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Design of experiments for the optimization of mefenamic acid microencapsulation in EC/HPMC cellulose derivatives and drug release improvement using *β*-cyclodextrin inclusion complex

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Abstract: Mefenamic acid (MA) is one of the low water soluble non-steroidal anti inflammatory drugs (NSAID), exhibiting poor wetting and poor dissolution. MA forms with β-cyclodextrin supramolecular inclusion complexes approving a drug solubility enhancement. The aim of the study is to combine the solubilization capability of a supramolecule MA:β-CD with the complementary functional properties of Ethylcellulose (EC) and hydroxylpropylmethylcellulose (HPMC) hybrid coat for the development of new MA controlled drug delivery systems, by using emulsion-solvent evaporation technique. First, a 2^2 factorial design is drawn to prepare and optimize pure MA/HPMC:EC microspheres, by studying and evaluating the effect of HPMC:EC ratio (1:1 and 1:4) and stirring speed of emulsion (600 and 1000 rpm) on the drug entrapment. The results showed that the main effect of variables is statistically significant, the drug entrapment was improved using HPMC and varied from 20 to 39%. Second, MA:β-CD inclusion complex was prepared by solvent co-evaporation method and subsequently encapsulated into EC and EC/HPMC hybrid matrices. The *in vitro* drug dissolution tests were performed in phosphate buffer solution with pH=7.4 at 37°C and the results showed that the drug release was effectively increased for MA:β-CD inclusion complex loaded microspheres.

Keywords: mefenamic acid; inclusion complex; β-cyclodextrin; DOE; microspheres.

INTRODUCTION

Mefenamic acid (MA) is a fenamate non-steroidal anti-inflammatory drug (NSAID) widely prescribed for the treatment of pains, inflammations, and primary

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dysmenorrheal.[1] Despite its well-established therapeutic efficacy, its clinical performance following oral administration remains limited because of its extremely poor aqueous solubility and slow dissolution rate. In BCS (the Biopharmaceutics Classification System), MA is classified as a Class II drug, exhibiting low solubility and high permeability, in which dissolution is the rate-limiting step governing drug absorption.[2]Consequently, improving the solubility and dissolution of MA remains a major challenge in pharmaceutical formulation development.[2-4]

Numerous formulation strategies have been explored to overcome the poor aqueous solubility of MA, including particle size reduction, crystallization, self-emulsifying, solid dispersions, lipid-based delivery systems, polymeric carriers, and cyclodextrin complexation.[5-8]

β-Cyclodextrin (β-CD) is extensively investigated owing to its biocompatibility, commercial availability, and remarkable inclusion capability. Several studies have demonstrated that MA:β-CD inclusion complexes significantly enhance the aqueous solubility and dissolution rate of MA by reducing drug crystallinity, improving wettability, and promoting molecular dispersion within the cyclodextrin cavity.[9-12]

Although inclusion complexation effectively enhances drug solubility, the resulting complexes are generally obtained as fine powders exhibiting poor flowability and limited suitability for downstream pharmaceutical processing. So, microencapsulation is emerging as an attractive strategy to overcome these limitations by improving powder handling, protecting the inclusion complex, and ensuring a more homogeneous distribution of the drug within a polymeric matrix.[13]

The performance of polymeric microspheres is largely governed by the physicochemical properties of the selected polymers. Cellulose derivatives such as Ethylcellulose (EC) and hydroxypropyl methylcellulose (HPMC) are largely explored in microencapsulation processes.[14,15] Ethylcellulose (EC) is a hydrophobic polymer widely used because of its excellent film-forming ability, mechanical strength, and chemical stability. However, its hydrophobic nature may limit water penetration into the polymeric matrix. In contrast, hydroxypropyl methylcellulose (HPMC) is a hydrophilic polymer capable of rapidly hydrating, that facilitates water diffusion and enhances drug wettability. The combination of EC and HPMC has attracted considerable interest for developing controlled-release microspheres because it provides an appropriate balance between structural integrity and matrix hydration, resulting in improved encapsulation efficiency and drug release characteristics. Khoukhi*et al*. (2016) successfully incorporated a piroxicam/β-cyclodextrin inclusion complex into EC/HPMC-based microspheres, demonstrating the feasibility of combining cyclodextrin complexation with cellulose derivative matrices to achieve controlled drug release.[16] Likewise, Assas*et al*. (2019)[17]and Badaoui *et al*. (2022)[18] further confirmed the suitability of

EC/HPMC-based microspheres as efficient sustained drug delivery systems for Niflumic acid and Repaglinide respectively.

Although previous studies have independently investigated MA:β-cyclodextrin inclusion complexes to improve drug solubility[9,10] and MA-loaded polymeric microspheres using chitosan, alginate, Eudragit RS100 and ethylcellulose to obtain controlled release formulations.[19-21]

However, the encapsulation of pure MA or MA:β-CD inclusion complex within an EC/HPMC hybrid polymeric matrix has not yet been investigated. Therefore, the present study aimed to develop novel polymeric drug delivery systems by combining the solubilization capability of a preformed MA:β-CD supramolecule with the complementary functional properties of EC/HPMC hybrid matrix.

In one hand, a 2^2factorial design is drawn to optimize MA microspheres, by studying and evaluating the effect of HPMC:EC ratio and stirring speed of emulsion on the drug entrapment and microparticles' characteristics. The significant effects of variables are determined by modeling using Minitab-DOE software.

In the other hand, MA:β-CD inclusion complex was prepared by the solvent co-evaporation method and subsequently encapsulated using the solvent evaporation technique in EC and EC/HPMC hybrid matrices. In this case, the effects of both β-CD inclusion and HPMC incorporation on the drug release are investigated.

EXPERIMENTAL

Materials

Mefenamic acid was purchased from Sigma–Aldrich (Germany), ethylcellulose (EC) (10 mPa s) and hydroxypropylmethylcellulose (HPMC) were purchased from Fluka analytical (USA) and Sigma–Aldrich (USA), respectively. Hydrolyzed polyvinylalcohol (PVA) 87–89 % (MW = 13000–23000) was purchased from Aldrich Fine Chemicals (USA) and *β*-cyclodextrin (*β*-CD) from Sigma–Aldrich (USA). Dichloromethane (DCM) (≥ 99 of purity) and absolute ethanol (99 % of purity) were purchased from Riedel–de Haën (USA).

Preparation of solid complexMA:β-CD

Solid inclusion complex of MA and *β*-CD was prepared in the initial (1:1) molar ratio of (MA:*β*-CD) by the solvent co-evaporation method.[9, 16]

MA (0.36 g, 0.0015 mol) and *β*-CD (1.700 g, 0.0015 mol) were dissolved in ethanol (75 ml), and distilled water (100 ml), respectively and the obtained solutions were merged in a flask and stirred for 2 h at 50 °C. Solvents were then evaporated under vacuum (5.6–5.1 kPa) using rotavapor AGCH- 9230 Flawil type: 1000147175 (Buchi Labortechnik, Switzerland) and the obtained MA:*β*-CD complex was dried at 40 °C.

Preparation of MA loaded microspheres

Microspheres are prepared by the solvent evaporation process as reported in our previous paper.[16] Briefly, polymers (EC or mixture of HPMC:EC) and drug (pure MA or MA:*β*-CD inclusion complex) are co-dissolved in 32 g of a water-immiscible organic solvent

(dichloromethane, DCM), the Pol./DCM ratio is kept at 5% (w/w). The organic solution is poured into 250 g of deionized water as an external phase containing 0.74% of PVA (w/w). The resulting oil in water (O/W) emulsion is continuously agitated in a glass reactor (600 mL, $\emptyset$ = 80 mm) using a six-blade turbine impeller stirrer (blade length = 50 mm, blade width = 10 mm, type VELP stirrer DLS, 0– 2000 min−1) at a constant stirring speed (600 or 1000 rpm) and at room temperature, for 6 hours. In all experiments, the percentage of pure MA against polymer matrices (%MA/Pol.(w/w) was 30% (w/w). The solidified microspheres were collected by filtration, washed several times with de-ionized water, and dried under vacuum in a desiccator containing $CaCl_2$ for at least 48 hours.

The drug microencapsulation optimization in HPMC and EC matrices is conducted using 2^2 factorial design, where the variables are HPMC:EC ratio and stirring speed of emulsion as indicated in Table I.

The effect of β-CD complex inclusion on the drug release is studied from other lots of microspheres which were prepared at 600 rpm and where the corresponding compositions are summarized in Table II.

TABLE I. 2^2 factorial design for MA microencapsulation optimization in HPMC:EC matrices

Lot	Variables			
	HPMC:EC ratio	Level	Stirring speed /rpm	Level
Lot a	1:4	-1	600	-1
Lot b	1:4	-1	1000	+1
Lot c	1:1	+1	600	-1
Lot d	1:1	+1	1000	+1

TABLE II. Composition of MA and β-CD:MA microspheres prepared at 600rpm of stirring speed.

Lot	HPMC mass /g	EC mass/g	MA mass/g	MA:β-CDcomplex mass/g
L1	-	1.6	0.48	-
L2	0.32	1.28	0.48	-
L3	0.32	1.28	-	1.752
L4	0.32	1.28	0.24	0.876

Drug entrapment and encapsulation efficiency

The drug content or entrapment (DE%) was determined by extraction in triplicate in ethanol, 5 mg of dried sample (microparticles or complex) were soaked in 20 mL of absolute ethanol under stirring in a corked bottle for 4h. The resulting solution was analysed by UV spectroscopy (Shimadzu UV-2401PC, Shimadzu, Japan); at the wavelength (λmax) of 284 nm where the molar extinction coefficient (ε) was equal to 81000 L mol^{-1} cm^{-1}. The drug entrapment and the encapsulation efficiency (EE%) were calculated from the following equations:

$$Drugentrapment(DE)\% = \left(\frac{m_{ofdrugextract}}{m_{particlesorcomplex}} \times 100\right) \quad (1)$$

$$Entrapmentefficiency(EE)\% = \left(\frac{m_{Actualdrug}}{m_{initialdrug}} \times 100\right) \quad (2)$$

Characterization of β-CD:MA inclusion complex and microspheres

Fourier transform infrared spectroscopy (FTIR)

The infra-red spectra of MA, *β*-CD, and MA:*β*-CD inclusion complex were recorded by FTIR spectroscopy (ATR ALPHA FT-IR spectrometer) (Bruker, Germany), the sample in its powder form was scanned for the wave number range of 4000–375cm^{-1}.

To confirm the effective MA encapsulation, microspheres and complex were characterized by infrared spectroscopy (ATR ALPHA FT-IR spectrometer) (Bruker, Germany). The samples in the powder form were scanned in the wave number range of 4000–400cm^{-1}.

X-ray powder diffraction

The infrared spectra and X-ray diffractograms of pure MA and *β*-CD were compared to those of the MA:β-CD complex.

To observe the physical state of drug in microspheres and the inclusion complex, the X-ray powder diffraction patterns were carried out at room temperature (25°C) using copper radiation on a Bruker D8 DISCOVER diffractometer (Germany) over the 2θ range of 1°to 40° or 60°, at the scanning rate of 0.020° min^{-1}. The X-ray diffractograms of pure MA, drug carriers were compared.

Particle size and morphology analysis

The mean diameters in micrometer and size distribution of microparticles (δ) were calculated from the results of optical microscopy (Optikam B1, Optika, Italy), by counting more than 500 microparticles using appropriate lenses. Each batch was analyzed in duplicate. The mean diameters were calculated from the following equations[22]:

$$d_{10} = \frac{\sum n_i d_i}{\sum n_i} \quad (3)$$

$$d_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (4)$$

$$d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (5)$$

$$\delta = \frac{d_{43}}{d_{10}} \quad (6)$$

Where di is the particle diameter, ni is the number of particles with diameter di; d_{10} is the average number diameter, d_{32} is the mean diameter of Sauter, and d_{43} is the volume weighted mean diameter.

The surface morphology of microspheres prepared with EC and/or HPMC, loaded either with pure MA or its complex, was examined using a scanning electron microscope (SEM, ZEISS EVO-15, Germany). The drug-loaded microspheres were mounted on a metal stub covered with a double-sided carbon adhesive label and then sputter-coated with a thin conductive gold–palladium layer in the nanometer range. The analysis was carried out under vacuum at an accelerating voltage of 20.

In vitro drug release study

The drug release kinetic experiments were carried out in duplicate. An amount of microspheres equivalent to 25 mg of MA is transferred into an appropriate dissolution reactor containing 900 mL of a buffered solution at pH = 7.4 ± 0.1, and plunged in a bath regulated at 37 ± 0.5°C, under a stirring rate of 250 rpm.

At selected time intervals, 3mL of sample is withdrawn and analyzed by UV spectroscopy (Shimadzu UV-2401 PC, Shimadzu, Japan); at the wavelength (λmax) of 385 nm, where ε=

9817 L mol^{-1} cm^{-1}. The taken volume is renewed by an equivalent volume of fresh dissolution fluid.

RESULTS AND DISCUSSION

Inclusion complex characterization

The results of inclusion of MA in β-CD by co-evaporation method showed that the drug entrapment was equal to 27.39% indicating a ratio of 1.77:1 of MA:*β*-CD. In fact, the results are getting closer to Sid *et al*. (2021) research[9] where it was demonstrated that the 2:1 ratio of MA:*β*-CD is the most stable inclusion complex.

The possible inclusion complex formation between β-cyclodextrin (β-CD) and MA can be confirmed through Fourier-transform infrared (FT-IR) spectroscopy and X-ray diffraction (XRD) analysis.

To elucidate the possible molecular interactions in the solid state, the FT-IR spectrum of the MA:*β*-CD inclusion complex was compared with the pure drug and *β*-CD spectra (Fig.1). Mefenamic acid exhibits characteristic absorption bands at 3307.17 cm^{-1} corresponding to N–H stretching, 1646.30 cm^{-1} for C=O stretching, 1574.22 cm^{-1} attributed to N–H bending, and 752.92 cm^{-1} associated with aromatic C–C vibrations indicative of ortho-substitution. Methyl group (–CH_3) vibrations are observed between 1470 and 1424 cm^{-1}, while the C–N stretching vibrations appear in the range of 1161–1254 cm^{-1}. Additionally, the band at 2915 cm^{-1} is assigned to the out-of-phase O–H stretching vibration.

FTIR spectrum of *β*-CD (Fig. 1) showed a prominent and large absorption band at 3263.12 cm^{-1}due to the O-H stretching vibration and C-H stretching vibration at 2924.52 cm^{-1}. The peaks appearing at 1020.32 cm^{-1} and 1151.95 cm^{-1} can be assigned to the stretching vibrations of C-OH and C-O-C glycosidic groups and the band at 757 cm^{-1} corresponded to pyranose ring deformation.[23]

The FT-IR analysis of the inclusion complex reveals a diminution of the MA bands intensities and minor shifts in the spectral peaks. Some characteristic peaks have been shifted, while others have disappeared altogether. For example, the bending and stretching vibrations of N-H bands initially observed at 1574.22 cm^{-1} and 3307.17 cm^{-1}in MA spectrum are now shifted in the complex spectrum to 1575.26 cm^{-1} and 3306.73 cm^{-1}, respectively, the C=O stretching band is shifted to 1647.04. Additionally, the characteristic peak of O-H bond in *β*-CD at 3263 cm^{-1} is completely absent in supramolecular complex, indicating a formal interactions between guest:host molecules.

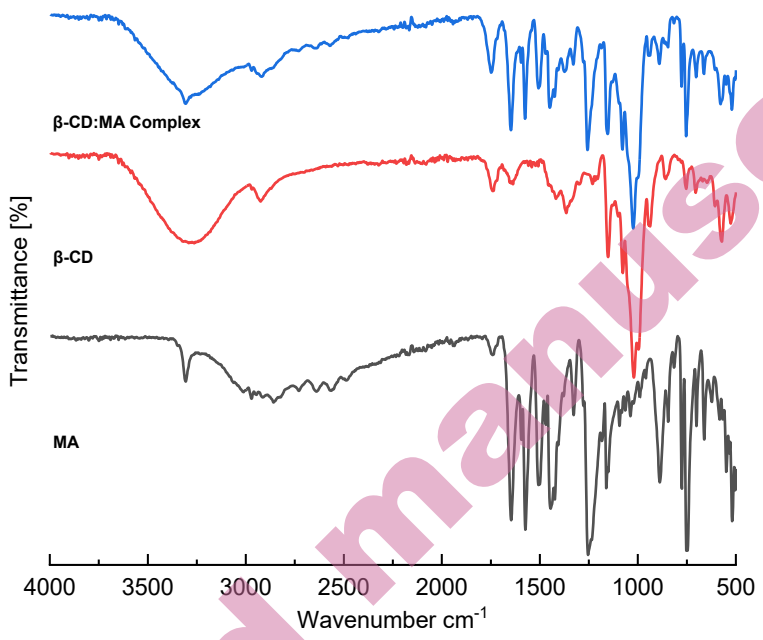

Fig 1. FTIR spectra of MA, β-CD and MA:β-CD inclusion complex

The X-ray diffraction analysis (Fig. 2) reveals a high degree of crystallinity for MA, β-CD and inclusion complex. Figure 2-a showed the presence of multiple diffraction peaks of MA indicating the crystalline nature of the drug. In fact, intense characteristic peaks of MA are observed at 6.4°, 16.0°,21.5°, 26.3°related to the form I polymorph of MA.[24]

The β-CD diffractogram displayed several sharp, distinct and intense peaks indicating the high crystalline nature of uncomplexed β-CD.[25]

In the XRD pattern of theMA:β-CD complex, almost of β-CD and MA peaks appeared but with low intensities. So, the loss of guest and host molecule crystallinity proves the formation of lower crystalline supramolecular structure.

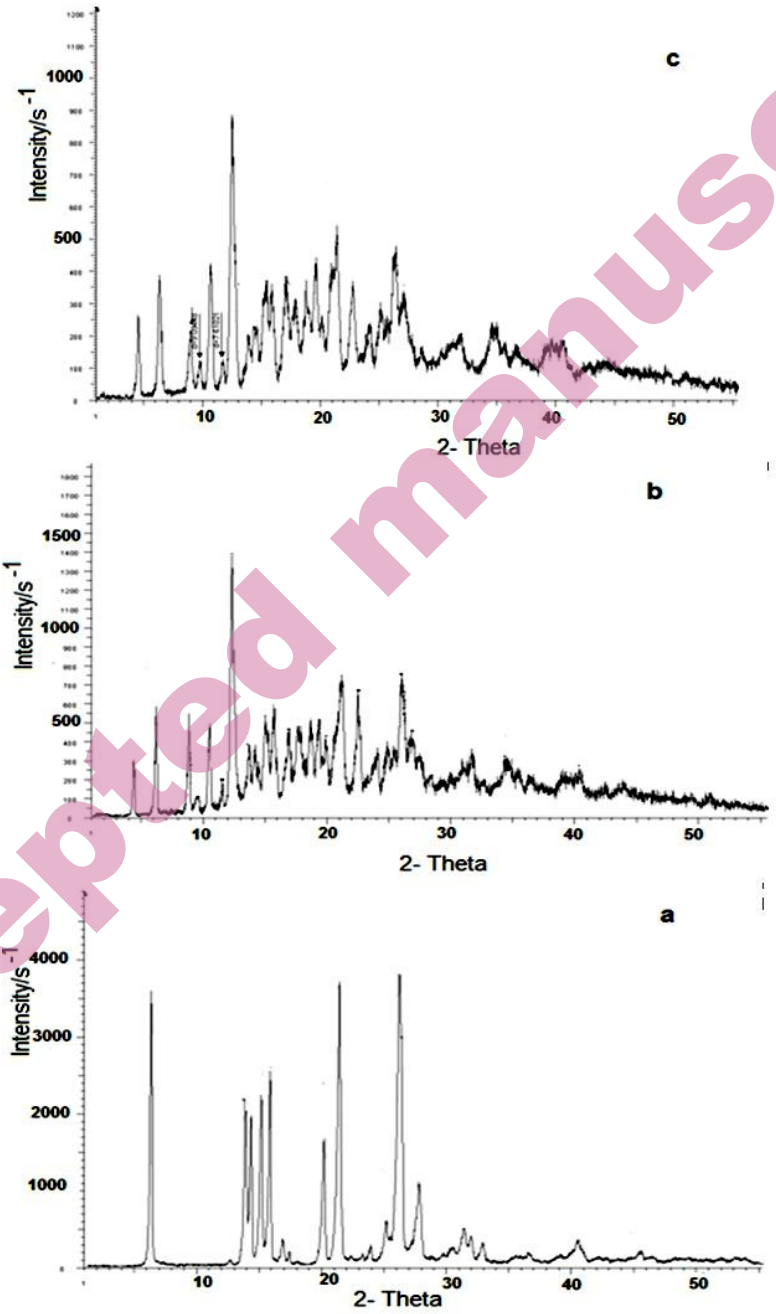

Fig 2. XRD patterns of a) MA, b) *β*-CD and c) MA:*β*-CD inclusion complex

Microparticles characterization

FTIR and XRD analysis

The IR spectra of different microparticle's formulations with polymers matrices and pure MA are given in Figure 3. The presence of some significant IR bands of drug is noticed in microspheres formulations spectra such as C=O stretching at 1646, C-N bending at 1254, and C-C aromatic vibration at 752cm^{-1}. This confirms the drug encapsulation and suggests the absence of any chemical interaction between the drug cellulose derivatives matrices since no new bands appeared.

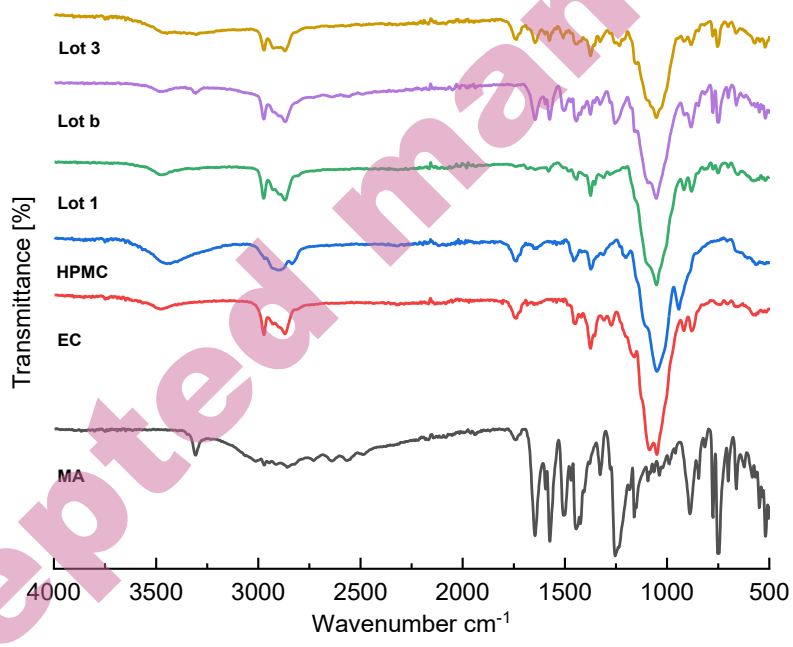

Fig 3. FTIR spectra of MA, EC, HPMC and microspheres lots (Lot1: composed of MA and EC, Lot b: composed of HPMC:EC and MA and Lot 3: composed of HPMC:EC and MA:β-CD inclusion complex)

The X-ray diffractogram of MA is compared to that of MAloaded microspheres (Fig4).It is noticeable that some characteristic peaks appropriated to MA appeared in X-ray diffraction patterns of microspheres but with a low intensity. The results indicated the diminution of the crystalline form of the drug in these formulations [26, 27]. This fact substantiates the enhancement of drug release (%) in various formulations. This amorphous state caused greater dissolution of drug from microspheres loaded with MA:β-CD complex than that of MA microspheres[28].

While, the X-ray diffraction patterns of microspheres showed typical broad diffraction peaks of cellulose at 2θ values in the range of 18°–23°, However, the EC sharp peak[29,30] at 2θ = 11° disappeared indicating the loss the corresponding crystalline zone of EC.

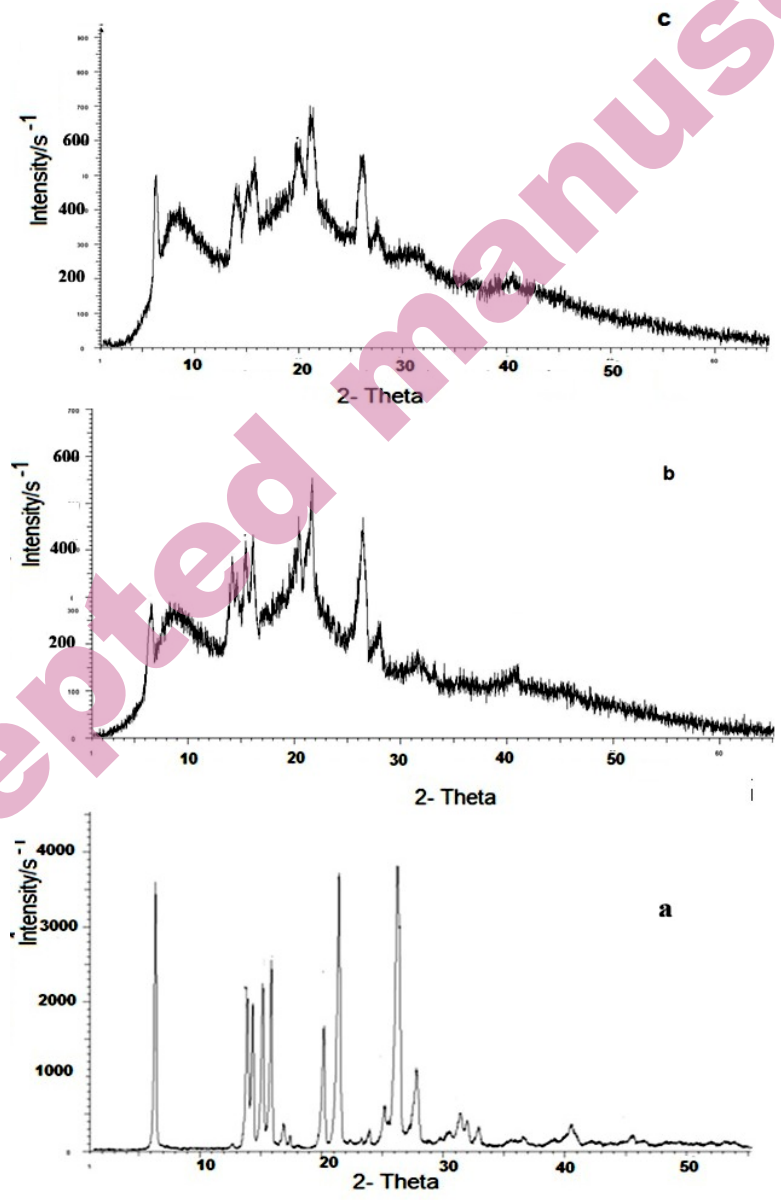

Fig 4. X-Ray diffractograms of (a) MA, (b) EC/MA microparticles (lot 1), and (c) EC/HPMC / (MA:β-CD microparticles (lot 3).

HPMC:EC microparticles optimization

In this section, an optimization of microencapsulation of pure MA in cellulose derivatives was conducted using design of experiments where two variables are chosen, namely HPMC:EC ratio and stirring speed of emulsion. The objective is to identify the significant effect of variables on the drug entrapment (DE%) using Minitab-DOE mathematical modeling. All data related to the 2^2 factorial design, are displayed in Table III.

TABLE III. Drug entrapment and HPMC:EC microspheres' size of 2^2 factorial design

Lot	Variables		Response in duplicate		DE%	d_{10}/µm	d_{32}/µm	d_{43}/µm	δ
	V1	V2	Y1: DE %		Mean±SD				
Lot a	-1	-1	31.48	30.52	31.00±0.48	68.8	77.1	80.0	1.16
Lot b	-1	+1	20.08	20.72	39.70±0.04	50.2	60.2	64.3	1.28
Lot c	+1	-1	39.66	39.75	20.40±0.32	54.8	71.0	77.6	1.42
Lot d	+1	+1	28.97	31.75	30.36±1.39	38.9	40.5	42.3	1.18

Coded value / Actual value	V_1: HPMC:EC ratio	V_2: Stirring speed of emulsion/rpm
-1	1:4	600
+1	1:1	1000

A statistical evaluation of the effects of the selected parameters is achieved using the factorial design and an interactive statistical first-order complete model (Equation7) is generated to identify statistically significant terms.

$$Y_i = a_0 + a_1V_1 + a_2V_2 + a_{12}V_1V_2 \quad (7)$$

where a_0 is the arithmetic mean response of 8 runs and a_i is the estimated coefficient for the factor V_i. The main effects of variables $V_1$and V_2representthe average result of changing one factor at a time from its low to high value (−1, +1). Though, the interaction betweenV1 and V2shows how the response (Yi)value changes when two factors are simultaneously changed.

The fitted equation related the response Yi=DE (%) to variables V1 and V2 is given by:

$$DE(\%) = 30.366 + 4.666V_1 - 4.986V_2 + 0.314V_1V_2 \quad (8)$$

R^2=98.80%, Adjusted R^2=97.90%

The result showed that the drug entrapment in these formulations varied from 20.4 to 39.7%. As shown in Figure 5a, a synergistic effect of V1 (HPMC:EC ratio) on the drug entrapment is observed meaning that DE% increased when HPMC mass increased; the same result was distinguished for the microencapsulation of niflumic acid.[18] However, an antagonist effect of V2 (stirring speed) on DE% is remarked (Fig 5a); increasing the stirring speed reduced the drug entrapment (DE); in fact when the stirring speed is higher, the drug transfer to the external phase becomes easier and then the drug entrapment decreased.[31]

The p value of both variables is <0.001 (p=0.000) indicating that the effects of the selected variables are significant. The p-value of the interactive effect V1V2 is 0.451 which is representative of a non-significant effect. In fact, the interactive plot (Fig5b), showed parallel lines indicating that the variables are independent and which is confirmed by the surface plot (Fig5c). The contour plot given in (Fig5d) can be useful for the prediction of response value or the suitable value of variables.

The number mean diameter d_{10} ranged from 38.9 to 68.8µm; it decreased when a stirring speed of emulsion increased which is in agreement with break-up theory and other research.[32-34] Also, the size dispersion (δ) ranged from 1.16 to 1.42 (Table III) demonstrating a narrow size distribution of microspheres.

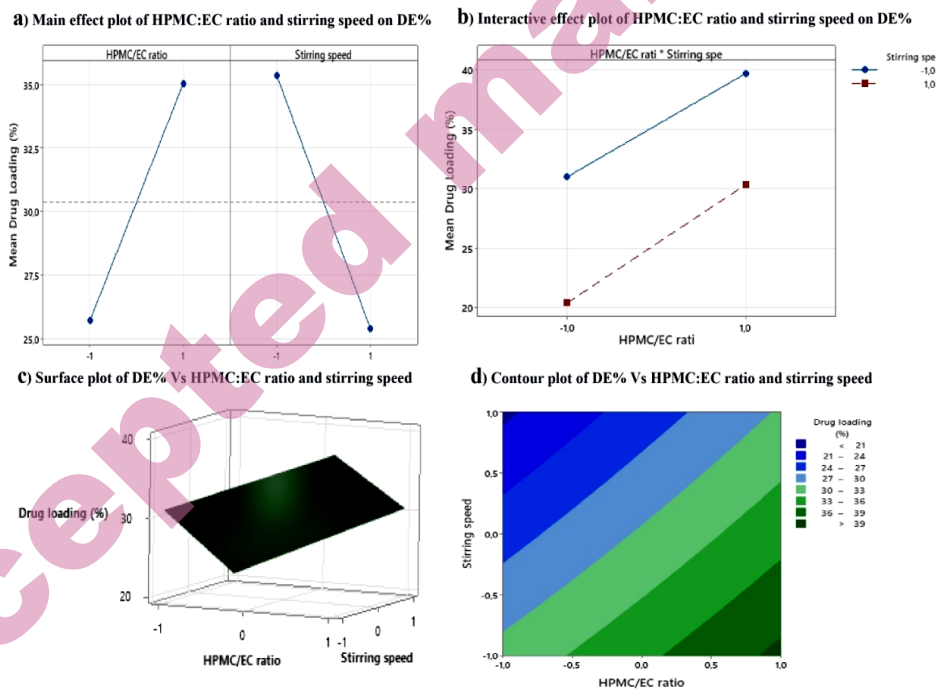

Fig 5. Graphical illustrations of DOE factoriel design: a) Main effect plot of HPMC:EC ratio and stirring speed on DE%, b) Interactive effect plot of HPMC:EC ratio and stirring speed on DE%, c) Surface plot of DE% VsHPMC:EC ratio and stirring speed, d) Contour plot of DE% VsHPMC:EC ratio and stirring speed

MA and MA:β-CD based microspheres

MA and MA:β-CD loaded microspheres are prepared at fixed stirring speed (600rpm) according to the composition given in Table II, in order to study the effect of matrices and drug form (pure and inclusion complex) on the

microspheres' characteristics and the drug release. The drug entrapment and encapsulation efficiency are provided in Table IV.

As reported, the content of MA in solid dispersion formula is found to be between 25 and 31 % and the EE% varied from 64 to 73 %. The drug loading and encapsulation efficiency are found to be dependent on the nature of the polymer and the drug formulation. Notably, the drug entrapment in the MA microspheres prepared with EC/HPMC combination (lot 2 and lot 4) was higher than those prepared using EC alone (lot 1). This can be attributed to the structural differences between types and solubility of polymer used in the formulation.[35]

However DE% decreased for microspheres based on the inclusion complex (lot 3). *β*-CD is fairly water soluble and can form water-soluble complexes with lipophilic guests that hide in the cavity of CDs.[36] So, the low DE% of the CD-containing microspheres can be caused by the water solubility of both HPMC and *β*-CD and their possible transfer to the external phase (water) during solvent evaporation.[16] In contrast, when the complex MA:β-CD was added to pure AM in formulation the encapsulation efficiency and drug content were improved.

Regarding the microspheres' size, depending on the microparticles composition, the number mean diameter (d10) of microparticle batches ranged between 68 and 108 µm. The results of the microparticle size showed that, the microparticles prepared with pure EC (lot1) exhibited a high mean diameter of 108 µm, whereas those prepared with EC/HPMC showed mean diameters ranging from 68 to 83 µm. An increase in ethyl cellulose concentration resulted in larger microsphere sizes. This can be attributed to the higher viscosity of the dispersed phase, which facilitates the coalescence of emulsified droplets.[37]

Moreover, the inclusion complex had no effect on the size of the microparticles, as indicated in the Table IV.

The size dispersion (δ) didn't exceed 1.42 demonstrating a relatively uniform and narrow microspheres' size distribution.

TABLE IV. L1-L4microspheres' characteristics and encapsulation results(%MA/Pol.= 30 %: w/w; %PVA/water=0.5%:w/w; %Pol./DCM=5%:w/w)

Lot (composition)	d_{10} (µm)	d_{32} (µm)	d_{43} (µm)	δ	DE % mean ± SD (n=3)	EE% mean ± SD (n=3)
1 (EC/MA)	108.2	137.2	150.2	1.39	28.15 ± 0.98	65.7 ± 2.31
2 (EC:HPMC/MA)	68.8	77.1	80	1.16	30.07 ± 1.37	65.15 ±2.98
3(EC:HPMC/ *β*-CD:MA)	80.3	102.8	113	1.42	25.09 ±1.48	64.3 ± 3.80
4 (EC:HPMC/MA/*β*-CD:MA)	86.3	114.3	125.4	1.2	31.67 ± 1.89	73.23 ± 4.38

Surface and morphology analysis were inspected by optical microscopy and scanning electron microscopy (Figures 6 and 7). The SEM and optical microscopy

micrographs showed that the prepared EC microspheres (Lot 1) were discrete and spherical, with a rough surface morphology characterized by small distinct pores. In contrast, microparticles containing HPMC or the *β*-CD/MA complex predominantly formed few aggregates with isolated microspheres, exhibiting an irregular, a rough and a highly porous surface (lot 2 and lot 3), which can improve wettability and consequently accelerate the dissolution rate of the active ingredient.

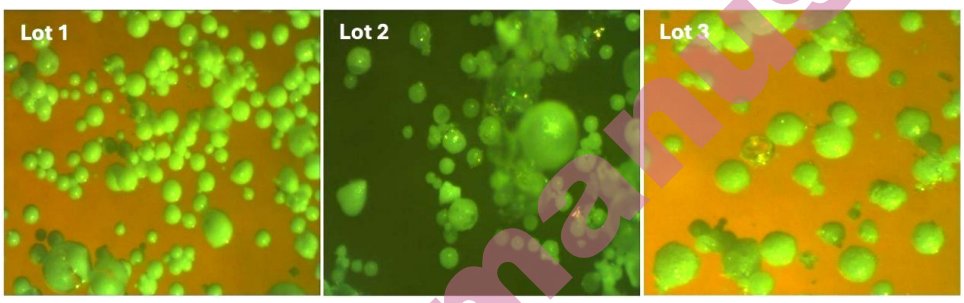

Fig 6. Optical microscopy micrographs of EC and EC/HPMC micropsheres (lots 1, 2 and 3)

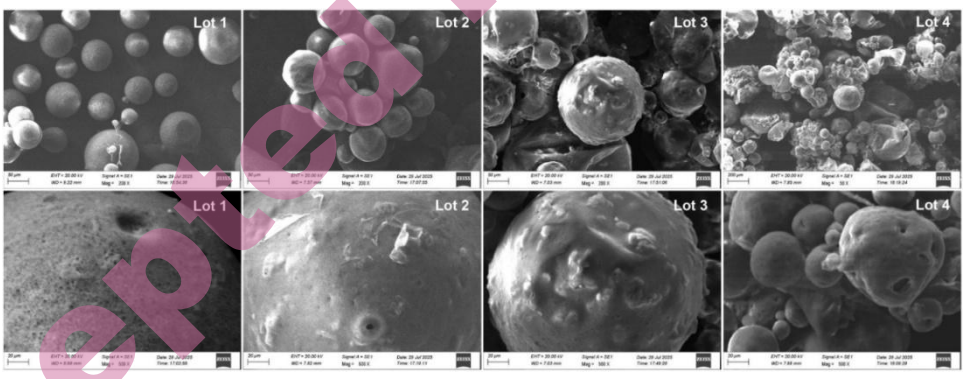

Fig 7. SEM micrographs of EC and EC/HPMC micropsheres batches (Lots 1-4)

Drug dissolution study

Dissolution behavior of MA in the microparticle samples was investigated in simulated intestinal medium at 37°C and pH=7.4. The drug release profiles are presented in Figure 8 and supported by drug release percentage values in Table V. The results showed that the *in-vitro* drug release from these formulations is influenced both by the nature of matrix (EC or HPMC:EC) and by the drug formulation.

As reported in literature,[6] the dissolution of mefenamic acid powder in phosphate buffer solution of pH=7.4 is very low, less than 5% dissolved in 1 hour. An increment in the dissolution from the MA:β-CD complex was well observed, as depicted in Fig.8, from the *β*MA:β-CD complex, 77% of drug was released after 1 hour with an initial burst release of 38%, and this fact proved that the higher

solubilization capability of substituted cyclodextrin favors the amorphous behavior of drug and dissolution.

A gradual and sustained drug release was observed regarding the MA loaded microspheres; the percentage release varied with the formulation compositions. In each formulation, ethyl cellulose was used as matrix base; the lot 1 is composed of only EC and pure MA whereas HPMC was included at 20% in the formulations of lot 2. The drug release profile of lot 1 showed a slow release; in fact, it is well shown that the microspheres prepared with ethyl cellulose alone showed sustained effect over an extended period due to its poor solubility, less permeability, and higher matrix thicknes.[38]

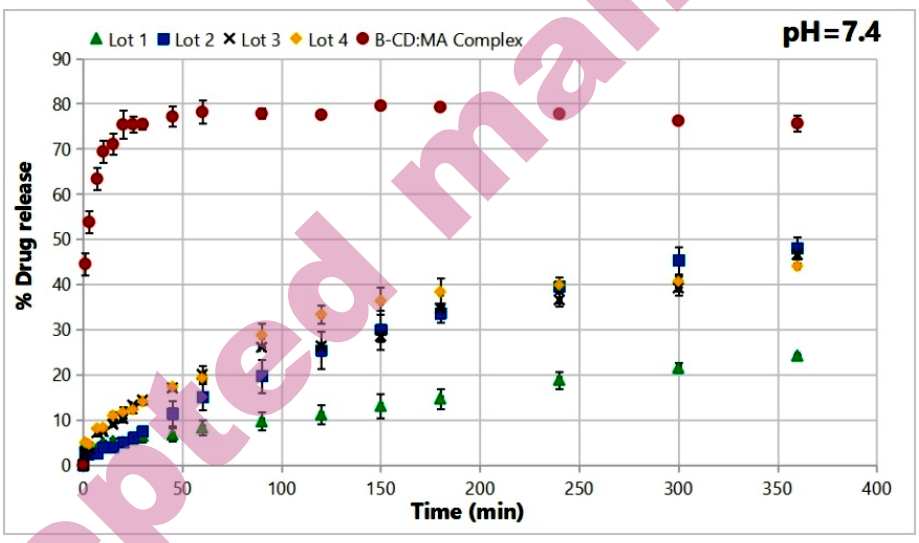

Fig 8. MA release profiles from MA:β-CD inclusion complex and microspheres (lot 1: EC/MA, lot 2: HPMC:EC/MA, lot 3: HPMC:EC/ MA:β-CD, lot4: HPMC:EC/MA/ MA:β-CD)

TABLE V. Percentage release data for MA measured at 30 min and 120, 360 and 1440 min from all formulation

Formulation	Time			
	30 min	120 min	360 min	1440 min
Lot 1	6.37± 1.3	11.10 ± 2.2	24.2 ±0.4	38.40 ±2.1
Lot 2	7.43 ± 1.1	25.39 ± 4.1	48.04 ± 2.4	78.9 ± 9.4
Lot 3	14.29 ± 0.5	26.28 ± 0.7	46.53 ± 1.8	59.59 ± 1
Lot 4	13.97 ± 0.2	33.31 ± 1.9	44.01 ± 0.1	60.39 ±0.6

Progressive and extended release profiles were obtained for lots 1-4. However, the addition of HPMC to EC in the MA incorporated microparticles improved the drug dissolution from 11% to 25% at 120 min by comparing lot 1 and lot 2 (Table

5). It's proved that the combination of HPMC and EC as a base matrix was shown to simultaneously enhance and control the release of poorly water soluble drugs[16]. This is due to the hydrophilic and hydrophobic properties of HPMC and EC.

Whereas, formulations based on HPMC:EC with inclusion complex (lot3) and inclusion complex +AM(lot4), exhibited an increase of dissolution from 26% to 33% after 2 hours of release (Table V). So, it is noted that the drug release rate was meaningfully increased when the microparticles were prepared with complex formulation comparing with pure drug. In fact; the drug dissolution was at once improved and controlled.[16,17]

In addition, the effect of particles size is also considered. It has been demonstrated that the size of microparticles influences the drug release rate or dissolution rate; smaller particles led to a fast drug release; this can be explained by the increase in surface contact that occurred and facilitated the drug release. The HPMC:EC microspheres are smaller than EC microspheres, therefore, an additional effect of size can also contributes in the drug release enhancing.

Release data analysis

The release mechanism of MA was identified by testing mathematical models in the earlier stage; 6 hours of release time. As detailed in paper by Paarakh *et al.*,[39] five models are explored for analysis, namely the zero order kinetic model which is related to a constant drug release independently of the concentration, first order describing a release mechanism where the release rate is concentration dependent, the Hixson Crowell cube root law exploring release from systems where there is a change in surface area anddiameter of particles, the Higuchi Fickian diffusion model of drug from insoluble matrix and Korsmeyer-Peppas model analyzing both Fickian and non-Fickian mechanisms of drug release from swelling as well as non-swelling polymeric delivery systems. The corresponding results are summarized in Table VI.

TABLE VI. Results of data analysis of drug release kinetics

Formulation	Korsmeyer-Peppas model			Higuchi		Hixson-Crowell		First order		Zeroorder	
	K_{KP}	n	r^2	K_H	r^2	K_{HC}	r^2	K_1	r^2	K	r^2
Lot 1	0.0136	0.461	0.964	1.203	0.971	2E-04	0.996	7E-04	0.997	6E-04	0.995
Lot 2	0.0051	0.801	0.992	0.993	0.993	6E-04	0.987	0.002	0.992	0.002	0.973
Lot 3	0.0244	0.501	0.991	2.382	0.990	5E-04	0.940	0.002	0.950	0.001	0.916
Lot 4	0.0273	0.493	0.984	2.465	0.964	5E-04	0.875	0.002	0.885	0.001	0.854

The drug release mechanism was evaluated based on the correlation coefficient values; the model exhibiting the highest r^2 value was considered the most suitable. The results showed that for lot 1 where slow drug release and concentration took place, the first order model is the most suitable model

describing the release mechanism, indicating that the release rate is concentration dependent, nevertheless, the regression coefficients of the other models are higher than 0.95; so a complex mechanism can be supposed in this case.

Regarding the lots 2, 3 and 4, the most appropriate kinetic models for the release of MA from these formulations were Higuchi and Korsmeyer–Peppas models indicating that the release mechanism of MA is governed by a diffusion phenomenon.

According to the results obtained from the Korsmeyer–Peppas model (Table VI), the values of the release exponent **n** range from approximately 0.493 to 0.801.The exponent value is $0.43<n<0.85$, so the release mechanism is considered as a non-Fickian diffusion or anomalous transport where both the diffusion and relaxation or erosion are involved.[39,40]

CONCLUSION

An integrated formulation strategy is expected in this research by combining the molecular solubilization capability of β-cyclodextrin with the hydrophilic properties of the HPMC matrix for the preparation of Mefenamic acid loaded microspheres, using emulsion-solvent evaporation technique. According to the factorial design analysis, HPMC:EC ratio and stirring speed had significant main effects on the drug entrapment; at a low level of stirring speed and a high level of HPMC:EC ratio, the DE exceeded 39%. SEM and optical microscopy showed spherical microspheres with a narrow size distribution and a number mean diameter (d10) ranging from 38.9 to 68.8µm and which increased to 108 µm when using EC alone and remained between 80 and 86 µm by the inclusion of MA:β-CD supramolecule. The FTIR, XRD characterization confirmed the effective drug encapsulation without chemical reaction. Also, the results showed that the microencapsulation of MA:β-CD supramolecule in HPMC:EC hybrid matrix improved the drug release. In addition, the formulation composed of both pure MA andMA:β-CD complex in HPMC:EC gave a high drug encapsulation with a high drug release where the mechanism is considered as a non-Fickian diffusion or anomalous transport according to Kosmeyer-Peppas model. Therefore, this formulation procedure represents a rational and innovative hybrid drug delivery platform for improving the pharmaceutical performance of mefenamic acid.

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И З В О Д

ДИЗАЈН ЕКСПЕРИМЕНАТА ЗА ОПТИМИЗАЦИЈУ МИКРОИНКАПСУЛАЦИЈЕ МЕФЕНАМИНСКЕ КИСЕЛИНЕ У ДЕРИВАТИМА ЦЕЛУЛОЗЕ EC/HPMC И ПОБОЉШАЊЕ ОСЛОБАЂАЊА ЛЕКА ПОМОЋУ ИНКЛУЗИОНОГ КОМПЛЕКСА СА β-ЦИКЛОДЕКСТРИНОМ

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Мефенаминска киселина (MA) је један од слабо растворљивих нестероидних антиинфламаторних лекова (NSAID), који показује лошу квашљивост и споро растварање. MA са β-циклодекстрином гради супрамолекулске инклузионе комплексе, што потврђује побољшање растворљивости лека. Циљ ове студије је комбиновање капацитета солватације супрамолекула MA:β-CD са комплементарним функционалним својствима хибридног омотача од етилцелулозе (EC) и хидроксипропилметилцелулозе (HPMC) за развој нових система за контролисано ослобађање мефенаминске киселине, коришћењем технике евапорације растварача из емулзије. Прво је примењен факторски дизајн 2^2 за припрему и оптимизацију микросфера чисте MA/HPMC:EC, проучавањем и евалуацијом утицаја односа HPMC:EC (1:1 и 1:4) и брзине мешања емулзије (600 и 1000 rpm) на инкапсулацију лека. Резултати су показали да је главни утицај променљивих статистички значајан; инкапсулација лека је побољшана коришћењем HPMC-а и варирала је од 20 до 39%. Друго, инклузиони комплекс MA:β-CD припремљен је методом коевапорације растварача и накнадно инкапсулиран у EC и EC/HPMC хибридне матрице. *In vitro* тестови растварања лека изведени су у фосфатном пуферу pH = 7,4 на 37 °C, а резултати су показали да је ослобађање лека ефективно повећано за микросфере пуњене инклузионим комплексом MA:β-CD.

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