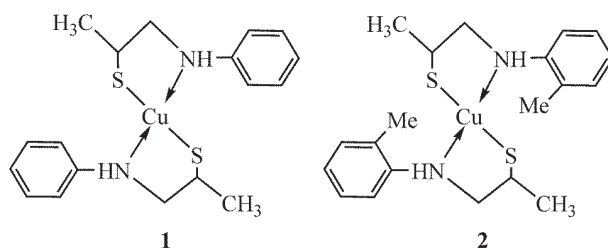
The stoichiometry of the complexes was determined using the Asmus straight-line method²⁹ and additionally confirmed by the equilibrium-shift and relative-yield methods.²⁹ The Asmus method is based on the analysis of the dependence of absorbance on the ratio of reagents under varying concentrations. In appropriately transformed coordinates, a linear relationship is obtained only for the true value of the stoichiometric coefficient, which allows the composition of the complex to be established. All applied methods consistently indicated a metal-to-ligand ratio of $\text{Cu}^{2+}:\text{L}^{1,2} = 1:2$, confirming the formation of neutral CuL_2 -type complexes, which is consistent with their high extractability into the organic phase.

To elucidate the coordination mode of the ligands toward copper(II), infrared spectroscopic studies were carried out for both the free ligands (**HL**¹ and **HL**²) and the corresponding complexes. The ligand molecules contain two potential donor atoms, nitrogen and sulfur, each capable of participating in coordination with Cu^{2+} . According to literature data, the IR spectra of the free ligands exhibit medium-intensity bands at approximately 2600 and 3400 cm^{-1} , which are assigned to $\nu(\text{SH})$ and $\nu(\text{NH})$ stretching vibrations, respectively.³⁰

Upon complex formation, the $\nu(\text{NH})$ band originally observed near 3400 cm^{-1} undergoes a red shift of $40\text{--}50\text{ cm}^{-1}$, indicating the involvement of the nitrogen atom in coordination with Cu(II) . Simultaneously, the absence of $\nu(\text{SH})$ bands in the IR spectra of the complexes suggests deprotonation of the thiol group during complexation. The appearance of new absorption bands in the low-frequency region at $270\text{--}285\text{ cm}^{-1}$, attributed to $\nu(\text{Cu-S})$ vibrations, provides direct experimental evidence for the coordination of the thiolate sulfur atom to Cu^{2+} .

In addition, the IR spectra of all complexes display bands in the region $1620\text{--}1640\text{ cm}^{-1}$ corresponding to the bending vibrations of water molecules. Intense bands observed in the $3350\text{--}3450\text{ cm}^{-1}$ region, assigned to $\nu(\text{OH})$ stretching vibrations, indicate the presence of crystallization water molecules within the complex structures.

Based on the combined results of stoichiometric analysis, aggregation calculations and IR spectroscopic characterization, the composition of the investigated compounds can be represented by the general formula $\text{Cu}(\text{L}^{1,2})_2$ (Scheme 3), in which the ligands coordinate to copper(II) through nitrogen and sulfur donor atoms, forming stable monomeric complexes.



Scheme 3. Suggested coordinations for the complexes in the study.

The thermal behavior of $\text{Cu}(\text{L}^{1,2})_2$ ($\text{C}_{20}\text{H}_{28}\text{N}_2\text{S}_2\text{Cu}$ (**1**) and $\text{C}_{18}\text{H}_{24}\text{N}_2\text{S}_2\text{Cu}$ (**2**), $\text{Cu}^{2+}:\text{L}^{1,2} = 1:2$) complexes containing ligands with thiol ($-\text{SH}$) and amine ($-\text{NH}-$) donor sites is determined by stepwise decomposition due to the periodic thermal stability of the Cu-S and Cu-N bonds. According to thermogravimetric (TG) and derivative thermogravimetric (DTG) analysis, the thermolysis process occurs in two main stages.

In the temperature range of $65\text{--}135\text{ }^\circ\text{C}$, the TG curves show an initial mass loss (2.78 % for complex **1** and 2.84 % for complex **2**), accompanied by a weak DTG peak, corresponding to the removal of physically adsorbed or weakly coordinated water. The low temperature and small DTG maximum indicate the absence of a strong bond between water molecules and the Cu(II) coordination center.

The main stage of development occurs in the $345\text{--}400\text{ }^\circ\text{C}$ region and is characterized by an intense DTG maximum, corresponding to the profound thermal degradation of the organic ligand. At this stage, the Cu-S and Cu-N coordination

bonds are cleaved, and the chelate ring is disrupted, corresponding to the mass differences of 87.35 % for complex **1** and 87.06 % for complex **2**. The position of the DTG maximum in the high-temperature region indicates a low thermal concentration of the complexes, due to the chelate effect and the use of sulfur- and nitrogen-containing donor atoms.

The final product of thermolysis in an oxidizing atmosphere is CuO, which corresponds to the residual mass and is consistent with literature data for Cu(II) coordination compounds with S,N-donor ligands.³¹

Photometric methods for determining Cu(II) using known reagents and **L**¹ and **L**² were compared (Table II). **L**¹ and **L**² offer several advantages over other reagents. Most published methods have a limited pH range (3–6).^{32,35,39} **L**¹ and **L**² are more versatile and can be used over a wide range of acidity and slightly alkaline environments, increasing their practical value. The wavelengths of maximum absorption of **L**¹ and **L**² are in the moderate visible range compared to modern spectrophotometric methods,^{32,33,36–38} which reduces potential interference from background absorption and improves analytical accuracy. Although the highest molar extinction coefficient ($\epsilon = 6.8 \times 10^4$) was observed for the method,³⁷ **L**¹ and **L**² combine relatively high sensitivity (ϵ of $(3.4\text{--}3.8) \times 10^4$) with the widest range of measurable concentrations. This is a key advantage: many methods are limited to low concentrations^{32–39} and are not suitable for samples with high copper content. **L**¹ and **L**² allow the determination of both trace and more concentrated levels of copper without the need for sample dilution, significantly expanding their practical application.

TABLE II. Comparative characteristics of spectrophotometric methods for determining Cu(II); SDA+SA – Schiff base derived from 2-aminothiophenol and salicylaldehyde; AAT – azoaminothiol reagent (S,N-donor system); TCA – thiohydrazone-based chelating agent; MT – modified thiosemicarbazone with electron-donating substituents; PAAT – pyridylazoaminothiol; SB+CPE – Schiff base + cloud point extraction; AIMM – azo dye immobilized on micellar medium; TBR – Thioamide-based reagent in micellar system

Reagent ^a	pH _{form.}	λ_{max} / nm	$\epsilon \times 10^4$	Beer's law range, $\mu\text{g mL}^{-1}$	Reference
SDA+ SA	3.5–5.5	465	2.80	0.05–3.5	32
AAT	4.0–6.0	490	3.60	0.04–4.0	33
TCA	2.5–4.5	455	3.10	0.06–3.0	34
MT	3.0–5.0	470	4.20	0.03–3.8	35
PAAT	5.0–7.0	510	3.95	0.05–5.0	36
SB+CPE	4.5–6.5	520	6.80	0.01–1.2	37
AIMM	6.0–8.0	540	5.40	0.02–2.0	38
TBR	3.0–4.5	480	4.90	0.02–2.5	39
L ¹ (TPT)	2.4–7.1	470	3.80	0.08–19	This work
L ² (PPT)	1.0–6.2	450	3.40	0.1–18	This work

As can be seen from Table II, the proposed method provides a wider linear range and comparable sensitivity relative to previously reported methods. In addition, the absence of a heating step and rapid extraction significantly improve the practicality and applicability of the method.

The influence of interfering ions

The selectivity of the proposed photometric method and the applicability of ligands **L**^{1,2} was evaluated by investigating the influence of common foreign ions on the determination of Cu(II). The experiments were carried out under the same conditions as those used for constructing the calibration curves, except that defined amounts of potentially interfering ions were added to the test solutions. A deviation of not more than $\pm 5\%$ in the determination of 40 μg of Cu(II) was taken as the criterion for the absence of interference. Alkali metal ions and the anions F^- , Cl^- and Br^- did not interfere at Cu(II): foreign ion ratios up to 1:1000. Mg^{2+} , PO_4^{3-} and CH_3COO^- were tolerated up to a ratio of 1:500. No significant interference was observed from Al^{3+} , Ba^{2+} , Ca^{2+} , Mn^{2+} , SO_4^{2-} and NO_3^- at a ratio of 1:100. Pb^{2+} , Ni^{2+} , J^- and $-\text{NH}_2\text{C}(\text{S})\text{NH}_2$ did not significantly affect the determination at a ratio of 1:7.5. Partial interference was observed in the presence of Sn^{2+} (1:4), Ti^{4+} (1:1) and Zn^{2+} (1:0.8). Pronounced interference was caused by Bi^{3+} , Co^{2+} and Fe^{2+} at a ratio of 1:1, as well as by Cd^{2+} and citrates, for which the allowable ratio did not exceed 1:0.5. The effects observed are attributed mainly to competitive complex formation with ligands **L**^{1,2} and, in some cases, to spectral overlap in the working wavelength region.

Analytical application

The selectivity data confirms the applicability of the proposed method for the photometric determination of Cu(II) in complex samples. The method was successfully applied to the analysis of various real samples, including alloys, food products, pharmaceutical preparations and natural waters. The obtained results showed good agreement with those obtained using the standard rubeanic acid spectrophotometric method,²⁸ which is widely recognized for its reliability and reproducibility (Tables III–V).

TABLE III. Results of copper determination in alloys ($n = 5$; $P = 0.95$); $\bar{X}$ mean value of n replicate measurements; n – number of determinations; P – confidence probability; t_p – Student's t -value at the given confidence level; S – standard deviation; S_r – relative standard deviation (RSD), calculated as $S_r = S / \bar{X}$. The term $\bar{X} \pm t_p S / \sqrt{n}$ represents the confidence interval of the mean

Sample	Cu content according to the passport, %	Method	$\bar{X} \pm \frac{t_p S}{\sqrt{n}}$
Alloy A 95-4	0.010	TPT	0.010 $\pm$ 0.003
		PPT	0.011 $\pm$ 0.002

TABLE III. Continued

Sample	Cu content according to the passport, %	Method	$\bar{X} \pm \frac{t_p S}{\sqrt{n}}$
Alloy A 95-5	0.015	TPT	0.016±0.004
		PPT	0.015±0.005
Alloy A 195-3	0.140	TPT	0.137±0.005
		PPT	0.142±0.002
Alloy A 195-4	0.110	TPT	0.109±0.003
		PPT	0.120±0.004
Alloy A 195-5	0.040	TPT	0.041±0.003
		PPT	0.039±0.006

TABLE IV. Results of analysis of copper content in food and pharmaceutical samples. ($n = 5$, $P = 0.95$); S_r – relative standard deviation (RSD), calculated as $S_r = S / \bar{X}$

Sample	Method	$\bar{X} / \mu\text{g kg}^{-1}$	S_r	$\bar{X} \pm \frac{t_p S}{\sqrt{n}}$
Beans	Rubeanic acid	5.42	0.056	5.42±0.03
	TPT	5.39	0.021	5.39±0.05
	PPT	5.40	0.023	5.40±0.07
Gelatin	Rubeanic acid	11.97	0.037	11.96±0.05
	TPT	11.94	0.045	11.94±0.08
	PPT	11.98	0.027	11.98±0.04
Wheat bran	Rubeanic acid	4.94	0.046	4.94±0.09
	TPT	4.98	0.031	4.98±0.07
	PPT	4.95	0.053	4.95±0.03
Kidneys	Rubeanic acid	12.93	0.055	12.93±0.03
	TPT	12.95	0.038	12.95±0.06
	PPT	12.89	0.033	12.89±0.05
Zincovit ^a (Apex)	Rubeanic acid	0.51	0.025	0.51±0.05
	TPT	0.52	0.071	0.52±0.07
	PPT	0.51	0.093	0.51±0.08
Multivitamin capsule ^b (A–Z)	Rubeanic acid	0.92	0.049	0.92±0.04
	TPT	0.88	0.035	0.88±0.07
	PPT	0.93	0.061	0.93±0.04

^aZincovit (Apex) – selenium (50 µg), manganese (0.9 mg), zinc (22 mg), copper (0.5 mg), chromium (25 µg), molybdenum (25 µg); ^bmultivitamin capsule A to Z ns (Alkem) – elemental copper (0.9 mg), elemental manganese (2 mg), elemental selenium (55 µg), elemental zinc (10 mg)

TABLE V. Results of copper determination in the water of the Kura River in the Republic of Azerbaijan ($n = 6$; $P = 0.95$)

Method	Cu found (initial), mg L ⁻¹	Cu added, mg L ⁻¹	Total found, mg L ⁻¹	S_r
Rubeanic acid	0.47	10.0	10.47±0.03	0.039
TPT	0.47	10.5	10.97±0.04	0.056
PPT	0.45	10.8	11.25±0.05	0.048

The initial copper concentration in the sample was determined prior to spiking. The “Cu added” column indicates the amount of copper introduced into the sample, while “total found” represents the measured concentration after spiking

CONCLUSION

The complexation of Cu(II) with aminothiol ligands **L**¹ (**TPT**) and **L**² (**PPT**) was investigated, and stable CuL₂-type neutral complexes were formed with a metal-to-ligand ratio of 1:2. The proposed extraction–spectrophotometric system demonstrated high extraction efficiency (up to ~98 %) and satisfactory stability over a wide acidity range, confirming its robustness for analytical applications. The developed methods exhibit good linearity in the concentration range of Cu(II), with low detection limits and satisfactory sensitivity, making them suitable for trace and higher-level copper determination without sample dilution. The selectivity studies confirmed that common ions do not significantly interfere under optimized conditions, except for certain heavy metal ions exhibiting competitive complexation. The methods were successfully applied to real samples, and the obtained results showed good agreement with those of the standard rubeanic acid spectrophotometric method, confirming the reliability and practical applicability of the proposed approach.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13793>, or from the corresponding author on request.

ИЗВОД

РАЗВОЈ ОСЕТЉИВЕ ЕКСТРАКЦИОНО–СПЕКТРОФОТОМЕТРИЈСКЕ МЕТОДЕ ЗА ОДРЕЂИВАЊЕ БАКРА(II) ЗАСНОВАНЕ НА КОМПЛЕКСИМА 1-(О-ТОЛИЛАМИНО)ПРОПАН-2-ТИОЛА И 1-(ФЕНИЛАМИНО)ПРОПАН-2-ТИОЛА

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Реакције бакра(II) са 1-(о-толиламино)пропан-2-тиола (**L**¹, **TPT**) и 1-(фениламино)-пропан-2-тиола (**L**², **PPT**) испитиване су екстракционо–фотометријском методом. Лиганди су окарактерисани применом IR и NMR спектроскопије. Испитиван је утицај рН водене фазе на формирање комплекса CuL₂, као и њихова термичка разградња. Као најбоље екстракционо средство изабран је хлороформ. Једноструком екстракцијом хлороформом издвојено је 98,6–97,9 % Cu(II). Максималан принос комплекса CuL₂ је био при $C_L = 9 \times 10^{-3}$ М и није долазило до његове разградње током 48 часа, нити током више од 45 дана након екстракције. Однос запремина фаза од 5:5 до 140:5 није утицао на рикавери. Оптимална киселост је се у опсегу рН_{опт} 3,1–5,3 (рН_{форм} 1,0–7,1). Моларни апсорпциони коефицијенти, $\epsilon_{450-470}$, износе $(3,4-3,8) \times 10^4$. Сагласност са основним законом апсорпције светлости постигнута је у опсегу концентрација $C_{Cu(II)}$ 0,08–19 $\mu\text{g mL}^{-1}$. Термичка разградња комплекса CuL₂ (C₂₀H₂₈N₂S₂Cu и C₁₈H₂₄N₂S₂Cu; Cu²⁺:L = 1:2) одвија

се постепено услед различите стабилности веза Cu–S и Cu–N. У температурном интервалу 65–135 °C уочава се губитак масе од 2,78–2,84 %, који се приписује уклањању слабо координоване воде. При температурама 345–400 °C долази до интензивне деструкције лиганда, праћене раскидањем координационих веза и разарањем хелатног прстена (губитак масе од 87,06 до 87,35 %). Предложене методе успешно су примењене за одређивање бакра у различитим сложеним узорцима.

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SUPPLEMENTARY MATERIAL TO
**Development of a sensitive extraction–spectrophotometric
method for Cu(II) based on 1-(*o*-tolylamino)- and
1-(phenylamino)propane-2-thiol complexes**

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*1-(*o*-tolylamino)-propane-2-thiol*

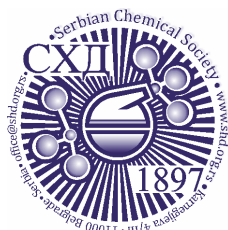
Yield: 1.3 g (80 %), bp 96 °C (0.3 mmHg), n_D^{20} 1.0350, R_f =0.52, n_D^{20} 1.5690, Found, %: C 66.25; S 17.76; H 8.34; N 7.69. Calculated, %: C 64.95; S 19.83; H 7.57; N 8.31. HRMS: m/z [M + H⁺] Calculated for C₁₀H₁₅NS: 181.112; Found: 167.098. HPLC-PDA: λ_{max} , nm: 270 nm, MeCN : MeOH (1 : 1), Rt: 3.07 min, 99.56 %. IR spectrum, ν , cm⁻¹: (KBr, cm⁻¹): 1448; 1513; 1585; 1604; 2561; 3017; 3035; 3055; 3407. ¹H NMR spectrum (300 MHz DMSO d₆), δ , ppm: 1.26–1.31 s (3H, CH₃), 2.1–2.3 s (3H, CH₃-Ph), 2.8–3.6 s (3H-CHCH₂), 6.1–7.3 s (4H, Ph), 1.6 s (1H-SH), 8.05 s (1H, NH). ¹³C NMR spectrum (75 MHz, DMSO d₆), δ , ppm: 21.3, 33.1, 34.2, 36.3, 60.7, 66.9, 113.4, 129.5, 144.7.

1-(phenylamino)-propane-2-thiol

Yield: 0.8 g (80 %), bp 80 °C (0.3 mm Hg), n_D^{20} 1.0800, R_f =0.56, n_D^{20} 1.5830, Found, %: C 64.63; S 19.28; H 7.83; N 8.48. Calculated, %: C 64.49; S 19.83; H 7.83; N 8.34. N 8.38, S 19.17. HRMS: m/z [M + H⁺] Calculated for C₉H₁₃NS: 167.211; found: 167.189. HPLC-PDA: λ_{max} , nm: 255 nm, MeCN : MeOH (1 : 1), Rt: 3.03 min, 99.18%. IR (KBr, cm⁻¹): 1418; 1454; 1496; 1506; 1514; 1587; 1613; 2554; 3057; 3028; 3222; 3404. ¹H NMR spectrum (300 MHz DMSO d₆), δ , ppm: 0.91 s (3H, CH₃), 3.18, 3.45 d (2H, CH₂), 6.59–7.24 s (5H, Ph), 1.6 s (1H, SH), 8.07 s (H, NH). ¹³C NMR spectrum (75 MHz, DMSO d₆), δ , ppm: 11.25, 28., 26, 38.56, 60.29, 13.32, 113.42, 129.64, 144.66.

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Dyeing of polyester fabric with disperse dye in a natural deep eutectic solvent as an eco-friendly medium

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Abstract: The present study proposed a sustainable, cleaner, alternative, safer and environmentally friendly method for dyeing polyester fabric using a natural deep eutectic solvent as a dyeing medium. Besides enabling water conservation and reducing wastewater generation, the process also successfully eliminated the use of toxic and environmentally harmful auxiliaries. Dyeing performance, measured by CIELab and *K/S* values, was compared to conventional dyeing. The NADES dyeing method achieved higher *K/S* values and eliminated the need for additional auxiliaries, such as leveling and dispersing agents. The polyester fabric structure remains intact, as confirmed by physical property results. SEM analysis shows that the surface morphology of all polyester samples is smooth and uniform with no visible changes, indicating the dyeing process did not affect the fiber surface. Fastness ratings are consistent across all dyed polyester samples.

Keywords: betaine; deep eutectic solvent; dyeing; polyester; sustainability.

INTRODUCTION

Polyester is the most hydrophobic synthetic fibre, with a compact crystalline structure and desirable qualities such as affordability, availability, softness, drapability, comfort and a lightweight feel against the skin. As a result, it is widely used in both wearable and decorative textiles and garments. However, its high hydrophobicity presents challenges in the common industrial dyeing process with disperse dyes in aqueous solutions, which requires high temperatures, pressure and a high fabric-to-solvent ratio to achieve optimal dyeing. As a result, residual disperse dyes and auxiliary chemicals remain in the water, producing large volumes of wastewater with high chemical and biochemical oxygen demand.^{1–3}

An alternative dyeing technique uses a specific class of solvents called deep eutectic solvents (DESs) as non-aqueous dyeing media. DESs are binary or ternary

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mixtures made from Lewis or Brønsted acids and bases. One component acts as a hydrogen bond donor (HBD) and the other as a hydrogen bond acceptor (HBA). When combined in a specific mole ratio, these components form a network of hydrogen bonds, leading to a significant reduction in the system's lattice energy. This leads to a substantial or, as commonly termed, “deep” – decrease in melting point, much lower than that of the pure components. If these components are naturally derived, the solvents are classified as natural deep eutectic solvents (NADESs).^{4–8}

NADESs represent the latest generation of “green” solvents prepared from natural, cheap, biodegradable, non-toxic, environmentally friendly and safe components, making them suitable for designing, tailoring, and modifying different technological processes. NADESs application in food, cosmetics and drug-related products is even declared acceptable or even desirable in comparison to some conventionally used organic solvents (especially volatile, toxic, expensive and strictly regulated).^{4–8}

Choline chloride, a quaternary ammonium salt acting as a hydrogen bond acceptor (HBA), is characterized by its low cost, biodegradability, non-toxicity and exceptional ability to form intermolecular interactions with various organic and inorganic compounds.

However, it is produced synthetically and is only available commercially as a synthetic product. Additionally, its strong hygroscopic nature poses challenges in handling and storage, often requiring the use of desiccants under certain conditions.

In recent years, betaine has emerged as an excellent, sustainable substitute for choline chloride. It is an inexpensive, non-toxic, biodegradable and renewable organic salt that exhibits high resistance to humidity. Commercially available betaine is bio-based, typically sourced as a by-product from sugar refining.^{9–12} Glycerol and urea were selected as hydrogen bond donors, since glycerol is a non-toxic, recyclable and renewable alcohol widely used in cosmetics and pharmaceuticals, while urea is a safe, cheap and readily available compound.^{7,8}

The main reason for selecting this specific ternary (three-component) system over more common binary mixtures lies in its synergistic effects. According to the literature, the formulation of ternary eutectic solvents by incorporating both an alcohol (glycerol) and an amide (urea) disrupts the crystalline lattice more effectively, which significantly lowers the viscosity and melting point of the solvent compared to binary alternatives like betaine-urea.^{13,14} Also, our previous work showed that this exact ternary system is highly effective for polyester fabric dyeing, achieving enhanced dye solubility and excellent kinetic penetration into the hydrophobic matrix of polyester fibres.¹⁵ Therefore, building directly upon our established findings, this study aimed to further explore and optimize the process parameters for this specific green solvent system.

Thus, the aims and novelties of the current study are:

a) developing a novel, greener and more sustainable technique for dyeing polyester fabric with Disperse red 60 using NADES – an eco-friendly liquid medium prepared from betaine hydrochloride, glycerol and urea;

b) characterizing the dyed fabric through colour strength determination (K/S values) and surface morphology testing.

By incorporating NADES into the dyeing process, two main goals were achieved simultaneously:

c) eliminating the need for toxic and environmentally harmful dyeing auxiliaries such as dispersants and carriers, reducing water consumption and the volume of wastewater generated.

EXPERIMENTAL

Materials and chemicals

Raw, unprocessed 100 % polyester fabric (surface mass $75 \text{ g} \cdot \text{m}^{-2}$) was kindly donated by Yumco (Vranje, Serbia). C.I. Disperse red 60 dye (chemical structure shown in Fig. 1) was purchased from Hoechst (Frankfurt, Germany). NADES components included betaine hydrochloride (99 %, Tokyo Chemical Industry, Tokyo, Japan; chemical structure in Fig. 1), glycerol (Ph Eur grade, Meilab, Belgrade, Serbia), and urea (99 %, Centrohem, Stara Pazova, Serbia). Other dyeing chemicals were glacial acetic acid (Zorka Pharma-Hemija DOO, Šabac, Serbia), distilled water, leveling agent Kollasol Lok, and dispersing agent CHT-Dispergator XHT-S (both from CHT GmbH, Tübingen, Germany). Sodium hydroxide and sodium hydrosulfite were obtained from Centrohem (Stara Pazova, Serbia), and non-ionic detergent Sarabid LDR from CHT GmbH (Tübingen, Germany).

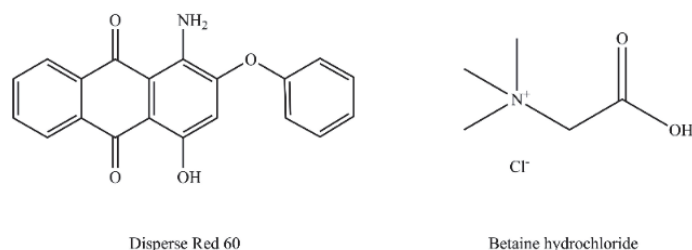


Fig. 1. Chemical structures of Disperse red 60 and betaine hydrochloride.

Preparation of NADES

An Erlenmeyer flask containing equimolar amounts of betaine hydrochloride, glycerol and urea was sealed with a glass stopper and heated to 40°C while stirring continuously at 1100 rpm. After 20 min, a homogeneous, stable, clear and transparent liquid formed. The prepared NADES solution was kept sealed and stored in a desiccator with CaCl_2 until further use.

Pre-dyeing processing

Polyester fabric was washed in a $2 \text{ g} \cdot \text{L}^{-1}$ solution of saturated detergent Sarabid LDR (CHT GmbH, Tübingen, Germany) for 30 min at 60°C with a 1:30 fabric-to-liquor ratio. After washing, the fabric was air-dried at room temperature for 24 h and ironed. It was then cut into $5 \times 5 \text{ cm}^2$ squares using a surgical scalpel.

Dyeing

Conventional dyeing method in Ahiba Nuance Top Speed dyeing machine (Allstates Textile Machinery Inc., Williamston, SC, USA) 0.6 % acetic acid, 1 % leveling agent, 1 % dispersing agent, temperature of 120 °C, duration of 110 min, bath ratio of 1:50, pH value of 4 and different dye concentrations (1, 2, 3, 4 and 5 mass %).

NADES dyeing method: NADES without any dyeing auxiliaries, temperature of 100 °C, duration of 60 min, pH value of 4, and different dye concentrations (1, 2, 3, 4 and 5 %).

Post-dyeing processing

After dyeing, the polyester fabrics were subjected to reductive cleaning using 2 g·L⁻¹ sodium hydroxide and 2 g·L⁻¹ sodium hydrosulfite at 60 °C for 15 min, followed by rinsing with cold water and drying in a laboratory oven at 100 °C for 10 min.

Evaluation of colour strength of dyed polyester fabric

The determination of the colour strength of dyed polyester fabric was carried out by *K/S* (Kubelka–Munk function) values using Datacolor Spectraflash SF600Ks (New York, USA). *K/S* values were calculated using the equation:¹⁶

$$K / S = (1 - R)^2 / 2R \quad (1)$$

where *K*, *S*, and *R* denote the adsorption coefficient, the scattering coefficient and the reflectance at complete opacity, respectively. Generally, the higher the *K/S* value, the higher the dye uptake on fabric, *i.e.*, the deeper the colour on the fabric.

Surface morphology test

Scanning electron microscopy (SEM) was used to analyse the morphology of undyed, conventionally dyed and NADES-assisted dyed samples. The samples were sprayed with an alloy of gold and palladium (85/15) under vacuum in a Fine Coat JEOL JFC-1100 ion sputter (JEOL Ltd., Tokyo, Japan). The metalized samples were scanned using a JEOL scanning electron microscope JSM-5300 (JEOL Ltd., Tokyo, Japan) under a magnification of 750 times, a voltage of 20 kV and a vacuum of 1.33×10⁻⁵ Pa.

Colour fastness of dyed polyester fabric

Adequate procedures provided by the specific ISO methods were employed for the determination of dyed fabrics' colour performance properties. The wash, light and rubbing fastness of dyed polyester fabric were measured by ISO 105-C06:2010, ISO 105-B02:2014 and ISO 105-X12:2016 protocols.¹⁷ The fastness of dyed polyester fabric was evaluated at standard depths of 1:1 and 2:1. The dyed sample specimen was subjected to a sublimation test according to the ISO 105-P01 1993 standard.¹⁸

Physical properties of polyester

Physical property (tensile strength and elongation at break) measurements of undyed, conventionally dyed and NADES-assisted dyed samples were performed on a MesdanLab strength tester dynamometer, in accordance with the standard ISO 13934-1:2013.¹⁹

RESULTS AND DISCUSSION

Comparison of colour strength and CIELab parameters of polyester fabric dyed by conventional and NADES-assisted processes

The *K/S* color strength values of conventionally and NADES-assisted dyed polyester samples in the range of $\lambda_{\max}$ 400–700 nm are presented in Fig. 2. The

maximum wavelength for the used disperse dye was $\lambda_{\max} = 524$ nm. Higher K/S values are noticeable for the samples dyed by the NADES-including dyeing technique. Moreover, the highest K/S values were observed at the highest dye concentration of 5 % for both dyeing procedures.

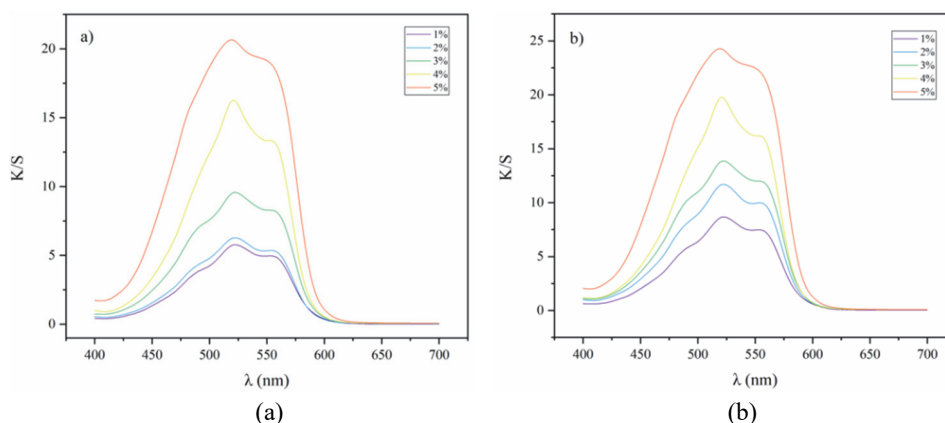


Fig. 2. Comparison of the K/S values obtained for conventionally (a) and NADES (b) dyed polyester fabrics at different dye concentrations.

CIELab parameters for conventionally and NADES-assisted dyed polyester samples are presented in Table I.

TABLE I. CIELab coordinates of undyed, conventionally dyed and NADES dyed polyester fabrics at different dye concentrations

Dye conc., %	Type of sample	L^*	a^*	b^*	C^*	H^*
—	Undyed	91.23	0.73	2.02	2.14	70.11
1	Conventionally dyed	61.85	45.71	3.58	45.85	355.52
	NADES-assisted dyed	47.94	58.58	6.56	58.66	356.98
2	Conventionally dyed	52.60	57.71	10.65	57.71	359.92
	NADES-assisted dyed	45.63	63.80	12.65	60.61	360.01
3	Conventionally dyed	50.26	57.78	11.52	57.80	360.23
	NADES-assisted dyed	44.45	60.33	13.52	64.03	362.25
4	Conventionally dyed	46.49	62.42	13.52	62.64	361.63
	NADES-assisted dyed	42.98	63.27	15.58	65.53	362.03
5	Conventionally dyed	45.65	63.42	15.23	64.52	362.52
	NADES-assisted dyed	40.19	65.72	17.08	65.02	362.75

The highest L^* parameter values are noticeable for the undyed sample, while the lowest are observed for the NADES-assisted dyed fabric with the maximum dye concentration (5 %), strongly indicating both a higher dye adsorption and darker achieved shades. Positive a^* parameter values for all dyed samples indicate

redness. Positive b^* parameter values in the raw sample and all dyed samples indicate yellowish. C^* values are higher in all dyed samples compared to the undyed samples, describing the distance from the black-white axis and good brilliance. Parameter H^* has the lowest values for the undyed sample, while the values for the dyed samples are similar.

These results can be explained by various factors. NADESs possess the potential for softening or partially dissolving various polymer-type matrices, thus facilitating dye penetration. Due to improved dye penetration, the fabric becomes darker. Therefore, careful choice of solvent and its adjustment according to the desired properties greatly affect the efficiency of the dyeing process.²⁰

As shown in Fig. 3, a visual comparison of samples at a 5 % dye concentration demonstrates that the introduction of NADES significantly enhances dyeing efficiency. This is supported by the highest K/S value and the visibly darker shade obtained for the NADES-assisted sample compared to the conventional one.

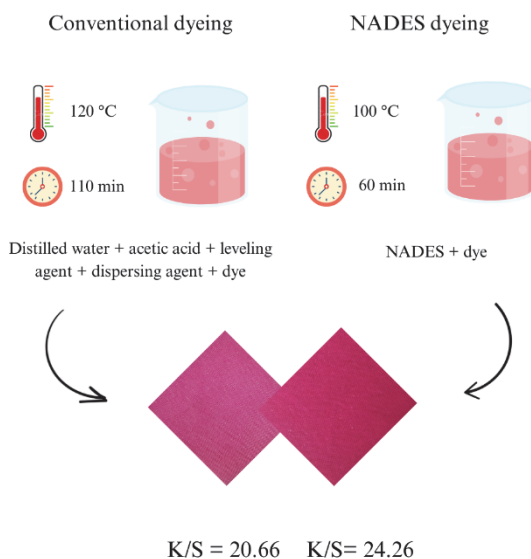


Fig. 3. Comparison between conventional and NADES-assisted dyeing at the maximum dye concentration.

SEM analysis/morphological properties of the polyester fabrics

SEM images for the undyed, conventionally dyed, and NADES-assisted dyed samples are shown in Fig. 4. Smooth surface morphology is noticeable, without any evident visual changes among all polyester fabrics.

Colour fastness properties (washing, light, rubbing and sublimation fastness) of dyed polyester

Colour fastness property ratings of conventional and NADES-assisted dyed samples are displayed in Table II. It is noticeable that these values are similar for

both dyeing processes, indicating there is no deterioration occurring when NADES-assisted dyeing is applied. Therefore, no dye residues were left on the polyester surface post-dyeing.

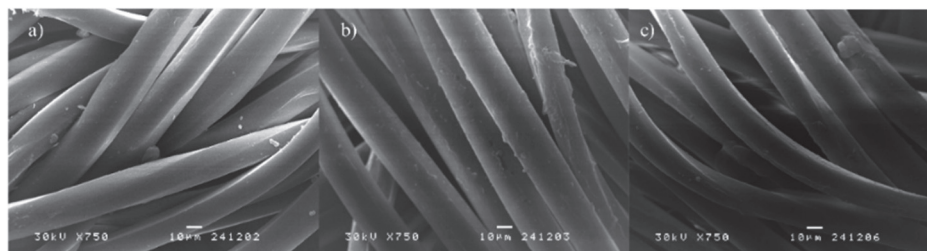


Fig. 4. SEM images of: a) undyed, b) conventionally dyed and c) NADES dyed polyester fabrics.

TABLE II. Wash, light, sublimation and rubbing fastness for the conventionally dyed and NADES dyed polyester fabrics

Type of sample	Wash fastness		Wet rubbing fastness		Dry rubbing fastness		Light fastness	Sublimation fastness
	Change	Staining	Change	Staining	Change	Staining		
Conventionally dyed	4-5	4-5	4	4	4-5	4-5	5-6	4-5
NADES dyed	4-5	4-5	4	4	4-5	4-5	5-6	4-5

Mechanical-physical properties of polyester

In order to provide a deeper understanding regarding the influence of NADES-containing dyeing solution on the strength of the polyester fabric, tensile strength and elongation at break measurements were conducted; results are presented in Table III. It is noticeable that the tensile strength values are the highest in the undyed sample compared to the conventionally dyed and NADES dyed samples. Conventional and NADES dyed samples have similar tensile strength values, the warp and weft directions. The breaking elongation values are the highest for the undyed sample, although similar values were obtained for the conventionally dyed and NADES dyed polyester fabric in the warp and weft direction.

TABLE III. Tensile strength and elongation at break values for undyed, conventionally dyed and NADES-dyed polyester fabrics

Type of sample	Tensile strength (Kgf)		Elongation at break, %	
	Warp	Weft	Warp	Weft
Undyed	85.04	50.17	42.5	28.1
Conventionally dyed	84.84	49.86	41.4	27.7
NADES dyed	84.64	49.66	40.2	27.1

CONCLUSION

A sustainable, eco-friendly technique for dyeing polyester fabrics with Disperse red 60 dye in the presence of betaine-based NADES is proposed. In the described process, NADES serves several roles: as a “greener” dyeing medium, dispersing agent, and homogenization agent for the dyeing bath. The incorporation of NADES in the process resulted in obtaining stable dyed fabric with higher *K/S* values and improved fastness properties, simultaneously excluding the need for adding a carrier or dispersant into the dyeing solution. Moreover, the structure of the polyester fabric was not degraded, which is confirmed by the results of the analysis of the physical properties of the fabric. Based on the SEM, all polyester fabric samples demonstrate a uniform and smooth surface morphology, without any noticeable visual alterations between the treated and untreated samples. The wash and light fastness of conventional and NADES dyed polyester are almost similar, although a slight advantage is given to NADES dyed polyester.

ИЗВОД

БОЈЕЊЕ ПОЛИЕСТАРСКЕ ТКАНИНЕ ДИСПЕРЗНОМ БОЈОМ У ПРИРОДНОМ ЕУТЕКТИЧКОМ РАСТВОРАЧУ КАО ЕКОЛОШКИ ПРИХВАТЉИВОМ МЕДИЈУМУ

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У раду је предложена одржива, чистија, алтернативна, безбеднија и еколошки прихватљива метода за бојење полиестарске тканине коришћењем природног дубоког еутектичког растварача као средства за бојење. Поред тога што омогућава уштеду воде и смањује стварање отпадних вода, процес је такође успешно елиминисао употребу токсичних и еколошки штетних помоћних средстава. Перформансе бојења, мерене CIELab и *K/S* вредностима, упоређене су са конвенционалним бојењем. Методом бојења NADES постигнуте су веће *K/S* вредности и елиминисана је потреба за додатним помоћним средствима, као што су средство за разливање и средство за дисперговање. Структура полиестарске тканине остала је нетакнута, што потврђују резултати физичких својстава. SEM анализа показује да је површинска морфологија свих узорака полиестра глатка и уједначена без видљивих промена, што указује на то да процес бојења није утицао на површину влакана. Оцене постојаности су сличне код свих обојених узорака полиестра.

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Optimizing fabrication of pharmaceutical capsule shell: A factorial design of corn-cob-MCC/carrageenan/starch biofilm

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Abstract: This study develops biodegradable soft-capsule films using carrageenan, tapioca starch, glycerol and microcrystalline cellulose (MCC) derived from corn-cob waste as a halal- and vegetarian-friendly alternative to gelatin. A 2³ factorial design evaluated the effects of carrageenan (15–25 wt. %), MCC (5–15 wt. %) and glycerol (10–20 wt. %) on tensile strength (*TS*), elongation (*EL*) and Young's modulus (*YM*). The produced biofilms were characterized using mechanical testing, FTIR, XRD, SEM, TGA, disintegration tests and ANOVA. Mechanical performance varied widely among samples. Among the synthesized biofilms, sample 2 exhibited the highest *TS* (6.565 MPa) and *YM* (97.029 MPa), while sample 5 showed the greatest ductility and the shortest disintegration time, reaching approximately 20 % and 12 min, respectively, indicating rapid water-induced matrix breakdown associated with the hygroscopic nature of the polysaccharide-based system. Regression models showed R² values of 84.48, 97.17 and 94.18 % for *TS*, *EL* and *YM*, respectively. XRD confirmed an MCC crystallinity of 62.6 %. The TGA results confirm the films' thermal stability and show moisture uptake and char residue consistent with those of the other analyses. Overall, the carrageenan–starch–MCC–glycerol system offers tuneable properties, although further optimization is required for moisture control and purity.

Keywords: biodegradable film; biomass utilization; medicine packaging; sustainable packaging.

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INTRODUCTION

Capsule shells play a vital role in oral dosage forms by encapsulating active pharmaceutical ingredients, protecting taste, enhancing swallowability and maintaining stability. Traditionally, soft and hard capsule shells depend heavily on gelatin, a biopolymer derived from partially hydrolysed animal collagen.¹ Gelatin's favourable dissolution properties and film-forming ability have made it the standard in the pharmaceutical industry.² However, the leading global sources of gelatin, namely porcine, contribute 46 %, bovine hides 29.4 % and pork and cattle skeletons 23.1 % of the total gelatin production, which limits its acceptance among certain groups, including Muslims, vegetarians and vegans.³

In response to these limitations, considerable research has focused on plant- or marine-derived polymers as potential materials for capsule shells. Polysaccharides such as carrageenan (extracted from red seaweed) and plant-derived starches have emerged as promising alternatives. Carrageenan offers gelation, film-forming, and stabilizing functions,⁴ while starch contributes to capsule biofilms by forming a continuous, biodegradable polymer network that can be tailored *via* its amylose/amylopectin ratio, plasticizers and co-polymers to provide mechanical strength, flexibility and suitable barrier properties for pharmaceutical use.^{5,6} Yet, simple carrageenan–starch systems may suffer from brittleness, limited flexibility or irregular morphology,^{7,8} which suggests the need for additional reinforcing or modifying agents.

One such candidate is microcrystalline cellulose (MCC), a semi-crystalline derivative of cellulose obtained through acid hydrolysis of α -cellulose,⁹ characterized by a typical particle diameter of 10–50 μm , a particle length of 100–1000 μm and crystallinity indices ranging from 60 to 80 %.¹⁰ MCC is widely used as a filler, binder and disintegrant in pharmaceutical tablet formulations, owing to its superior mechanical performance and compressibility.¹¹ When incorporated into film-forming matrices, MCC can act as a reinforcing particulate, reducing brittleness and improving the flexural and tensile strength of polymer films.¹² Furthermore, corncob, as an abundant agricultural by-product in Indonesia, with approximately 33.5 wt. % of annual corn production becoming residue,¹³ represents a sustainable source of α -cellulose, reported at around 30 wt. %.¹⁴ Among the plasticizers used to increase film flexibility, sorbitol and glycerol are the most commonly used. However, glycerol can enhance elongation at break by more than 25 % compared to sorbitol, and it also exhibits a moisture uptake rate that is roughly twice as high.¹⁵

In this study, a composite biofilm system based on tapioca starch, carrageenan, corncob-MCC and glycerol is proposed as a sustainable alternative for pharmaceutical capsule shells. The carrageenan–starch matrix provides the necessary gelation and film-forming behaviour, while MCC and glycerol enhance mechanical strength, flexibility and durability. Systematically, a two-level factorial design (2³)

is applied to screen the main and interaction effects of carrageenan, MCC and glycerol on tensile strength, elongation and Young's modulus. Biofilms prepared under controlled casting conditions will be assessed through ANOVA and subjected to morphological, chemical, thermal and disintegration analyses. The development of a mechanically robust, halal-compliant and vegetarian-compliant capsule material is expected to contribute meaningfully to advancing sustainable pharmaceutical excipient technology.

EXPERIMENTAL

Materials

The materials employed in this research comprised corncobs as the biomass feedstock, along with sodium hydroxide, sodium hypochlorite and hydrochloric acid as chemical reagents. Carrageenan, tapioca starch and glycerol were utilized as biopolymers, matrix components and plasticizers, respectively. All preparations and experimental procedures were conducted using distilled water to ensure the purity and consistency of the reactions.

Raw material preparation

The collected corncobs were thoroughly washed and subsequently milled to obtain a fine powder, which was later utilized as the feedstock for α -cellulose isolation. To eliminate residual impurities, the powdered material was pretreated with distilled water at 70 °C for 1 h under constant heating. The preheated sample was then filtered and oven-dried at 60 °C for 24 h.

Isolation of α -cellulose from corncob

Dried corncob powder (20 g) was treated with 600 mL of 0.5 M NaOH solution under ultrasonic agitation at 40 °C for 30 min. Following sonication, the suspension was filtered through a mesh bag and thoroughly rinsed with distilled water until the filtrate reached a neutral pH. The resulting material was then immersed in 250 mL of 5.25 vol. % NaOCl solution and allowed to stand for 30 min. Subsequently, the mixture was heated at 50 °C for 30 min, re-filtered, washed and oven-dried at 60 °C for 18 h to obtain the pretreated corncob sample.

Preparation of microcrystalline cellulose

Microcrystalline cellulose was synthesized through the acid hydrolysis of purified α -cellulose using hydrochloric acid. In this procedure, 50 mL of 3 M HCl was reacted with the α -cellulose at 80 °C for 30 min to initiate depolymerization. After the reaction, the resulting MCC was repeatedly rinsed with distilled water until a neutral pH was achieved, ensuring the removal of residual acid. The neutralized material was subsequently filtered and oven-dried at 60 °C for 4 h, yielding a fine white MCC powder suitable for further processing.

Preparation of soft capsule biofilm

Carrageenan, MCC and tapioca starch were thoroughly blended and dispersed in 100 mL of distilled water to obtain a uniform mixture. The suspension was subsequently heated to 70–80 °C under continuous stirring for approximately 5 min to ensure complete dissolution. Thereafter, glycerol was incorporated into the mixture and stirred for an additional 5 min. The resulting solution was carefully poured into moulds and oven-dried at 60 °C for 18 h.

Sample characterization

Analytical techniques included Fourier transform infrared spectroscopy (FTIR, Shimadzu, Japan), X-ray diffraction (XRD, Bruker D8 Advance, USA), scanning electron microscopy

(SEM, Quanta 250, Netherlands) and thermogravimetric analysis with differential scanning calorimetry (TGA/DSC, LINSEIS STA PT-1600, Germany). FTIR was used to identify functional groups in corn cob, MCC and capsule films, while XRD was used to evaluate crystalline structures. SEM was used to examine the surface morphology, and TGA was used to assess moisture-related mass loss, hygroscopic behaviour and thermal stability. Mechanical properties were measured using a universal testing machine (HZ-1007A, Lyxian, China) in accordance with ASTM D683-14.

Analytical methods

The analysis focused on a 2^3 factorial design implemented using Design Expert v. 25.0 to evaluate the percentage composition of carrageenan (15, 20 and 25 wt. %), MCC (5, 10 and 15 wt. %), glycerol (10, 15 and 20 wt. %), and tapioca starch (by difference) with respect to the responses tensile strength (*TS*), elongation (*EL*) and Young's modulus (*YM*) of the capsule shell biofilm. According to Eqs. (1–3), tensile strength was determined as the tensile force divided by the cross-sectional area, elongation as the ratio of the increase in length to the initial length, and Young's modulus as the ratio of stress to strain. ANOVA was performed to determine the optimal values for each film characteristic. The composition of each sample is presented in Table I:

$$TS(\sigma) = \frac{F}{A} \quad (1)$$

$$EL(\varepsilon) = 100 \frac{\Delta L}{L} \quad (2)$$

$$YM(\gamma) = \frac{\sigma(\text{stress})}{\varepsilon(\text{strain})}_{ss} \quad (3)$$

TABLE I. Sample composition

Run	Carrageenan, %	MCC, %	Glycerol, %	Starch, %
1	25	5	20	50
2	15	5	10	70
3	25	5	10	60
4	15	15	20	50
5	25	15	10	50
6	20	10	15	55
7	15	5	20	60
8	25	15	20	40
9	20	10	15	55
10	15	15	10	60

RESULTS AND DISCUSSION

Based on laboratory observations, 10 film samples were prepared according to Table I (and shown in Fig. S-1 of the Supplementary material to this paper) to serve as candidate materials for drug capsule shells. The samples were subsequently evaluated to confirm the successful synthesis of MCC, determine the optimal film formulation suitable for capsule fabrication and assess their initial physical characteristics prior to further analyses, including FTIR, SEM, TGA and

disintegration testing in accordance with the Indonesian Pharmacopoeia¹⁶ and statistical evaluation using ANOVA.

Mechanical test

The mechanical test results for each sample are shown in Table II. Analysis of variance (ANOVA) revealed that glycerol significantly influences tensile strength. Elongation is affected by carrageenan, MCC, glycerol and their interaction effects. Meanwhile, Young's modulus is governed by the main and interaction effects of carrageenan, MCC and glycerol. The statistical model derived from the ANOVA results is presented in Tables S-I–S-III of the Supplementary material, and the fit statistics for each response are tabulated in Table S-IV of the Supplementary material.

TABLE II. Mechanical test results (dual repetition)

Sample	TS / MPa		EL / %		YM / MPa	
	1	2	1	2	1	2
1	3.374	3.728	10.324	9.198	32.681	40.531
2	5.082	6.565	5.753	6.766	88.337	97.029
3	6.015	5.080	11.623	11.661	51.751	43.564
4	2.073	2.209	8.569	7.720	24.192	28.614
5	4.848	4.682	20.192	18.774	24.010	24.939
6	5.39	3.842	10.09	11.272	53.419	34.084
7	2.638	3.035	8.758	10.260	30.121	29.581
8	0.981	1.623	8.803	7.565	11.144	21.454
9	5.626	4.459	10.626	10.486	52.946	42.523
10	4.794	4.900	7.403	6.179	64.758	79.301

Based on Table II, the mechanical test results show clear variations in strength, ductility, and stiffness across the 10 samples. Sample 2 demonstrates the highest overall performance, with *TS* reaching 6.565 MPa and *YM* up to 97.029 MPa, indicating a strong and stiff material; however, its low elongation at break (~6 %) suggests limited flexibility and more brittle behaviour. At the opposite end, sample 8 shows the lowest *TS* (0.981–1.623 MPa) and *YM* (11.144–21.454 MPa), indicating a weak, highly compliant material. Sample 5 achieves the greatest elongation at break (18–20 %), offering superior ductility despite only moderate strength. These differences illustrate the trade-offs among strength, stiffness and flexibility, with some formulations favouring rigidity (sample 2), others favouring deformability (sample 5), and some exhibiting the lowest structural performance (sample 8). Biofilms for soft capsule applications intended to replace porcine gelatin should exhibit a tensile strength within the range of 2.4–63.25 MPa and an elongation at break of 4.4–90.55 %.¹⁷ Based on these criteria, samples 4 and 8 do not meet the required specifications because their tensile strength values fall below the acceptable range.

The statistical summary provides a solid basis for evaluating the validity of the regression models (Table S-IV). The coefficient of variation (*C.V.*), defined as the ratio of the standard deviation to the mean, is used to assess data dispersion, precision, and reproducibility. In empirical studies, a *C.V.* of < 10 % is considered indicative of high precision.¹⁸ In this study, *C.V.* values for *TS* (18.91 %) and *YM* (15.52 %) reflect moderate variability, suggesting that subtle differences between treatments might approach the limit; by contrast, *EL* shows a *C.V.* of 7.68 %, indicating relatively high precision under the experimental conditions.

The regression models for *TS*, *EL* and *YM* demonstrate strong explanatory and predictive performance. *EL* and *YM* show high adjusted R^2 values (0.9552 and 0.9149), capturing over 90 % of the response variability, while *TS* has an adjusted R^2 of 0.7543, explaining about 75 %. Adjusted R^2 is preferred because it accounts for multiple predictors and reduces the risk of overfitting. The predicted R^2 values (0.9112 for *EL*, 0.8748 for *YM* and 0.6776 for *TS*) fall within 0.20 of their respective adjusted R^2 values, meeting established reliability criteria and confirming good predictive capability within the design space.¹⁹

Furthermore, the adequate precision values (signal-to-noise ratios) for all three models exceed the minimum threshold of 4, indicating acceptable model discrimination and response-surface reliability.²⁰ The high signal-to-noise ratios confirm that the coded regression models reliably capture the response trends and are suitable for probing factor interactions and guiding formulation optimization. Overall, the models (Eqs. (4)–(6)) provide a robust representation of how carrageenan (*A*), MCC (*B*) and glycerol (*C*) influence *TS*, *EL* and *YM*. The predicted vs. actual plots are illustrated in Figs. S-2–S-4 of the Supplementary material for each response:

$$TS = 4.05 - 0.06A - 0.59B - 1.39C - 0.17AB + 0.03AC - 0.15BC - 0.22ABC \quad (4)$$

$$EL = 10.10 + 2.30A + 0.68B - 1.07C + 0.89AB - 2.22AC - 1.41BC - 0.94ABC \quad (5)$$

$$YM = 43.25 - 11.99A - 8.45B - 15.96C - 2.42AB + 11.15AC + 2.51BC - 1.79ABC \quad (6)$$

The 3D surface and contour plots show how *A*, *B* and *C* affect *TS*, *EL* and *YM*, and which combinations maximize each response. Fig. 1 shows their effect on the *EL* response. The *AC* interaction critically governs the balance between stiffness and ductility in the films. At low to moderate carrageenan contents, increasing glycerol significantly enhances elongation at break by increasing chain mobility and weakening intermolecular hydrogen bonding within the polysaccharide matrix. However, at higher carrageenan levels, the reinforcing effect of carrageenan increasingly restricts chain motion, reducing glycerol's plasticization efficiency and

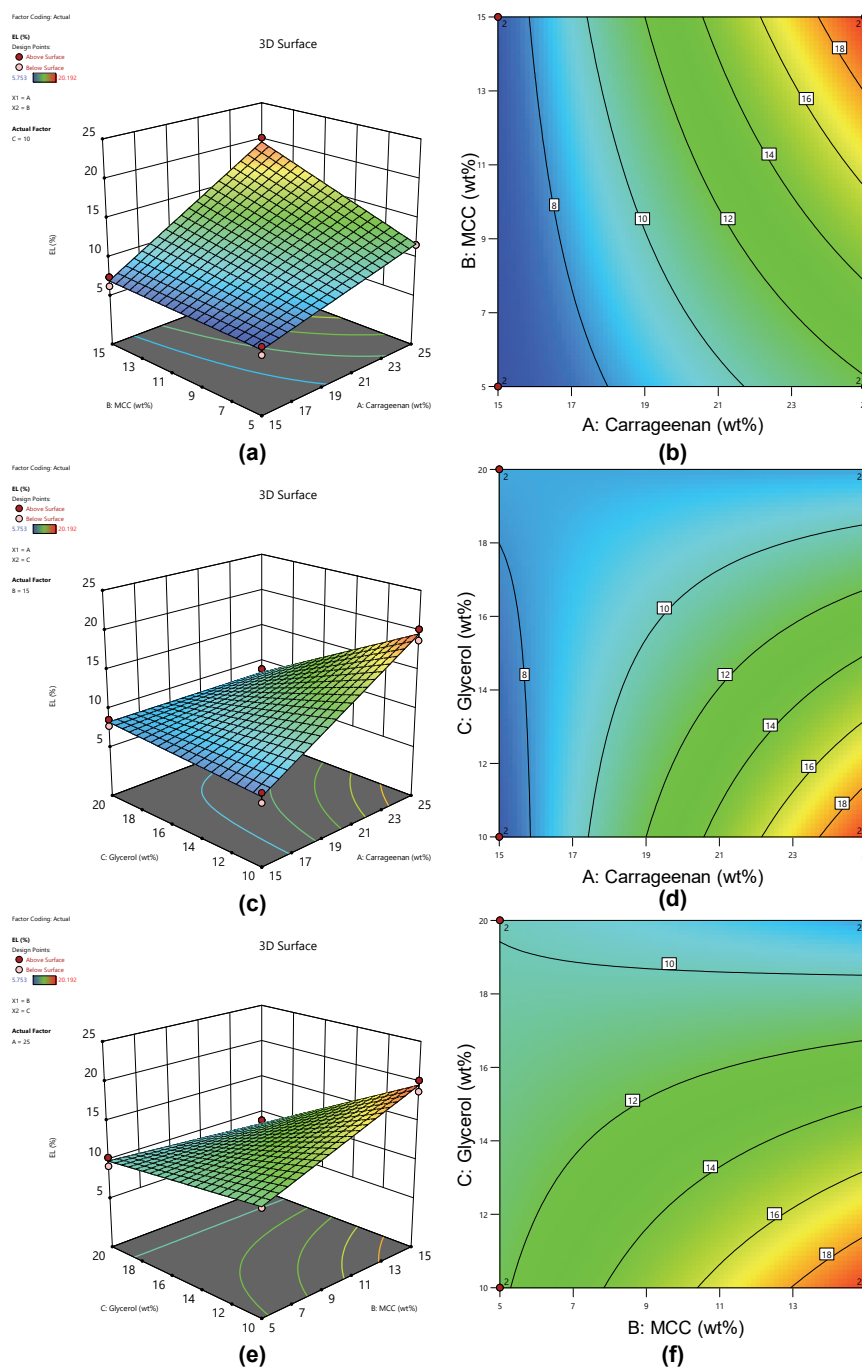


Fig. 1. Surface and contour plots showing the effect of factors on *EL*: a,b) carrageenan and MCC, c,d) carrageenan and glycerol and e,f) MCC and glycerol.

leading to greater stiffness. This antagonistic behavior explains the strong *AC* interaction observed for *YM*, where stiffness is controlled by the competition between matrix reinforcement and plasticizer-induced flexibility. For *TS* (Fig. S-5 of the Supplementary material), the *BC* interaction further defines the strength–ductility trade-off, where MCC enhances tensile strength through rigid reinforcement and efficient stress transfer at low glycerol levels, whereas increasing glycerol progressively disrupts filler–matrix interactions, reducing tensile strength. At higher MCC loadings, elongation at break is reduced. Excessive glycerol addition results in visibly wet, tacky films, indicating matrix oversaturation that compromises mechanical integrity and underscores the need for controlled plasticizer content to maintain an optimal balance among strength, flexibility, and dimensional stability. The highest *YM* was observed when *A*, *B* and *C* were kept low (Fig. S-6 of the Supplementary material).

Disintegration test

As reported in a previous study, the mean disintegration time (*DT*) in 0.1 M HCl was approximately 18 min.¹⁷ The experimental outcomes, summarized in Table III, reveal distinct variations among the tested samples. Notably, samples 3 and 5 demonstrated the fastest disintegration (*DT* < 20 min), achieving complete disintegration within 16 and 12 min, respectively, which is markedly faster than the average and significantly lower than the times observed for other formulations, several of which exceeded 30 min. According to the Indonesian Pharmacopoeia,¹⁶ immediate-release tablets must disintegrate within 30 min, while antifungal and anticholinergic products require *DT* of < 20 and < 15 min, respectively. In this context, sample 5 exhibits enhanced disintegration under acidic conditions, sample 3 closely meets antifungal specifications, and the slower-disintegrating samples 7 and 10 are more consistent with delayed- or extended-disintegration formulations. The disintegration behaviour of the films is governed by the hygroscopic polysaccharide–glycerol matrix, which facilitates rapid water uptake upon immersion. Absorbed water disrupts intermolecular hydrogen bonding, induces matrix swelling, and leads to swelling-induced structural breakdown of the film.²¹ Variations in disintegration time among formulations reflect differences in glycerol content and network rigidity, consistent with the observed mechanical and thermal properties. Therefore, sample 5 was selected for further characterization as it exhibited the fastest disintegration while maintaining the mechanical performance, making it the most representative formulation.

TABLE III. Disintegration test results in 0.1 M HCl solution

Sample	1	2	3	5	7	9	10
<i>DT</i> / min	> 30	> 30	16	12	26	> 30	21

Scanning electron microscopy (SEM)

The SEM observations of the MCC revealed that, under lower magnifications ($100\times$ and $250\times$), the particles exhibited a generally homogeneous distribution. However, occasional larger fragments were still discernible, likely as a consequence of incomplete removal during the filtration stage. With increasing magnification ($500\times$ and $1000\times$), the surface morphology displayed distinct microstructural features, such as pores, fissures and inter-aggregate voids, indicating the presence of interfibrillar regions that may enhance the material's capacity for adsorption and controlled release of active compounds. Based on Fig. 2a and b, the MCC particle size was $< 200\text{ }\mu\text{m}$, which aligns with the standard dimensional range reported for MCC grades PH 101, PH 102, PH 103, PH 112, PH 113, PH 200, PH 301 and PH 302 ($50\text{--}180\text{ }\mu\text{m}$).²² The isolated sample demonstrated conformity within acceptable limits, confirming the consistency of the particle size.

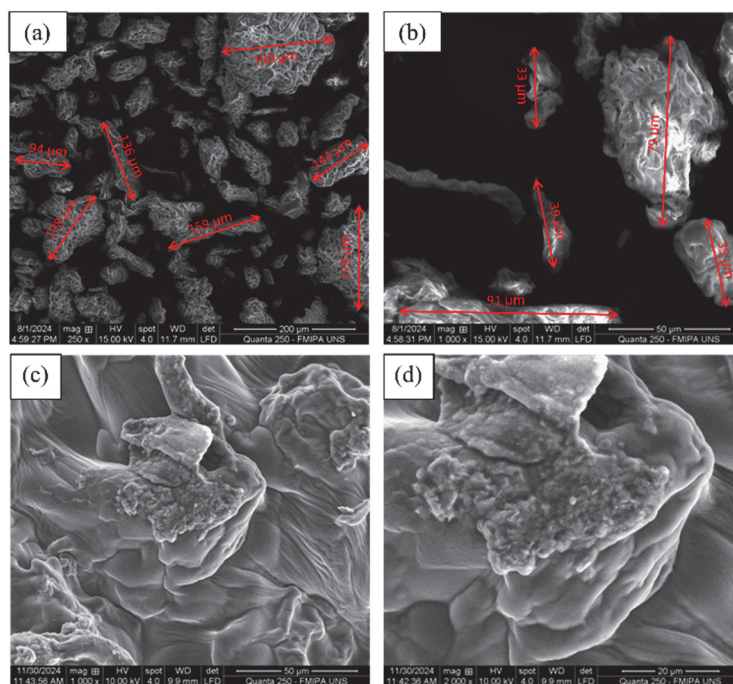


Fig. 2. SEM images of MCC: a) $250\times$ and b) $1000\times$ magnifications and of the biofilm: c) $1000\times$ and d) $2000\times$ magnifications.

The SEM micrographs of the biofilm exhibited a distinctly layered morphology at $1000\times$ and $2000\times$ magnifications (Fig. 2c and d). The surface evolved from heterogeneous aggregated clusters to partially disentangled fibrous structures, ultimately revealing well-defined layered networks that indicate the coexistence of

amorphous domains and successful incorporation of microcrystalline cellulose within the biofilm matrix.

Fourier transform infrared analysis

According to Fig. 3a, the FTIR spectra of corncob, MCC and the capsule biofilm show distinct compositional differences despite their overall similarity. All samples display broad O–H stretching bands at $3500\text{--}3200\text{ cm}^{-1}$, with MCC showing the sharpest band, indicating a higher abundance of O–H groups. The C–H stretching band at $3000\text{--}2800\text{ cm}^{-1}$ appears clearly in corncob and MCC, reflecting cellulose and hemicellulose structures.^{25,26} In contrast, the biofilm exhibits a pronounced $1750\text{--}1600\text{ cm}^{-1}$ carbonyl band linked to C=O groups from glycerol, starch and carrageenan.²⁷ The corncob spectrum shows stronger lignin-associated C=C vibrations at $1600\text{--}1500\text{ cm}^{-1}$,²⁴ whereas this band is reduced in MCC after delignification.²⁴ Signals near 1250 cm^{-1} confirm C–O stretching from cellulose and hemicellulose, indicating successful cellulose isolation, while the $1000\text{--}1100\text{ cm}^{-1}$ band, characteristic of carrageenan, highlights C–O–C glycosidic linkages.²⁸ Overall, FTIR spectroscopy successfully identified functional groups associated with cellulose-based materials and polymer components in the samples. The spectra show absorption bands corresponding to O–H, C–H and C–O functional groups, indicating the presence of cellulose-related structures and the incorporation of carrageenan, glycerol and starch in the film matrix. No new absorption bands were observed, suggesting that the film formation is governed by physical interactions rather than chemical modification.

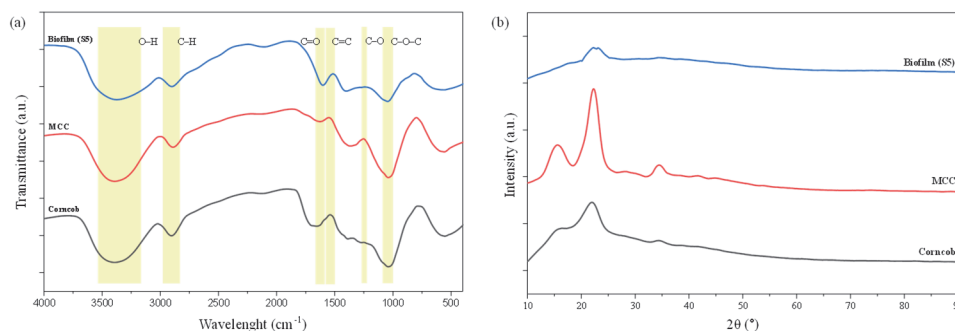


Fig. 3. Spectra analyses of corncobs, capsule biofilms and MCC: a) FTIR and b) XRD.

X-Ray diffraction

X-ray diffraction analysis reveals that the MCC isolated from corncob exhibits sharp, well-defined peaks at 2θ 15.5° , 22.2° and 34.4° , corresponding to a crystallinity index of 62.6 % (Fig. 3b). In contrast, the untreated corncob exhibits broader, less intense peaks, indicating a more amorphous structure and a lower crystallinity

of 49.8 %. Sample 5, in contrast, exhibits very diffuse diffraction patterns and markedly diminished peak intensity, indicating substantial disruption of the ordered crystalline domains. This loss of long-range order is likely caused by the incorporation of starch and carrageenan, which interfere with the tight packing of cellulose chains and increase the free volume within the matrix. Additionally, the incorporation of glycerol into the starch-based matrix is expected to interfere with intermolecular hydrogen-bonding interactions.²⁹ Although no pronounced shift or intensity change is observed in the O–H stretching region ($3500\text{--}3200\text{ cm}^{-1}$) in Fig. 3a, a slight band broadening and subtle variations in band shape can be discerned. This behavior suggests the formation of new hydrogen bonding interactions between glycerol and polysaccharide chains, which partially replace the native polymer–polymer interactions rather than significantly altering the overall hydroxyl vibrational profile. Consequently, these interactions suppress crystalline domain formation and lead to reduced crystallinity as observed from the decreased XRD diffraction intensity (Fig. 3b), resulting in a more flexible and amorphous film structure. From a functional perspective, the marked reduction in crystallinity in sample 5 may confer several advantages for pharmaceutical biofilm or capsule applications. Amorphous polymer systems typically offer greater flexibility and better resistance to stress fracture.³⁰ However, because amorphous domains generally have higher molecular mobility and a greater tendency to recrystallize over time, it will be essential to assess the physical stability of sample 5 during storage, including its potential for aging or recrystallization, to ensure long-term performance. Similar phenomena (loss of crystallinity after blending with plasticizers and biopolymers) have been reported in other polysaccharide/glycerol films.³¹

Thermogravimetric analysis

For polysaccharide-based capsule materials, low-temperature mass loss is particularly relevant, as it reflects absorbed and bound water that directly influences storage stability and disintegration performance.³² While the primary thermal degradation occurs above $250\text{ }^{\circ}\text{C}$, the early-stage mass loss at lower temperatures reflects the film's hygroscopic behaviour, which plays a more critical role in determining its storage stability and functional performance in pharmaceutical use.³³ According to Fig. 4, both TGA (T_{max}) and DSC results confirm a high cellulose fraction, consistent with the characteristic thermal decomposition of cellulose occurring between 300 and $350\text{ }^{\circ}\text{C}$: MCC exhibited the highest T_{max} , while sample 5 showed a slightly lower T_{max} and broader thermal transitions, consistent with reduced crystallinity.

However, the primary limitation for pharmaceutical use is not thermal stability but hygroscopicity, which poses risks for moisture-sensitive drugs. To overcome this issue, the partial replacement of glycerol with less hygroscopic plasticizers, such as tributyl citrate, together with mild crosslinking to reduce the number of

free hydroxyl groups, thereby decreasing moisture uptake, may be considered. Additionally, the relatively high char residue (~16 % at 700 °C) warrants attention. Similar trends have been reported for MCC derived from corncob and for carrageenan-based capsules, which can yield 11.8–17.7 % char, indicating the persistence of non-volatile structural components or trace impurities even after purification.³⁴

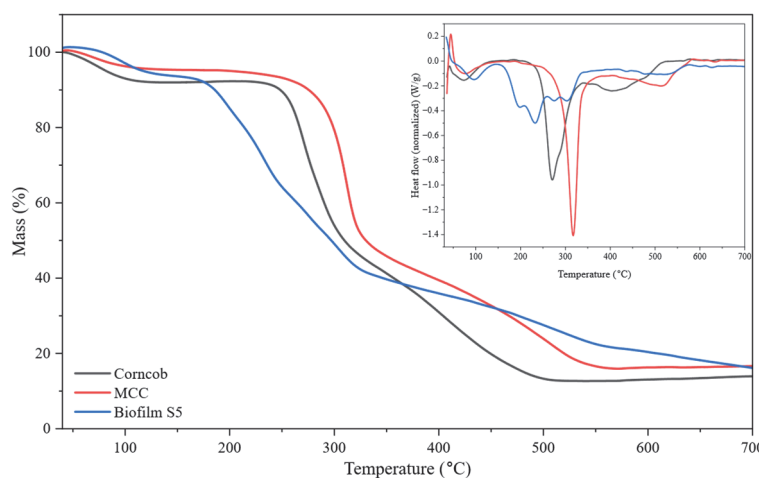


Fig. 4. TGA and DSC spectra.

CONCLUSION

This study quantitatively elucidates the individual and interaction effects of carrageenan, MCC and glycerol on the mechanical performance of bio-based capsule films using a 2^3 factorial design. Statistical analysis shows that glycerol is the dominant factor controlling tensile strength ($p < 0.01$), while MCC enhances stiffness and load-bearing capacity. Elongation at break is governed by both main and interaction effects ($p < 0.05$), demonstrating the coupled roles of polymer matrix composition and plasticization in regulating film ductility. This study observed *TS* values ranging from 1.0 to 6.6 MPa, *EL* values ranging from 5.8 to 20.2 %, and *YM* values ranging from 11.1 to 97 MPa. *TS* is maximized by increasing MCC content while minimizing glycerol, elongation is enhanced by higher carrageenan and glycerol contents with reduced MCC loading, while Young's modulus is maximized when carrageenan, MCC and glycerol are all kept at low levels. Despite moderate experimental variability, the models exhibited strong predictive capability. The disintegration time results are in accordance with the Indonesian Pharmacopoeia, demonstrating that the hygroscopic polysaccharide–glycerol matrix facilitates water uptake and swelling-induced structural breakdown. Combined XRD, FTIR and TGA/DSC analyses demonstrate that glycerol-induced disruption of hydrogen bonding reduces film crystallinity and thermal stability, increasing the

amorphous character and hygroscopicity. These findings provide practical guidance for formulating plant-based pharmaceutical capsule films by balancing mechanical performance, disintegration behaviour and moisture stability.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13666>, or from the corresponding author on request.

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ИЗВОД

ОПТИМИЗАЦИЈА ИЗРАДЕ ОМОТАЧА ФАРМАЦЕУТСКЕ КАПСУЛЕ: ФАКТОРИЈАЛНИ ДИЗАЈН БИОФИЛМА КУКУРУЗНА МИКРОЦЕЛУЛОЗА/КАРАГЕНАН/СКРОБ

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У овој студији су развијани биоразградиви филмови меких капсула користећи карагенан, тапиока скроб, глицерол и микрокристалну целулозу (МСС), која је добијена из отпада кукурузног клипа као халал и вегетаријанска алтернатива желатину. 2³ факторијални дизајн је коришћен за процену утицаја удела карагенана (15–25 теж. %), МСС (5–15 теж. %) и глицерола (10–20 теж. %) на затезну чврстоћу (TS), издужење (EL) и Јангов модул еластичности (YM). Произведени биофилмови су окарактерисани применом механичких испитивања, FTIR, XRD, SEM, TGA, тестова дезинтеграције и ANOVA. Механичке перформансе су се значајно разликовале међу узорцима. Узорак 2 је показао највећу затезну чврстоћу (6,565 МПа) и Јангов модул еластичности (97,029 МПа), док је узорак 5 показао највећу дуктилност и време растварања, достижући приближно 20 % и 12 min, редом, што указује на брзу разградњу матрице под дејством воде услед хигроскопне природе полисахаридог система. Регресиони модели су били веома поуздани, са R² вредностима од 94,02, 97,96 и 96,78 % за TS, EL и YM, редом. Кристалинност МСС, одређена XRD анализом, износила је 62,6 %. Резултати TGA су потврдили термичку стабилност филмова и показали апсорпцију влаге и заостали угљеник у складу са осталим анализама. Генерално, својства система карагенан–скроб–МСС–глицерол се могу лако подешавати, при чему је потребна даља оптимизација за контролу влаге и чистоће.

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SUPPLEMENTARY MATERIAL TO

**Optimizing fabrication of pharmaceutical capsule shell:
A factorial design of corncob-MCC/carrageenan/starch biofilm**

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Table S-I. ANOVA parameter for tensile strength response

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	38.27	7	5.47	9.33	0.0005	significant
A-Carrageenan	0.0582	1	0.0582	0.0993	0.7581	
B-MCC	5.53	1	5.53	9.44	0.0097	
C-Glycerol	31.09	1	31.09	53.06	< 0.0001	
AB	0.4621	1	0.4621	0.7885	0.3920	
AC	0.0136	1	0.0136	0.0233	0.8813	
BC	0.3514	1	0.3514	0.5996	0.4537	
ABC	0.7634	1	0.7634	1.30	0.2760	
Residual	7.03	12	0.5860			
Lack of Fit	3.06	1	3.06	8.46	0.0142	significant
Pure Error	3.97	11	0.3613			
Cor Total	45.31	19				

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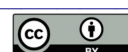


Table S-II. ANOVA parameter for elongation response

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	247.89	7	35.41	58.86	< 0.0001	significant
A-Carrageenan	84.33	1	84.33	140.17	< 0.0001	
B-MCC	7.37	1	7.37	12.26	0.0044	
C-Glycerol	18.39	1	18.39	30.57	0.0001	
AB	12.59	1	12.59	20.93	0.0006	
AC	79.06	1	79.06	131.41	< 0.0001	
BC	32.00	1	32.00	53.19	< 0.0001	
ABC	14.15	1	14.15	23.51	0.0004	not significant
Residual	7.22	12	0.6016			
Lack of Fit	1.34	1	1.34	2.50	0.1419	
Pure Error	5.88	11	0.5346			
Cor Total	255.11	19				

Table S-III. ANOVA parameter for Young modulus response

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	9755.36	7	1393.62	30.50	< 0.0001	significant
A-Carrageenan	2300.62	1	2300.62	50.35	< 0.0001	
B-MCC	1142.15	1	1142.15	25.00	0.0003	
C-Glycerol	4075.90	1	4075.90	89.20	< 0.0001	
AB	93.98	1	93.98	2.06	0.1771	
AC	1990.57	1	1990.57	43.56	< 0.0001	
BC	100.82	1	100.82	2.21	0.1632	
ABC	51.33	1	51.33	1.12	0.3101	not significant
Residual	548.34	12	45.69			
Lack of Fit	19.88	1	19.88	0.4138	0.5332	
Pure Error	528.46	11	48.04			
Cor Total	10303.70	19				

Table S-IV. Fit statistics of each response

Parameter	TS	EL	YM
Standard deviation	0.7655	0.7756	6.79
Mean	4.05	10.10	43.75
C.V. %	18.91	7.68	15.52
R ²	0.8448	0.9717	0.9418
Adjusted R ²	0.7543	0.9552	0.9149
Predicted R ²	0.6776	0.9112	0.8748
Adequate precision	9.3391	26.9560	18.1185

Based on Table S-IV, The C.V. values for tensile strength (18.91 %) and Young's modulus (15.52 %) indicate moderate experimental variability, which is commonly observed in mechanically tested polymer-based and biopolymer films due to inherent material heterogeneity, minor thickness variations, non-uniform

plasticizer distribution, and sensitivity to testing conditions. Despite this variability, the mechanical trends and statistically significant factor effects remain consistent, indicating acceptable methodological reproducibility for a factorial design. Model adequacy was assessed using ANOVA significance, lack-of-fit tests, and residual diagnostics; although one of the three responses exhibited a significant lack of fit, this suggests limitations of the linear model for that specific response rather than invalidating the overall trends, and higher-order models or additional experiments would be required only if greater predictive accuracy were sought.

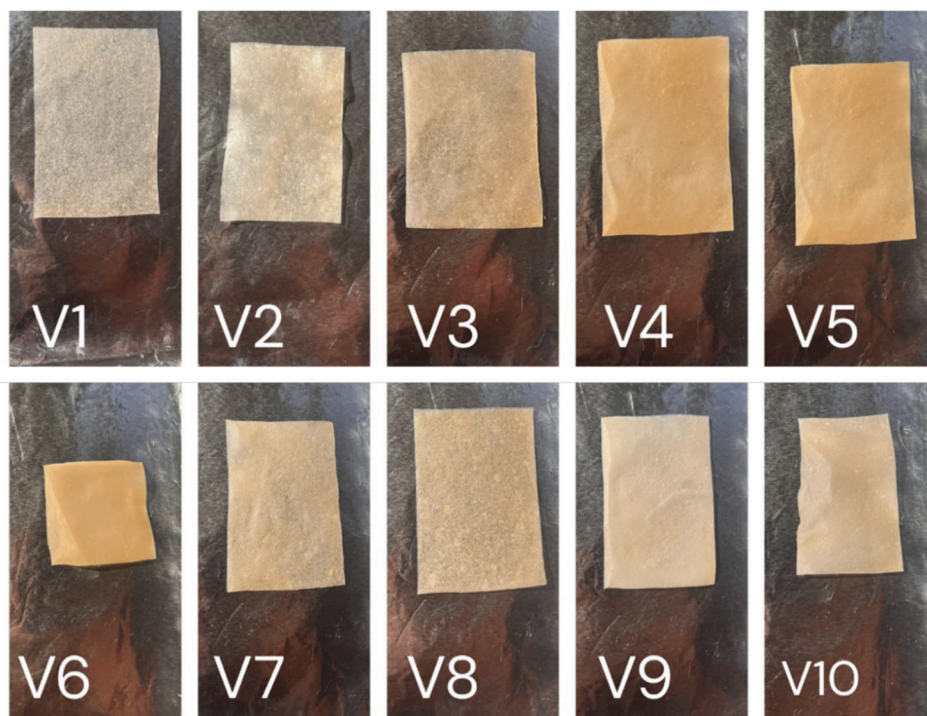


Fig. S-1. Appearance of the sample produced

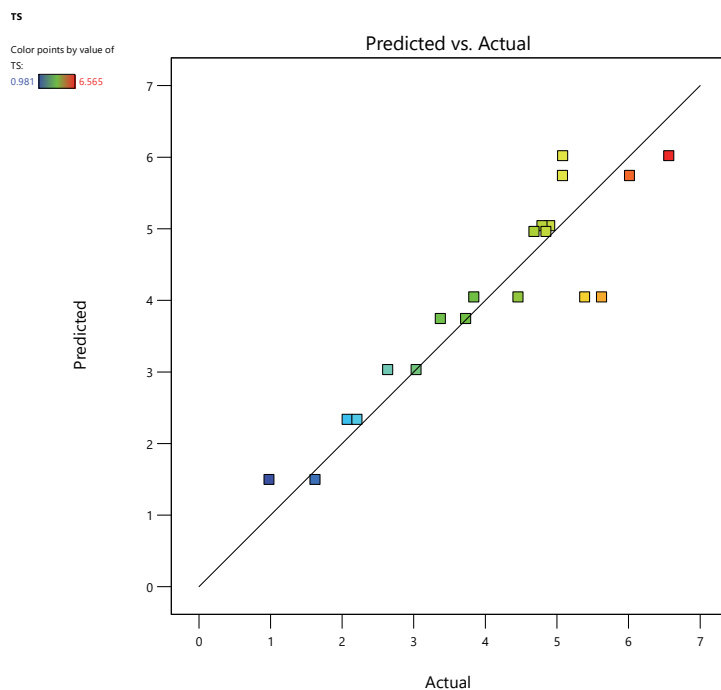


Fig. S-2. Predicted vs. actual scatter plot of TS response

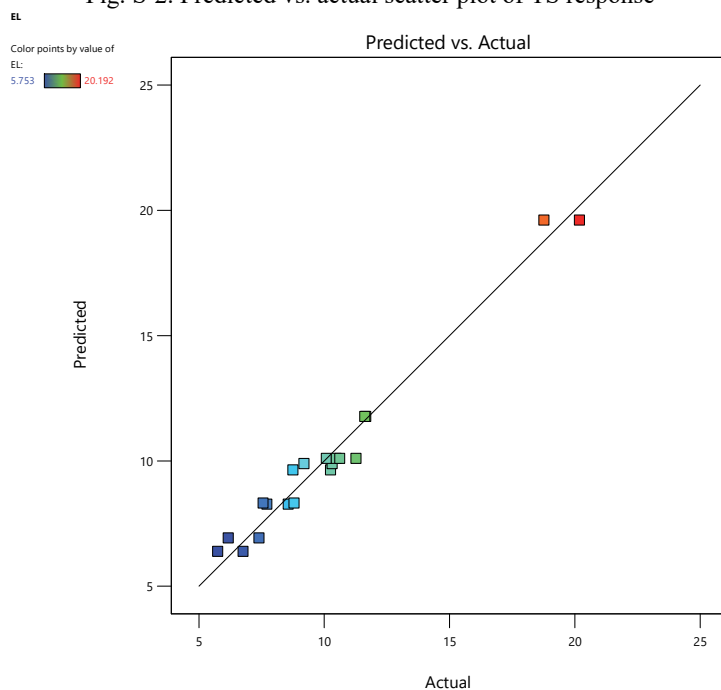


Fig. S-3. Predicted vs. actual scatter plot of EL response

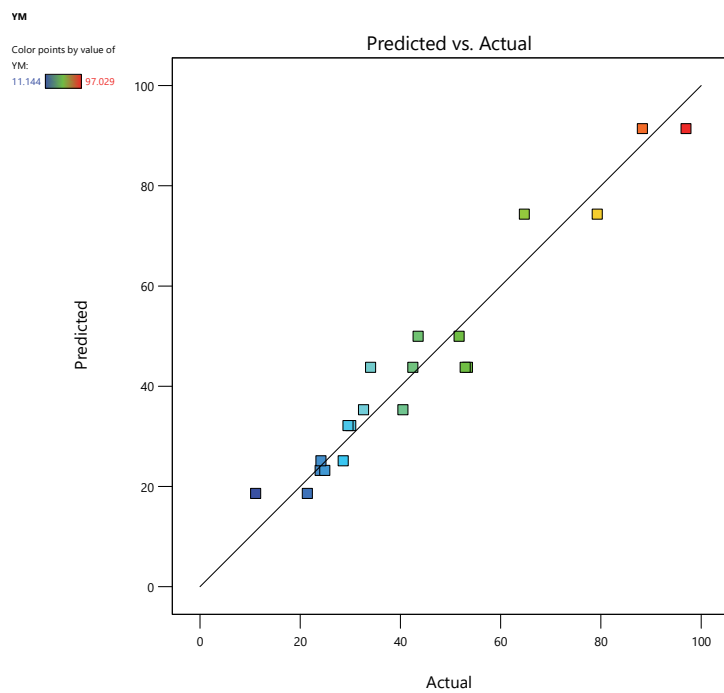


Fig. S-4. Predicted vs. actual scatter plot of TS response

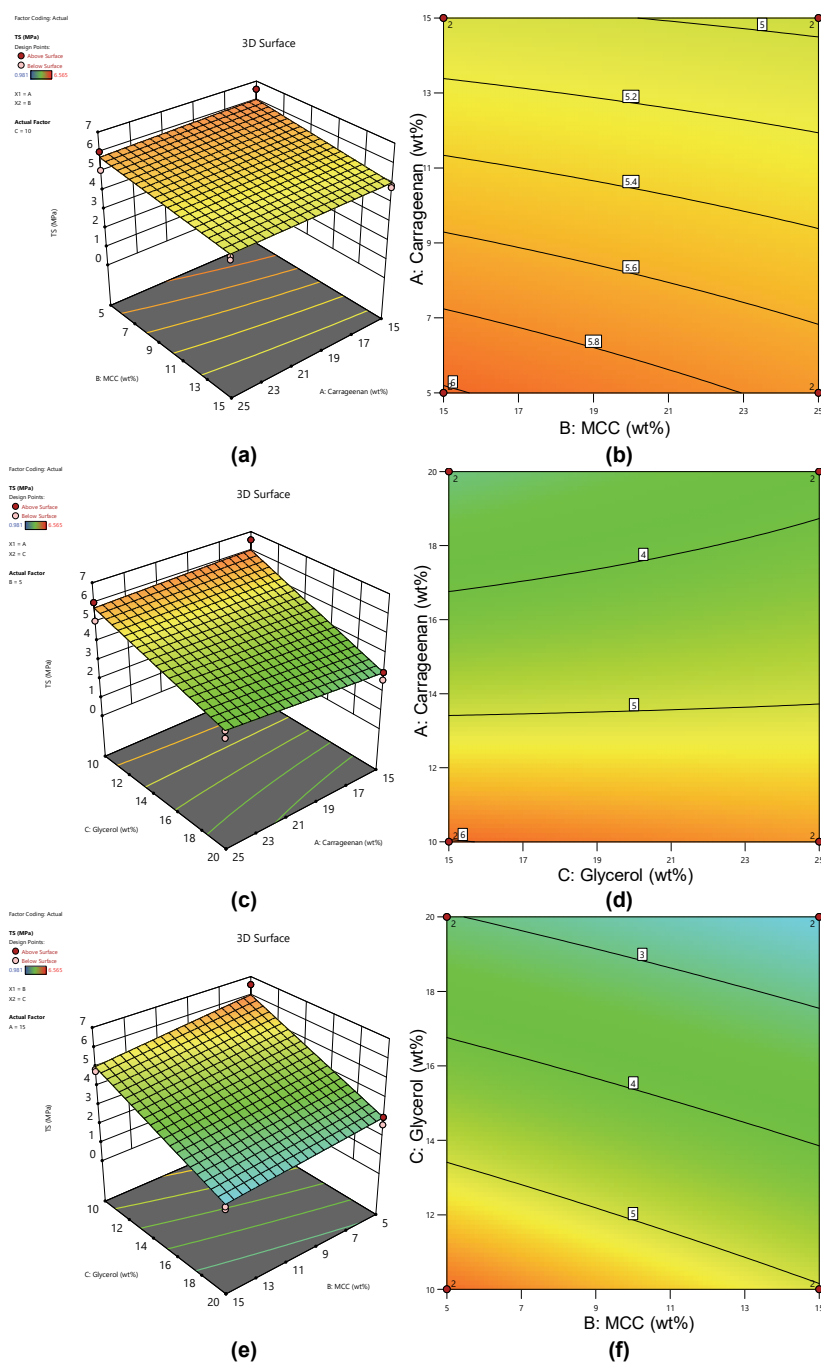


Fig. S-5. Surface and contour plots showing the effects of factors on TS: (a,b) carrageenan and MCC, (c,d) carrageenan and glycerol, and (e,f) MCC and glycerol..

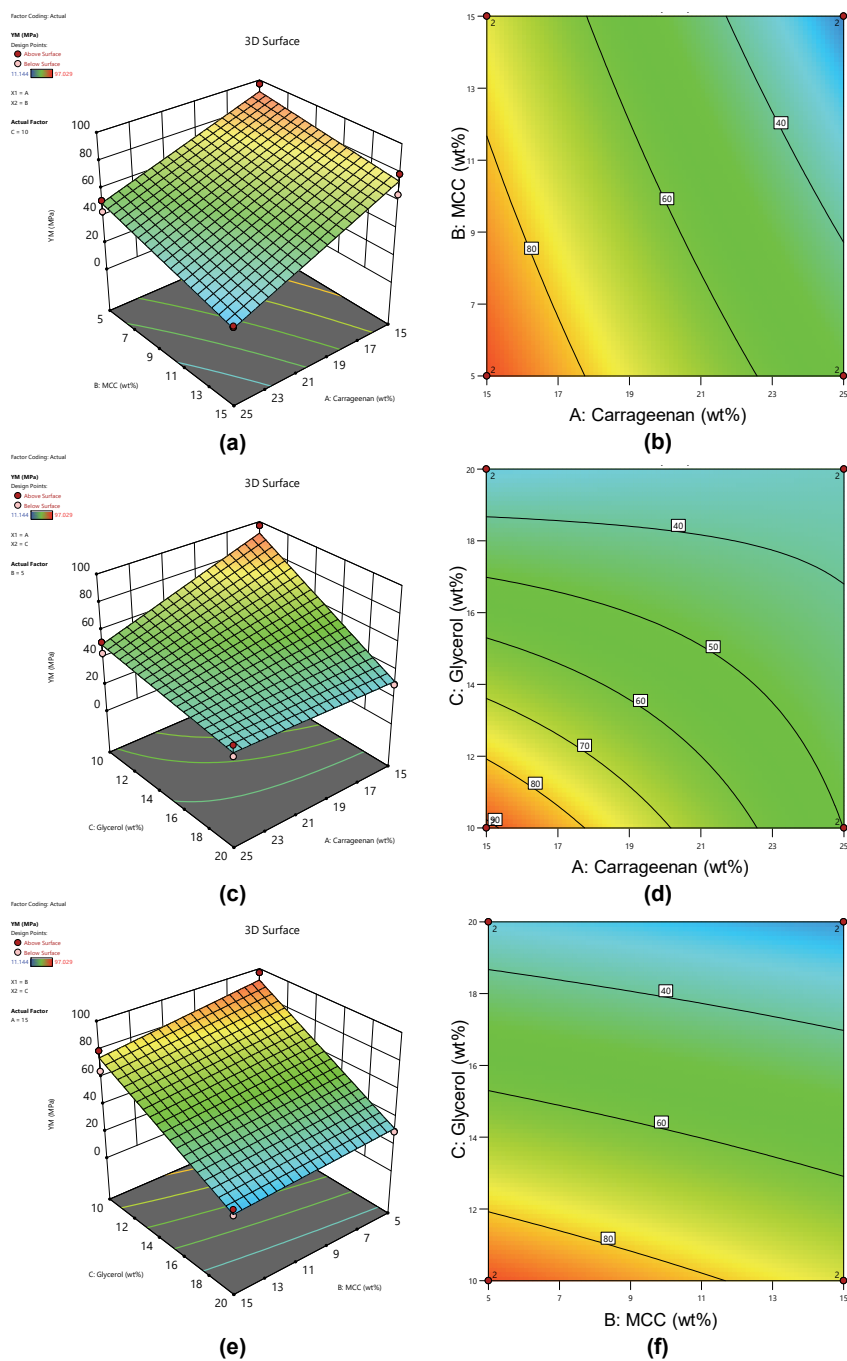


Fig. S-6. Surface and contour plots showing the effects of factors on YM: (a,b) carrageenan and MCC, (c,d) carrageenan and glycerol, and (e,f) MCC and glycerol.



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Impact of silica gel G on mechanical and microstructural properties of magnesium oxychloride

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Abstract: Magnesium oxychloride cement (MOC), a type of Sorel's cement, has gained renewed attention as a sustainable building material due to its low carbon footprint and energy consumption. However, its application in construction remains limited, primarily due to its inadequate moisture resistance and reduced early-age strength under elevated temperature conditions. This research investigates the impact of silica gel G (SG) on the properties of MOC. SG was incorporated into MOC at varying proportions (0–20 wt. % MgO) to form MOC–SG composites. The study evaluated the physical and mechanical properties of these composites, including setting time, moisture ingress resistance and compressive strength. Additionally, FTIR, PXRD, SEM-EDX and TGA analyses were conducted to examine the crystalline phases of the composites. SG acts as a binding agent, enhancing the strength and durability of the composite. This research demonstrates the potential of MOC–SG composites as promising sustainable building material. Results indicate that the addition of 5 % SG significantly improves the properties of MOC.

Keywords: cement; silica gel G; setting time; water resistance; compressive strength.

INTRODUCTION

Cement, a foundational material in human civilization and infrastructure development, has been utilized for centuries.¹ In recent years, there has been a growing interest in low-carbon cement alternatives, such as magnesium oxide-based cement. Magnesium oxychloride cement (MOC) was first developed in 1867 by Sorel.² In recent years, MOC is gaining attention due to its low energy consumption and reduced CO₂ emissions.^{3–7}

In comparison with ordinary Portland cement (OPC), MOC exhibits several noteworthy performance advantages, including high early and ultimate strength,

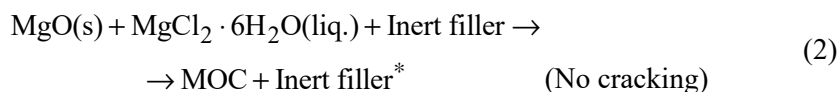
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strong interfacial bonding, rapid setting, low thermal conductivity, enhanced wear resistance, favorable mechanical properties, dimensional stability and excellent cohesiveness in lightweight composite panels.^{8,9} Additionally, its inherent fire resistance and the absence of the requirement for humid curing further contribute to its applicability across diverse construction contexts.¹⁰

MOC is frequently referred to as a green or eco-friendly cement due to its lower energy demand during synthesis, requiring neither high-temperature processing nor external heat or light. The CO₂ emissions associated with MOC production are typically 40–50 % lower than those associated with OPC. Moreover, MOC continues to sequester CO₂ from the atmosphere after curing and does not release heat during curing, making it a viable and cost-effective substitute for energy-intensive OPC.

MOC is produced through the direct reaction between lightly calcined MgO and a concentrated MgCl₂ solution. This reaction is exothermic and the heat generated can induce microcracking during the setting and curing stages. Such thermal microcracks facilitate water ingress, ultimately compromising the compressive strength and durability of the cement. To mitigate this issue, an inert filler is incorporated into the MOC matrix. Acting through a collision-based heat absorption mechanism, this filler dissipates thermal energy without participating in the primary cementation reactions.¹¹ In the present study, dolomite powder was used as an inert filler to decrease thermal shock and minimize crack development in MOC:



Temperature, along with the mole ratios and relative concentrations of MgO, MgCl₂ and H₂O, plays a critical role in governing the hydration and mineral composition of MOC. These parameters strongly influence the formation, stability and proportion of hydration products, typically represented as $x\text{Mg(OH)}_2 \cdot y\text{MgCl}_2 \cdot z\text{H}_2\text{O}$, which in turn exert a direct effect on the resulting compressive strength of the material.^{12,13}

Several distinct phase assemblages are known to form within the MgO–MgCl₂–H₂O system, including phase-3 (3Mg(OH)₂·MgCl₂·8H₂O), phase-5 (5Mg(OH)₂·MgCl₂·8H₂O), phase-9 (9Mg(OH)₂·MgCl₂·8H₂O) and phase-2 (2Mg(OH)₂·MgCl₂·8H₂O), which are typically stable over temperatures ranging from approximately 30 to 120 °C. The specific hydrate phases present in the hardened matrix are largely determined by the MgO/MgCl₂ mole ratio, which controls the reaction pathways and ultimate phase composition.

Under ambient conditions, phase-5 and phase-3 are the predominant and most thermodynamically stable hydration products in MOC systems, as represented in reactions (3) and (4):



These phases contribute significantly to strength development, durability and overall performance of MOC.

Although MOC exhibits a wide range of favorable mechanical and engineering properties, its large-scale industrial application remains significantly constrained. The primary limitations arise from its poor resistance to moisture and its susceptibility to reduced early-age strength when exposed to elevated temperatures. These drawbacks lead to excessive moisture uptake, dimensional instability and structural deformability under humid or high-temperature conditions. Consequently, despite its promising performance attributes, the broader commercial adoption of MOC has been limited. Owing to its many advantageous characteristics, extensive research has been devoted to magnesium oxychloride cement, with numerous studies aiming to further enhance its performance and broaden its applicability. A wide range of inorganic, organic, and hybrid additives have been incorporated into MOC systems with the aim of enhancing properties such as moisture resistance, mechanical strength, dimensional stability and overall durability.^{14–25}

Numerous studies have demonstrated that incorporating silica-based materials such as silica fume, nano-silica and others, either individually or in combination with supplementary additives like fly ash and phosphoric acid, significantly enhances the water resistance and overall performance of MOC. Silica fume, owing to its ultrafine particle size and high amorphous SiO_2 content, improves MOC mainly by filling micropores, microstructure densification and promoting the formation of magnesium–silicate–hydrate or Mg–Cl–Si–H type gels, which have superior stability in moist environments compared with conventional MOC hydration phases.²⁶ Hybrid systems incorporating silica fume and fly ash often demonstrate synergistic effects, with improved long-term retention of strength after water immersion while maintaining adequate mechanical properties.^{27,28} Nano-silica, owing to its high reactivity and surface area, further accelerates hydration, promotes secondary reactions of unreacted MgO, refines the pore structure and markedly reduces permeability. These effects translate into notable improvements in compressive strength and durability under wet conditions.²⁹ When nano-silica is combined with fly ash or phosphate-based modifiers, additional improvements arise owing to the formation and stabilization of water-resistant binding gels.³⁰ Collectively, the literature indicates that silica-based additives play a crucial role in mitigating the inherent water sensitivity of MOC by altering its hydration chemistry and microstructure.

Silica gel G (SG) is an amorphous form of SiO_2 containing approximately 13 % gypsum, which contributes to its binding capability. This research study aims to introduce SG as a novel additive for MOC and evaluate its effect on the material's

physicochemical and mechanical properties. The investigation also examines dolomite as an inert filler alongside SG, considering its role in improving particle packing, dimensional stability and resistance to shrinking-induced microcracking. The key objective is to determine whether the combined use of SG and dolomite can refine the MOC microstructure, lower capillary porosity, stabilize hydration products, and promote water-resistant binding phases, thereby improving durability and reducing the well-known susceptibility of MOC to strength loss under water exposure.

EXPERIMENTAL

Materials

In this study, magnesium oxide (MgO), magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), dolomite and silica gel G (SG) were used as the raw materials.

Magnesium oxide. Lightly calcined commercial grade MgO was procured from Suksha Exports, Jaipur, Rajasthan, India. The chemical composition of MgO was: MgO = 89.90 %, SiO_2 = 4.19 %, CaO = 1.40 %, Fe_2O_3 = 0.12 % and Al_2O_3 = 0.88 %. The loss of ignition, brightness and whiteness of MgO were 3.08, 88.20 and 91.60 %, respectively.³¹

Magnesium chloride (as $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$). Technical grade magnesium chloride was procured from Alum Scientific Suppliers, Jaipur, Rajasthan, India. It is a colorless crystalline and hygroscopic compound, which has a purity of 96.60 %.³²

Dolomite. In this study, commercial grade dolomite was used as an inert filler and procured from Ases Chemical Works, Jodhpur, Rajasthan, India. The chemical composition of dolomite was: CaO = 28.00 %, MgO = 18.00 %, SiO_2 = 10.98 %, Fe_2O_3 = 0.71 % and Al_2O_3 = 0.10 %. The LOI, brightness and whiteness of dolomite were 41.80, 89.40 and 92.70 %, respectively.³³

Silica Gel G. In this study, SG was employed as a chemical admixture and procured from CDH Fine Chemicals, New Delhi, India.

Methods

The methods for the preparation of the dry-mix composition, the gauging solution and the wet-mix composition with the incorporation of SG are as follows.

Preparation of dry-mix composition and incorporation of silica gel G. To prepare the dry-mix, MgO and dolomite were gently mixed together in an equal weight ratio. Powdered SG was also mixed into this dry-mix composition in varying weight proportions of 5, 10, 15 and 20 % with respect to MgO. A control dry-mix was also prepared with no addition of SG.

Preparation of gauging solution. To prepare the gauging solution, a sufficient amount of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ powder was added to lukewarm water (37 °C) with continuous agitation until saturation point was reached. This saturated solution of MgCl_2 is referred to as the gauging solution for MOC. The concentration of saturated gauging solution was obtained approx. 31 °Be on Baume Scale. A solution of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ of 25 °Be concentration was used as the gauging solution in the present research work.

Preparation of wet-mix. To form the wet-mix, the gauging solution was blended into the dry-mix to get a workable wet-mix consistency. The incorporation of SG into the optimal cementing composition of MOC was investigated with this wet-mix composition. To obtain a uniform and workable paste, the solid-to-solution ratio was optimized experimentally for each mix. For the compressive-strength specimens, the total solid mass (MgO + dolomite) was fixed at 540 g (MgO:Dolomite = 1:1) for compressive strength analysis (Table S-1 of the Supplementary

material to this paper) and at 200 g (MgO:dolomite = 1:1) for setting time analysis, and the volume of MgCl_2 solution required for workable consistency varied with the SG content (Table S-II of the Supplementary material).

In this study, each experimental procedure was repeated five times and the average value was taken into account to get accurate readings. All experiments were performed under controlled environmental conditions, maintaining a constant temperature of 30 °C and a relative humidity of approximately 90 % throughout the testing period.

Following procedures were conducted to investigate the effect of SG in MOC.

Setting time investigation

To evaluate the effect of SG on setting characteristics of MOC, different percentages (5, 10, 15 and 20 %) of SG were mixed with dry-mix composition (MgO:dolomite = 1:1) in varying proportions. This mixture was then gauged with gauging solution to form a workable wet-mix. A standard procedure was adopted according to the Indian Standard (IS 10132-1982) specification to determine standard consistency. A Vicat needle apparatus was used to determine the initial and final setting times.³³ Setting time blocks of simple MOC (M0, only MgO and gauging solution) and control MOC (M1, MgO:dolomite = 1:1 with gauging solution) were also prepared for comparative analysis. Results are reported in Fig. 1.

Water ingress investigation

Moisture ingress tests were conducted on all previously prepared setting-time specimens to assess the effect of SG on the soundness of MOC. After a curing period of 60 days, the specimens (M0, M1 and various MOC–SG formulations) were subjected to boiling-water exposure, a procedure essential for evaluating their comparative moisture-sealing performance. A time-dependent assessment was then performed to determine the relative moisture resistance of each composition at successive intervals across a total duration of 30 h. The measurements were recorded at the time intervals of 0–5, 5–10, 10–15, 15–20, 20–25 and 25–30 h, to systematically monitor the progressive changes in the system. The comparative results of moisture-resistance performance for all specimens are summarized in Table S-III of the Supplementary material.

Compressive strength

To evaluate the influence of SG on the compressive strength of MOC, standard cube specimens of dimensions of 70.6 mm×70.6 mm×70.6 mm with a surface area of 50 cm² were fabricated using various compositions (M0, M1 and MOC–SG formulations containing 5, 10, 15 and 20 % of SG).³³ The dry mixtures were homogenized and subsequently gauged with the prepared gauging solution before being cast into molds. After remaining in the molds for 24 h, the specimens were demolded and subjected to a 28-days curing period under controlled environmental conditions (temperature around 30 °C, and relative humidity above 90 %). Compressive strength testing was performed using a universal testing machine in accordance with standard procedures (Fig. S-3 of the Supplementary material). The results of the compressive strength measurements are presented in Fig. 2.

Spectroscopic analysis

The effect of SG on the MOC block was also investigated through the following spectroscopic techniques using the best modified MOC–SG composite.

Fourier transform-infrared spectroscopy (FT-IR). FT-IR spectra of MOC and SG-incorporated MOC–SG composite cement were recorded using an MRC-28 FT-IR analyzer in the range from 400 to 4000 cm⁻¹. For FT-IR analysis, the MOC cement samples were prepared in dehydrated potassium bromide.

Powder X-ray diffraction (PXRD). PXRD diffraction patterns of MOC and SG-incorporated MOC–SG composite cement were recorded using a Panalytical X Pert Pro Diffractometer (conditions for diffraction pattern: 2θ 5–70°, CuK α radiation, $\lambda = 0.15406$ Å).

Microscopic analysis

Field emission-scanning electron microscopy (FE-SEM) images of MOC and SG-incorporated MOC–SG composite were recorded using a Jeol JSM- 7610F Plus FE-SEM Instrument. An EDS was also performed for elemental analysis of MOC and MOC–SG composite samples.

Thermogravimetric analysis

Thermogravimetric analysis (TGA) of MOC and SG-incorporated MOC–SG composite cement was performed using a Perkin Elmer STA 6000 to evaluate the thermal stability. MOC and MOC–SG composite samples were heated under a nitrogen environment from room temperature to 800 °C at a rate of 10 °C per min to obtain the results.

RESULTS AND DISCUSSION

Setting time analysis

Fig. 1 illustrates the influence of varying SG concentrations (0–20 %) on the initial and final setting times of the resulting composites. The exothermic reaction between MgO and the MgCl₂ solution is responsible for the rapid setting of MOC. Hence the shortest initial and final setting times were observed for M0 without dolomite and additive.

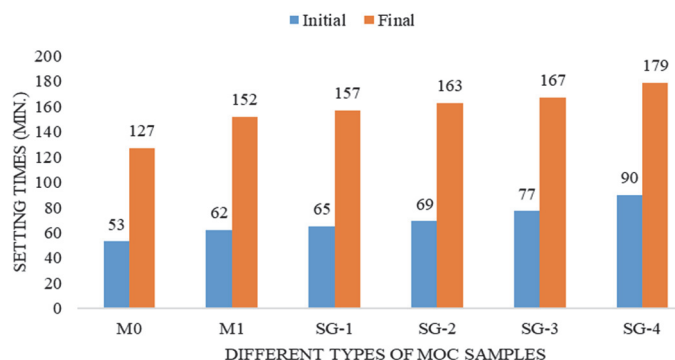


Fig. 1. Setting times of M0, M1, and different types of MOC-SG samples; M0 = MgO + gauging solution; M1 = (MgO:dolomite = 1:1) + gauging solution; SG-1 = (MgO:dolomite = 1:1) + 5% SG + gauging solution; SG-2 = (MgO:dolomite = 1:1) + 10 % SG + gauging solution; SG-3 = (MgO:dolomite = 1:1) + 15% SG + gauging solution; SG-4 = (MgO:dolomite = 1:1) + 20 % SG + gauging solution.

In contrast, the control sample (M1) with 50 % dolomite replacement exhibited relatively longer setting time than M0 due to the reduced exothermic reaction. Dolomite works as an inert filler, being responsible for absorption of thermal

shocks *in situ* in MOC by the three-body collision mechanism, as represented in reactions (1) and (2).

However, the addition of SG, a hydrophilic material, delays the setting process. SG's strong hydrogen bonding capacity leads to increased water retention, hindering the hydration reaction and extending both the initial and final setting times. As the SG concentration rises, the water retention capacity increases, further delaying the setting process and leading to a more controlled setting profile.

Analysis of water resistance

The water resistance characteristics of the MOC specimens are presented in Table S-III. Owing to the highly exothermic reaction between magnesium oxide and the magnesium chloride solution, visible cracks developed in the M0 sample during the curing stage (Fig. S-1). During the subsequent water-resistance test, these cracks facilitated rapid water penetration, leading to their further widening and propagation. The increased permeability significantly accelerated water absorption and consequently diminished the overall water resistance of the M0 composition, rendering it unsuitable for structural applications.

In contrast, neither the M1 sample nor the SG-modified MOC specimens exhibited cracking during curing (Fig. S-2). The improved resistance to crack formation and water absorption can be attributed to the presence of dolomite, which effectively absorbs a portion of the heat generated during the exothermic reaction of the reactive components. By moderating the temperature rise, dolomite limits internal stress development and prevents crack initiation. The absence of cracks significantly restricts water ingress, thereby enhancing the overall durability of the cement matrix. Furthermore, dolomite contributes to improved water resistance by occupying internal voids and micropores within the hardened matrix. This pore-filling effect reduces the permeability of the cement, impeding water migration through the material and promoting the formation of a denser and more structurally sound matrix.

Compressive strength analysis. Fig. 2 illustrates the compressive strength of MOC-SG composite blocks after a 28-day air curing period. The M0 sample, exhibited significant cracking during curing due to the exothermic reaction between MgO and MgCl₂, rendering it unsuitable for construction applications (Fig. S-1). The M1 sample and different types of SG samples were free from cracks due to the presence of dolomite (Fig. S-2). The addition of SG resulted in a notable enhancement in the compressive strength of the composite blocks. Specifically, the SG-1 sample demonstrated the highest compressive strength, surpassing both the control sample (M1) and samples with higher SG concentrations. The strong hydrogen bonding between the hydroxyl groups of SG and the MOC matrix likely contributed to the formation of interlocking crystals, leading to improved mechanical properties. However, excessive SG incorporation was found to be detrimental

to the compressive strength. Therefore, the optimal SG concentration for enhancing the mechanical properties of MOC–SG composites was determined to be 5 % by weight of MgO. Building upon the physical analysis results, M1 and SG-1 composite blocks were subjected to in-depth characterization using FT-IR and PXRD spectroscopic analyses.

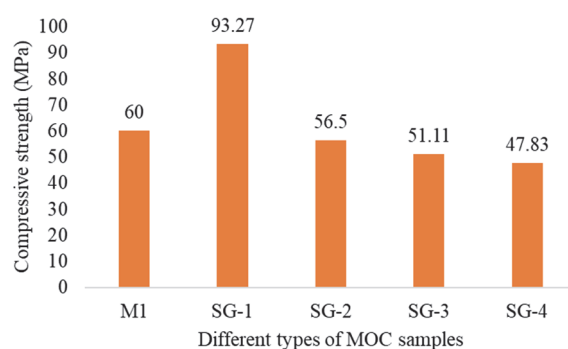


Fig. 2. Effect of silica gel G on compressive strength characteristics of MOC–SG composite blocks.

FT-IR analysis. FT-IR spectra of both M1 and SG-1 composite samples were recorded using KBr pellets in the range of 400–4000 cm^{-1} at ambient conditions. Fig. 3 illustrates the FT-IR spectra of M1 and SG-1. Both M1 and SG-1 exhibited sharp, intense peaks in the 3600–3800 cm^{-1} region, characteristic of the stretching vibrations of water molecules.^{34,35} However, the incorporation of SG resulted in broader, less intense peaks in this range, indicating a decrease in water content. This suggests that SG enhances the water resistance of the MOC–SG composite. The stretching vibrations of crystalline hydroxyl groups were observed at 3695 (M1) and 3698 cm^{-1} (SG-1), which are attributed to the stretching vibrations of the –OH group in brucite ($\text{Mg}(\text{OH})_2$) and suggest a low presence of $\text{Mg}(\text{OH})_2$ in both samples. This is beneficial for the mechanical strength and moisture resistance of the SG-1 composite sample.

The peaks at 1622 (M1), 1611 (SG-1), 3435 (M1) and 3428 cm^{-1} (SG-1) originate from the crystalline phases, specifically phase-5 ($5\text{Mg}(\text{OH})_2 \cdot \text{MgCl}_2 \cdot 8\text{H}_2\text{O}$) and phase-3 ($3\text{Mg}(\text{OH})_2 \cdot \text{MgCl}_2 \cdot 8\text{H}_2\text{O}$), of the MOC matrix. The peak at 1385 cm^{-1} corresponds to the linear C–O bond vibration in carbonates. The peak at 1445 (M1) and 1439 cm^{-1} (SG-1) associated with asymmetric C=O stretching, becomes broader and more intense in SG-1, indicating an interaction between the polar C=O group and the functional groups of SG. The out-of-plane bending of CO_3^{2-} is represented by peaks at 881 (SG-1) and 880 cm^{-1} (M1).³ The absorption bands at 726 and 730 cm^{-1} (M1 and SG-1), 530 (SG-1) and 542 cm^{-1} (M1), are attributed to the stretching and bending vibrations of Si–O, Mg–O and Mg–Cl, respectively.

Peaks at 1611 (SG-1) and 1622 cm^{-1} (M1) can be attributed to O–H bending vibrations. The presence of dolomite is also shown by the carbonate absorption peak at 2530 (SG-1) and 2527 cm^{-1} (M1). The reduction in O–H peak intensity suggests the formation of hydrogen bonds between the hydroxyl groups of MOC phases and silica gel G. This hydrogen bonding, facilitated by SG, creates a protective coating that enhances the bonding strength of the MOC.

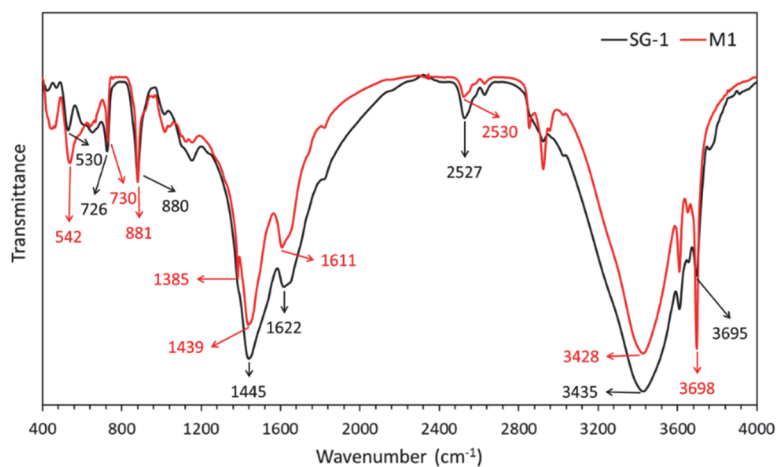


Fig. 3. FT-IR spectra of M1 and silica gel G (SG) incorporated SG-1 composite samples.

Powdered XRD analysis. PXRD spectra of M1 and SG-1 composite samples were recorded at ambient conditions and are presented in Fig. 4. As evidenced by the diffraction patterns, the primary components of the MOC cement are phase-5 ($5\text{Mg}(\text{OH})_2 \cdot \text{MgCl}_2 \cdot 8\text{H}_2\text{O}$), unreacted MgO, brucite and minor carbonated MgO. The characteristic reflections of MgO are indicated by peaks at 2θ 43 and 62.5°, indicating incomplete MgO hydration in both systems. The incorporation of 5 % SG does not significantly introduce any new crystalline phases into the MOC composite matrix. The overall phase assemblage of SG-1 remains similar to that of M1, indicating that SG does not participate in crystallization as a distinct crystalline phase. However, a noticeable change in peak characteristics is observed. Compared to M1, SG 1 pattern exhibits slightly reduced peak intensities accompanied by moderate peak broadening, which suggests refinement of the crystalline size and/or an increase in structural disorder. These changes are consistent with a modification of the hydration and crystallization process rather than a change in phase composition.

A particularly prominent peak at 2θ 31°, associated with the inert filler dolomite, is present in both samples with comparable intensity. This suggests that dolomite remains unreactive during hydration and functions as a heat-absorbing filler, without participating in the formation of hydration products. No distinct dif-

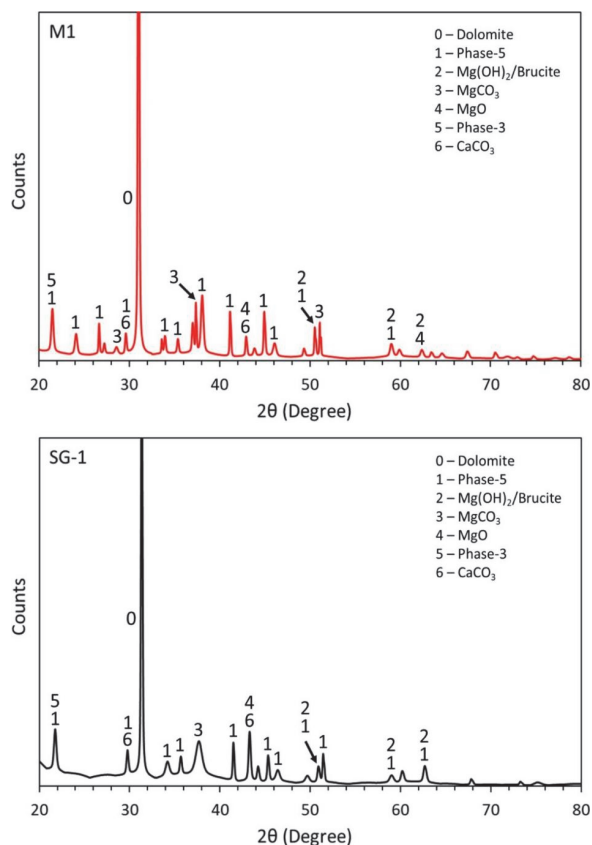


Fig. 4. PXRD spectra of M1 and SG-1 samples.

fraction peaks attributable to SG are detected in SG-1. This observation is expected, as SG is predominantly amorphous in nature and therefore does not contribute sharp crystalline reflections in PXRD. The absence of additional peaks also confirms that SG does not form new crystalline reaction products with the MOC phases within the detection limits of PXRD. Instead, its presence is suggested to influence the nucleation and growth kinetics of phase-5, which is also supported by the observed extension in setting time. The qualitative comparison of peak positions and relative intensities suggests that the incorporation of SG modifies the microstructural packing and crystallization behavior of the MOC matrix. This microstructural refinement is consistent with the experimentally observed improvements in water resistance and thermal stability of SG-1. The mechanical enhancement observed for SG-1 is therefore attributed to microstructural densification and improved phase interlocking, rather than an increase in the absolute amount of phase-5.

SEM-EDX analysis. SEM-EDX analyses of both M1 and SG-1 were recorded and are shown in Figs. 5 and 6. The incorporation of silica gel G helped the rod-like phase-5 crystals (M1) convert into a gel-like amorphous phase-5 structure (SG-1), which could notably reinforce the mechanical properties. It is also clear that the needle-like shape of the crystals was clearer in the SG-1 composite material than in M1. Due to the needle-like crystals, SG-1 exhibited more interlocking

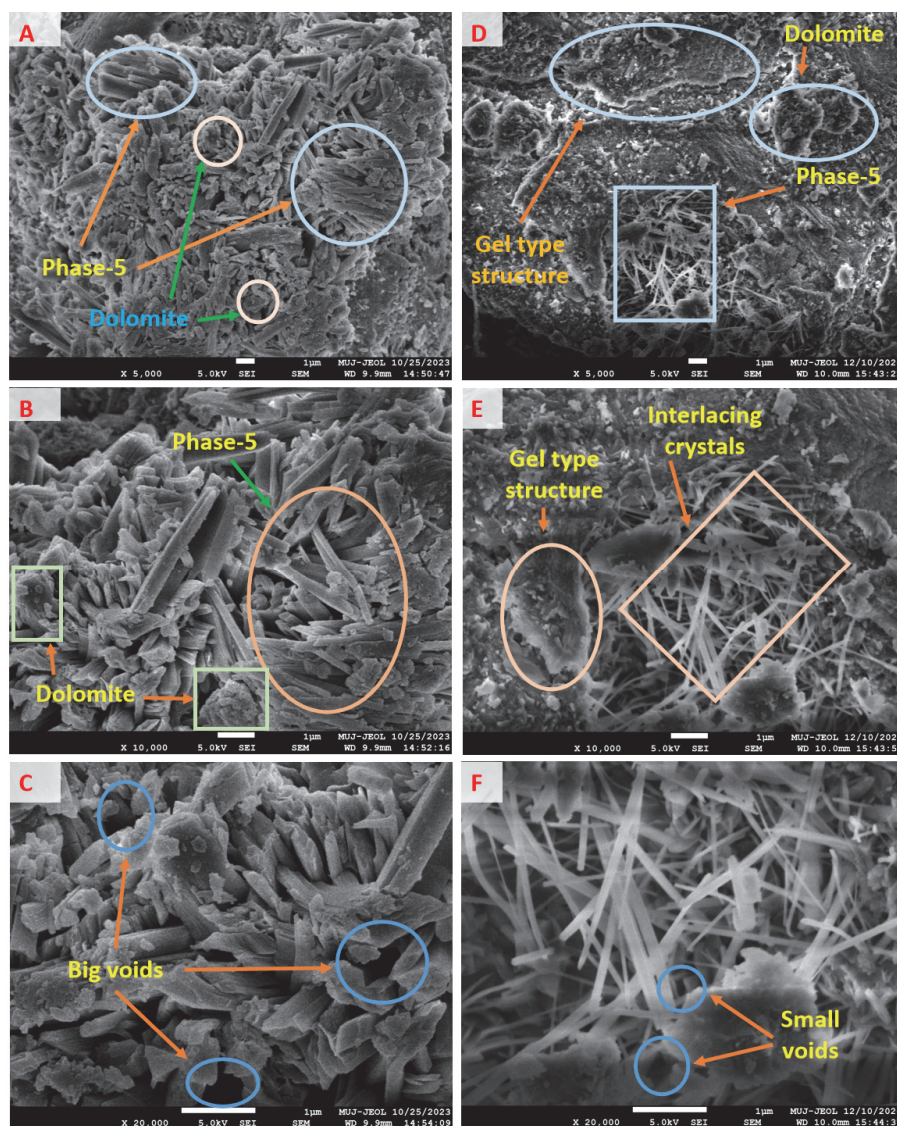


Fig. 5. SEM images of M1 (A–C) and SG-1 (D–F) MOC-SG composite samples at different magnifications.

phase-5, which can be explained by the smaller void gaps in SG-1 composite (Fig. 5F) as compared to the larger void gaps in M1 (Fig. 5C). Therefore, SG-1 showed more interlocking phase-5 than M1, supporting enhanced strength and durability. In addition, the transformation of phase-5 resulted in smaller voids in SG-1, thereby increasing the water-resistance of MOC.

The SEM–EDX analysis (Fig. 6) shows distinct differences in the elemental ratios of Mg, Cl and O between M1 and SG-1. The mole ratio in M1 is 10.45:1.00:28.59, whereas SG-1 exhibits a ratio of 1.13:1.00:2.41. Rather than indicating the presence of amorphous phases, these differences reflect the relative proportions of magnesium-chloride-based hydration products formed in each mix. The higher Mg and Cl content in SG-1 suggests a greater formation of phase-5-type MOC hydration products, consistent with the known role of silica additives in promoting phase-5 development. This interpretation is in agreement with literature reports, which demonstrate that silica gel enhances both the quantity and crystallite size of phase-5.³⁵ In contrast, the higher oxygen proportion in M1 is attributed to the larger amount of carbonate minerals present in the mix, rather than to any amorphous hydration product.

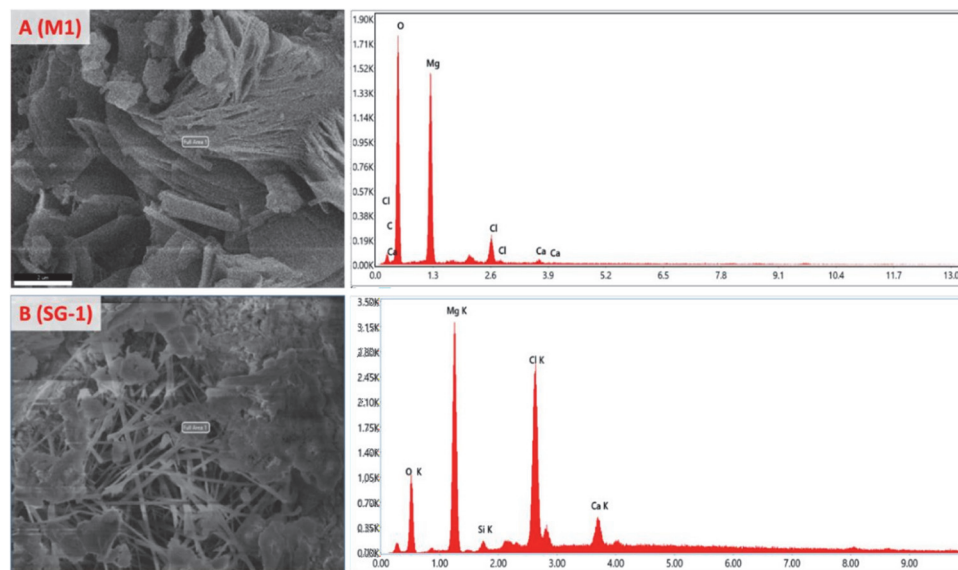


Fig. 6. SEM-EDX of M1 (A) and SG-1 (B) MOC–SG composite samples.

Thermogravimetric analysis. The thermal decomposition of M1 and SG-1 can be seen from the TGA curves in Fig. 7. The decomposition takes place in three stages (Eqs. (5)–(7)). Both M1 and SG-1 samples exhibit mass loss as a result of the slow evaporation of moisture (free water molecules), crystalline water, structural water and hydrochloric acid content throughout the heating process.^{36,37}

The weight loss in the first stage is nearly the same in both M1 and SG-1 samples, but slightly less weight loss was observed in SG-1. In the second stage, the weight loss increases in both M1 and SG-1 compared to the first stage weight loss and M1 exhibits greater weight loss compared to SG-1. In the third stage, the weight loss decreases as compared to both the first and second stages in M1. SG-1 has comparatively more weight loss in third stage as compared to M1. It was indicated that SG-1 is more thermally stable in the lower temperature range (30 to 430 °C), but undergoes rapid decomposition in the temperature range of 430–730 °C and becomes constant after 730 °C. The overall weight loss in M1 and SG-1 is 44.80 and 38.04 %, respectively, and therefore, SG-1 sample is thermally more stable than M1:

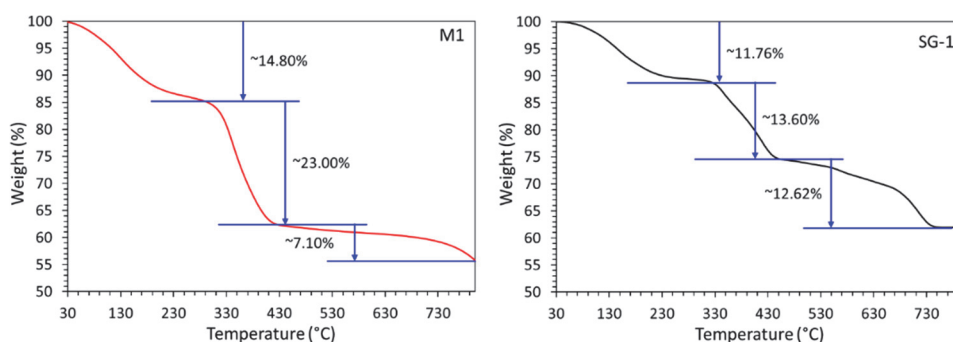
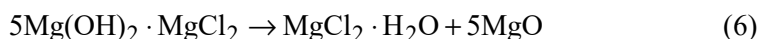


Fig. 7. TGA of M1 and SG-1 sample.

CONCLUSION

This research investigated the potential of silica gel G as an additive in magnesium oxychloride cement to enhance its properties and sustainability. The incorporation of SG in varying proportions (0–20 % by weight of MgO) led to notable improvements in the physical and mechanical properties of the resulting MOC–SG composites. The mixing of SG into MOC significantly enhanced its physical and mechanical properties.

The key findings include:

Accelerated setting time; the addition of SG reduced the initial and final setting time of MOC, likely due to the decreased availability of MgO for the hydration reaction.

Enhanced mechanical strength; SG contributed to an increase in the compressive strength of MOC–SG composites, reaching an optimum value at around 5 % SG content. This improvement is attributed to the formation of additional hydration products and the filling of pores by SG particles.

Improved thermal stability; the incorporation of SG enhanced the thermal stability of MOC–SG composites. This is likely due to the formation of a more stable crystalline structure and the reduced porosity of the composite.

Overall, the results of this study demonstrate the potential of silica gel G as a valuable additive for improving the performance of magnesium oxychloride cement. Further research is recommended to optimize the dosage of silica gel G and explore its impact on other properties, such as durability and fire resistance.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13681>, or from the corresponding author on request.

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ИЗВОД

УТИЦАЈ СИЛИКА-ГЕЛА G НА МЕХАНИЧКА И МИКРОСТРУКТУРНА СВОЈСТВА МАГНЕЗИЈУМ-ОКСИХЛОРИДА

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Магнезијум-оксихлоридни цемент (МОС), врста Сорел цемента, поново је добио на значају као одрживи грађевински материјал због малог угљеничног отиска и мале потрошње енергије. Међутим, примена овог цемента у грађевинарству остаје ограничена, првенствено због неадекватне отпорности на влагу и мале почетне чврстоће на повишеним температурама. У овом раду је испитиван утицај различитих количина силика гела G (0–20 мас. % SG) на својства добијених композита МОС–SG. Анализирана су физичка и механичка својства ових композита, укључујући време везивања, отпорност на влагу и чврстоћу на притисак. Поред тога, спроведене су и FTIR, XRD, SEM-EDX и TGA анализе како би се испитале кристалне фазе композита и структурне карактеристике. SG делује као везивно средство, повећавајући чврстоћу и издржљивост композита. Ово истраживање показује потенцијал композита МОС–SG као обећавајућег одрживог грађевинског материјала. Резултати показују да додавање 5 % SG значајно побољшава својства МОС.

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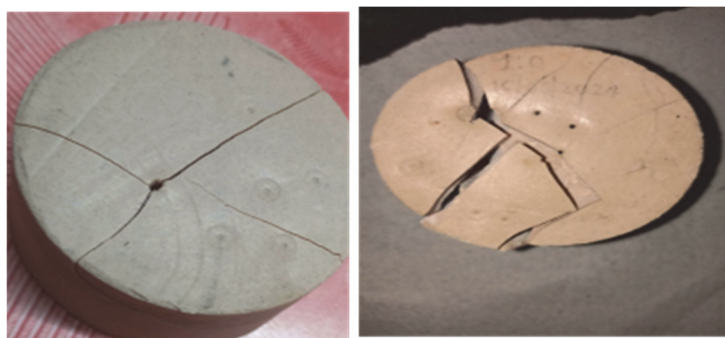
SUPPLEMENTARY MATERIAL TO
Impact of silica gel G on mechanical and microstructural properties of magnesium oxychloride

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(a)



(b)

Fig. S-1. Cracking in setting time blocks (a), and in compressive strength blocks (b).

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Fig. S-2. Compressive and setting time blocks without cracking.



Fig. S-3. Compressive strength testing of MOC block by UTM machine.

Table S-I: Compressive strength sample preparation.

Sample no.	Silica Gel G (SG)		MgO (g)	Dolomite (g)	Gauging solution (mL)
	(%)	(g)			
M0	0%	0	540	0	209.3
M1	0%	0	270	270	169.5
SG1	5%	13.50	270	270	207.50
SG2	10%	27.00	270	270	220.00
SG3	15%	40.50	270	270	239.90
SG4	20%	54.00	270	270	248.10

Table S-II: Setting time sample preparation.

Sample no.	Silica Gel G (SG)		MgO (g)	Dolomite (g)	Gauging solution (mL)
	(%)	(g)			
M0	0%	0	200	0	78.9
M1	0%	0	100	100	72.2
SG1	5%	5.00	100	100	76.00
SG2	10%	10.00	100	100	79.60
SG3	15%	15.00	100	100	84.20
SG4	20%	20.00	100	100	100.60

Table S-III: Water resistance of M0, M1, and different types of MOC-SG samples.

Composition	Different types of MOC blocks immersed in boiling water for					
	0-5 hours	5-10 hours	10-15 hours	15-20 hours	20-25 hours	25-30 hours
M0	During curing M0 was cracked					
M1	N	N	N	N	N	N
SG -1	N	N	N	N	N	N
SG- 2	N	N	N	N	N	N
SG- 3	N	N	N	N	N	N
SG- 4	N	N	N	N	N	N

N = Not affected



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Process optimization and economic calculation of photocatalytic degradation of methylene blue over sulfur-doped g-C₃N₄

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Abstract: Sulfur doping has been considered an effective strategy to enhance the photocatalytic activity of graphitic carbon nitride (g-C₃N₄). However, there are no reports on the process optimization and economic calculation to determine the optimal photocatalytic parameters and compare the operating costs, respectively. In this work, sulfur-doped g-C₃N₄ (S/g-C₃N₄) was prepared *in situ* by a one-pot pyrolysis approach using thiourea as the precursor and sulfur source. S/g-C₃N₄ was characterized by various instrumental techniques. Response surface methodology (RSM) was employed to determine the optimal photocatalytic conditions for the degradation of Methylene blue (MB) over S/g-C₃N₄. A central composite design (CCD) with four factors at five levels was established, comprising 30 experimental runs. The optimal conditions were determined as an initial MB concentration of 27.6 mg L⁻¹, a catalyst dosage of 4.1 g L⁻¹, a solution pH of 8.7 and a reaction time of 127 min. Under these conditions, the predicted degradation efficiency could reach 99.94 %, while the experimental value was 99.5 %. Cost accounting indicated that the optimization substantially reduced direct costs, greatly improved equipment utilization, and enhanced operational flexibility. This work will lay key foundation for cost control and efficiency improvement in the scale-up application of g-C₃N₄-based photocatalysts.

Keywords: graphitic carbon nitride; photocatalyst; response surface methodology; cost accounting.

INTRODUCTION

Resource shortages and environmental pollution are closely related to our quality of life and health conditions, and have drawn considerable attention from governments around the world. Among the two, the issues caused by environmental pollution are more significant.¹ Among various kinds of environmental pollution, organic dye wastewater has attracted the most attention because of its high

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concentration, diverse composition, difficult decolorization, widespread pollution and complex structure. At present, there are many technologies to treat organic dye wastewater, including adsorption, membrane separation, biodegradation, extraction, oxidation and so on.² In recent years, the development of advanced oxidation technologies represented by photocatalytic oxidation has attracted considerable attention. The use of photocatalytic materials to purify water has become a high-tech environmental purification technology of global concern.³

In recent decades, a variety of novel visible-light-response photocatalytic materials have been developed, such as bismuth oxyhalides, metal-organic frameworks, ZnIn_2S_4 , CuFeS_2 , WO_3 , CuWO_4 , Cu_2SnS_3 and others. These materials have high visible-light catalytic stability and activity.^{4–7} However, the precious metals in the components increase the preparation and production costs of photocatalytic materials. At the same time, they have certain toxicity and the potential to pollute the water environment. Therefore, these materials are not suitable for large-scale practical applications. Graphitic carbon nitride ($\text{g-C}_3\text{N}_4$) is a new type of non-metallic semiconductor with a band gap of about 2.7 eV, which can achieve visible light response and make full use of solar energy. Moreover, $\text{g-C}_3\text{N}_4$ has a stable structure, controllable performance, a non-toxic and harmless nature, acid and alkali resistance, and light corrosion resistance. It has become a hotspot in the research field of photocatalytic materials.^{8,9} However, the shortcomings of $\text{g-C}_3\text{N}_4$, such as fast electron-hole recombination and a small specific surface area, limit its practical application. Therefore, heterojunction construction, doping modification, microstructure regulation and other methods have been employed to improve the photocatalytic activity of $\text{g-C}_3\text{N}_4$.^{10,11} Among them, doping with non-metallic elements could effectively change the electronic band structure of $\text{g-C}_3\text{N}_4$, thereby improving its photocatalytic activity.¹² Xu and coworkers successfully prepared $\text{g-C}_3\text{N}_4$ with nitrogen defects and sulfur doping *via* a cold plasma technique. S-doping could offer more active sites for the photocatalytic process and enhance the stability of N defects. The visible light photocatalytic activity of S-doped $\text{g-C}_3\text{N}_4$ was 11.25 times higher than that of pure $\text{g-C}_3\text{N}_4$.¹³ Ma *et al.* used the dielectric barrier discharge (DBD) plasma to obtain an S-doped $\text{g-C}_3\text{N}_4$ photocatalyst under an H_2S atmosphere. They found that DBD plasma treatment could make more sulfur be doped into the $\text{g-C}_3\text{N}_4$ lattice than traditional roasting approach. The as-prepared S-doped $\text{g-C}_3\text{N}_4$ exhibited much higher photocatalytic degradation efficiency of rhodamine B (RhB).¹⁴ In addition, a porous S-doped $\text{g-C}_3\text{N}_4$ was successfully prepared *via* one-step calcination method by Yang and coworkers. The as-prepared porous S-doped $\text{g-C}_3\text{N}_4$ displayed approximately 6.2 times higher peroxymonosulfate activation capacity for the degradation of RhB than pristine $\text{g-C}_3\text{N}_4$ under visible light irradiation.¹⁵ Song *et al.* employed urea and thiourea as precursors to prepare S-doped $\text{g-C}_3\text{N}_4$ nanosheets through physical steam activation. The as-prepared photocatalyst exhibited 2.1 and 5.52 fold higher

decomposition efficiency and reaction rate for the photodegradation of Methylene blue (MB) dye than bulk g-C₃N₄, respectively.¹⁶ Overall, S doping has been proven to be an efficient strategy to improve light absorption and photogenerated electron–hole separation, consequently boosting the photocatalytic activity of g-C₃N₄.¹⁷ However, there are few reports on the process optimization of photocatalytic degradation of organic pollutants over S-doped g-C₃N₄. Response surface methodology (RSM) based on a central composite design (CCD) is a widely used process parameter optimization method.^{18–20} This methodology includes experimental condition design and parameter optimization. The specific steps of this methodology can be divided into the following four aspects:^{21,22} 1) determination of the variables and experimental design, 2) evaluation of the parameters for mathematical model regression analysis, 3) prediction of the model response and 4) confirmation of the accuracy of the model. In addition, as far as we know, the economic calculation of photocatalytic degradation of organic pollutants over S-doped g-C₃N₄ before and after process optimization has not been investigated.

Therefore, in this work, S-doped g-C₃N₄ was prepared by a facile one-pot pyrolysis approach using thiourea as the precursor and sulfur source to realize *in situ* S doping and achieve high photocatalytic activity. Based on the characterization analyses and photocatalytic performance evaluation of S-doped g-C₃N₄, the RSM method was employed for the first time to optimize the process of MB photodegradation over S-doped g-C₃N₄ to determine the optimal operating parameters. Moreover, the economic calculations before and after optimization were performed for the first time. Therefore, this work will lay an engineering and cost foundation for the large-scale practical applications of g-C₃N₄-based photocatalysts.

EXPERIMENTAL

Preparation of S-doped g-C₃N₄

A facile one-pot pyrolysis approach was employed to prepare *in situ* S-doped g-C₃N₄ using thiourea as the precursor and sulfur source.²³ The specific procedures are as follows: 15 g of thiourea was weighed and put into a 100 mL crucible, and then heated from room temperature to 550 °C at a heating rate of 20 °C/min in a muffle furnace. After being kept for 4 h, the sample was cooled to room temperature in the furnace. Finally, it was ground into powder for subsequent use.

Characterization techniques

X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM) and UV–Vis diffuse reflectance spectroscopy (UV–Vis DRS) were used to characterize the as-prepared samples. The detailed information is provided in the supplementary file.

Photocatalytic experiments

The photocatalytic performance of S-doped g-C₃N₄ was evaluated using MB dye as the target pollutant. The experimental procedure is provided in the Supplementary material to this paper.

RSM optimization method

In this work, four parameters were selected as variables, namely the initial MB concentration (mg L^{-1}), catalyst dosage (g L^{-1}), solution pH and reaction time (min), which were labeled as X_1 , X_2 , X_3 and X_4 , respectively. At the same time, the degradation rate of MB dye was used as the output response (Y). The detailed descriptions of experimental design, mathematical modeling and response optimization are provided in the Supplementary material. In addition, the range and the level of independent variables are shown in Table S-I of the Supplementary material.

Economic calculation method

Generally, the standardized economic calculation for a wastewater treatment process included three main parts: raw material cost, operation cost and integrated cost.^{24–26} Their calculation methods are provided in the Supplementary material.

RESULTS AND DISCUSSION

Characterization of S-doped $\text{g-C}_3\text{N}_4$

Fig. 1A presents the XRD pattern of the S-doped $\text{g-C}_3\text{N}_4$ sample. The XRD pattern shows two prominent diffraction peaks located at 2θ 13 and 27.5° . The peak at 13° belongs to the in-plane ordering of triazine units and is indexed to the (100) crystal plane of $\text{g-C}_3\text{N}_4$, whereas the peak at 27.5° corresponds to the inter-layer stacking of aromatic layers and is assigned to the (002) crystal plane of $\text{g-C}_3\text{N}_4$.²⁷ The FT-IR spectrum of S-doped $\text{g-C}_3\text{N}_4$ is displayed in Fig. 1B. The absorption bands in the $1225\text{--}1640\text{ cm}^{-1}$ region are assigned to stretching vibrations of C–N and C=N bonds within the N-containing ring framework, together with exocyclic C–N stretching.²⁸ The broad band at 3135 cm^{-1} is associated with N–H stretching vibrations,²⁹ while the band at 796 cm^{-1} is attributed to the bending vibration of the C–N ring.³⁰

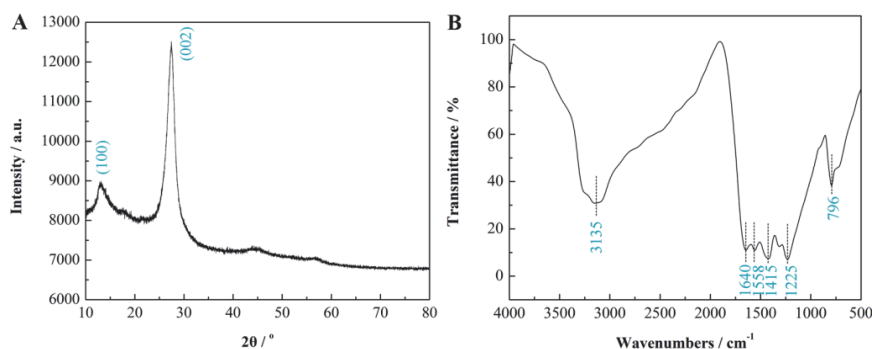


Fig. 1. A) XRD pattern and B) FTIR spectrum of S-doped $\text{g-C}_3\text{N}_4$.

Fig. 2A gives an FESEM image of S-doped $\text{g-C}_3\text{N}_4$, displaying a non-uniform particulate morphology in which the particles are irregularly agglomerated, consistent with the typical morphology of $\text{g-C}_3\text{N}_4$.³¹ Fig. 2B shows a TEM image of the

S-doped g-C₃N₄ sample. It can be seen that the as-prepared sample has a typical thin-layer structure with some wrinkles, similar to graphene. This thin-layer structure will further increase the specific surface area of the S-doped g-C₃N₄ sample, thereby improving the adsorption capacity and photocatalytic activity of the sample.³² From the energy-dispersive spectrum (EDS) of S-doped g-C₃N₄ (Fig. 2C), it can be clearly seen that the as-prepared sample contains three main elements C, N and S, which indicates that S has been successfully introduced into g-C₃N₄. In addition, no characteristic diffraction peak or absorption peak belonging to S was found in the XRD pattern and the FTIR spectrum, which implies that S might enter the crystal structure of g-C₃N₄ and replace C or N, rather than as impurities.^{13–16}

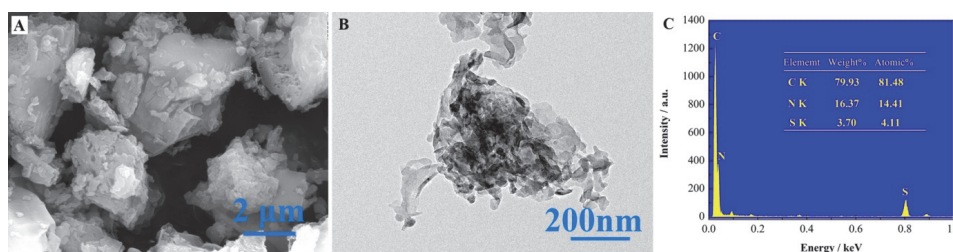


Fig. 2. A) FESEM, B) TEM and C) EDS images of S-doped g-C₃N₄.

Fig. 3A shows the UV–Vis DRS spectrum of S-doped g-C₃N₄, from which it can be observed that the S-doped g-C₃N₄ sample has strong visible-light absorption and an absorption edge is located at approximately 412 nm. According to the extrapolation of $(F(R)-h\nu)^{1/2}$ curve vs. photon energy $h\nu$ (Fig. 3B), the band gap (E_g) of S-doped g-C₃N₄ is estimated to be 2.63 eV, in agreement with literature reports.³³ Notably, the as-prepared sample exhibits an additional absorption feature near 645 nm in the UV–Vis DRS spectrum, corresponding to an E_g of 1.81 eV. This might be associated with an S-induced impurity energy level in the band structure,³⁴ which can facilitate the enhancement of photocatalytic performance.

Process optimization of MB photodegradation over S-doped g-C₃N₄

To evaluate the photocatalytic performance of S-doped g-C₃N₄, MB with analytical purity was used as the target pollutant under the conditions of 10 mg L⁻¹ MB, 5.0 g L⁻¹ S-doped g-C₃N₄, pH 7 and room temperature. As shown in Fig. 4A, after 60 min in the dark to reach adsorption equilibrium, the removal rate of MB is only 9.4 %, suggesting that the contribution of adsorption to MB removal can be neglected. However, after 120 min of photocatalytic irradiation, the corresponding degradation rate increased to 95.8 %. Accordingly, the as-prepared S-doped g-C₃N₄ sample exhibits stronger photocatalytic performance toward MB degradation. Additionally, this photocatalytic process follows the pseudo-first-order kinetic model (Fig. 4B), with a reaction rate constant of 0.02296 min⁻¹. The UV–Vis absorption spectra of the MB solution during different photocatalytic periods are

presented in Fig. 4C, where it can be seen that the main absorption peak of the MB dye molecule at 650 nm disappeared after 120 min of photocatalytic reaction, indicating that the dye molecule has been decomposed. In addition, the removal percentage of total organic carbon (TOC) after 120 min of photocatalytic irradiation was measured as 78.4 %. Therefore, it can be supposed that the MB dye was decomposed into CO_2 , H_2O , and other low-molecular-weight substances.

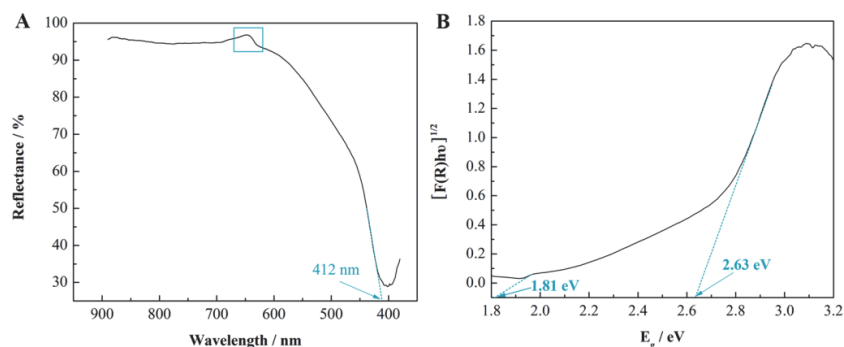


Fig. 3. A) UV-Vis DRS spectrum and B) $(F(R)-h\nu)^{1/2}$ versus $h\nu$ curve of S-doped $\text{g-C}_3\text{N}_4$.

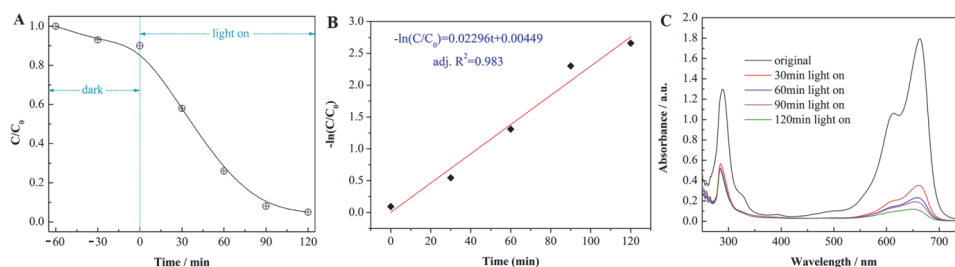


Fig. 4. A) Photocatalytic performance toward MB degradation over S-doped $\text{g-C}_3\text{N}_4$; B) fitting line of the pseudo-first-order kinetic model; C) UV-Vis absorption spectra of the MB solution during different photocatalytic periods.

Based on the above results, the effects of different experimental parameters on the degradation rate of MB dye were evaluated by RSM. According to the CCD, an experimental scheme with 4 factors and 5 levels was designed, and a total of 30 sets of experiments were carried out. The CCD experimental matrix is listed in Table S-II of the Supplementary material, along with the experimental and predicted values of the response Y . Among them, six groups of experiments are replicate experiments at the center point, which ensures that the relative error of the experiment is small. Their close response values mean higher experimental accuracy.³⁵

To predict the response value, the experimental values were fitted by a second-order polynomial regression model, and the results are shown as:

$$\begin{aligned}
 Y = & 66.46 + 5.82X_1 - 13.35X_2 + 7.62X_3 + 12.70X_4 - 2.74X_1X_2 + \\
 & + 1.69X_1X_3 + 1.70X_1X_4 + 0.79X_2X_3 + 0.21X_2X_4 - 1.17X_3X_4 - \\
 & - 2.48X_1^2 - 3.90X_2^2 - 10.22X_3^2 - 2.61X_4^2
 \end{aligned} \quad (1)$$

From the analysis of variance (ANOVA, Table S-III of the Supplementary material), it can be seen that the F value is 8.09, which is much higher than the standard value $F_{0.05} = 2.42$, which means that the second-order polynomial model is highly significant. In addition, as listed in Table S-II, the experimental and predicted values of the response Y are relatively close, with a correlation coefficient of $R^2 = 0.9588$. This is the main criterion for evaluating the correlation between the experimental and predicted values.³⁶

Moreover, the predicted response values are in good agreement with the experimental values (Fig. S-2 of the Supplementary material). Furthermore, the $Prob > F$ value is less than 0.0500, which means that the influence of the variable is significant, whereas the value greater than 0.1000, indicates that the influence of the variable is not significant.³⁷ Therefore, in this work, it can be seen from Table S-III that X_1 , X_2 , X_3 , X_4 , X_1X_3 , X_1X_4 , X_1^2 , X_3^2 and X_4^2 are the significant influencing variables during the MB photodegradation over S-doped g-C₃N₄. In addition, the statistical analysis of the experimental data indicates that the initial MB dye concentration, S-doped g-C₃N₄ dosage, solution pH and reaction time have significant effects on MB photodegradation.

The three-dimensional (3D) response surface plots of the interaction effect between two factors are shown in Fig. 5, including the interactions between initial MB concentration and S-doped g-C₃N₄ dosage, initial MB concentration and solution pH, initial MB concentration and reaction time, S-doped g-C₃N₄ dosage and solution pH, S-doped g-C₃N₄ dosage and reaction time and solution pH and reaction time. It can be seen that the degradation rate of MB increases with the decrease in the initial MB concentration and the increase in the S-doped g-C₃N₄ dosage (Fig. 5A); increases with the decrease in the initial MB concentration and the increase in the solution pH (Fig. 5B); increases with the decrease in the initial MB concentration and the increase of the reaction time (Fig. 5C); increases with the increase of the S-doped g-C₃N₄ dosage and the solution pH (Fig. 5D); increases with the increase in the S-doped g-C₃N₄ dosage and the reaction time (Fig. 5E); increases with the increase in the reaction time and the solution pH (Fig. 5F). According to the prediction model, the process parameters of MB photodegradation over the S-doped g-C₃N₄ photocatalyst are optimized as follows: the initial MB concentration is 27.6 mg L⁻¹, the S-doped g-C₃N₄ dosage is 4.1 g L⁻¹, the solution pH is 8.7, and the reaction time is 127 min. Under these optimal conditions, the predicted degradation rate of MB can reach 99.94 %. Under the identical conditions, the experimental value of the MB degradation rate is 99.5 %, showing excellent agreement with the model prediction.

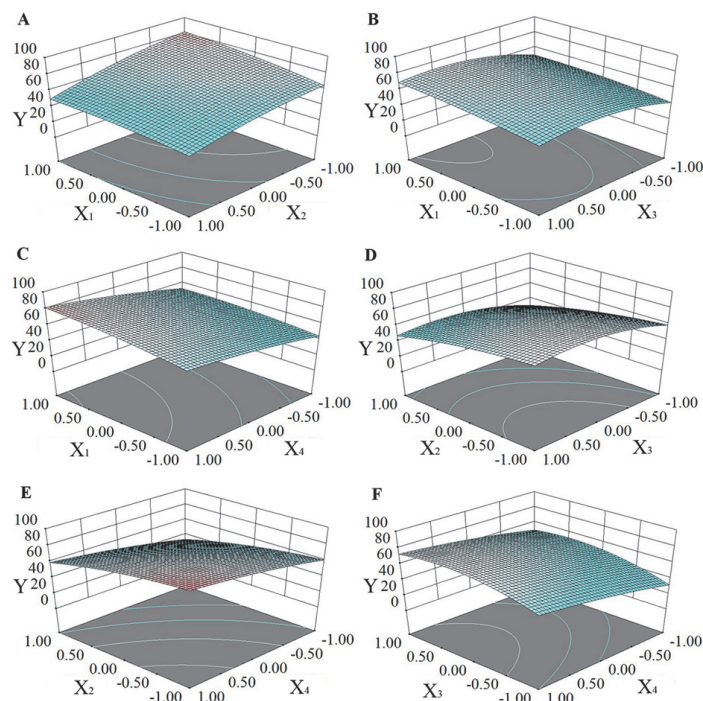


Fig. 5. 3D response surface of MB photodegradation (Y) over S-doped $g\text{-C}_3\text{N}_4$ with the interaction effect between: A) initial MB concentration (X_1) and S-doped $g\text{-C}_3\text{N}_4$ dosage (X_2), B) initial MB concentration (X_1) and solution pH (X_3), C) initial MB concentration (X_1) and reaction time (X_4), D) S-doped $g\text{-C}_3\text{N}_4$ dosage (X_2) and solution pH (X_3), E) S-doped $g\text{-C}_3\text{N}_4$ dosage (X_2) and reaction time (X_4) and F) solution pH (X_3) and reaction time (X_4).

Economic calculation of MB photodegradation over S-doped $g\text{-C}_3\text{N}_4$

1) *Comparative analysis of raw material cost.* Table S-IV of the Supplementary material provides the comparative analysis of raw material cost before and after optimization. Before optimization, the catalyst cost for treating 1 m^3 of MB wastewater was $5\text{ kg} \times 80\text{ CNY kg}^{-1} = 400\text{ CNY}$. After optimization, the corresponding cost for treating 1 m^3 of MB wastewater was $4.1\text{ kg} \times 80\text{ CNY kg}^{-1} = 328\text{ CNY}$. Therefore, the catalyst cost for treating 1 m^3 wastewater was reduced by 72 CNY, with a decrease of 17.8 %. Moreover, after optimization, the pH value of MB solution was adjusted from 7.0 to 8.7, so sodium hydroxide needed to be added to adjust the pH value. Based on the calculation of the acid-base balance, 0.12 kg of sodium hydroxide was required to adjust the pH from 7.0 to 8.7 for 1 m^3 wastewater, and the corresponding cost was $0.12\text{ kg} \times 2.5\text{ CNY kg}^{-1} = 0.3\text{ CNY}$. After optimization, although the acid-base adjustment cost was slightly increased, combined with the significant reduction in catalyst cost, the integrated raw material

cost still achieved a net saving of 71.7 CNY for treating 1 m³ wastewater, with a reduction of 17.7 %.

2) *Comparative analysis of operation cost.* Table S-V of the Supplementary material gives the comparative analysis of operation cost before and after optimization. The energy consumption of photocatalytic reaction mainly came from the xenon lamp (300 W) and the stirrer (50 W). The energy consumption was positively correlated with the reaction time. Before optimization, the energy consumption for treating 1 m³ wastewater was (300 W + 50 W) × 3 h = 1.05 kWh and the corresponding energy cost was 1.05 kWh × 0.6 CNY kWh⁻¹ = 0.63 CNY. After optimization, the reaction time was 127 min (2.12 h). Therefore, the energy consumption for treating 1 m³ wastewater was (300 W + 50 W) × 2.12 h = 0.742 kWh, and the corresponding energy cost was 0.742 kWh × 0.6 CNY kWh⁻¹ = 0.445 CNY. Therefore, after optimization, the energy consumption cost for treating 1 m³ wastewater was reduced by 0.185 CNY, with a decrease of 29.4 %.

With regard to the labor cost, before optimization, assuming that the operation time of a single batch treatment of 1 m³ wastewater was about 3.5 h, the labor cost was 3.5 h × 30 CNY h⁻¹ = 105 CNY. After optimization, the operation time was shortened to 2.5 h, and the labor cost was 2.5 h × 30 CNY h⁻¹ = 75 CNY. Therefore, the labor cost for treating 1 m³ wastewater was directly reduced by 30 CNY, with a decrease of 28.6 %.

3) *Comparative analysis of integrated cost.* Based on the raw material and operation costs, the integrated cost for treating 1 m³ MB wastewater was calculated. Before optimization, the integrated cost for treating 1 m³ MB wastewater was (400 + 0 + 0.63 + 105) CNY = 505.63 CNY. After optimization, the integrated cost for treating 1 m³ MB wastewater was (328 + 0.3 + 0.445 + 75) CNY = 403.745 CNY. Therefore, after optimization, the integrated cost for treating 1 m³ MB wastewater was reduced by 101.885 CNY, with a decrease of 20.0 %.

CONCLUSION

In this work, S-doped g-C₃N₄ was successfully prepared by a facile one-step pyrolysis approach, using thiourea as the precursor and sulfur source. The as-prepared sample was characterized by XRD, FTIR, FESEM, TEM, EDS and UV-Vis DRS. The sample exhibited a wide visible-light absorption region, and its E_g was estimated to be 2.63 eV. Furthermore, an additional absorption feature near 645 nm was observed in the UV-Vis DRS spectrum, corresponding to an E_g of 1.81 eV. This might be associated with an S-induced impurity energy level in the band structure. The as-prepared sample exhibited stronger photocatalytic performance toward MB degradation. After 120 min of photocatalytic irradiation, the MB degradation rate reached 95.8 %. Based on RSM under CCD, the process optimization of MB photodegradation over the S-doped g-C₃N₄ photocatalyst was

conducted. Based on the economic cost calculations, after optimization, the integrated cost for the photodegradation of 1 m³ MB wastewater over S-doped g-C₃N₄ was reduced from 505.63 to 403.745 CNY, with a decrease of 20.0 %.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13775>, or from the corresponding author on request.

ИЗВОД

ОПТИМИЗАЦИЈА ПРОЦЕСА И ЕКОНОМСКИ ПРОРАЧУН ФОТОКАТАЛИТИЧКЕ ДЕГРАДАЦИЈЕ МЕТИЛЕНСКОГ ПЛАВОГ КОРИШЋЕЊЕМ g-C₃N₄ ДОПИРАНОГ СУМПОРОМ

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Допирање сумпором сматра се ефикасном стратегијом за побољшање фотокаталитичке активности графитног нитрида угљеника (g-C₃N₄). Међутим, у литератури нису присутни извештаји о оптимизацији овог процеса, као ни о економском прорачуну при одређивању оптималних фотокаталитичких параметара. У овом раду, g-C₃N₄ допиран сумпором (S/g-C₃N₄) припремљен је *in situ* методом пиролизе у једном кораку, уз коришћење тиоуреа као прекурсора и извора сумпора. У раду је извршена карактеризација S/g-C₃N₄ различитим инструменталним техникама. За одређивање оптималних фотокаталитичких услова за деградацију метиленског плавог (МВ) коришћењем S/g-C₃N₄ коришћена је метода одзивне површине (RSM). Успостављен је дизајн експеримента са четири фактора на пет нивоа, који је обухватио 30 експерименталних понављања. Добијени су следећи оптимални услови: почетна концентрација МВ од 27,6 mg L⁻¹, доза катализатора 4,1 g L⁻¹, рН раствора 8,7 и време реакције 125 min. Под овим условима, предвиђена ефикасност деградације достиже 99,94 %, док је експериментална вредност износила 99,5 %. Анализа трошкова показала је да оптимизација значајно смањује директне трошкове, у великој мери побољшава искоришћеност опреме и повећава оперативну флексибилност. Овај рад поставља кључну основу за контролу трошкова и повећање ефикасности при примени фотокатализатора на бази g-C₃N₄ у индустријским размерама.

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SUPPLEMENTARY MATERIAL TO
Process optimization and economic calculation of photocatalytic degradation of methylene blue over sulfur-doped g-C₃N₄

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EXPERIMENTAL

Characterization techniques

The crystal phase composition of the product was determined by X-ray diffraction (XRD), conducted on a Rigaku D/max-3B X-ray diffractometer (Japan). The functional groups of the sample were analyzed by Fourier transform infrared spectroscopy (FTIR), operated on a Nicolet Nexus infrared spectrometer (United States). The microscopic morphology of the sample was observed by field emission scanning electron microscopy (FESEM), realized on a FEISirion 200 scanning electron microscope (Netherlands). The microstructure of the sample was observed by transmission electron microscopy (TEM), obtained on a JEOL JEM-2010 transmission electron microscope (Netherlands). The optical absorption edge and band gap of S-doped g-C₃N₄ were analyzed by UV-vis diffuse reflectance spectroscopy (UV-vis DRS), recorded on an Ocean Optics USB4000 UV-visible diffuse reflectance spectrometer with integrating sphere, using a South African optical standard template was used as the reference. The content of total organic carbon (TOC) was measured by a XPERT-TOC/TNb TOC analyzer (Netherlands).

Photocatalytic experiments

The photocatalytic performance of S-doped g-C₃N₄ was evaluated under a 300W xenon lamp (100 mW/m²), using MB dye as the target pollutant. The experimental procedures are as follows: 10 mg L⁻¹ MB dye solution was prepared, and the absorbance of the standard solution was measured by a 722-type spectrophotometer, marked as A₀. 100 mL MB working solution was put into a 250 mL glass breaker. Then, a certain amount of S-doped g-C₃N₄ sample was added to this solution. After being stirred for 60 min, the adsorption equilibrium of the dye was obtained, and then the lamp was turned on. The schematic diagram of photocatalytic reactor is displayed in Fig. S1. During the photocatalytic reaction, about 5 mL of suspension was extracted at a specific reaction time. The supernatant was centrifuged and separated. The absorbance of the supernatant was measured and marked as A_i. The MB degradation rate was calculated by the following equation: $Y(\%) = 100(A_0 - A_i)/A_0$. Meanwhile, under identical experimental conditions, random experiments were carried out to verify the repeatability of the experimental results.

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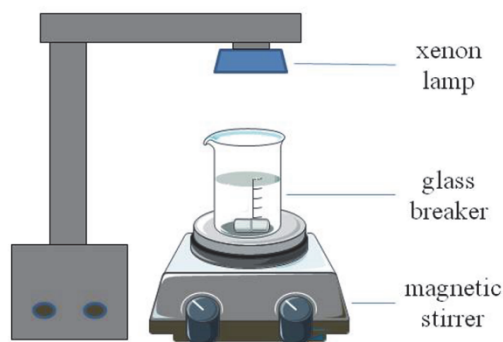


Fig. S-1. Schematic diagram of the photocatalytic reactor.

RSM optimization method

In this work, four parameters were selected as variables, namely initial MB concentration (mg L^{-1}), catalyst dosage (g L^{-1}), solution pH, and reaction time (min), which were labeled as X_1 , X_2 , X_3 , and X_4 , respectively. At the same time, the degradation rate of MB dye was used as the output response (Y). The experimental design, mathematical modeling and response optimization were completed by Design Expert 8.0.7.1 software (Stat-Ease, Inc.). For statistical calculation, the variable X_i was encoded as x_i according to the equation (S1):

$$x_i = \frac{X_i - X_0}{\delta X} \quad (\text{S-1})$$

Where, x_i is the coded value, X_i is the uncoded value, X_0 is the center point value of X_i , and δX represents the step size. In addition, a second-order regression model was used to determine and analyze the response of independent variables. The formula is shown in Equation (S2):

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_i \sum_j \beta_{ij} X_i X_j \quad (\text{S-2})$$

Where, Y is the response value (MB degradation rate), X_i and X_j are the variables that affect the response value, β_i is the first-order regression coefficient, β_{ii} is the second-order regression coefficient, and β_{ij} is the interaction coefficient of two independent variables. Finally, the significance and correlation coefficient of the mathematical model were determined by analysis of variance (ANOVA). The quality and predictive reliability of the polynomial were determined by the correlation coefficient (R^2). The range and level of independent variables for MB photodegradation over S-doped $\text{g-C}_3\text{N}_4$ are shown in Table S-I.

TABLE S-I. Variables and their codes with corresponding real experimental values

Variables	Coded levels				
	-2	-1	0	1	2
X_1 (mg L^{-1})	3	9	15	21	27
X_2 (g L^{-1})	1	2	3	4	5
X_3	3	5	7	9	11
X_4 (min)	30	60	90	120	150

*Economic calculation method**(1) Cost calculation of raw materials*

The cost of raw materials mainly included the preparation cost of S-doped g-C₃N₄ and the consumption cost of acid-alkaline regulating reagents, which were the core component of the fixed cost of the photocatalytic process over S-doped g-C₃N₄.

The unit preparation cost of S-doped g-C₃N₄ included raw material procurement, energy consumption, and equipment depreciation, and was determined to be 80 CNY kg⁻¹. The preparation cost for treating 1 m³ of MB dye wastewater was calculated as follows: Catalyst cost = catalyst mass (kg) × 80 CNY kg⁻¹.

The pH adjustment required sodium hydroxide (or hydrochloric acid), with an industrial cost of approximately 2.5 CNY kg⁻¹. The acid/alkali reagent cost for treating 1 m³ of MB dye wastewater was calculated as follows: acid/alkali reagent cost = acid/alkali reagent dosage (kg) × 2.5 CNY kg⁻¹.

(2) Cost calculation of operation

The operating costs mainly included energy consumption (irradiation and stirring) and labor costs. As the main variable-cost component, operating cost depended directly on the reaction time and treatment scale.

Energy consumption in this photocatalytic process primarily arose from the light source and the stirrer. A 300 W xenon lamp was used for irradiation, with an electricity tariff of 0.6 CNY kWh⁻¹, and stirring was provided by a 50 W mixer. As the energy consumption increased with reaction time, the energy-consumption cost for treating 1 m³ of methylene blue wastewater was calculated as: energy cost = [light source power (kW) + stirrer power (kW)] × reaction time (h) × 0.6 CNY kWh⁻¹.

The labor cost mainly included wastewater monitoring, parameter adjustment, equipment maintenance, and other labor costs. The per capita wage of industrial production was set as 6,000 CNY month⁻¹, equivalent to 30 CNY hour⁻¹. Assuming that the operation time was positively correlated with the reaction time, the labor cost for treating 1 m³ MB dye wastewater was calculated as follows: labor cost = reaction time (h) × 30 CNY h⁻¹.

(3) Integrated cost calculation

Combined with raw material cost and operation cost, the integrated cost for treating 1 m³ MB dye wastewater could be calculated as follows: integrated cost = (raw material cost + operation cost) = (catalyst cost + acid/alkali reagent cost + energy cost + labor cost).

RESULTS AND DISCUSSION

TABLE S-II. Experimental matrix designed by CDD and experimental and predicted values of Y response

runs	X ₁	X ₂	X ₃	X ₄	Y (%)	
					experimental	predicted
1	0.000	0.000	0.000	0.000	66.78	66.39
2	-1.000	-1.000	1.000	1.000	62.91	61.26
3	0.000	0.000	0.000	0.000	66.24	66.39
4	2.000	0.000	0.000	0.000	63.4	60.13
5	-1.000	1.000	-1.000	-1.000	11.02	5.64
6	0.000	2.000	0.000	0.000	16.78	17.38
7	-1.000	1.000	-1.000	1.000	31.93	31.90
8	-2.000	0.000	0.000	0.000	40.84	42.36
9	-1.000	1.000	1.000	1.000	60.29	60.22
10	1.000	1.000	-1.000	1.000	34.64	38.95
11	1.000	-1.000	1.000	1.000	98.72	95.11
12	0.000	0.000	0.000	-2.000	21.47	23.43
13	0.000	0.000	0.000	2.000	81.74	79.24
14	1.000	-1.000	-1.000	1.000	73.5	69.18
15	-1.000	-1.000	-1.000	1.000	58.38	59.48
16	0.000	-2.000	0.000	0.000	76.11	75.89
17	0.000	0.000	0.000	0.000	66.27	63.18
18	0.000	0.000	-2.000	0.000	25.59	25.78
19	0.000	0.000	2.000	0.000	16.76	16.49
20	0.000	0.000	0.000	0.000	66.17	66.36
21	0.000	0.000	0.000	0.000	66.87	66.36
22	1.000	1.000	-1.000	-1.000	16.53	16.59
23	-1.000	1.000	1.000	-1.000	31.56	32.13
24	0.000	0.000	0.000	0.000	66.43	66.36
25	-1.000	-1.000	1.000	-1.000	62.88	61.86
26	-1.000	-1.000	-1.000	-1.000	29.54	29.13
27	1.000	-1.000	1.000	-1.000	70.79	71.00
28	1.000	-1.000	-1.000	-1.000	39.89	40.16
29	1.000	1.000	1.000	1.000	67.48	67.16
30	1.000	1.000	1.000	-1.000	41.42	41.10

Table S-III. ANOVA analysis for second-order polynomial regression.

Source	Sum of squares	Degree of freedom	Mean square	F value	p value (Prob > F)	
model	13659.70	14	975.69	8.09	0.0001	significant
X ₁	811.77	1	811.77	6.73	0.0204	
X ₂	4277.34	1	4277.34	35.45	< 0.0001	
X ₃	1394.77	1	1394.77	11.56	0.0040	
X ₄	3869.94	1	3869.94	32.07	< 0.0001	
X ₁ X ₂	120.56	1	120.56	1.00	0.3334	
X ₁ X ₃	45.83	1	45.83	0.38	0.5469	
X ₁ X ₄	46.24	1	46.24	0.38	0.5452	
X ₂ X ₃	9.99	1	9.99	0.083	0.7775	
X ₂ X ₄	0.72	1	0.72	0.006	0.9393	
X ₃ X ₄	21.90	1	21.90	0.18	0.6761	
X ₁ ²	168.67	1	168.67	1.40	0.2555	
X ₂ ²	416.88	1	416.88	3.45	0.0828	
X ₃ ²	2862.65	1	2862.65	23.72	0.0002	
X ₄ ²	186.64	1	186.64	1.55	0.2327	
residual	1810.03	15	120.67			
R ²	0.9588					
adjusted R ²	0.9189					

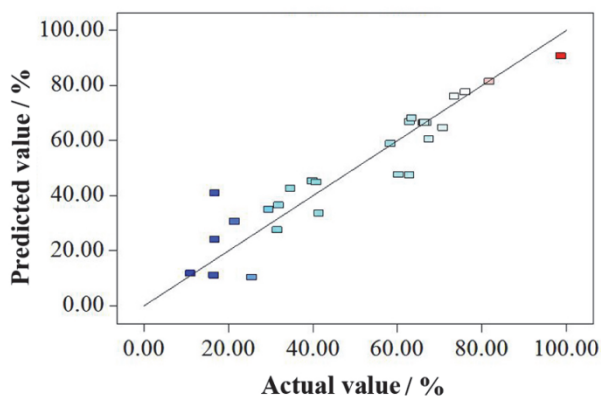


Fig. S-2. The plots of predicted values versus actual values.

Table S-IV. Comparative analysis data of raw material cost for treating 1 m³ MB wastewater before and after optimization.

	catalyst cost data		acid-alkaline cost data		net saving (CNY)
	dosage (kg)	cost (CNY)	dosage (kg)	cost (CNY)	
before optimization	5	400	0	0	
after optimization	4.1	328	0.12	0.3	71.7
saving cost	—	72	—	-0.3	

Table S-V. Comparative analysis data of operation cost for treating 1 m³ MB wastewater before and after optimization.

	energy cost data		labor cost data		net saving (CNY)
	energy consumption (kWh)	cost (CNY)	operation time (h)	cost (CNY)	
before optimization	1.050	0.630	3.5	105	
after optimization	0.742	0.445	2.5	75	30.185
saving cost	—	0.185	—	30	

Silica modified with choline chloride/urea DES for ligand-free Cu(II) adsorption

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Abstract: In this study, silica modified with a hydrophilic deep eutectic solvent (DES) composed of choline chloride and urea (DES-Si) was synthesized and evaluated for ligand-free Cu(II) removal from aqueous solutions. The DES-Si was successfully characterized using Fourier transform infrared spectroscopy, thermogravimetric analysis, nuclear magnetic resonance and elemental analysis, confirming the effective immobilization of DES onto the silica surface. Batch adsorption experiments demonstrated that DES-Si achieved a Cu(II) removal efficiency exceeding 95 % under optimal conditions (pH 8, initial Cu(II) concentration 5 mg L⁻¹, contact time 45 min, at 25 °C). The maximum adsorption capacity obtained from the Langmuir model was 17.54 mg g⁻¹, indicating strong affinity toward Cu(II) ions. Kinetic studies revealed that the adsorption process followed the pseudo-second-order model ($R^2 = 0.9964$), suggesting that the rate-limiting step is governed by surface interactions. Equilibrium data were well described by the Langmuir, Freundlich and Temkin isotherm models, with the Temkin model providing the best fit ($R^2 = 0.9914$). Comparative studies showed that Cu(II) removal using DES-Si without a chelating ligand was significantly more efficient than in the presence of 1,10-phenanthroline, highlighting the effectiveness of ligand-free adsorption. These findings demonstrate that DES-Si is a promising and environmentally friendly adsorbent for efficient Cu(II) removal from aqueous media.

Keywords: deep eutectic solvent; silica modification; copper adsorption; ligand-free removal.

INTRODUCTION

Copper is a metal known for its excellent thermal and electrical conductivity, ease of fabrication and good corrosion resistance. As a result, it is widely used in

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industries such as electroplating, mining and smelting, brass manufacturing and petroleum refining. Cu(II) is also essential for living organisms in ultra-trace amounts, playing key roles in antioxidant defense, cellular iron metabolism and cellular respiration.¹ However, excessive levels of Cu(II) are potentially toxic to aquatic life, humans and the environment.² Therefore, the removal of Cu(II) from industrial effluents is critical to protect water resources.² Adsorption technology is one of the most promising methods for Cu(II) removal.³ Various materials, including activated carbon, natural bentonite, montmorillonite and rice husk, have been employed for this purpose.^{4–7} However, many of these adsorbents suffer from limitations such as long equilibrium times, low removal capacities and selectivity and poor thermal stability.⁷ To overcome these drawbacks, silica-based adsorbents have been explored due to their large specific surface area, uniform pore structure, and easily modifiable surface properties.⁹ Organically modified silicas, in particular, have attracted considerable attention as promising adsorbents capable of enhancing the removal efficiency of heavy metals.¹⁰

Deep eutectic solvents (DESs) are a class of environmentally friendly solvents created by combining quaternary ammonium salts with hydrogen bond donors. These unique mixtures possess several beneficial characteristics, such as minimal vapor pressure, low toxicity, affordability and excellent biodegradability.¹¹ Due to these advantages, DESs have gained increasing recognition and are now employed in a wide range of scientific and industrial areas, including organic synthesis, electrochemical applications, materials engineering, biological studies, separation processes and chemical analysis.¹² A pioneering example of their use was demonstrated by Abbott *et al.*, who applied a DES made from chlorocholine chloride and urea (ChCl–urea) to modify cellulose structures.¹²

Recently, ionic liquids (ILs)-modified silica were successfully applied as stationary phases in liquid chromatography and as adsorbents.¹³ However, it is challenging to prepare large-scale ILs in industry, because of complicated synthetic processes and the expensive raw material chemicals.¹⁴ Hence, the low cost of deep eutectic solvents (DESs), which are ionic liquids analogues, was introduced.¹⁵ DESs are formed by mixing a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) at a certain ratio and temperature.¹⁵ DES appear to have useful properties, such as being easily prepared with high purity, nontoxic, inert in water and biodegradable.¹⁵ The properties of DESs can be customized to a particular type of chemistry.¹⁵ Owing to these attractive characteristics, attention had been given to DES as an alternative, environmentally friendly solvent and media for organic and inorganic reactions and separations.¹⁶

In recent years, DES-based mesoporous silica has been explored as an alternative stationary phase and adsorbent for size-exclusion chromatography columns.¹⁷ However, their application as adsorbents for heavy metal removal, parti-

cularly Cu(II), remains underexplored. In this study, the feasibility of DES-modified silica (DES-Si) for Cu(II) removal from aqueous solutions was investigated. The DES was composed of choline chloride (HBA) and urea (HBD) and was immobilized onto silica to form DES-Si. This material was then used for Cu(II) adsorption without the addition of a chelating agent.¹⁸ Although ligands such as 1,10-phenanthroline (phen) are commonly used for trace metal separation due to their specificity and selectivity, despite their toxicity character is far from environmentally friendly criteria.¹⁸ As a comparison, the removal of Cu(II) was constructed using DES-Si with the presence of 1,10-phenanthroline (phen) in aqueous solution. The optimum conditions, such as pH, contact time and initial concentration of Cu(II), had been investigated to obtain efficient removal. In addition, the kinetic and isotherm studies were also conducted to identify the mechanism of adsorption.

EXPERIMENTAL

Chemicals

All reagents and chemicals used for the synthesis of DES-Si were analytical grade and used without further purification. Urea was purchased from Fluka-BioChemika ($\geq 99.5\%$). Choline chloride (ChCl) was acquired from Sigma-Aldrich ($\geq 98\%$). 1,10-Phenanthroline monohydrate (phen) was obtained from Systerm Chemicals (Shah Alam, Selangor, Malaysia). Ultrapure water type I was filtered from an ultrapure water system (Division of Millipore, USA).

Instrumentations

The Fourier transform infrared spectroscopy (FT-IR) was obtained from the Systerm 2000 PerkinElmer set, with the wavenumber between 400 and 4000 cm^{-1} at a scan rate of 8 scans per min. The thermogravimetric analysis (TGA) was collected from PerkinElmer STA 6000. The nuclear magnetic resonance (NMR) spectrum was analyzed using a Bruker Avance III 500 MHz. The carbon, hydrogen and nitrogen contents of silica, DES and DES-Si were determined by elemental analysis using PerkinElmer CHN elemental analyzer 2400 Series II. Atomic absorption spectrometer (AAS) analysis was obtained from a fully computer-controlled PerkinElmer absorption spectrometer Analyst 400.

Preparation and characterization of DES and DES-Si

The DES was prepared as previously reported by mixing choline chloride (ChCl) and urea in a 1:2 mole ratio, followed by constant heating and stirring at 80 °C for 2 h until a clear homogeneous liquid was obtained.¹⁹ Silica has been activated to react with DES. The synthesis schemes for DES and DES-Si are shown in Fig. 1a and b, respectively. The crude product of DES-Si was obtained by heating at 80 °C for 15 h. Then, the DES-Si was filtered, washed with ultrapure water to remove impurities and unreacted DES, and dried at 100 °C for 6 h. Both DES and DES-Si were characterized using FT-IR, TGA, NMR and elemental analysis.

Preparation of standard and working solutions

100 mg L^{-1} Cu(II) solution was prepared freshly from 1000 mg L^{-1} of Cu(II) standard solution. A series of standard solutions for calibration analysis was prepared with concentrations of 1, 2, 3, 4 and 5 mg L^{-1} diluted with ultrapure water from a 100 mg L^{-1} solution. 100 mg L^{-1} of Cu(II) working solution was prepared in 100 mL volumetric flask by dissolving $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ with ultrapure water.

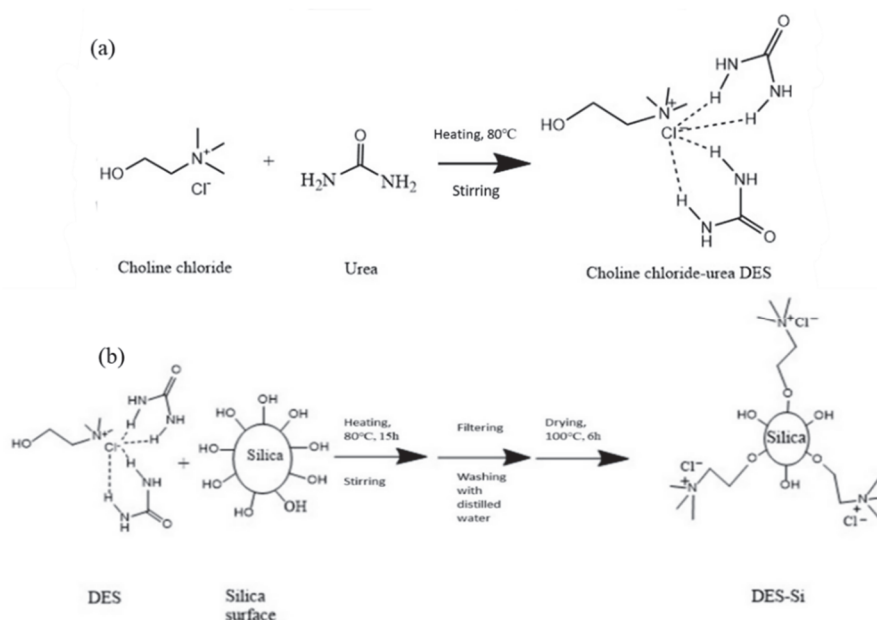


Fig. 1 Synthesis scheme of: a) DES and b) DES-Si.

Optimization parameters for the removal of Cu(II) ions

Effect of pH. A series of Cu(II) working solutions (20 mg L^{-1}) was adjusted to the desired pH range (2–8) using 0.1 M NaOH and 0.1 M HCl at 25°C . For each pH, 20 mL of solution was mixed with 0.05 g of DES-Si and vortexed to ensure uniform dispersion. The mixture was allowed to equilibrate for 30 min (contact time) to enable Cu(II) adsorption. Afterward, the mixture was centrifuged to separate the solid adsorbent from the solution. The supernatant was filtered and analyzed using AAS. For comparison, 0.01 M phen was added to the Cu(II) solutions of varying pH, and the same procedure was followed.

Effect of contact time. A series of working solutions (20 mg L^{-1}) with optimum pH were mixed with 0.05 g DES-Si and allowed to contact at various time intervals (5, 10, 20, 30, 40 and 45 min) at 25°C using a vortex and a centrifuge. The solutions were filtered and analyzed using AAS. Meanwhile, 0.01 M phen was added to each Cu(II) solution at the optimum pH, and the solutions were centrifuged for various times.

Effect of initial concentration. The initial concentrations of Cu(II) solutions were prepared as follows: 5, 10, 15, 20, 25 and 30 mg L^{-1} . Each solution was adjusted to the optimum pH, mixed with 0.05 g DES-Si and allowed to contact for the optimum time using a vortex and a centrifuge. The solutions were filtered and analyzed using AAS. Meanwhile, 0.01 M of phen was added to Cu(II) solutions with various concentrations and proceeded with the same procedure as above.

RESULTS AND DISCUSSION

Characterization

FT-IR. The FT-IR spectra exhibit various absorption bands, indicating different functional groups in DES and DES-Si. The functional groups and their

corresponding wave numbers (cm^{-1}) are listed in Table I. Fig. 2 shows the FT-IR spectra of ChCl, urea, activated silica, DES and DES-Si. The appearance of bands associated with ChCl, such as $\nu_{\text{as}}(\text{OH})$, $\delta_{\text{as}}(\text{OH})$, $\rho(\text{CH}_3)$, $\rho(\text{CH}_2)$, $\nu(\text{C-O})$, $\nu_{\text{as}}(\text{CCO})$ and $\nu_{\text{s}}(\text{N-CH}_3)$ in the spectrum of DES confirms that no destruction of Ch^+ structure occurs.²⁰ The absorption peaks of urea at 3430 and 3332 cm^{-1} were shifted towards the lower regions (3317 and 3190 cm^{-1}) and gained broader peaks due to the formation of additional hydrogen bonds, such as OH-O, OH-N and OH-OH, between ChCl and urea.²⁰ From Fig. 2, the spectrum for DES-Si shows a broad peak for $\nu_{\text{as}}(\text{OH})$, δ_{as} of NH_2 , Si-O-C and Si-O-Si strong absorption bands, $\nu_{\text{as}}(\text{CCO})$ and $\nu_{\text{s}}(\text{N-CH}_3)$ at 3460, 1639, 1103, 970 and 802 cm^{-1} , respectively. The presence of these peaks confirms the attachment of DES to the activated silica.¹⁹ The spectrum for activated silica shows the existence of bands associated with Si-O-Si and OH at 1099 and 3441 cm^{-1} , respectively. These bands indicate the presence of an active site on activated silica, where the modification process can occur.²¹

TABLE I. Wave numbers (cm^{-1}) and their assignment for FT-IR spectra; ν = stretching; δ = bending; ρ = rocking; ω = weak

Assignments	Adsorbent	
	DES	DES-Si
$\nu_{\text{as}}(\text{OH})$	3317	3460
$\nu_{\text{s}}(\text{NH}_2)$	3190	—
$\delta_{\text{s}}(\text{NH}_2)$	1663	—
$\nu(\text{C=O})$	1608	—
$\delta_{\text{as}}(\text{OH})$	3025	—
$\delta_{\text{as}}(\text{NH}_2)$	—	1639
$\rho(\text{CH}_3)$	1476	—
$\rho_{\text{s}}(\text{NH}_2)$	1433	—
$\nu_{\text{as}}(\text{CN})$	1169	—
$\nu_{\text{as}}(\text{Si-O-Si})$	—	1103
$\nu_{\text{as}}(\text{Si-O-C})$	—	—
$\rho(\text{CH}_2)$	1082	—
$\nu(\text{C-O})$	1005	—
$\nu_{\text{as}}(\text{CCO})$	952	970
$\nu_{\text{s}}(\text{N-CH}_3)$	865	—
$\omega(\text{C=O})$	783	—
$\nu_{\text{s}}(\text{N-CH}_3)$	—	802
$\delta(\text{N-H})$	692	—

TGA. TGA analysis was performed to investigate the decomposition patterns of DES and DES-Si. The TGA analysis for all compounds is shown in Fig. 3. For the DES sample, TGA shows the first composition at 61 °C due to the loss of water. Meanwhile, there were compositions of ChCl and urea at 220 and 306 °C, respectively.²² After immobilization onto the silica, the weight of DES-Si did not change

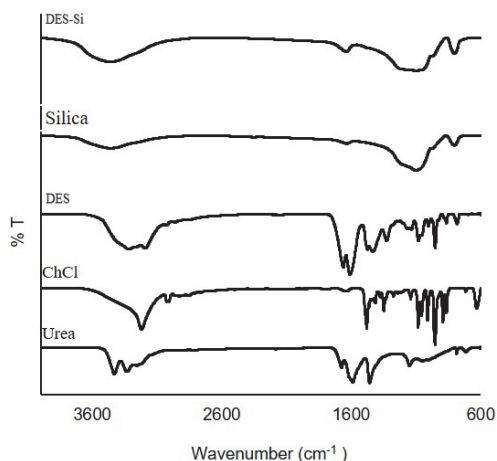


Fig. 2. FT-IR spectra for urea, ChCl, DES, activated silica and DES-Si.

significantly, thus making it stable at high temperatures. The total weight loss of DES-Si upon heating from 250 to 700 °C was less than 2 %, due to the desorption of water and the degradation of organic components before TGA.¹⁹ Therefore, only a small amount of fly ash was released from the compounds in the TGA analysis.

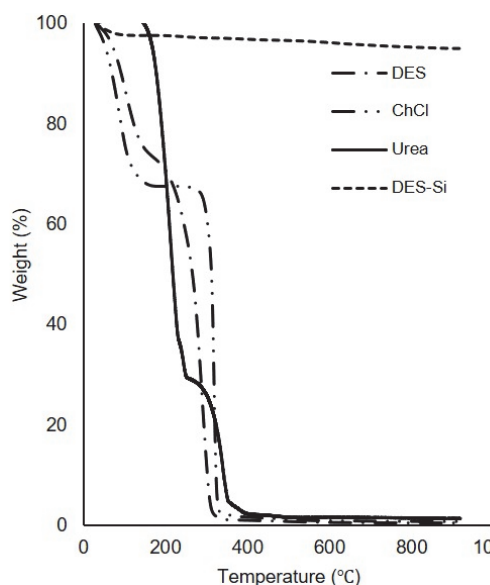


Fig. 3. TGA analysis of urea, ChCl, DES, and DES-Si.

NMR. The structure and spectra of DES, urea, and ChCl were deduced from ¹H-NMR (refer to Fig. S-1a–c of the Supplementary material to this paper) with tabulated assignment of functional groups in Table II. The spectrum of DES (Fig. S-1a) consisted of multiplet peaks for the NH₂ (δ 5.76 ppm, 8H) and a singlet peak

for the deuterium hydroxide oxide (HDO) (δ 3.70 ppm, 1H),²³ attributed to the urea. The presence of HDO is due to the trace of water detected in DES given by its hygroscopic nature.²³ The spectrum of DES also shows the peaks of choline chloride through the existence of a singlet peak for the OH (δ 5.60 ppm, 1H), multiplet peaks for CH₂ (δ 3.80 ppm, 2H; δ 3.43 ppm, 2H), and multiplet peaks for (CH₃)₃ (δ 3.14 ppm, 9H).

TABLE II. ¹H-NMR analysis of urea, ChCl and DES

Sample	Assignment	δ / ppm
Urea	HDO	3.38
	R(-NH ₂) ₂	5.45
ChCl	OH	5.70
	CH ₂ (2)	3.81
	CH ₂ (1)	3.45
	N(CH ₃) ₃	3.16
	R(-NH ₂) ₂	5.76
DES	OH	5.60
	HDO	3.70
	CH ₂ (2)	3.80
	CH ₂ (1)	3.43
	N(CH ₃) ₃	3.14

CHN analysis. From Table III, the distribution of the elements detected was 1.32 (C), 0.97 (H) and 0.54 % (N) in DES-Si, 0.14 (C), 0.76 (H) and 0.08 % (N) for activated silica and 28.2 (C), 8.81 (H) and 22.92 % (N) in DES. The presence of nitrogen in DES-Si confirms that the DES was successfully immobilized onto silica. The detection of small amounts of nitrogen and carbon in silica may be due to contamination during sample preparation, which led to the adsorption of impurities onto the silica surface. Although DES-Si shows a significant increase in elemental content compared to unmodified silica, its C, H and N percentages are substantially lower than those of pure DES. This is expected, as only a thin layer of DES is coated onto the silica surface during immobilization, rather than forming a bulk DES material. Therefore, the comparatively lower elemental percentages in DES-Si reflect this partial surface coverage. Such a thin coating not only confirms efficient immobilization but also helps preserve the silica support's porosity and structural integrity.

TABLE III. Elemental analysis of DES-Si, silica and DES

Sample	Element content, %		
	C	H	N
DES-Si	1.32	0.97	0.54
Silica	0.14	0.76	0.08
DES	28.2	8.81	22.92

Optimization parameters for the removal of Cu(II)

The percentage removal of Cu(II) was calculated as:

$$\text{Removal} = 100 \frac{c_0 - c_e}{c_0} \quad (1)$$

where c_0 is the initial adsorbate concentration (mg L^{-1}) and c_e is the equilibrium concentration of the adsorbate (mg L^{-1}).

Effect of pH. The pH of the metal solution plays a crucial role in adsorption studies. In this experiment, the removal of Cu(II) using DES-Si and DES-Si-phen was carried out over a pH range of 1 to 8. The removal of Cu(II) (Fig. 4), with and without ligand, was found to increase as the pH of the solution increased from 4 to 8. This result indicates that Cu(II) removal using DES-Si is favored under alkaline conditions. At lower pH, the concentration of H_3O^+ greatly exceeds that of Cu(II), leading to competitive binding at the active sites on DES-Si. At higher pH, the concentration of H_3O^+ is lower, allowing a greater number of Cu(II) ions to bind to the surface. The adsorption mechanism primarily involves electrostatic interactions and surface complexation between divalent Cu^{2+} and negatively charged functional groups present on the DES-modified silica surface. These interactions are more favorable at higher pH values, where proton competition is reduced. The H_3O^+ is replaced by metal ion and metal species such as Cu^{2+} , CuOH^+ and $\text{Cu}_2(\text{OH})_2^{2+}$. However, an increase in pH may also promote the precipitation of Cu(II) as metal hydroxides. In this study, the pH was adjusted to 8 and precipitation was monitored throughout to ensure none occurred. The solution at pH 8 was confirmed to be free from precipitation. Based on Fig. 4, the removal efficiency of Cu(II) using DES-Si reached 83.67 %, compared to 55.52 % for DES-Si-phen at the same pH. Therefore, the optimal pH for Cu(II) adsorption in this system is pH 8.

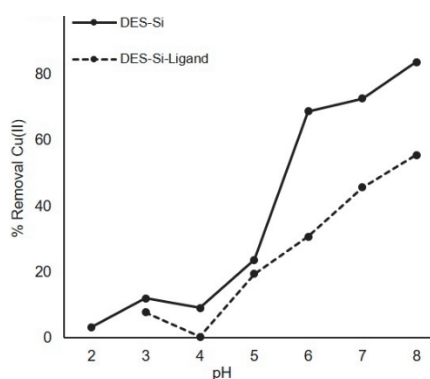


Fig. 4. Effect of pH on adsorption of Cu(II) (conditions: sorbent: 50 mg; initial concentration: 30 mg L^{-1} ; volume: 20 mL; contact time: 30 min; temperature: 25 $^{\circ}\text{C}$).

Effect of contact time. Contact times ranging from 5 to 45 min were investigated for the removal of Cu(II) using DES-Si and DES-Si-phen. The result in Fig. 5 shows that the removal percentage increases with increasing contact time

for DES-Si and DES-Si-phen. The percentage removal of Cu(II) was slightly constant from 5 min and increased to 90.29 % for DES-Si and 53.15 % for DES-Si-phen at 45 min contact time. Overall, the result shows that the percentage removal of Cu(II) aqueous assisted phen is lower than the removal of Cu(II) without ligand. The lower removal of Cu(II) when phen is present can be explained by how it interacts with the Cu(II) ion. Phen forms strong, stable complexes with Cu(II) in the solution. Because of this, fewer free Cu(II) ions are available to bind to the DES-Si surface. These Cu-phen complexes might also have different charges or shapes, making them less likely to interact with the functional groups on DES-Si. As a result, the presence of phenanthroline interferes with the adsorption process rather than aiding it. The optimum contact time of 45 min was used for the next optimization for the removal of Cu(II).

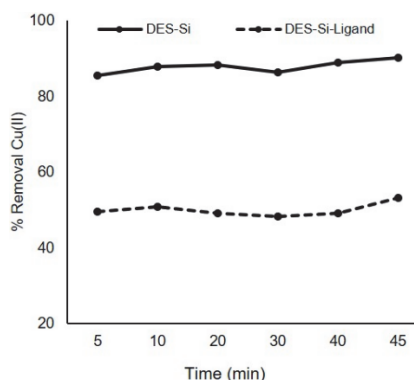


Fig. 5. Effect of contact time on adsorption of Cu(II) (conditions: pH: 8; sorbent: 50 mg; initial concentration: 30 mg L⁻¹; volume: 20 mL; temperature: 25 °C).

Effect initial concentration of Cu(II). The effect of the initial Cu(II) ion concentration on the adsorption performance of DES-Si and DES-Si-ligand was systematically investigated over a concentration range of 5–30 mg L⁻¹ to evaluate their removal behavior. As illustrated in Fig. 6, DES-Si exhibited consistently high

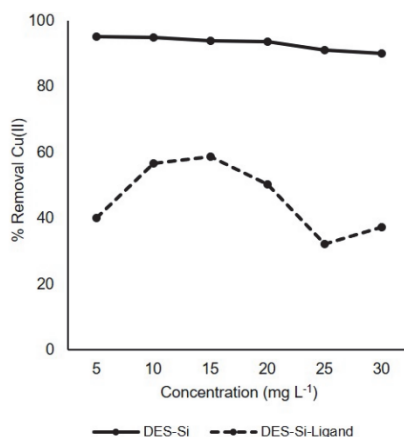


Fig. 6. Effect of initial concentration of Cu (II) (conditions: pH: 8; sorbent: 50 mg; contact time: 45 min; volume: 20 mL; temperature: 25 °C).

Cu(II) removal efficiency across the studied concentration range, with only a slight decrease at higher concentrations. This observation indicates the presence of sufficient active adsorption sites and a strong affinity between Cu(II) ions and the DES-Si surface. In contrast, the removal efficiency of DES-Si–ligand increased with increasing initial Cu(II) concentration up to 15 mg L⁻¹, after which a noticeable decline was observed at higher concentrations. This trend can be attributed to the saturation of available adsorption sites and increased competition among Cu(II) ions at elevated concentrations. Overall, DES-Si demonstrated superior adsorption performance compared to DES-Si–ligand throughout the investigated concentration range, highlighting its enhanced effectiveness for Cu(II) removal.

Batch adsorption studies

Adsorption kinetics. Adsorption kinetics is defined as the residence time of the sorbate onto the interface of the solid sorbent, controlled by the removal rate of the solute. The purpose of this study is to investigate the adsorption mechanism and the rate-controlling steps. Pseudo-first and second-order kinetic equations (Supplementary material) were applied to describe the adsorption of the adsorbate in an aqueous system onto the solid surface of the adsorbent.^{8,26}

Δq (%) and RE (%) are the normalized standard deviation value and relative error, respectively, which were calculated to determine the stability and the fitness of the model to describe the kinetics of adsorption. The smaller the value of Δq and RE , the better the model fits.

By comparing the correlation coefficient (R^2) values of PFO and PSO (Table IV), it can be concluded that both sets of experimental data follow the PSO involving a chemisorption mechanism. In this mechanism, adsorption is driven by a chemical reaction at the exposed surface.^{24,25}

TABLE IV. Details of the parameters and correlation coefficient of adsorption kinetic models

Kinetic model	Parameter	Removal Cu (II)	
		DES-Si	DES-Si–ligand
Pseudo first order	$q_{e,exp} / \text{mg g}^{-1}$	6.604	3.776
	$q_{e,calc} / \text{mg g}^{-1}$	0.6714	0.2667
	k_1	0.04813	–0.01888
	R^2	0.2343	0.4577
	$\Delta q / \%$	40.17	41.56
	$RE / \%$	89.83	92.94
Pseudo second order	$q_{e,calc} / \text{mg g}^{-1}$	6.333	3.560
	$k_2 / \text{g mg}^{-1} \text{min}^{-1}$	–2.899	0.3933
	$h / \text{mg g}^{-1} \text{min}^{-1}$	–116.3	4.985
	$t_{1/2} / \text{min}$	–0.4578	0.1105
	R^2	0.9964	0.9873
	$\Delta q / \%$	1.84	2.56
	$RE / \%$	4.10	5.72

Adsorption isotherm. An adsorption isotherm is a model that explains the affinity for the adsorbent and the surface properties. The result obtained can be used to explain the interaction that occurred between the adsorbate and the adsorbent. The adsorption mechanism of Cu(II) on DES-Si was studied using adsorption models, namely Langmuir, Freundlich, Temkin and Dubinin–Radushkevich.^{8,26,27} The details are given in the Supplementary material. The parameters obtained from the isotherm models were represented in Table V.

TABLE V. Details of parameters and correlation coefficient of adsorption isotherm models for adsorption of Cu (II) ion onto DES-Si

Isotherm model	Parameter	Value
Langmuir	$q_m / \text{mg g}^{-1}$	17.54
	$b / \text{L mg}^{-1}$	0.5231
	R_L	0.09911
	R^2	0.9887
Freundlich	n	1.4395
	$K_F / \text{mg g}^{-1}$	5.5424
	R^2	0.976
Temkin	$b_T / \text{J mol}^{-1}$	711.17
	$S_T / \text{L g}^{-1}$	6.4887
	R^2	0.9914
Dubinin–Radushkevich	$q_m / \text{mg g}^{-1}$	9.5172
	$\beta / \text{mol}^2 \text{kJ}^{-2}$	0.1031
	$E / \text{kJ mol}^{-1}$	2.2022
	R^2	0.9663

Based on the results obtained in Table V, it can be found that the value of R^2 is 0.9663, suggesting that these experimental data did not fit the Dubinin–Radushkevich isotherm model, indicating that the adsorption process does not follow this model. Result showed the mean free energy, E , of $2.2022 \text{ kJ mol}^{-1}$ indicates that adsorption Cu(II) ions onto DES-Si is mainly physisorption because when a mean free energy is less than 8 kJ mol^{-1} , physisorption controls the adsorption mechanism, while chemisorption prevails when the mean free energy is in the range of 8 and 16 kJ mol^{-1} .²⁴

CONCLUSIONS

In this work, DES-Si was successfully synthesized and able to remove >95 % of Cu(II) from aqueous solution at an optimum pH of 8, 5 mg L^{-1} of initial concentration of Cu(II), and 45 min of contact time. The removal efficiency of Cu(II) using DES-Si was compared to that using DES-Si in the presence of a ligand. A higher removal percentage was achieved with DES-Si alone, indicating that the addition of the ligand reduced adsorption efficiency. This decrease is attributed to the formation of stable Cu(II) ligand complexes in solution, which reduce the availability of free Cu(II) ions for adsorption. The adsorption mechanism is primarily

governed by electrostatic interactions, surface complexation and possible hydrogen bonding between the Cu(II) ions and functional groups (such as amine and hydroxyl) on the DES-Si surface. The data obtained from adsorption kinetic and isotherm studies showed that the adsorption of Cu(II) onto DES-Si is best fitted with PSO, Langmuir, Freundlich and Temkin models.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13520>, or from the corresponding author on request.

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ИЗВОД

СИЛИКА МОДИФИКОВАНА ХОЛИН-ХЛОРИД/УРЕА ДУБОКИМ ЕУТЕКТИЧКИМ РАСТВОРАЧЕМ ЗА АДОРПЦИЈУ Cu(II) БЕЗ ЛИГАНДА

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У овој студији синтетисана је силика модификована хидрофилним дубоким еутектичким растварачем (DES) састављеним од холин-хлорида и урее (DES-Si) и испитана за уклањање Cu(II) јона из водених раствора без употребе лиганата. DES-Si је успешно окарактерисана применом инфрацрвене спектроскопије са Фуријеовом трансформацијом, термогравиметријске анализе, нуклеарне магнетне резонанце и елементалне анализе, чиме је потврђена ефикасна имобилизација DES на површини силике. Експерименти адсорпције у шаржном режиму показали су да DES-Si остварује ефикасност уклањања Cu(II) већу од 95 % под оптималним условима (pH 8, почетна концентрација Cu(II) 5 mg L⁻¹, време контакта 45 min, 25 °C). Максимални адсорпциони капацитет добијен из Langmuir модела износио је 17,54 mg g⁻¹, што указује на снажан афинитет према Cu(II) јонима. Кинетичка испитивања показала су да процес адсорпције прати модел псеудо-другог реда ($R^2 = 0,9964$), што сугерише да је брзина процеса одређена површинским интеракцијама. Равнотежни подаци добро су описани Langmuir, Freundlich и Temkin изотермним моделима, при чему је Temkin модел показао најбоље слагање са експерименталним подацима ($R^2 = 0,9914$). Упоредне студије показале су да је уклањање Cu(II) помоћу DES-Si без хелатног лиганда значајно ефикасније него у присуству 1,10-фенантролина, што потврђује ефикасност адсорпције без лиганата. Ови резултати указују да је DES-Si обећавајући и еколошки прихватљив адсорбент за ефикасно уклањање Cu(II) из водених медијума.

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SUPPLEMENTARY MATERIAL TO
**Silica modified with choline chloride/urea DES for ligand-free
Cu(II) adsorption**

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The equation for pseudo-first order (PFO) is described as follows:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \quad (\text{S-1})$$

where k_1 is the rate constant of the pseudo-first-order model (min^{-1}), q_e is the amount of metal adsorbed at equilibrium (mg g^{-1}), and q_t is the amount of metal adsorbed at time t (mg g^{-1}). The value of k_1 and q_e was obtained from the plotted graph of $\log(q_e - q_t)$ against t .

Pseudo-second-order (PSO) equation by Ho and Mckay (1999) is as followed:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (\text{S-2})$$

where k_2 is the rate constant of pseudo-second order ($\text{g mg}^{-1} \text{min}^{-1}$). A graph of t/q_t vs t was plotted to find the initial adsorption rate, h ($\text{mg g}^{-1} \text{min}^{-1}$), and the half-equilibrium time, $t_{1/2}$ (min).

$$h = k_2 q_e^2 \quad (\text{S-3})$$

$$t_{1/2} = \frac{k_2}{q_e} \quad (\text{S-4})$$

The Langmuir isotherm describes the monolayer adsorption of an adsorbate onto the surface of an adsorbent containing a finite number of active sites. There will be no further sorption once an active site is filled up and the surface reaches a saturation point where the maximum adsorption is achieved.

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The isotherm is represented as:

$$\frac{C_e}{q_e} = \frac{1}{q_m} C_e + \frac{1}{b q_m} \quad (\text{S-5})$$

where C_e is the equilibrium concentration of the adsorbate (mg L^{-1}), q_m is the adsorption capacity (mg g^{-1}), and b is the rate of adsorption (L mg^{-1}). The features of the parameter for Langmuir adsorption isotherm can be used to predict the affinity between the adsorbate and adsorbent using a dimensionless constant called the separation factor or equilibrium parameter (R_L), which is expressed by the following relationship:

$$R_L = \frac{1}{1 + b C_0} \quad (\text{S-6})$$

Where C_0 is the initial adsorbate concentration (mg L^{-1}), R_L stipulates the favourability of the adsorption nature, either irreversible ($R_L = 0$), favorable ($0 < R_L < 1$), linear ($R_L = 1$) or unfavorable ($R_L > 1$). The values of linear regression coefficient (R^2) is 0.9887, demonstrating that the experimental data fitted well with the Langmuir isotherm equation. The R_L value in the present investigation was found to be 0.0911, indicating that the adsorption of the Cu (II) ions onto DES-Si is favorable, with the maximum adsorption capacity (q_m) of 17.54 mg g^{-1} .

The Freundlich isotherm describes the heterogeneous adsorption between adsorbate and adsorbent with a non-uniform distribution of heat of adsorption. The Freundlich isotherm is represented by the following Equation:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (\text{S-7})$$

Adsorption capacity, K_F , (mg g^{-1}) and Freundlich exponent, n can be found by plotting graph of $\log q_e$ versus $\log C_e$. The n value indicated the type of sorption process to be chemical process if $n < 1$ and physical process if $n > 1$. As shown in Table V, it can be found that the value of R^2 is 0.976. In the present study, since n value is larger than 1, it indicates the physical adsorption of Cu (II) ions onto DES-Si.

The Temkin isotherm is a model that assumes the heat of adsorption (function of temperature) of the adsorbate in the layer would decrease linearly with the surface coverage due to adsorbent–adsorbate interactions. The equation of Temkin model is written as:

$$q_e = \frac{RT}{b_T} \ln S_T + \frac{RT}{b_T} \ln C_e \quad (\text{S-8})$$

Where b_T (J mol^{-1}) is the Temkin constant related to the heat of adsorption and S_T (L g^{-1}) is the Temkin isotherm equilibrium binding constant. As shown in Table V, it can be found that the value of R^2 is 0.9914, suggesting that these

experimental data fit very well with the Temkin isotherm model. The following values were estimated: $S_T = 6.4887 \text{ L g}^{-1}$, $b_T = 711.17 \text{ J mol}^{-1}$, which are indications of the heat of sorption and physical adsorption process, respectively.

Dubinin-Radushkevich isotherm is a model that is commonly used to describe the mechanism of adsorption onto a heterogeneous surface and can be applied to determine the mean of adsorption energy (E) of the adsorbate. This adsorption energy is used to remove the adsorbate from the sorption space using the equation below:

$$E = \frac{1}{\sqrt{2\beta}} \quad (\text{S-9})$$

where β is the DR model constant ($\text{mol}^2\text{kJ}^{-1}$). The value of E is important as it can be used to determine the type of adsorption of metal ions, either physical or chemical adsorption. The linear equation of this model is represented as:

$$\ln q_e = \ln q_m - \beta E^2 \quad (\text{S-10})$$

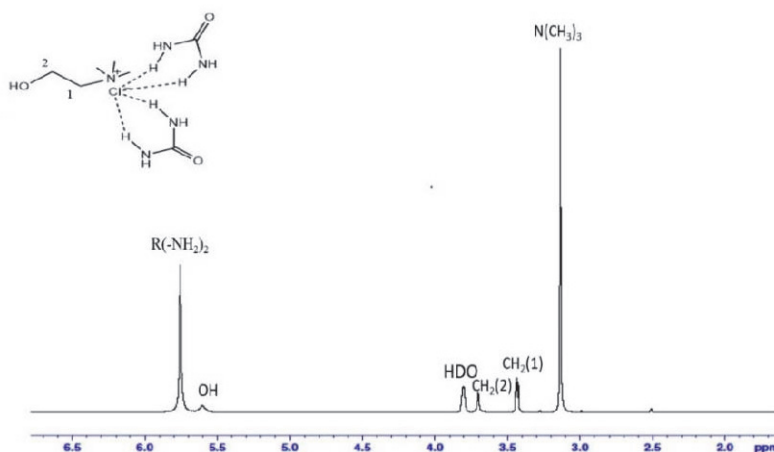
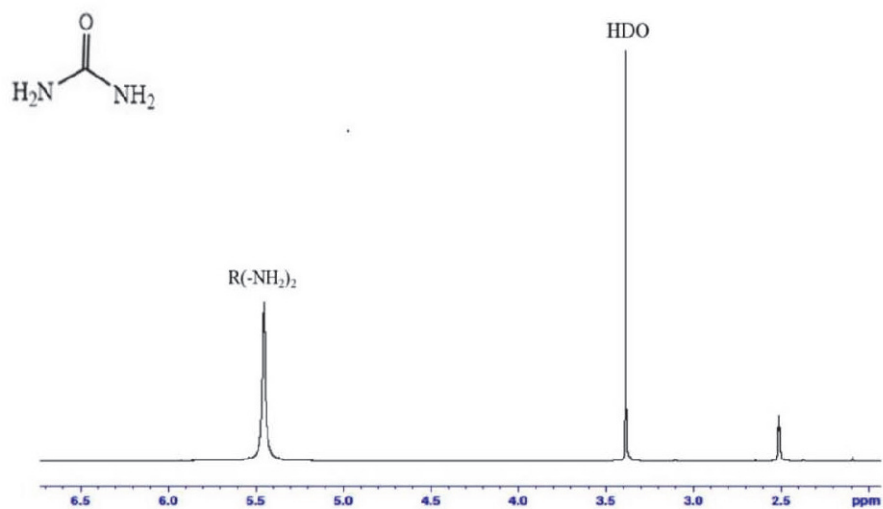
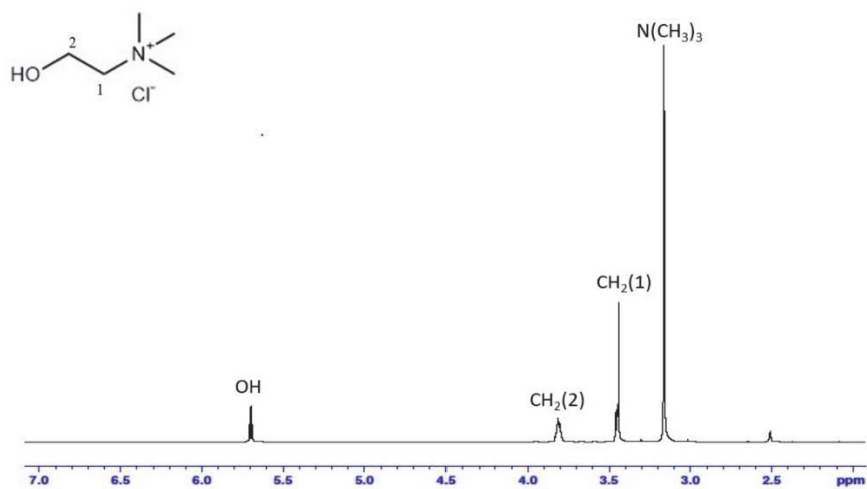


Fig. S1(a) ^1H -NMR spectrum of DES

Fig. S1(b) ¹H-NMR spectrum of ureaFig. S1(c) ¹H-NMR spectrum of choline chloride.



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Magnetic *Ambrosia artemisiifolia*-based biosorbent for Congo red removal from water: A circular economy approach

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Abstract: Biomass-based biosorbents have demonstrated significant potential for removing synthetic organic dyes from water. This study explores the use of a magnetic biosorbent (Fe₃O₄/MB) derived from *Ambrosia artemisiifolia*, as a novel material for the removal of Congo red (CR) dye from aqueous solutions. The biosorption process was investigated by varying key parameters: pH, Fe₃O₄/MB dosage, CR concentration and temperature, followed by kinetic and equilibrium analyses. The structural properties of Fe₃O₄/MB were characterized using Fourier transform infrared spectroscopy. The CR concentration was monitored *via* UV–Vis spectrophotometry. Kinetic studies indicated that the biosorption of CR followed a PSO model ($R^2 = 0.99$). Isotherm analysis revealed that the Langmuir model best described the biosorption of CR ($R^2 = 0.97$), with a maximum adsorption capacity ($Q_{\max}$) of 33.56 mg g⁻¹. The implementation of Fe₃O₄/MB supports sustainable water management and contributes to the development of greener and more circular industrial practices.

Keywords: biomass; biosorption; synthetic organic dyes; water treatment.

INTRODUCTION

Water pollution has become an escalating global problem. Synthetic organic dyes, used in the textile, paper and leather industries, significantly impact the environment and contribute to water contamination.¹ Many of these dyes are toxic, carcinogenic and resistant to degradation.² Reports indicate that about 700 tons of dye-laden industrial wastewater are released daily into the world's water bodies. Research reveals that even low concentrations of these compounds can be harmful to the environment.³ Congo red (CR) is a widely used benzidine-based anionic azo dye, characterized by two azo groups, a long linear chain and two symmetrical

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naphthylamine moieties, with good water solubility and poor biodegradability. CR is primarily used in the textile and rubber industries and can enter the body through the skin, eyes, respiratory system or gastrointestinal tract, affecting vital organs and the immune system, including carcinogenic and mutagenic effects on human health.⁴ Therefore, effectively removing CR from industrial wastewater is important for environmental protection.

Numerous techniques, such as adsorption, ozonation, advanced oxidation, reverse osmosis, flocculation, ion exchange, membrane separation, photocatalytic degradation and biodegradation, have been implemented to remove synthetic organic dyes from wastewater in order to reduce environmental pollution. Adsorption is the most widely used due to its operational simplicity, low cost, high efficiency and minimal sludge generation.^{5,6} Various adsorbent materials, like synthetic polymers, nanocomposites, clays, modified adsorbents, carbon-based materials, *etc.*, have been used for dye removal.⁷ Among the many materials for dye sorption, biomass-based materials are an excellent choice because of their non-hazardous nature, wide availability in sufficient quantities, sorption efficiency and cost-effectiveness. These materials are not harmful to the environment and their use in environmental protection has become both an ecological and economic opportunity, representing an effective eco-friendly solution. Many researchers have investigated the use of biomass for wastewater treatment.^{8–10} Biomass is composed of cellulose, lignin, hemicellulose, lipids and proteins, which contain functional groups (hydroxyl, carboxyl, amino and phenolic groups), making them effective for dye removal. A variety of biosorbents have been utilized in dye biosorption, such as microorganisms, agricultural wastes or weeds, forestry and food residues, wood wastes, *etc.*^{11–13}

Ambrosia artemisiifolia is an invasive weed plant that has spread extensively across Europe, the Americas and Asia. In Europe alone, about 44 million people suffer from allergies caused by *Ambrosia*, resulting in high medical costs, with impaired quality of life for those patients. Beyond its allergenic effects, *A. artemisiifolia* can cause significant crop yield losses, exerting generally negative impacts on the environment.¹⁴ This weed is particularly widespread in the northern regions of the Republic of Serbia, and the maximum recorded pollen concentration reached 12356 grains m⁻³, making it the dominant airborne allergen.¹⁵ Several researchers have explored the potential use of *A. artemisiifolia* as a biosorbent for removing some hazardous compounds, while its use in the removal of synthetic organic dyes remains largely unexplored.^{16–18} Therefore, the literature on this topic remains limited, presenting opportunities for further investigation. In our previous study, a magnetic biosorbent derived from *A. artemisiifolia* biomass showed great potential for the removal of Malachite green (MG), a cationic dye, from aqueous

solutions.¹⁹ The aim of this study was to examine the potential of the same magnetic biosorbent, Fe₃O₄/MB, for the sorption of a different type of dye, the anionic dye CR, while also considering a circular economy aspect of this research.

EXPERIMENTAL

Chemicals

The chemicals used in this research were: iron(II) chloride (FeCl₂·4H₂O, ≥98 %), iron(III) chloride (FeCl₃·6H₂O, ≥97 %), Congo red (C₃₂H₂₂N₆Na₂O₆S₂), sodium hydroxide (NaOH), sodium chloride (NaCl), acetone ((CH₃)₂CO), ethanol (C₂H₅OH), methanol (CH₃OH) and hydrochloric acid (HCl). All chemicals were purchased from Sigma–Aldrich.

Biosorbent material and characterization

The sample collection, biomass pre-treatment, preparation of magnetic biosorbent Fe₃O₄/MB, and its characterization (SEM, EDS, XRD and pH_{pzc}) were thoroughly described in detail in our previous paper.¹⁹ The structure of Fe₃O₄/MB, before and after biosorption of CR dye, was characterized in this study using a Nicolet Summit FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), in ATR mode over the range 4000–400 cm^{–1}.

Biosorption study in a batch system

Determination of the optimal conditions for the efficient biosorption of CR with Fe₃O₄/MB in a batch system, was achieved by monitoring the effects of biosorbent dose (1–5 g L^{–1}), contact time (0–180 min), dye concentration (10–300 ppm), pH (2–10) and temperature (298–318 K). For monitoring the biosorption process, 5 g L^{–1} of Fe₃O₄/MB was added to a vial flask containing 20 mL of a 50 ppm CR solution, based on the experience from our previous study.¹⁹ The samples were shaken for 180 min at 450 rpm, in an orbital shaker (Orb-Pro Labbox Labware, Barcelona, Spain). After the biosorption process, the Fe₃O₄/MB was separated using an external magnet and the remaining CR concentration was measured using a UV–Vis spectrophotometer, at λ_{max} = 498 nm. UV–Vis spectroscopy (Novel-102S, COLOLab Experts, Polje ob Sotli, Slovenia) was used for determining the CR concentrations. The removal efficiency (*R* in %), and the biosorption capacity (*Q_t*, in mg g^{–1}) of CR were calculated as:²⁰

$$R = 100 \frac{c_0 - c_e}{c_0} \quad (1)$$

$$Q_t = \frac{(c_0 - c_t)V}{m} \quad (2)$$

where *c*₀, *c_e* and *c_t* (ppm) represent the CR concentrations in the aqueous solution at the initial, equilibrium and any time *t*; *V* (L) is the water solution volume; *m* (g) is the biosorbent mass.

For the desorption experiment Fe₃O₄/MB (5 g L^{–1}) was added to the CR dye solution (*c*₀ = 50 ppm; pH 3; *T* = 298 K; contact time = 180 min; shaking speed = 450 rpm). After CR biosorption, the CR-loaded Fe₃O₄/MB was separated using an external magnet. Desorption agents (20 mL), including absolute EtOH,²¹ 0.1 M HCl,²¹ 0.1 M NaOH,²¹ distilled water,²² acetone,²³ MeOH and 10 % NaCl:MeOH (volume ratio 3:7),²⁴ were used for desorption of CR from the CR-loaded Fe₃O₄/MB, and shaken for 60 min at 450 rpm. The samples were separated using an external magnet and the concentrations of CR were measured using a UV–Vis spectrophotometer at λ_{max} = 498 nm. The desorption percentage (*D* in %) of CR was determined using as:²⁵

$$D = 100 \frac{Q_d}{Q_a} \quad (3)$$

where Q_d and Q_a (mg g^{-1}) are the desorbed and adsorbed quantities of CR.

RESULTS AND DISCUSSION

FTIR characterization of $\text{Fe}_3\text{O}_4/\text{MB}$

The Fourier transform infrared spectroscopy (FTIR) spectrum of $\text{Fe}_3\text{O}_4/\text{MB}$, before and after biosorption of CR, given in Fig. 1, shows the presence of many functional groups responsible for dye biosorption. The broad band at 3331.8 cm^{-1} is characteristic of the stretching vibration of hydroxyl groups ($-\text{OH}$) and primary amines stretching ($-\text{NH}$), probably connected to hydrogen bonds within cellulose and lignin.^{26,27} The peaks at 2919.1 and 2850.9 cm^{-1} can be attributed to C–H stretching vibrations in $-\text{CH}_2$ and $-\text{CH}$ groups. The absorption peaks at 1641.5 and 1419.6 cm^{-1} indicate the presence of $-\text{COO}$ of carboxyl and C–O groups on the $\text{Fe}_3\text{O}_4/\text{MB}$ surface.^{22,28} The peak at 1260.9 cm^{-1} can refer to stretching of the COC group, while the peak at 1026.5 cm^{-1} corresponds to the CO stretching vibration of primary alcohols.²⁹ The spectrum after CR biosorption didn't show significant changes in peaks intensities in the region from 3300 – 2000 cm^{-1} , but in the region from 2000 – 500 cm^{-1} changes in intensities and appearance of a new peaks at 1602.5 , 1421.2 , and 1041.1 cm^{-1} were evident, due to the dye biosorption on the $\text{Fe}_3\text{O}_4/\text{MB}$ surface.³⁰ The peak at 587.7 cm^{-1} is related to magnetite, incorporated into the structure of magnetic $\text{Fe}_3\text{O}_4/\text{MB}$.³¹ According to these results, the functional groups potentially involved in the biosorption of CR dye include hydroxyl, carboxyl and amine groups. The shifting of peak frequency and intensity in the FTIR spectrum of CR-loaded $\text{Fe}_3\text{O}_4/\text{MB}$, compared to the $\text{Fe}_3\text{O}_4/\text{MB}$ could be assigned to the biosorption of CR on the $\text{Fe}_3\text{O}_4/\text{MB}$ surface. In general, the biosorption of CR with $\text{Fe}_3\text{O}_4/\text{MB}$ occurs through hydrogen bonding, electrostatic

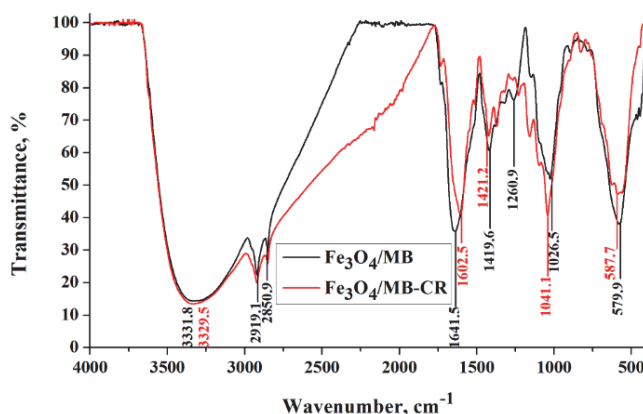


Fig. 1. FTIR spectra of $\text{Fe}_3\text{O}_4/\text{MB}$ and $\text{Fe}_3\text{O}_4/\text{MB-CR}$.

interaction, π - π interactions and ion exchange. The presence of cellulose, hemicellulose and lignin in the biosorbent structure, with a large number of hydroxyl, carbonyl and carboxyl functional groups, allows CR biosorption from aqueous solutions.²⁷

Effect of pH on biosorption

The pH of the CR solution plays a crucial role in determining the biosorption capacity, because it directly affects the surface charge of the $\text{Fe}_3\text{O}_4/\text{MB}$ and the ionization of the CR. The removal efficiency of CR from aqueous solutions using $\text{Fe}_3\text{O}_4/\text{MB}$ was evaluated across a range of pH values (2–10), while maintaining constant experimental conditions ($c_0 = 50$ ppm, $\text{Fe}_3\text{O}_4/\text{MB}$ dose = 5 g L^{-1} , $T = 298 \text{ K}$, contact time = 180 min and shaking speed = 450 rpm). The pH of the CR solutions was adjusted using a pH meter and by adding 0.1 M NaOH or 0.1 M HCl solutions. $\text{Fe}_3\text{O}_4/\text{MB}$ was added to each solution, after which the samples were shaken. After the biosorption process at different pH, the samples were separated using an external magnet, and the concentrations of CR were measured using a UV–Vis spectrophotometer at $\lambda_{\text{max}} = 498 \text{ nm}$. As illustrated in Fig. 2a, the CR removal percentage increased from 32.23 % at pH 10 to a maximum of 82.88 % at pH 3. At pH values below the point of zero charge ($\text{pH}_{\text{pzc}} 4.8$),¹⁹ the $\text{Fe}_3\text{O}_4/\text{MB}$ surface is positively charged, leading to electrostatic attraction between the $\text{Fe}_3\text{O}_4/\text{MB}$ and the anionic CR dye, which increased biosorption efficiency (Fig. 3). In contrast, at pH values above 4.8, the $\text{Fe}_3\text{O}_4/\text{MB}$ surface becomes negatively charged, leading to a decrease in CR biosorption.

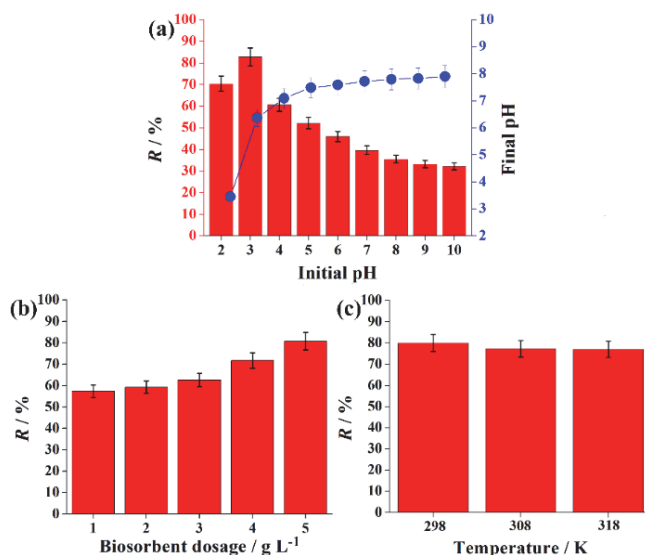


Fig. 2. The effects of the experimental parameters: a) initial pH (2–10) and final pH, b) $\text{Fe}_3\text{O}_4/\text{MB}$ dose (1–5 g L^{-1}) and c) T (298–318 K), on CR biosorption onto $\text{Fe}_3\text{O}_4/\text{MB}$.

The pH analysis of CR solutions before and after biosorption of CR with $\text{Fe}_3\text{O}_4/\text{MB}$ revealed a dependence on the initial acidity of the solution. Under acidic conditions, the final pH values increased relative to the initial pH, whereas in basic conditions, the pH remained largely unchanged, reaching a plateau. Notably, at the optimal pH 3, a pronounced change in pH was observed (from 3 to 6.37), corresponding to the highest CR removal. Hence, acidic conditions were more favorable for the biosorption process of CR onto $\text{Fe}_3\text{O}_4/\text{MB}$, which is in accordance with literature data.^{32,33}

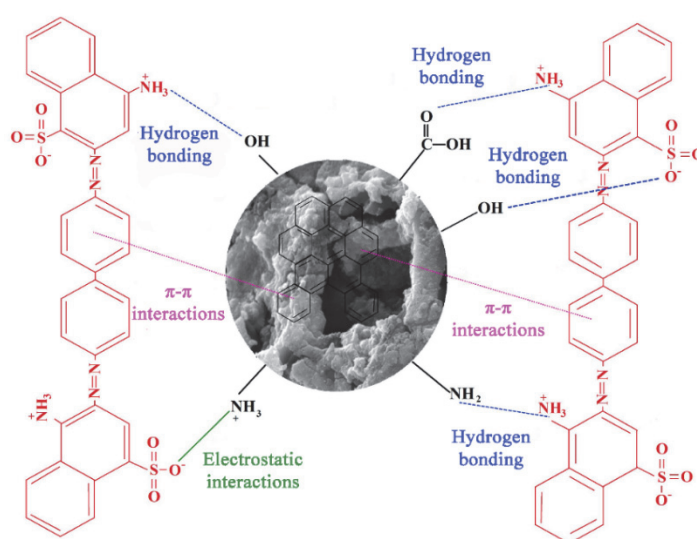


Fig. 3. Proposed biosorption mechanisms of CR biosorption onto $\text{Fe}_3\text{O}_4/\text{MB}$ at pH 3.

Effect of biosorbent dose

The effect of varying $\text{Fe}_3\text{O}_4/\text{MB}$ doses ($1\text{--}5\text{ g L}^{-1}$) on the removal efficiency of CR was investigated while keeping all other experimental parameters constant ($c_0 = 50\text{ ppm}$, pH 3, $T = 298\text{ K}$, contact time = 180 min, and shaking speed = 450 rpm). The results indicate that CR removal efficiency increased from 57.39 to 80.72 % as the $\text{Fe}_3\text{O}_4/\text{MB}$ dose was raised from 1 to 5 g L^{-1} (Fig. 2b). This is attributed to the greater availability of active sites on the $\text{Fe}_3\text{O}_4/\text{MB}$ surface at higher doses, which facilitates dye biosorption. Based on these results, an $\text{Fe}_3\text{O}_4/\text{MB}$ dose of 5 g L^{-1} was selected as the optimal dose for CR removal.

Biosorption thermodynamics

The thermodynamic parameters – Gibbs energy (ΔG°), enthalpy (ΔH°) and entropy (ΔS°) changes for the biosorption of CR onto $\text{Fe}_3\text{O}_4/\text{MB}$ were evaluated at three different temperatures (298, 308 and 318 K), while maintaining constant

experimental conditions ($c_0 = 50$ ppm, $\text{Fe}_3\text{O}_4/\text{MB}$ dose = 5 g L^{-1} , pH 3, contact time = 180 min, and shaking speed = 450 rpm). The reduction in CR uptake capacity of the $\text{Fe}_3\text{O}_4/\text{MB}$ at higher temperatures might be due to the weakening of interactions between the dye molecules and the active sites on the $\text{Fe}_3\text{O}_4/\text{MB}$ surface (Fig. 2c). The negative values of ΔG° (Table I), in the temperature range 298–318 K suggest that the biosorption of CR with $\text{Fe}_3\text{O}_4/\text{MB}$ is spontaneous. The negative values of ΔH° and ΔS° (Table I) indicate the exothermic nature of the biosorption process, associated with decreased randomness and disorder at the solid–liquid interface.³⁴ The thermodynamic parameters were determined using the Van't Hoff Eqs.:^{8,35}

$$\Delta G^\circ = -RT \ln K_d \quad (4)$$

$$K_d = \frac{Q_e}{c_e} \quad (5)$$

$$\log K_d = \frac{\Delta S^\circ}{2.303R} - \frac{\Delta H^\circ}{2.303RT} \quad (6)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (7)$$

where R is the ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), T is the Kelvin temperature of biosorption, K_d is the linear sorption distribution coefficient, Q_e (mg g^{-1}) is the equilibrium adsorption capacity representing the mass of CR adsorbed per unit mass of the biosorbent and c_e (mg L^{-1}) is the equilibrium concentration of the dye in solution.

TABLE I. Thermodynamic parameters for the biosorption of CR onto $\text{Fe}_3\text{O}_4/\text{MB}$

$\Delta H^\circ / \text{kJ mol}^{-1}$	$\Delta S^\circ / \text{J mol}^{-1} \text{ K}^{-1}$	$\Delta G^\circ / \text{kJ mol}^{-1}$		
		298 K	308 K	318 K
−6.97	−25	−0.56	−0.98	−1.06

Biosorption kinetics

The influence of contact time on the removal efficiency of CR by $\text{Fe}_3\text{O}_4/\text{MB}$ is illustrated in Fig. 4. The biosorption process demonstrated a relatively rapid uptake, reaching ~83 % removal within 120 min. Initially, the biosorption rate was high due to the abundance of available binding sites on the $\text{Fe}_3\text{O}_4/\text{MB}$ surface. Over time, the rate of CR biosorption decreased, suggesting saturation of the binding sites. For a better understanding of the biosorption mechanism, the experimental data were fitted to the linearized forms of four kinetic models: pseudo-first-order (PFO), pseudo-second-order (PSO), Elovich and intra-particle diffusion (IPD) model (Table S-I of the Supplementary material to this paper). According to the results (Table II; Fig. 5), the PSO model provided the best fit, with a correlation coefficient (R^2) of 0.99. Furthermore, the calculated equilibrium biosorption cap-

acity from the PSO model (8.68 mg g^{-1}) closely matched the experimentally determined value (Fig. 4; 8.36 mg g^{-1}), confirming the suitability of the PSO model for describing the biosorption kinetics of CR onto $\text{Fe}_3\text{O}_4/\text{MB}$.

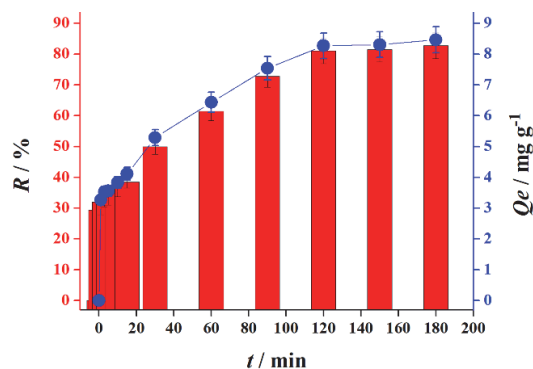


Fig. 4. Effect of contact time on the removal of CR onto $\text{Fe}_3\text{O}_4/\text{MB}$ ($c_0 = 50 \text{ ppm}$; $\text{Fe}_3\text{O}_4/\text{MB}$ dose = 5 g L^{-1} ; $T = 298 \text{ K}$; pH 3; shaking speed = 450 rpm).

TABLE II. Kinetic parameters of various models for the biosorption of CR onto $\text{Fe}_3\text{O}_4/\text{MB}$

Parameter	Value
$Q_e^{\text{exp}} / \text{mg g}^{-1}$	8.36
PFO	
$Q_e^{\text{cal}} / \text{mg g}^{-1}$	5.83
k_1 / min^{-1}	0.02
R^2	0.97
PSO	
$Q_e^{\text{cal}} / \text{mg g}^{-1}$	8.68
$k_2 / \text{g mg}^{-1} \text{min}^{-1}$	0.01
R^2	0.99
Elovich model	
$\alpha_E / \text{mg g}^{-1}$	7.20
$\beta_E / \text{g mg}^{-1}$	0.89
R^2	0.88
IPD model	
$k_3 / \text{g mg}^{-1} \text{min}^{-1}$	0.46
R^2	0.98

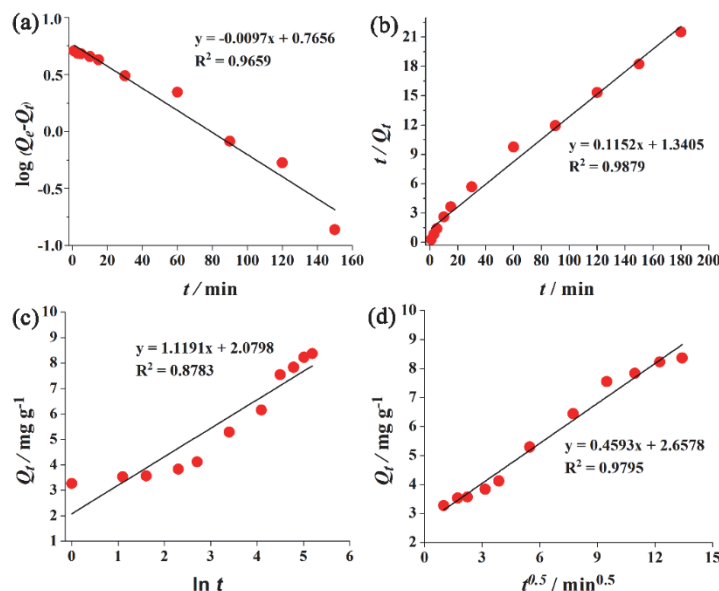


Fig. 5. Linear plots of kinetics models: (a) PFO, (b) PSO, (c) Elovich, and (d) IPD model, for the biosorption of CR onto $\text{Fe}_3\text{O}_4/\text{MB}$.

Biosorption isotherms

Fig. 6 illustrates the biosorption of CR onto $\text{Fe}_3\text{O}_4/\text{MB}$ at varying initial dye concentrations (10–300 ppm), using a constant $\text{Fe}_3\text{O}_4/\text{MB}$ dose of 5 g L^{-1} , with contact times ranging from 0 to 180 min, at 298 K and pH 3. The experiments were conducted under constant experimental conditions. The results demonstrate that increasing the initial CR concentration leads to an increase in the biosorption capacity of $\text{Fe}_3\text{O}_4/\text{MB}$. The experimental data were fitted to the Langmuir, Freundlich, Temkin, Elovich and Dubinin–Radushkevich isotherm models (Table S-II of the Supplementary material). The Langmuir model, which assumes monolayer adsorption on a homogeneous surface with uniform energy sites, showed the best fit, as indicated by a high coefficient of determination ($R^2 = 0.97$, Table III; Fig. 7). The theoretical maximum adsorption capacity calculated from the Langmuir model was 33.56 mg g^{-1} , which is close to the experimentally determined value of 29.64 mg g^{-1} . The Dubinin–Radushkevich model is important for determining of the adsorption energy (E) and underlying sorption mechanisms (physical or chemical), responsible for CR removal. The adsorption processes with E levels $< 8 \text{ kJ mol}^{-1}$ reflect physical, between 8 and 16 kJ mol^{-1} reflect combination of physical chemical and higher than 16 kJ mol^{-1} reflect chemical-based mechanisms.³⁶ The E values detected in this study $> 8 \text{ kJ/mol}$ ($11.28 \text{ kJ mol}^{-1}$) aligns with the energies for cooperative processes involving physical and chemical interactions (8–16 kJ

mol⁻¹), indicating that ion exchange is the dominant process of absorption of CR onto Fe₃O₄/MB,³⁷ which is consistent with other results.

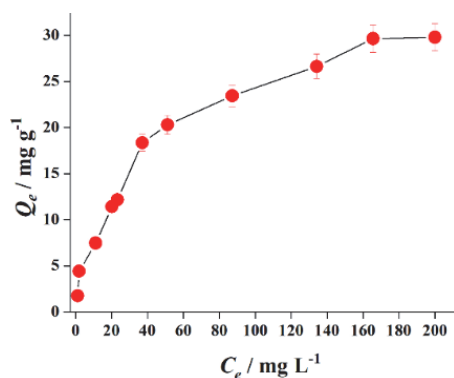


Fig. 6. Influence of the initial concentration of CR and contact time on the CR biosorption capacity onto Fe₃O₄/MB.

TABLE III. Parameters for various isotherm models of CR biosorption onto Fe₃O₄/MB

Parameter	Value
Langmuir model	
$Q_m / \text{mg g}^{-1}$	33.5
$K_L / \text{L mg}^{-1}$	0.03
R^2	0.97
Freundlich model	
n_F	1.89
$K_F / \text{L g}^{-1}$	2.26
R^2	0.96
Temkin model	
$A_T / \text{L mg}^{-1}$	0.73
R^2	0.91
Elovich model	
$Q_m / \text{mg g}^{-1}$	12.58
$K_E / \text{L g}^{-1}$	0.12
R^2	0.88
Dubinin–Radushkevich	
$K_{D-R} / \text{mol}^2 \text{kJ}^{-2}$	3.93×10^{-6}
$E / \text{kJ mol}^{-1}$	11.28
$Q_{D-R} / \text{mg g}^{-1}$	162.32
R^2	0.97

Comparison studies

The maximum biosorption capacity of Fe₃O₄/MB for CR was evaluated by comparison with other biosorbents reported in the literature (Table IV). Based on the Langmuir isotherm model, in this study, the calculated maximum capacity was 33.56 mg g⁻¹, indicating that Fe₃O₄/MB exhibits competitive efficiency and promising potential for application in the treatment of dye-contaminated wastewater.

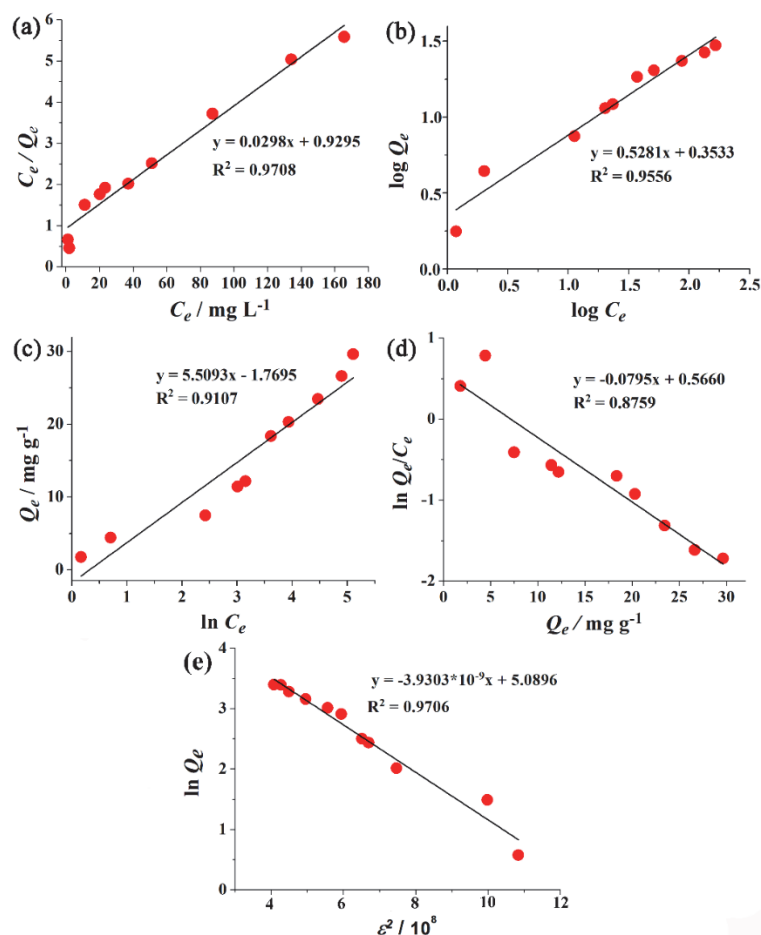


Fig. 7. Linear plots of isotherm models: a) Langmuir, b) Freundlich, c) Temkin, d) Elovich and e) Dubinin–Radushkevich model for the biosorption of CR onto $\text{Fe}_3\text{O}_4/\text{MB}$.

In addition to its notable performance, the application of $\text{Fe}_3\text{O}_4/\text{MB}$ has several advantages: it is a minimally modified, inexpensive and readily available biomaterial, making it attractive for practical applications and contributing to the reduction of the harmful impact of weeds on the environment and human health. Moreover, this biosorbent has not been previously analysed for CR removal and, to date, no studies have reported its application for this purpose in the available literature. Although these results are favourable compared to many low-cost and biomass-based biosorbents, it is important to note that the referenced data were obtained under different experimental conditions. Variations in initial dye concentration, pH, biosorbent dosage, contact time and temperature can significantly influence

the results. Therefore, to enable a more accurate comparison, further studies under standardized conditions are recommended.

TABLE IV. Comparison of CR removal using different biosorbents

Biosorbent	Conc. ppm	pH	Biosorbent dose, g L ⁻¹	Time min	Temp. K	Q _{max} mg g ⁻¹	Ref.
Fe ₃ O ₄ /MB	50	3	5	180	298	33.50	–
Garlic peel	25	5	7	15	298	15.22	38
Oyster shells	72.34	3.3	0.1	84.44	298	84.77	3
Cotton calyx–Fe ₃ O ₄	25	1–10	24	60	293	20.66	39
<i>Vangueria infausta</i> fruit pericarp	10	2	14	180	328	7.91	40
<i>Pleurotus mutilus</i>	50	3.5	1	150	300	36.68	41
Wood biomass	50	7	0.008	360	298	8.00	27
Modified egg shell membrane	100	4.5	10	180	298	117.65	42

Regeneration of Fe₃O₄/MB

A regeneration study of Fe₃O₄/MB was conducted using seven different desorption agents: 0.1 M HCl, 0.1 M NaOH, absolute EtOH, acetone, MeOH and 10 % NaCl:MeOH (volume ratio 3:7). The results, shown in Fig. 8a, indicate that 10 % NaCl:MeOH was the most effective desorbing agent after the first adsorption/desorption cycle, and it was used for all subsequent regeneration tests. As depicted in Fig. 8b, the desorption efficiency of CR decreased significantly from 98.32 to 46.0 % over five regeneration cycles. Several factors could explain the significant decline. Repeated exposure of the biosorbent to the CR likely causes physical or chemical degradation, which negatively impacts its performance across multiple adsorption/desorption cycles. Also, it is possible that the regeneration procedure used in this study isn't effective in achieving total removal of all CR molecules from the biosorbent surface, leading to fewer available active sites after each cycle. The Fe₃O₄/MB particles may have agglomerated during the biosorption process, reducing the overall biosorption capacity over time. To address these

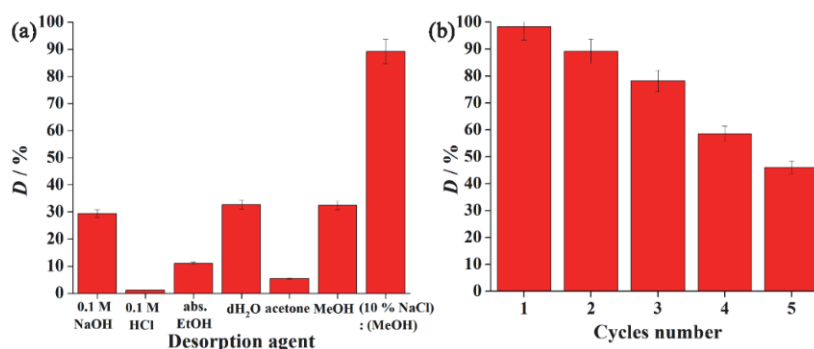


Fig. 8. A regeneration/reusability study of Fe₃O₄/MB: a) Examined CR desorption agents; b) number of CR desorption cycles.

challenges and improve the reusability of the $\text{Fe}_3\text{O}_4/\text{MB}$, it is essential to optimize the regeneration conditions. This involves identifying the ideal protocol that guarantees complete CR desorption while minimizing any possible interruption.⁹

Circular-economy approach

The circular economy is a production/consumption model that involves 4R's, including repairing, reusing, refurbishing and recycling of existing materials and bioproducts.⁴³ Adopting a circular economy approach in wastewater treatment emphasizes the reuse of natural, renewable resources to minimize waste and environmental impact. Utilizing biomass-based biosorbents such as $\text{Fe}_3\text{O}_4/\text{MB}$, which is derived from *A. artemisiifolia*, a widely distributed weed species whose collection incurs no cost, aligns with this strategy by transforming low-value plant material into an effective tool for pollutant removal. This not only reduces dependence on synthetic or energy-intensive adsorbents but also adds value to biological waste, thereby closing the loop between resource use and waste generation. Implementing such biosorption systems supports sustainable water management and contributes to the development of greener, more circular industrial practices.⁴⁴

CONCLUSION

This study demonstrated the strong potential of $\text{Fe}_3\text{O}_4/\text{MB}$, derived from *Ambrosia artemisiifolia* biomass, for the biosorption of Congo red (CR) in a batch system. Kinetic analysis confirmed that the PSO model best describes the biosorption process of CR, indicating that chemisorption is the rate-limiting step. Isotherm analysis revealed that the Langmuir model accurately fit the experimental data, suggesting monolayer adsorption of CR on the biosorbent surface. Under optimal experimental conditions ($c_0 = 50$ ppm; $\text{Fe}_3\text{O}_4/\text{MB}$ dose = 5 g L^{-1} ; $T = 298 \text{ K}$; $t = 180 \text{ min}$; pH 3), the maximum CR adsorption capacity was 33.56 mg g^{-1} . These findings indicate that $\text{Fe}_3\text{O}_4/\text{MB}$ is an economical, environmentally friendly and effective alternative for CR removal from wastewater.

Compared to our previous study, $\text{Fe}_3\text{O}_4/\text{MB}$ proved to be effective for the sorption of anionic dyes, but with a slight advantage for cationic dyes, as the biosorption efficiency (91.81 % for MG; 83.68 % for CR) was higher at shorter sorption times (60 min for MG; 180 min for CR). Both studies observed that the dye uptake using the same biosorbent was best fit to the Langmuir isotherm and the PSO kinetic model. Thermodynamic modelling revealed that both biosorption processes were spontaneous and exothermic. In summary, we presume that the CR and MG uptakes by the $\text{Fe}_3\text{O}_4/\text{MB}$ were achieved by a combination of electrostatic attraction, π - π electron-donor interaction and hydrogen bonds. Therefore, $\text{Fe}_3\text{O}_4/\text{MB}$ could serve as an alternative adsorbent/biosorbent for the treatment of contaminated water that contains different synthetic organic dyes. Future research will explore $\text{Fe}_3\text{O}_4/\text{MB}$ applicability for removing a broader range of contaminants

such as pesticides and pharmaceuticals, as well as scaling-up from laboratory to industrial applications. This will require a thorough assessment of technological feasibility and economic viability.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: <https://www.shd-pub.org.rs/index.php/JSCS/article/view/13530>, or from the corresponding author on request. This work is related to the implementation of the United Nations Sustainable Development Goal 6 – Clean water and sanitation.

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ИЗВОД

МАГНЕТИЧНИ БИОСОРБЕНТ НА БАЗИ БИОМАСЕ *Ambrosia artemisiifolia* ЗА УКЛАЊАЊЕ КОНГО ЦРВЕНЕ ИЗ ВОДЕ: ЦИРКУЛАРНО-ЕКОНОМСКИ ПРИСТУП

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Биосорбенти на бази биомасе показали су значајан потенцијал за уклањање синтетичких органских боја из воде. Ова студија заснива се на употреби магнетичног биосорбента (Fe₃O₄/MB) на бази биомасе *Ambrosia artemisiifolia*, као новог материјала за уклањање конго црвене (CR) боје из воденог раствора. Процес биосорпције је испитиван варирањем кључних параметара: pH, доза биосорбента, концентрација боје и температура, након чега су уследиле кинетичке и равнотежне анализе. Структурна својства Fe₃O₄/MB су окарактерисана Фуријеовом трансформационом инфрацрвеном спектроскопијом. Концентрација боје праћена је UV–Vis спектрофотометријом. Кинетичке анализе су показале да биосорпција боје прати PSO модел ($R^2 = 0,99$). Ленгмиров модел изотерми показао је најбоље слагање са експерименталних подацима ($R^2 = 0,97$), са максималним капацитетом адсорпције ($Q_{\max}$) од 33,56 mg g⁻¹. Примена Fe₃O₄/MB подржава одрживо управљање водама и доприноси развоју еколошки прихватљивих и циркуларних индустријских пракси.

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SUPPLEMENTARY MATERIAL TO

Magnetic *Ambrosia artemisiifolia*-based biosorbent for Congo red removal from water: A circular economy approach

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RESULTS AND DISCUSSION

Biosorption kinetics

Table S-I. Kinetic models applied to analyze CR biosorption with Fe₃O₄/MB.¹

Model	Equation	Plot	Parameter
Pseudo-first-order (PFO)	$\log(Q_e - Q_t) = \log(Q_e) - \frac{k_1}{2.303}t$	$\log(Q_e - Q_t)$ vs. t	Q_e, k_1
Pseudo-second-order (PSO)	$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{t}{Q_e}$	$\frac{t}{Q_t}$ vs. t	Q_e, k_2
Elovich	$Q_t = \frac{1}{\beta} \ln(\alpha\beta) + \frac{1}{\beta} \ln(t)$	Q_t vs. $\ln(t)$	α, β
Intra-particle diffusion (IPD)	$Q_t = k_3 t^{0.5} + C_{id}$	Q_t vs. $t^{0.5}$	k_3

where Q_e and Q_t (mg g⁻¹) are the adsorption capacity's at equilibrium and at time t , k_1 (min⁻¹) and k_2 (g mg⁻¹ min⁻¹) are the rate constant; α (mg g⁻¹) is the initial adsorption rate, β (g mg⁻¹) is related to the extent of surface coverage; k_3 (g mg⁻¹ min⁻¹) is intra-particle diffusion rate constant

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*Biosorption isotherms***Table S-II.** Isotherm models applied to analyze CR biosorption with Fe₃O₄/MB.^{2,3}

Model	Equation	Plot	Parameter
Langmuir	$\frac{C_e}{Q_e} = \frac{1}{k_L Q_{\max}} + \frac{C_e}{Q_{\max}}$	$\frac{C_e}{Q_e}$ vs. C_e	$Q_{\max}, k_L$
Freundlich	$\log Q_e = \log k_F + \frac{1}{n} \log C_e$	$\log Q_e$ vs. $\log C_e$	k_F, n
Temkin	$Q_e = \frac{RT}{b_T} \ln A_T + \frac{RT}{b_T} \ln C_e$	Q_e vs. $\ln C_e$	A_T, b_T
Elovich	$\ln \frac{Q_e}{C_e} = \ln K_E Q_m - \frac{Q_e}{Q_m}$	$\ln \frac{Q_e}{C_e}$ vs. Q_e	Q_m, K_E
Dubinin-Radushkevich	$\ln Q_e = \ln Q_{D-R} - K_{D-R} \epsilon^2$ $\epsilon = RT \ln \left[1 + \frac{1}{C_e} \right]$ $E = \left[\frac{1}{\sqrt{2K_{D-R}}} \right]$	$\ln Q_e$ vs. ϵ^2	Q_{D-R}, K_{D-R}

where Q_e (mg g⁻¹) and C_e (mg L⁻¹) are the adsorption capacity at equilibrium and adsorbate equilibrium concentration respectively; k_L (L mg⁻¹) is Langmuir constant; Q_m (mg g⁻¹) is the maximum monolayer adsorption capacity; n and k_F (L g⁻¹) are Freundlich constants related to adsorption and intensity of adsorption driving force or surface heterogeneity, respectively; A_T is Temkin constants (L mg⁻¹), T is the temperature (K), R is the gas constant (8.314 J mol⁻¹ K⁻¹); K_E (L g⁻¹) is Elovich equilibrium constant. Q_{D-R} is the Dubinin-Radushkevich maximum adsorption capacity (mg g⁻¹), K_{D-R} is the activity coefficient (mol² kJ⁻²), ϵ is the Polanyi potential, R is the gas constant (8.314 J mol⁻¹ K⁻¹), and E is adsorption energy (kJ mol⁻¹).

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Errata*

Article “Adsorption of copper ions onto acid-modified *Aframomum africanum* shell: isotherm and kinetic studies” by Yane Chimbilima, Murali Dadi and Tanweer Ahmad, printed in *J. Serb. Chem. Soc.* **91**(6) 383–392 (2026) (<https://doi.org/10.2298/JSC250603010C>), contained errors in the printed version. The wrong reference list was printed. Here is the correct list of references for that article:

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