PREPARATION OF MICRO AND NANOSTRUCTURES OF CHITOSAN BY ULTRASONIC COALESCENCE OF W/O EMULSIONS

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Abstract
In the paper, the preeliminary study of chitosan micro and nanospheres precipitation due to two reverse emulsions (W/O) coalescence is described. The composition conditions, which must be filled to obtain stable reverse emulsion is pointed out. Furthermore, the parameters of ultrasonic emulsification and coalescence technique are presented and finally, morphological study of obtained micro and nanospheres are discussed.

Key words: *chitosan, microspheres, nanospheres, ultrasonic emulsification, precipitation.*

1. Introduction

Emulsions are compositions of two coexisting immiscible liquids called: water phase (W) and oil phase (O), which reveal macroscopic homogeneity and microscopic heterogeneity. Indispensible condition of emulsion appearance is the addition to initial mixture the known amount of surfactant (Surface Active Agent), and often even a cosurfactant [**1**].

The basic classification of emulsions reflects characteristics of both phases of the medium. Thus, there are oil in water emulsions (O/W) or water in oil emulsions (W/O, so called – reverse emulsions). Emulsions can be additionally classified regarding mean diameter of inner phase droplets (d) and stability of whole composition: macroemulsions (d > 500 nm; rather unstable), miniemulsions (500 nm < d > 10 nm, mean stability) and microemulsions (d < 10 nm, stability ~ hours, somethimes even months / years) [**2**].

Applicability of emulsions, especially miniemulsions and microemulsions, in fuel, cosmetic, pharmaceutic and agrochemic industries is uncontested. Emulsions, due to their extremely developed interface, are ideal substances for inorganic particles production as well as macromolecular synthesis conduction [**3]**.

Several methods of chitosan nanoparticles preparation has been established up to date, including coagulation of diluted chitosan solutions with NaOH, ionic gelation (polyelectrolyte complexation) with TPP, O/W emulsification with subsequent solvent diffusion or reverse micellization followed by droplet coallescence **[4]**.

Lately, a few papers on chitosan microparticles and nanoparticles preparation by means of W/O reverse emulsification / micellization techniques has been presented.

Indian research group obtained chitosan microparticles as a result of W/O emulsification followed by crosslinking and precipitation with KOH **[5]**. Hungarian authors also used a water-in-oil emulsion crosslinking method to produce chitosan microparticles. The chitosan inner phase droplets were solidified and hardened by glutaraldehyde crosslinking agent [**6**].

Recently, a complex process called spontaneous reverse emulsification was evaluated for chitosan nanoparticles production **[7]**. Moreover, S.M. Anto et al. from India proposed the W/O emulsion droplet coalescence technique to obtain chitosan nanoparticles as a drug carriers in cancer therapy **[8]**.

The aim of the presented preeliminary research was the optimisation of process parameters and conditions of ultrasonic emulsification with coalescence technique to obtain chitosan nanospheres and/or microspheres characterized by narrow size distribution.

Potential applications of chitosan nano/microspheres prepared by this method are: antimicrobial agent in cosmetics and novel carriers of drugs or therapeutic genes [**9, 10**].

2. Materials and Methods

2.1. Materials

Two separate aqueous phases were prapared:

- chitosan phase: 1.5% chitosan (Vanson 0171, DD=74.8%) in 0.9% lactic acid (POCH S.A., Poland, CAS no.: 598-82-3);
- coagulant phase: NaOH (POCH S.A., Poland, CAS no.: 1310-73-2) or sodium tripolyphosphate (TPP, Sigma-Aldrich, CAS no.: 7758-29-4) aqueous solutions.

Various nonpolar liquids were selected as an oil phase of emulsions, i.e. canola oil, olive oil, linen oil, isopropyl mirystate and liquid paraffin (medical grade).

Several chemical compounds were chosen to stabilise W/O emulsions. Among them, Volpo L3 (ethoxylated fatty alcohol, HLB=8.0, Croda Chemicals, UK), Brij O2 (PEG-2 oleyl ether, HLB=4.9, Croda Chemicals, UK) and Lecithin (mixture of triglycerides and glico/phospholipids, HLB=4.0, Cargill Poland) seemed to be appropriate surfactants. Moreover, isopropyl alcohol (cosurfactant) was added to pre-formed emulsions to obtain additional reduction of surface tension.

2.2. Emulsification and ultrasonic coalescence processes

Ultrasonic W/O emulsification followed by coalescence and chitosan precipitation is a complex process composed of several subsequent steps. ***Figure 1.*** presents schematic illustarion of chitosan nano- and microspheres preparation.

Firstly, two non-stable W/O emulsions were prepared by means of mechanical stirring (IKA RW14 basic, 600 RPM) of mixtures of water phase (chitosan or coagulant aqueous

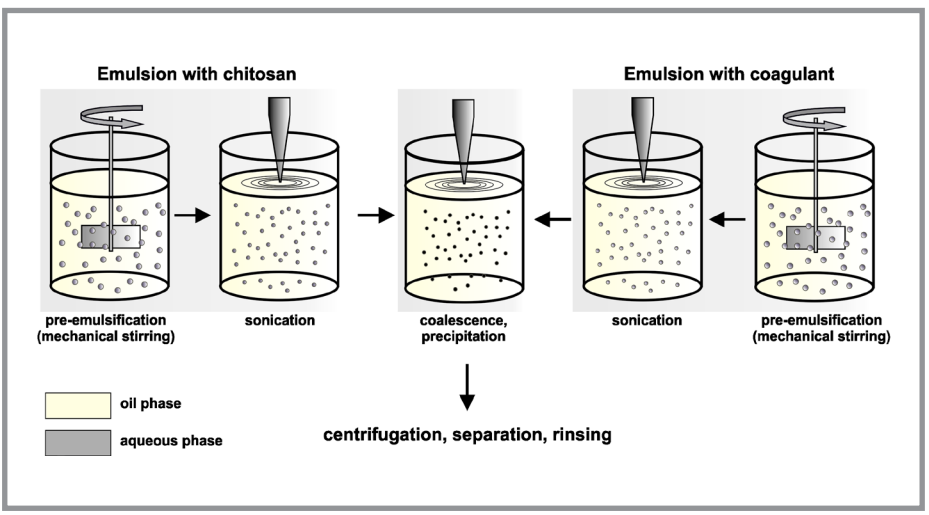

Figure 1. *Preparation process of nano- and microstructures of chitosan.*

solutions), oil phase and additives (surfactant, cosurfactant) This process was carried out at ambient temperature for 10 minutes. Next, both emulsions underwent sonication with Hielsher UP 200S probe, 24 kHz, Amplitude: 70 μm (time = 1 min). As a result of sonication process, stable miniemulsions appeared. Next, W/O miniemulsions of chitosan and coagulant were mixed together while being sonicated with the same ultrasounds frequency. This step lasted for 5 minutes. During this process, coalescence, precipitation and agglomeration of chitosan nano- and microspheres occurred. At last, obtained dispersion of agglomerates were centrifuged (MPW 340, 4200 RPM), and sedimented chitosan structures were collected and rinsed several times with distilled water.

2.3. Nano- and microstructures characterization

Chitosan nano- and microspheres morphology and sizes were observed by means of optical microscopy and scanning electron microscopy SEM (FEI Quanta 200).

3. Results and discussion

It is a challenging task to prepare a stable emulsion [**11**]. Complexity of two phase liquid systems results in a lot of experiments being conducted before the colloidal system appears.

In ***Figure 2.A.*** a common schematic pseudo-ternary phase diagram of emulsion systems is shown. Three characteristic areas can be distinguished:

- Winsor emulsions are heterogenic systems in which a sequence of equilibria between a few phases is set. They are mostly present at low surfactant concentration.
- O/W mini- and microemulsions are likely to be formed, when there is an excess of surfactants in relation to non-polar liquid.
- Finally, W/O mini- and mircoemulsions, also called reversed mini-/microemulsions, tend to appear in systems in which, there is an excess of surfactants in relation to an aqueous component.

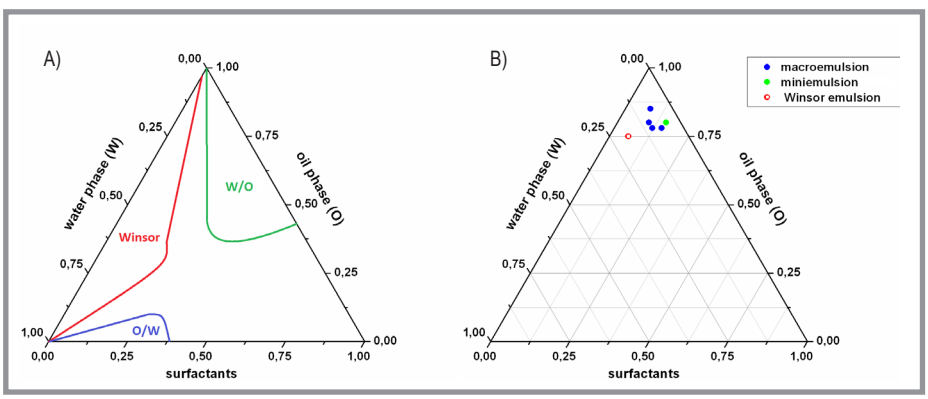

Figure 2. *Pseudo-ternary phase diagram of emulsion systems: A. Common diagram taken from literature [1]; B. Diagram with experimental data (for composition see Table 1)*

Table 1. *Chitosan emulsions composition: where: oil phase – liquid paraffin (medical grade); water phase – 1.5% chitosan solution; surfactants – Brij O2 with an addition of isopropanol (10% v/v).*

Emulsion no.	Oil phase (weight fraction)	Water phase (weight fraction)	Surfactants (weight fraction)	Emulsion system
1	0.780	0.070	0.150	Unstable macroemulsion
2	0.780	0.099	0.121	Unstable macroemulsion
3	0.800	0.100	0.100	Unstable macroemulsion
4	0.850	0.070	0.080	Unstable macroemulsion
5	**0.800**	**0.046**	**0.154**	**Stable miniemulsion**
6	0.750	0.190	0.060	Winsor emulsion

Figure 2.B. shows an pseudo-ternary phase diagram with experimental points, which represents different emulsion systems with chitosan obtained during research. Their composition is presented in ***Table 1.***

Furthermore, three specific emulsion systems are presented in ***Figure 3***. Only third picture (***Figure 3.C***) shows stable water-in-oil miniemulsion (Emulsion no. 5 in ***Table 1***). Miniemulsion is an opaque pseudo-homogenic liquid with inner phase droplets diameters in the range of dozens and hundreds of nanometer.

Stable chitosan miniemulsion and corresponding coagulant miniemulsion were mixed together under ultrasonic agitation (time of process = 3.5 min). During sonication, aqueous droplets of chitosan solution and coagulant collided, which in turn resulted in chitosan coagulation and precipitation in a form of nano- and microspheres.

A microphotograph of precipitated chitosan structures in miniemulsion is presented in ***Figure 4***. One can see individual microspheres as well as agglomerates of chitosan structures.

Chitosan precipitate was centrifuged and rinsed with distilled water. The process of nano- and microspheres purification and collection was repeated twice.

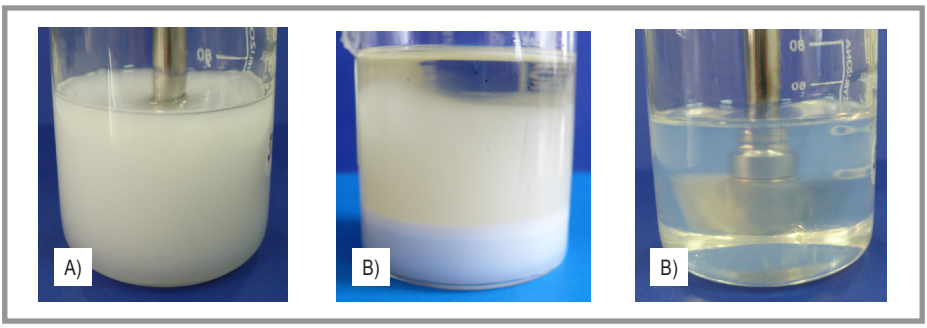

Figure 3. *Photographs of obtained emulsion systems: A. Macroemulsion; B. Winsor emulsion; C. Stable W/O microemulsion.*

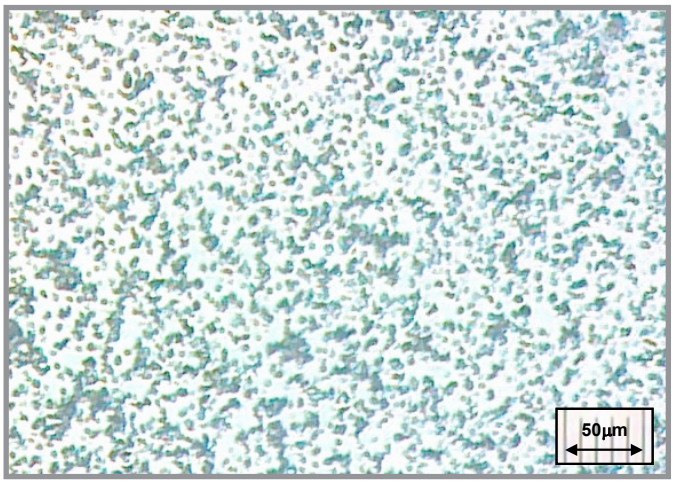

Figure 4. *Microphotograph of chitosan precipitate after W/O microemulsions coalescence.*

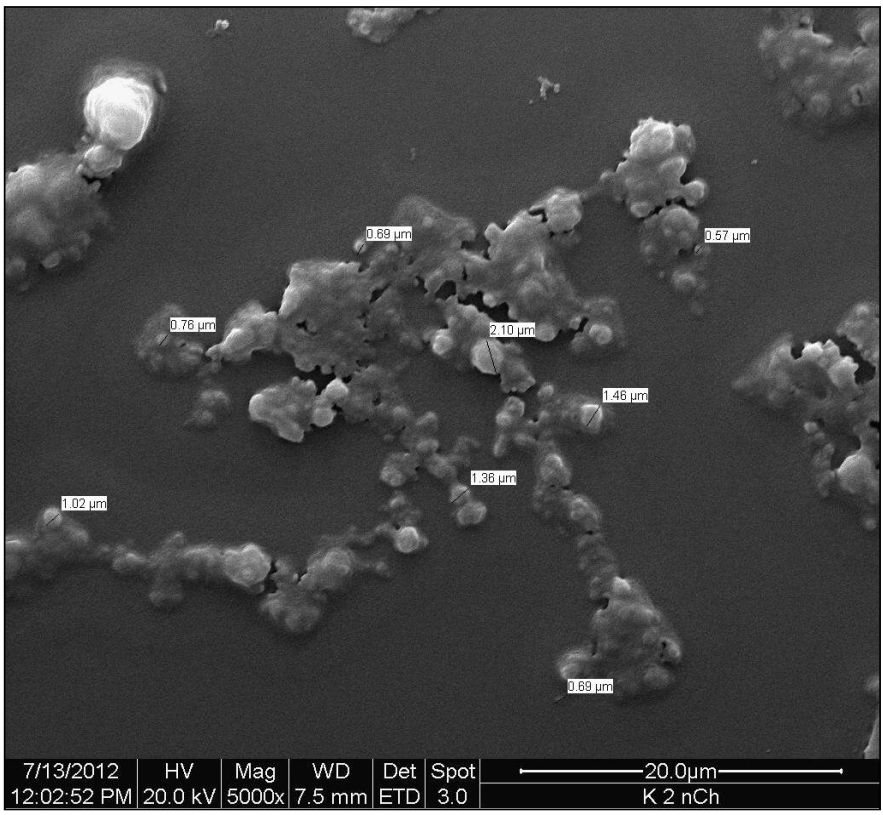

Figure 5. *SEM microphotograph of chitosan nano- and microspheres.*

Finally, chitosan structures were dispersed in distilled water once again. Then, the droplet of dispersion was put on a microscope glass and left to water evaporation at ambient temerature. After that, the sample underwent SEM analysis.

Figure 5 shows nano- and microspheres of chitosan in a form of agglomerates. Obtained structures are sphere-shaped and their sizes range from 500 nm up to dozens of micrometers.

4. Conclusions

Preeliminary study on nano- and microstructures of chitosan prepared by means of complex method of ultrasonic coalescence of W/O emulsions demonstrated, that spherical chitosan structures of diameters ranging from 0.5 μm up to dozens of micrometers can be successfully obtained. Water-in-oil stable opaque miniemulsions were prepared. However, during sonicated coalescence, random collisions of aqueous nanodroplets resulted in nano- and microspheres formation. Further studies should be focused on the improvement of process efficiency as well as control of coalescence phenomena, which will result in the narrowing of chitosan structures size distribution. Another key issue is to evaluate an influence of oil phase and surfactants composition on emulsion stability as well as chitosan particles size distribution.

5. References

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