



*Dedicated to Professor Ionel Haiduc  
on the occasion of his 75<sup>th</sup> anniversary*

## MICROPARTICLES OBTAINED BY BIOPOLYMER-SURFACTANT INTERACTION: INFLUENCE OF PRESSURE

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Interaction between chitosan (CS), a biopolymer, and an anionic surfactant, sodium bis (2-ethyl hexyl) sulfosuccinate (AOT) generates gel membranes in form of microparticles. The formation of CS-AOT complex is evidenced by Fourier Transform Infrared (FTIR) Spectroscopy. Microparticles formed by the CS-AOT complex can be synthesized at atmospheric pressure or at high pressure. Microparticles are obtained at atmospheric pressure by dripping the chitosan solution into the anionic surfactant solution at optimal concentrations. The phase diagrams are developed to determine the domains of stable microparticles formation at different protonation degrees of CS. An experimental apparatus equipped with a high pressure cell is used in order to obtain ultrafine particles. The biopolymeric solution in contact with high pressure CO<sub>2</sub> is sprayed into surfactant solution bath, through a stainless steel capillary nozzle. Ultrafine particles are formed at pressure higher than 2 MPa, while under this value wires are obtained. The microparticles obtained at high pressure are quasi-spherical in aqueous medium and irregular with many pores and a rough surface after freeze-drying.

### INTRODUCTION

The interaction of polymers and surfactants can generate complexes that may exist in the system as soluble entities, particles of colloidal size or gels with good mechanical properties.<sup>1</sup> Chitosan (CS) is deacetylated chitin, that is a natural cationic polymer which is extracted from crab and shrimp shells.<sup>2</sup> The basic unit of chitosan is 2-deoxy-2-acetyl amino glucose. These units are bound in positions 1-4 and form a linear chain polymer, with the chemical structure: 2-Amino-2-deoxy-(1→4)-β-D-glucopyranan. Chitosan is relatively reactive and can be produced in various forms such as

powder, paste, film and fiber.<sup>3,4</sup> The protonation of amino groups makes chitosan water soluble and the two hydroxyl groups can be chemically modified to give useful products in different fields of applications.<sup>5</sup>

Compared to many other natural polymers, chitosan has positive charges and acts as a chelating agent that selectively binds trace metals,<sup>6</sup> being used as coagulant in wastewater treatment,<sup>7</sup> or in fabrication of composite materials.<sup>8</sup> Due to its nontoxicity and biodegradability, chitosan is a useful material for drug delivery systems.<sup>9,10</sup> Chitosan is relatively reactive and can be produced in various forms such as powder, paste, film, fiber,

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etc.<sup>3,4</sup> Different methods as emulsion cross-linking,<sup>11</sup> coacervation/precipitation,<sup>12</sup> spray-drying<sup>13</sup> and anionic surfactant gelation<sup>14-16</sup> have been used to prepare chitosan particulate systems.

Sodium bis-(2-ethyl hexyl) sulfosuccinate (AOT) is an anionic surfactant which contains two tails in the lipophilic part of molecule that can aggregate forming bilayers.<sup>17</sup> The amino groups of chitosan serve as reaction sites for ionic interaction with anionic surfactants. It may be at least possible that the protonated CS in acetic acid solution is neutralized by the dissociated negative AOT ions through the electrostatic interaction, like in the case of the interaction between chitosan and sodium dodecyl sulfate.<sup>18, 19</sup> The chitosan-anionic surfactant complexes can exist as either homogenous clear solution or precipitate or as membranes depending on the molar ratio between the polymer structural units and the anionic surfactant and the protonation degree of polymer.<sup>15</sup>

The aim of present study is to obtain and to characterize the ultrafine particles obtained by interaction between chitosan and AOT at atmospheric and high pressure.

## RESULTS AND DISCUSSION

Homogeneous solutions, precipitates or gelled membranes that separate the two aqueous solutions are formed, by dripping the protonated chitosan solution into the AOT aqueous solution at atmospheric pressure. The membranous gel at the interface is the result of insoluble chitosan-AOT complex formation by the diffusion of surfactant in the polymeric solution, followed by a cross-linking mechanism implying electrostatic as well as hydrophobic interactions.

The microparticles form instantaneously when the chitosan solution droplets reach the surface of the surfactant solution. The microparticles evolution was followed through video enhanced

microscopy method with a Logitech videocamera, for 1 hour and then the observations regarding the aspect of chitosan-AOT complex were used to draw the chitosan surfactant phase diagrams. Phase diagrams were drawn by using samples whose concentrations in chitosan increased from each other by 0.01mole/L and 0.001mole/L in the case of AOT.

The phase diagram exhibits several domains of existence which can be characterized by specific statements by the molar ratio between the structural units of chitosan and by the surfactant, namely: i) homogeneous solutions, monophasic and transparent within domain C; ii) precipitates, preceded by the appearance of an advanced turbidity within domain PP; iii) formation of gel-type membranes able to separate the two aqueous solutions of polymer and surfactant within domains MEC (microcapsule that expels its content – Fig. 1b), MP (microparticles – Fig. 1a) and DM (shrunken microcapsule – Fig. 1c).

When one analyzes transition from the homogeneous solutions in the domain C to the precipitates in the domain PP, it can be looked as the solubility limit of a chitosan-surfactant complex. The transition from domain C to domain PP (C→PP) in the case of CS-AOT system for different protonation degrees (1% and 2.5% wt acetic acid) presents two characteristics branches (see Fig. 2 a, b).

Certain dependence is observed for solubility vs. surfactant concentration curve on the protonation degree. In the case of AOT surfactant, the minimal concentration where microparticles (MP) domain appears is much higher than the critical micellar concentration (CMC) of the surfactant in water ( $2 \times 10^{-3}$  mole/L) or in water-ethyl alcohol mixtures.<sup>20</sup> The MP domain of the phase diagrams in which microparticles are formed, are the most interesting for subsequent applications.

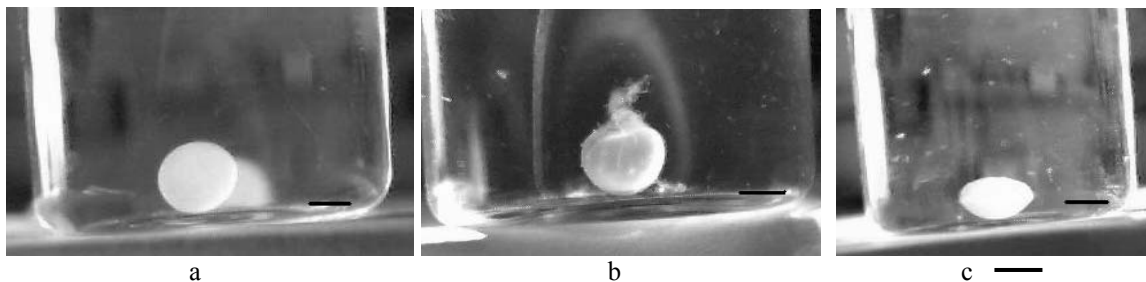


Fig. 1 – Microparticles (a), microcapsule that expels its content (b), shrunken microcapsule (c); bar = 1000  $\mu\text{m}$ .

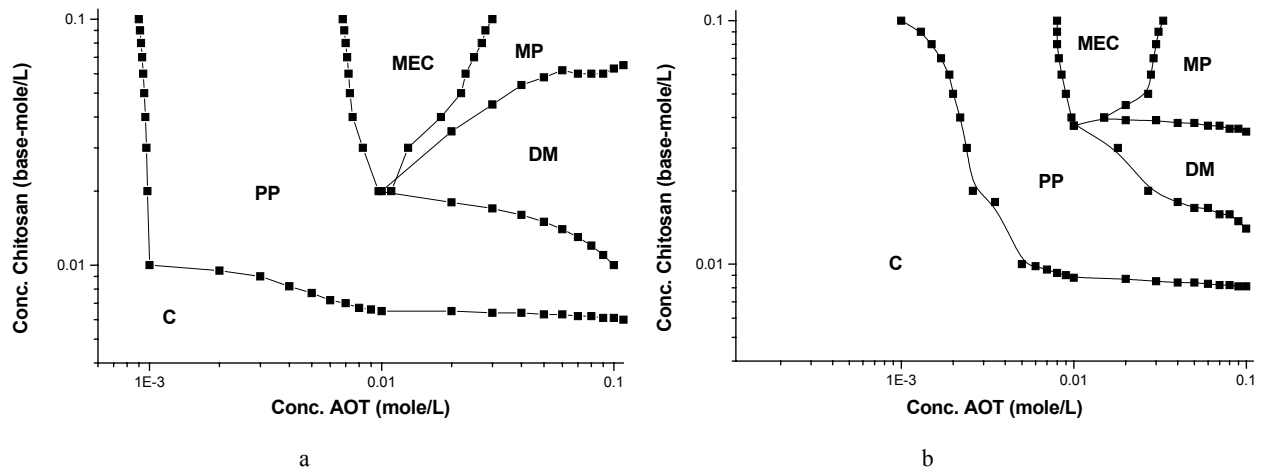


Fig. 2 – Domains of solubility and formation of complex-gel in the chitosan-AOT protonated with: 1% wt acetic acid (a), 2.5% wt acetic acid (b).

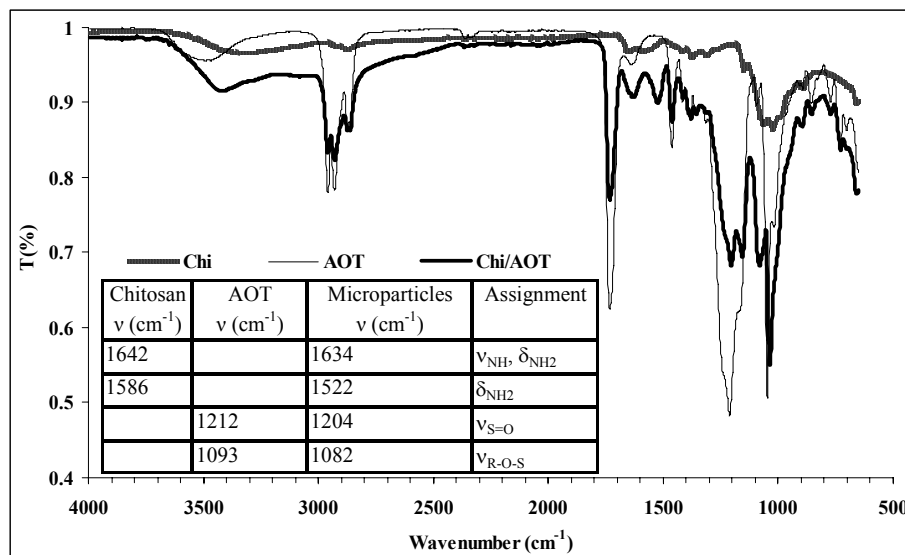


Fig. 3 – FTIR spectra of AOT, chitosan, and CS-AOT microparticles.

The interaction between the biopolymer and surfactant was studied by Fourier Transform Infrared (FTIR) spectroscopy. Fig. 3 shows the FTIR spectra of AOT, chitosan, and CS-AOT particle. The wavenumber values and the assignments of characteristic peaks of AOT, Chi, and CS-AOT particles are presented in the inset.

The characteristic peaks of chitosan appear at 1642 and 1586  $\text{cm}^{-1}$ . They are shifted to 1634 and 1522  $\text{cm}^{-1}$  in CS-AOT microparticles. The sulfonate vibration peaks of AOT at 1212 and 1093  $\text{cm}^{-1}$  are shifted to 1204 and 1082  $\text{cm}^{-1}$  in the spectrum of particles, which proves the interaction between the amino groups of chitosan and the sulfonate ones of AOT.

The technique to obtain microparticles has been improved by using an experimental apparatus for spraying at high-pressure. Ultrafine particles of

different sizes were obtained depending on pre-expansion pressure of  $\text{CO}_2$  applied to spray the chitosan and distance between the needle and the liquid surface. We observe that with increasing the spraying pressure of polymer, the size of the particles decreases.

The polymer-surfactant complex is synthesized in the form of microparticles or wires depending on the applied pre-expansion pressure in the synthesis step: ultrafine particles that form irregular aggregates are made at  $\text{CO}_2$  pressure higher than 2 Mpa, but below this value, CS-AOT wires are obtained.

Optical microscopy images of polymer-surfactant complex after 30 minutes from spraying chitosan into AOT solution are presented in Fig. 4 a and b.

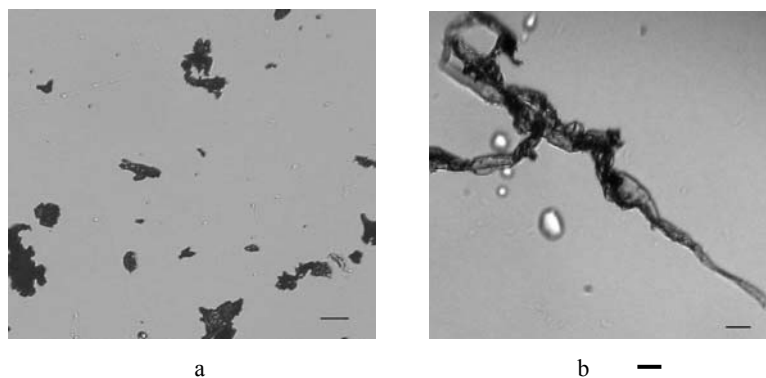


Fig. 4 – Polymer-surfactant complex: microparticles (a), wires (b); bar = 500  $\mu\text{m}$ .

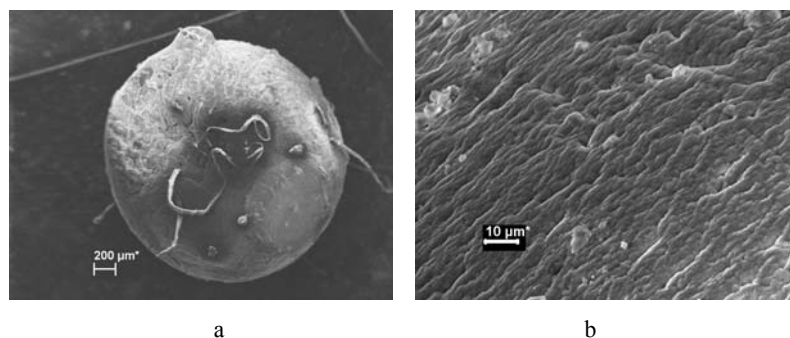


Fig. 5 – CS-AOT microparticle in wet conditions.

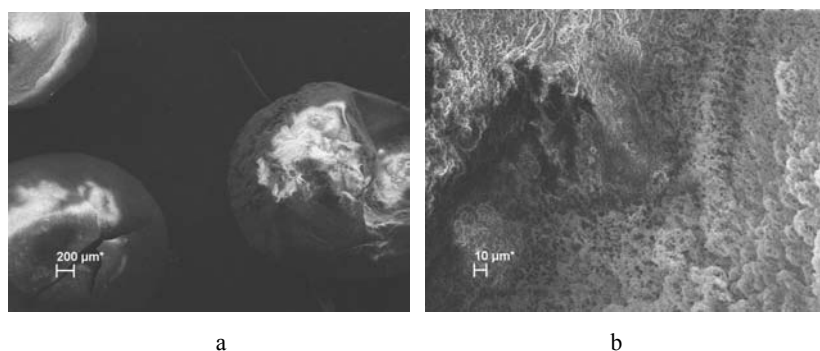


Fig. 6 – SEM photos: lyophilized CS-AOT microparticles (a), surface of microparticle (b).

To study the complex morphology of the chitosan-AOT particles synthesized at high pressure we used the SEM microscopy. Fig. 5 shows the typical morphology of chitosan-AOT wet microspheres, having an average diameter of 1000  $\mu\text{m}$ , and a relatively smooth surface with a granulated texture and significant porosity.

The photomicrograph of the Fig. 6a shows typical freeze-dried particles. The lyophilized particles present pores, and a good delimited surface with an internal porous pattern. The freeze-dried particles have a much bigger surface roughness and porosity. They are highly porous and collapse very easily under pressure.

The morphology of synthesized particles recommends them for possible applications in adsorption of organic and inorganic substances from aqueous medium.

## EXPERIMENTAL

Chitosan (CS) with medium molecular weight ( $M_w = 120 \pm 70$  kDa) was supplied from Aldrich and used without purification. The sodium bis (2-ethyl hexyl) sulfosuccinate (AOT) 98% and acetic acid 99% from Sigma were used as received. Carbon dioxide with 99.9% purity was purchased from Linde Gas Romania. The water used in the experiments was Millipore filtered.

The CS solution was obtained by dissolving 1g of chitosan in 50 cm<sup>3</sup> of 0.5% (v/v) aqueous acetic acid solution under magnetic stirring at 350 rpm for 24h at room temperature.

At atmospheric pressure, the complex of chitosan-anionic surfactant is formed instantaneously when drops from a syringe, the biopolymer into AOT solution. The visual observations concerning the appearance of the chitosan-surfactant complex performed for one hour were used to plot the phase diagrams. Turbidity determinations were made by means of a UV-Vis Varian Cary 100 Bio, to note the passage from the field of solutions to the field of the precipitates.

In order to obtain microparticles at high pressure an experimental apparatus equipped with a high pressure cell was used. The CS-AOT microparticles are instantaneously formed by spraying 1 mL CS solution 2% (wt/wt) preheated 1h at 40°C, into 10 mL AOT 0.03M aqueous solution through a stainless steel capillary nozzle of 30 mm length and 0.4 mm diameter. The CO<sub>2</sub> pre-expansion pressure was 1-5 MPa and the distance from the nozzle tip to surfactant solution interface from lower cell was of about 15 mm.

The Fourier Transform Infrared (FTIR) spectra was recorded on a Nicolet iN10 FT-IR microscope with iZ 10 external module, in the 650-4000 cm<sup>-1</sup> wavenumber range, at a spectral resolution of 4 cm<sup>-1</sup>. The size and morphology of particles were determined by optical microscopy on a Magnum Trinocular and by scanning electron microscopy (SEM) using a Quanta 3D FEG 200/400 apparatus.

## CONCLUSIONS

Two methods to obtain ultrafine particles, by the interaction between chitosan and anionic surfactant sodium bis (2-ethyl hexyl) sulfosuccinate, at atmospheric and high pressure, were developed.

Chitosan-surfactant complex synthesized at atmospheric pressure may take the form of stable microparticles at optimal concentrations of the reactants.

An experimental apparatus equipped with a high pressure cell for spraying the polymer into the anionic surfactant solution, was successfully used for produce either ultrafine particles or wires depending on the spraining pressure.

Optical microscopy and scanning electron microscopy showed that the particles obtained at atmospheric pressure have a relatively smooth surface with a granulated texture and significant porosity. The interaction between chitosan and AOT is proved by FTIR spectroscopy.

Chitosan-AOT microparticles which have undergone to the freeze-drying process present a higher roughness and porosity than the wet particles.

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