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Chitosan/sodium dodecyl sulphate microcapsules preparation: Influence of applied method

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Abstract: This study aims to compare and analyse the synthesis of microcapsules stabilized with chitosan/sodium dodecyl sulphate complex according to emulsion preparation methods. For this purpose, 20 % oil-in-water emulsions were obtained in two ways: by emulsifying the oil phase in aqueous solution of chitosan and its mixtures with anionic surfactant ("method I") and by subsequently dropping chitosan into an already prepared emulsion stabilized by anionic surfactant ("method II"). Good stability, positive zeta potential of the emulsions and uniform droplet size distribution obtained with both methods, enabled the preparation of chitosan-based microcapsules which were separated by spray drying and investigated in terms of yield, moisture content, particle mean diameter and size distribution. The results showed a uniform particle size distribution and approximately equal mean diameters of emulsion droplets (≈ 8 μm), *i.e.*, suspension particles (≈ 5μm), while the microcapsule yields and moisture content for method I were 15 and 1.14 %, and for method II were 1.2 and 2.85 %, respectively. These results indicate that method I may be more suitable for use in the pharmaceutical and food industries for the production of chitosan microcapsules with oil content. The study confirmed that small variations in preparation can lead to large changes in the microencapsulation process and microcapsule structure.

Keywords: chitosan; sodium dodecyl sulfate; emulsion; microcapsules.

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INTRODUCTION

Microcapsule is a functional barrier between the core and the shell material to avoid chemical and physical reactions and to maintain the biological, functional and physicochemical properties of the core materials. Shell material properties determine the chemical and physical changes during capsule wall formation. Also, the shell material ultimately determines the choice of preparation method and the application direction of functional microcapsules.[1,2] Much attention has also been given to the use of chitosan, deacetylated chitin which is typically found in exoskeletons of arthropods and crustaceans and also in fungal cell walls, as polymer shell of the microcapsules. It's a polycationic polysaccharide compound that tends to interact with other substances, most often with the oppositely charged ones,[3–9] resulting in different morphology of novel microcapsules.[10] Since chitosan possesses a combination of properties such as biocompatibility, biodegradability, nontoxicity, as well as bioactivity: hypocholesterolemic, hypolipidemic, antimicrobial, antiviral, anticoagulant and antifungal, it has many applications in medicine, cosmetics and food industries.[11] Chitosan-based microcapsules have great potential for the development and applications of natural products that are widespread in the food, cosmetics and pharmaceutical industries.[1]

The capsule shell formation principle is based on the interaction between shell materials which include electrostatic interactions, covalent bonds and hydrogen bonds. Researchers have continuously explored a variety of preparation methods in the preparation process, such as the emulsification method, layer-by-layer (LBL) assembly method, sonochemical method, interface polymerization and spray drying method.[12–15]

Last couple of years, the most used microcapsule preparation and separation technique in the industry is spray drying. It consists of the rapid evaporation of the water phase from an emulsion by atomization with a pressure nozzle or a centrifugal wheel. The wide variety of materials that can be used, the simplicity of the procedure, its low cost and accessible equipment make spray drying the easiest and cheapest option to produce microencapsulated materials.[16] However, when using this method, great attention should be paid to choosing the shell material that could act as a barrier and protect the encapsulated bioactive compounds against oxygen, water, light and contact with other ingredients.[17] Furthermore, the characteristics of shell materials influence the controlled release of encapsulated bioactive compounds into the surrounding medium.[18,19]

Emulsions are colloid systems where one fluid is broken into small droplets within the other fluid and are often used in microencapsulation processes of liquid active components. The main problem occurring in emulsions is a phase separation that is due to flocculation and coalescence.[20] The emulsion stabilized by the polymer/surfactant complex is generally more stable than the one stabilized by surfactant only because it is stabilized not only by the electrostatic repulsion of the

droplets but also by the steric hindrance of the bulky oppositely charged polymer/surfactant complexes.[21] Emulsion stability, which is related to the size distribution of dispersed droplets and rheological properties of the continuous phase, can be expressed using the creaming index, where higher creaming index values indicate lower emulsion stability.[22] Also, achieving the emulsion droplets decrease, as well as attaining sufficiently high viscosity in the continuous phase contribute directly to emulsion stability and encapsulation efficiency of the core material at the subsequent drying process for microencapsulation.[23]

Methods for the preparation of emulsions used in the encapsulation process significantly affect the core stability, process efficiency and degree of protection of active components. Most of these methods involve preparing primary emulsion and then adding the polymer,[24–28] but this method may not be suitable for every polymer/surfactant or polymer/polymer system. To this end, based on the relevance of emulsion properties to efficient microencapsulation, this study compared and analysed the synthesis of microcapsules stabilized with chitosan/sodium dodecyl sulphate complex according to emulsion preparation methods.

EXPERIMENTAL

Materials

Low molecular weight chitosan (LMWCh) (product number: 448869) was obtained from Sigma–Aldrich. LMWCh degree of deacetylation, determined by potentiometric titration, is found to be 81.8 %.[29] Sodium dodecyl sulphate (SDS), purity >99 %, was purchased from Merck. Medium-chain triglycerides (caprylic/capric triglyceride, Saboderm TCC, Comcen, Zemun, Serbia) were used as oil phase. In all experiments buffered water was used as a solvent and pH was adjusted using 0.2 M water solution of acetic acid (Zorka-Pharma, Šabac, Serbia) and 0.2 M water solution of sodium acetate (Centrohem, Stara Pazova, Serbia). Aerosil 200, silicon dioxide, was provided by Carl Roth (Karlsruhe, Germany).

Preparation of solutions

The experiments were carried out at pH 4. pH was measured at room temperature using 827 lab pH-meter (Metrohm, Herisau, Switzerland). Stock solution of 0.2 mass % LMWCh was prepared by dissolving a given mass of polymer in the buffered water while stirring and after relaxation at room temperature during 24 h, pH value of the solution was checked. SDS stock solution of 1 mass % was prepared using the same procedure. LMWCh:SDS mixtures were prepared by mixing required volumes of LMWCh and SDS stock solutions. Before measurements, mixtures were left for 24 h at room temperature.

Preparation of oil-in-water emulsions

Oil-in-water (O/W) emulsions were prepared at the oil:water mass ratio 20:80 (20 % O/W emulsions) at 30 °C in two ways. The first method (“method I”) of preparation was the emulsification of semi-synthetic oil – Saboderm TCC in the aqueous phase by homogenizer Ultra Turrax T25. Solution of 0.1 % LMWCh, solutions of 0.001, 0.2 and 1 % SDS and mixtures of LMWCh:SDS at mass ratios 100:1, 1:2 and 1:10 were used as the aqueous phase of the emulsions. Before the start of emulsification, the aqueous phase was homogenized for 5 min at 5000 rpm by homogenizer Ultra Turrax T25, then during the first minute of emulsification the oil

phase was gradually added and the emulsification was continued for up to 10 min under the same conditions.

The second method ("method II") involved emulsion preparation by method I but with SDS solution as a water phase and then dropping a solution of chitosan into an already formed emulsion with SDS. This method was used to obtain a 20 % O/W emulsion with a mass ratio of LMWCh:SDS 1:2.

Spray drying procedure

In order to avoid agglomeration, 2 % of Aerosil was added to the emulsions. Mini spray dryer (Büchi 190, Flawil, Switzerland) with a standard 0.7 mm nozzle was used to convert the emulsions into microcapsules powders. The inlet temperature was kept at 160 °C and the outlet at 100 °C. The drying parameters during the process, such as aspiration (0.6 m^3/min) and feeding (2.2 ml/min) were controlled.

Zeta potential measurements

Zeta potential of emulsions was determined using Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Viscosity (0.88 mPas) and refractive index (1.33) of the solvent at room temperature were used for data analysis. All measurements were carried out in triplicate.

Droplet/particle size distribution

Droplet size distribution of the emulsions and particle size distribution of the suspensions of microcapsules in water was assessed by microphotography which was taken on optical microscope, Biooptica BEL-3000 (Milano, Italy) at 40× magnification and analyzed using BELView software. Droplet mean diameter as well as particle mean diameter, expressed as volume-surface mean value, d_{vs} (μm) and standard deviation σ (μm) were calculated from the experimental data, respectively:

$$d_{vs} = \sum n_i d_i^3 / \sum n_i d_i^2 \quad (1)$$

$$\sigma = (\sum n_i (d_i - d_{vs})^2 / \sum n_i)^{1/2} \quad (2)$$

where d_i is droplet/particle diameter (μm) and n_i is number of droplets/particles. Droplet/particle size distribution curves were obtained by fitting the experimental data with the gamma-equation:

$$Y_p = G x^m e^{(-ax)} \quad (3)$$

Emulsion stability test

For stability test, the emulsions were transferred into 10 ml graduated cylinders and stored at room temperature for 7 days. The emulsions were observed for the changes in homogeneity and phase separation during storage. The total height of the emulsion (H_0) and the height of the serum layer (H_s) were also measured over time. The extent of creaming was characterized by the creaming index, H:

$$H = 100 H_s / H_0 \ (\%) \quad (4)$$

The higher the creaming index the worse the emulsion stability. All experiments were carried out in triplicate and reported as average values plotted with standard deviation errors.

Yield analysis

The microcapsule powders collected from the processing equipment were stored in tightly closed dark container, at 4 °C, before further analysis. The product yield was calculated as the ratio between the mass of the output powders, recovered from the equipment at the end of the process, and the mass of the solid content of the initial emulsion, fed to the spray-dryer chamber.

Moisture content

The moisture content of microcapsules was determined gravimetrically by oven drying at 105 °C up to a constant weight.[30] One gram of powder was used and the moisture was expressed as a percentile value. All of the experiments were conducted in triplicate.

RESULTS AND DISCUSSION

The possibility of using chitosan (0.1 %) and SDS (0.001, 0.2 or 1 %) to stabilize the medium-chain triglycerides emulsion in water was first investigated. The appearance of the obtained emulsions 24 h after preparation is shown in Fig. 1.

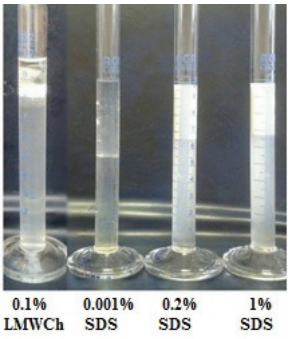

Fig. 1. O/W emulsions stabilized with LMWCh and SDS, 24 h after preparation.

The concentrations of SDS were selected based on the previous study[31] to cover the area before LMWCh/SDS coacervate formation (100:1), the coacervation area (1:2), as well as the area after coacervate formation (1:10), *i.e.*, 0.001, 0.2 and 1 % SDS, respectively. It can be noticed that emulsification properties of LMWCh and 0.001 % SDS are poor, after a few minutes there was a complete emulsion phase separation. This behavior of LMWCh is completely expected, due to its weak interface activity, as shown by our previous study.[29,31] Also, concentration of 0.001 % SDS is below critical micelles concentration of SDS[29] and insufficient for the formation of a stabile adsorption layer at the water-oil interface.

The stability of emulsions with 0.2 and 1 % SDS were monitored by visual observation of changes within the samples during 140 min of storage at room temperature and creaming index was determined. Results are presented in Fig. 2.

Concentrations of 0.2 and 1 % SDS were sufficient to provide a stable emulsion. In addition, the sample with 1 % SDS presented a slightly lower creaming index, suggesting that higher concentration of SDS provides a more stable emulsion. By observing the emulsions with 0.2 and 1 % SDS under a microscope (Fig. 2), immediately after preparation, it was noticed that they have spherical drops of different diameters. The mean droplet diameters were determined and they were 8.72±0.753 μm and 5.81±0.357 μm for emulsions with 0.2 and 1 % SDS, respectively.

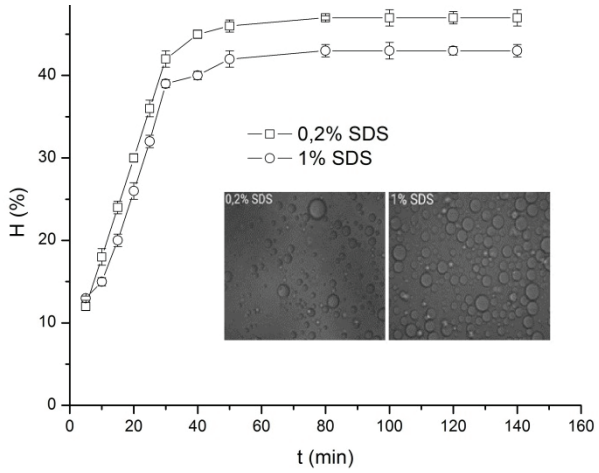

Fig. 2. Creaming index *H* and microphotograph of O/W emulsions stabilized by SDS.

It can be seen that the prepared emulsions stabilized with 0.2 and 1 % SDS have a droplet size below 9 μm and both emulsions had uniform droplet size distribution with low standard deviation. As expected, an emulsion with 1 % SDS has a lower value of mean droplet diameter than one with 0.2 % SDS, *i.e.*, as the concentration SDS increases, the droplet size decreases. This is ascribed to the enhanced water solubility of the oil molecules induced by the addition of ionic surfactants.[32]

To investigate the possibility of using LMWCh/SDS complexes for emulsion stabilization by method I, 20 % medium-length triglyceride emulsions in water were prepared which included a continuous phase with LMWCh:SDS solution mixture, and their appearance is shown in Fig. 3.

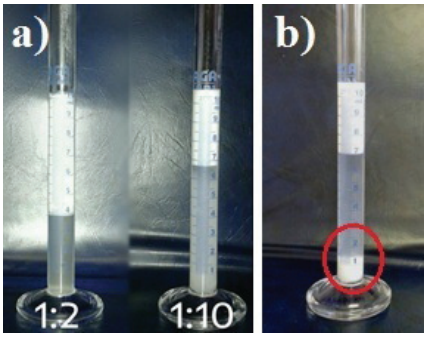

Fig. 3. Appearance of: a) O/W emulsions stabilized by LMWCh/SDS complex of different mass ratio 24 h after preparation; b) O/W emulsion stabilized by LMWCh/SDS complex in mass ratio of 1:10, 7 days after preparation.

Fig. 3a shows that stratification of emulsion to the cream and serum layer occurs due to the difference in density between the continuous and dispersed phase. Comparing the visual transparency of serum layers, it is observed that serum layer of the emulsion stabilized by LMWCh/SDS complex in mass ratio 1:10 was more

turbid and had higher creaming index than the other one. The creaming index of emulsions with LMWCh/SDS complexes in mass ratios 1:2 and 1:10 was monitored within 4 h and results are shown in Fig. 4.

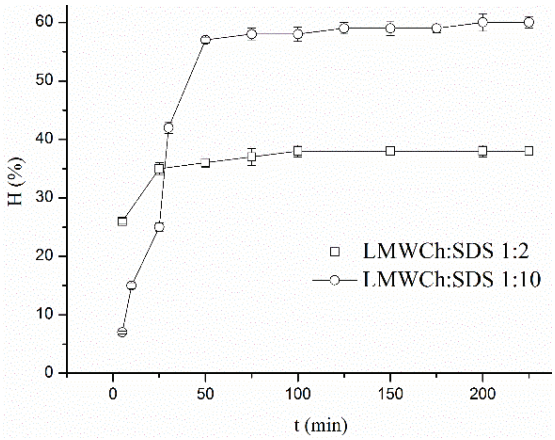

Fig. 4. Creaming index *H* of emulsions monitored within 4 h.

Changes in emulsion creaming index *H* with time (Fig. 4) indicate rapid separation of layers for emulsion stabilized with LMWCh/SDS complex in the mass ratio 1:10 during the first two hours of storage. Emulsion stabilized by LMWCh/SDS complex in the mass ratio 1:2 has slow separation of layers during the first hour of storage, which suggests better stability for this emulsion. After those times creaming index in booth emulsions remains almost unchanged.

Zeta potential of emulsion stabilized by LMWCh/SDS complex in mass ratio 1:2 was 22.3±4.05 μV and the droplets had a positive charge, while emulsion stabilized by LMWCh/SDS complex in mass ratio 1:10 had a negatively charged (–3.45±0.77 μV) which indicates that SDS molecules prevail in the adsorption layer. Based on our previous study,[29,31] the behavior of LMWCh/SDS complexes at interfaces and in the bulk was investigated in detail. When it comes to water solution the LMWCh/SDS mass ratio 1:2 complex is neutral and completely precipitated in the form of coacervates, while in emulsion containing the same complex, droplets bear positive charge. This phenomenon could be explained by share--induced reorganization in structure of formed complexes during emulsification process.[7,33] The reorganization during emulsification is a dynamic process where the emulsifier molecules adapt to changes in droplet size, phase interactions and surface coverage to stabilize the emulsion. In the context of emulsions, the polyelectrolyte complex can interact with both the oil phase and the aqueous phase, leading to a more complex behavior and potential changes. The reorganization of the polyelectrolyte complex at the interface during emulsification is critical to maintaining the stability and homogeneity of the emulsion.[34] Based on the general

knowledge polyelectrolyte complex interfacial behavior, two mechanisms are possible: the complex coacervates reorganize into a continuous protecting layer surrounding the oil droplets[35] or the coacervates adsorb on the oil droplet surface and stabilize the emulsion by a Pickering effect.[36,37]

As it can be seen from Fig. 3b emulsion stabilized by LMWCh/SDS complex in mass ratio 1:10 showed LMWCh/SDS coacervate sedimentation at the bottom of cylinder 7 days after preparation. Such findings along with negative charge of the droplets confirm that only SDS molecules are adsorbed at the interface.

Based on the above, for preparation of microcapsules with method II which is the usual method in literature,[24–26,28,38] 0.2 % SDS was selected for emulsion stabilization and LMWCh solution was dropped until LMWCh:SDS mass ratio of 1:2 was obtained.

Zeta potential of the emulsion prepared by method II was 17.1±3.29 μV, *i.e.*, the drops have a positive charge. The presence of a coacervate phase around the oil droplets (Fig. 5A and B) can be noticed in both emulsions, prepared by method I and method II, but their charge is not neutral. Positively charged emulsions have more advantages in the pharmaceutical and cosmetics industry due to potentially better bioavailability and biocompatibility.[39]

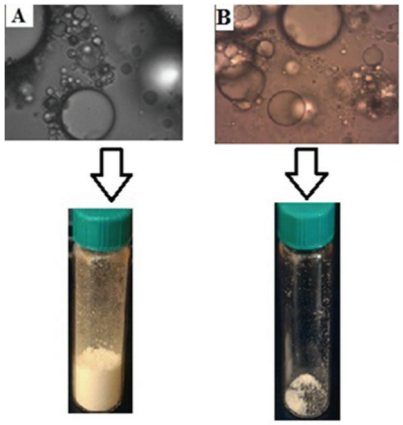

Fig. 5. Microphotograph of O/W emulsions stabilized by LMWCh/SDS complex in mass ratios 1:2 immediately after their preparation; A) method I and B) method II and obtained yield of microcapsules.

Microcapsules prepared by method I and method II and separated by spray drying were investigated in terms of yield, moisture content, particle mean diameter and size distribution.

Microcapsule production yield shows how much powder mass is produced after spray drying process and it is expressed by the percentage of core and shell materials present in the initial emulsion. Microcapsule yield was 15 % for microcapsules obtained by method I and 1.2 % for microcapsules obtained by method II. During the collection process after drying, the microcapsule powder obtained

in the method II was sticky and greasy probably due to the weak mechanical properties of their shell material.

Also, the low yield values obtained by method II are probably caused by the microcapsule preparation process itself. In the process of obtaining microcapsules by the method II it is most likely that stable coacervate did not form on the boundary surface, and it was partially formed in the continuous phase, as well. This is probably due to the weak affinity of LMWCh with SDS molecules at the boundary surface, which is a consequence of its weak surface activity, *i.e.*, before LMWCh reaches the boundary surface it binds to free SDS molecules in the continuous phase. For these reasons during the drying process the drops broke, which together with the coacervate from the continuous phase stuck to the walls of the drying chamber, *i.e.*, the powder adheres to the interior surface of the drying chamber due to high adhesive ability of LMWCh.[40]

The moisture content is an important variable related to the shelf life of powders and is often conducted to check on the spray-drying process. The moisture content of the microcapsules obtained by method I was 1.14 and 2.85 % for microcapsules obtained by method II. Its values are following the results from other studies.[30] This lower value for moisture content for microcapsules obtained by method I contributes to the powder stability during the storage and prevention of changes in physical and chemical characteristics.

For easier viewing, Table I shows a comparative analysis of the parameters tested for emulsions produced by both methods, microcapsules obtained from them by spry drying, as well as the mean diameters of re-dispersed microcapsules, which will be discussed below. The distribution parameters of the mean particle diameters of emulsion and re-dispersed microcapsules were obtained by processing microphotographs of their suspensions in water using BELview7 software. The particle size distribution curves of microcapsules that were obtained by fitting the experimental data with gamma equation are shown in Fig. 6.

TABLE I. Comparative analysis investigated parameters for both methods of emulsion preparation

Sample	Method I	Method II
Zeta potential of emulsion, μV	22.3±4.05	17.1±3.25
Particle mean diameter of emulsion, μm	8.72±0.753	7.32±1.052
Particle mean diameter suspensions of microcapsules, μm	5.12±0.372	5.70±0.819
Yield of microcapsules, %	15	1.2
Moisture content, %	1.14±0.275	2.85±0.652

As seen from the Table I and Fig 6, emulsions and suspensions of microcapsules were produced with mean diameters from 5.12 to 8.72 μm which is in accordance with the results of other studies.[41] Namely, after spray drying process all suspensions of microcapsules show slightly lower mean diameter and decreased

polydispersity compared to the starting emulsion. The spray drying process of emulsions affects the size and size distribution of the microcapsules, which is unsurprising taking into account that during drying process small droplets can be aspirated and large droplets can burst if the wall is not sufficiently resistant.[14] Also, both samples of microcapsules had uniform particle size distribution with low standard deviation. These results indicate equal properties of the microcapsule wall for both methods of their preparation. However, considering the appearance (sticky and oily), yield and moisture content of the microcapsule powders, it can be concluded that microcapsules are formed in smaller quantities when using method II. It is clear that yields of microcapsules and stability of the microcapsule shell as well as their characteristics were directly influenced by the emulsion preparation method. From all of the above method I can be considered better for the micro-encapsulation of oil in the system chitosan/sodium dodecyl sulphate.

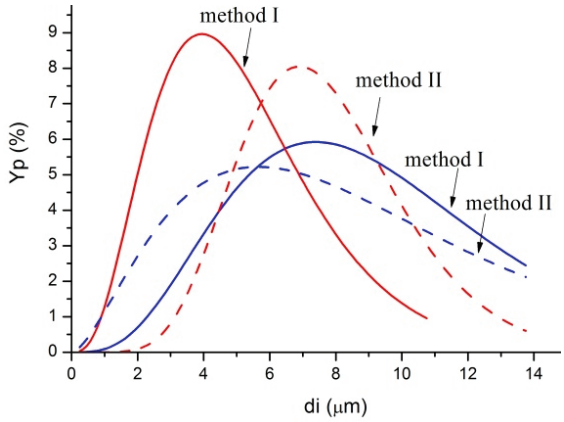

Fig. 6. Droplet and particle size distribution of the emulsions (blue) and suspensions (red) of microcapsules.

CONCLUSION

In this study, two preparation methods were proposed and demonstrated (method I: homogenization of oil in the already formed LMWCh/SDS complex and method II: subsequent dropping of chitosan into the already prepared emulsion with SDS) for chitosan-based microcapsules whose wall consisted of LMWCh/ /SDS complex. Microcapsules were obtained in powder form using spray drying as a method for their separation. Chitosan microcapsules were successfully prepared by both methods of preparation. For both methods, it was determined that the mean diameters suspensions of microcapsules have similar values (5.12±0.372 μm for method I, 5.70±0.819 μm for method II). However, the yield of the drying process was 15 % for microcapsules obtained by method I and 1.2 % for the method II of preparation, which indicates a small influence of the spray drying

method and a dominant influence of emulsions preparation method on formation stable or unstable shells of microcapsules. Also, a lower value of moisture content for microcapsules obtained by method I (1.14 %) could indicate their better stability during the storage by preventing changes in their physical and chemical characteristics. The results showed that method II, more commonly used in the literature for microencapsulation of oil in most polymer/surfactant and polymer/ /polymer systems, would not be proved to be the method of choice for the system chitosan/SDS. Therefore, method I gives a much higher yield of microcapsules and a lower moisture content than method II, which gives it an advantage in selection for obtaining oil-core microcapsules for the cosmetic and pharmaceutical industries. The study also confirmed that small variations in preparation can lead to large differences in the microcapsule structure.

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ИЗВОД

ПРИПРЕМА МИКРОКАПСУЛА ХИТОСАН/НАТРИЈУМ-ДОДЕЦИЛ-СУЛФАТ: УТИЦАЈ МЕТОДЕ ПРИПРЕМЕ

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Циљ овог рада је упоредна анализа микрокапсула стабилизованих комплексом хитозан/натријум-додецил-сулфат у зависности од методе припреме емулзија. У том циљу, 20 % емулзије уље у води добијене су на два начина: емулговањем уљне фазе у воденом раствору хитозана и његових смеша са анјонским сурфактантом („метод I") и накнадним укапавањем хитозана у већ припремљену емулзију стабилизовану помоћу анјонског сурфактанта („метод II"). Добра стабилност, позитиван зета потенцијал и уједначена расподела величина капи емулзија добијених обема методама, омогућили су припрему хитозанских микрокапсула које су издвојене сушењем распршивањем и којима је одређен принос, садржај влаге, средњи пречник и расподела величине честица. Резултати су показали уједначену распподелу честица по величини и приближно једнаке средње пречнике капи емулзија (≈ 8 μm), односно суспензија (≈ 5 μm), док су приноси микрокапсула и садржај влаге за метод I били 15 и 1,14 %, а за метод II 1,2 и 2,85 %, редом. Добијени резултати указују да метод I може бити погоднији за производњу хитозанских микрокапсула са уљним језгром у фармацеутској и прехрамбеној индустрији. Студија је показала и да мале варијације у припреми могу довести до великих промена у процесу микрокапсулирања и структури микрокпсула.

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