Formation of positively charged microcapsules based on chitosan-lecithin interactions

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The formation of microcapsules which contain rosemary oil, is herewith described. The process is based on two steps: (a) formation of oil-in-water emulsions, by using lecithin as emulsifier, thus imparting negative charges on the oil droplets; (b) addition of a cationic biopolymer, chitosan, in conditions that favor the formation of an insoluble chitosan-lecithin complex. Zeta potential measurements revealed that addition of very low concentrations of chitosan to lecithin stabilized emulsions, led to reversal of charge. At a suitable pH range the chitosan precipitated around the oil droplets, forming positively charged microcapsules. The chitosan-lecithin insoluble complex is composed of a 1 : 1 molar ratio of the chitosan monomeric unit and lecithin, as evaluated by elementary analysis and turbidity measurements.

Keywords: Chitosan, lecithin, emulsion, microcapsule, zeta potential.

Introduction

Chitosan is a biodegradable high molecular weight polymer, which is composed of acetyl-D-glucosamine and is soluble in various organic acids, but becomes insoluble at pH values >6·5 (Berkeley *et al.* 1979). At low pH, the chitosan is present as a cationic polymer, with very high charge density, and therefore may function as a good flocculant for negatively charged particles (Strom 1985, Agerkvist 1993). In the past, chitosan was used for microencapsulation by interacting the positively charged chitosan with a negatively charged polymer, such as CMC (Toshiaki and Cho Kyun 1989) and sodium alginate (Li *et al.* 1993). For example, anti inflammatory drugs were encapsulated in aqueous solution with chitosan, which caused changes in bioavailability and ulcerogenic activity. Chitosan was also suggested (Meshali *et al.* 1989) for the preparation of beads, which may contain various substances such as nano-particles (Bodmeier *et al.* 1989) or biomaterials (Seo and Kinemura 1989).

We have recently shown that microcapsules of oil droplets can be formed by interacting a positively charged protein, gelatin, with a negatively charged surfactant, SDS, at the surface of an emulsion droplet (Magdassi *et al.* 1995). In the present research an attempt was made to form microcapsules of oil droplets by interacting the chitosan with a negatively charged surfactant, lecithin. We used rosemary oil as the dispersed phase, as a prototype for a new slow-release

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natural lice-repellent formulation (Magdassi *et al.* 1996, Mumcuoglu *et al.* 1996).

Experimental

Materials

Chitosan, having 72% deacetylation, was obtained from Sigma. Commercial grade lecithin powder, Centrolex P was obtained from Central Soya, USA. Rosemary oil was obtained from Frutarom, Israel. All materials were used without further purification.

Methods

Emulsification. A 1% w/w lecithin solution in water was prepared at 4°C, and the final pH was adjusted to 4·5, by addition of citric acid. To this solution, the rosemary oil was added dropwise to yield a final concentration of 30% w/w, with homogenization at 900 rpm (Ultra-Turrax T-25, Junke and Kunkel GMBH), for 20 min. Separately, a 2% chitosan solution was prepared by dissolving the chitosan in a 10% citric acid solution, and mixing for two hours. The final pH of this solution was 2·2. This solution (or after dilution to required concentration in 10% citric acid) was added slowly to the previously prepared emulsion, in a 1 : 1 weight ratio, and homogenized for 30 min. The final pH was 2·5. The final composition of the emulsion was: oil 15% w/w, chitosan 0–1% w/w, citric acid 5% w/w, lecithin 0·35% w/w and water 78·65–79·65% w/w.

Microencapsulation. The emulsions which were prepared in the previous step were diluted by 2·5 × with distilled water and the pH was raised by adding NaOH solution (10% w/w) to a final value of pH 3·5.

Zeta potential measurements. Were performed by a Zeta meter (Zeta Meter, Inc.) or a Zeta Master-model ZEM 5002 (Malvern), after diluting the sample in a citric acid solution at pH = 3·5.

Evaluation of emulsions and microcapsules. The emulsions and microcapsules were evaluated by using light microscopy (Nikon Optiphot, equiped with a camera), and scanning electron microscopy (JEOL, JSM-35). Particle size distributions were analyzed by Galai CIS-1 counter (Israel).

Turbidity. Turbidity of chitosan/lecithin systems was measured by a Hach Turbidimeter (Ratio XR model) 1 min after mixing a 0·14% w/w chitosan solution with lecithin solutions at various concentrations.

Phosphorous and nitrogen content. The phospherous and nitrogen content in the insoluble complex were determined after filtration of the precipitate by elementary analysis.

Results and discussion

Preliminary experiments were conducted in order to evaluate the possibility of forming a stable oil-in-water emusion, by using only lecithin or chitosan. We found that in both cases it was possible to form emulsions, but they were both unstable. Phase separation was observed 1 h after preparation of chitosan stabilized emulsions. The lecithin-stabilized emulsion was more stable and phase separation was not observed for several days (in accelerated stability tests, storage at 45°C, phase separation occurred after 3 days). According to these findings, it was decided to first form the emulsion by using lecithin. The emulsion was expected to be negatively charged, and therefore, it was assumed that the chitosan molecules would be capable of interacting with the adsorbed lecithin at the oil-water interface, leading to a more stable system. As shown in figure 1, increasing the concentration of chitosan indeed led to enhanced stability: no oil separation was observed even after 35 days at 45°C, in the system which contained 0·5% w/w chitosan and 0·35% lecithin (pH = 2·5). This system was also stable at room temperature, without any change in the mean size of the droplets for at least 8 months. Microscopic observation revealed that while the pH of the emulsion is kept below 2·8, only spherical droplets, which are typical of simple emulsions, are present without any aggregation. However, upon increasing the pH to 3·5, the droplets become non-uniform, as would be expected from an increased rigidity of the coating layer, which is typical for microcapsules. It is interesting to note that the dispersed particles showed birefringency at this pH, indicating a degree of structural order in the coating layer. In addition, at this pH, aggregation of the particles occurred due to precipitation of the adsorbed chitosan and the free chitosan, which was dissolved in a solution at lower pH. A similar aggregation was observed by Bergenstahl *et al.* (1995), but at a higher pH, for soya oil emulsion stabilized by lecithin and chitosan, in the presence of bile salts. The aggregation and formation of rigid particles upon increasing the pH, also made filtration possible, in contrast

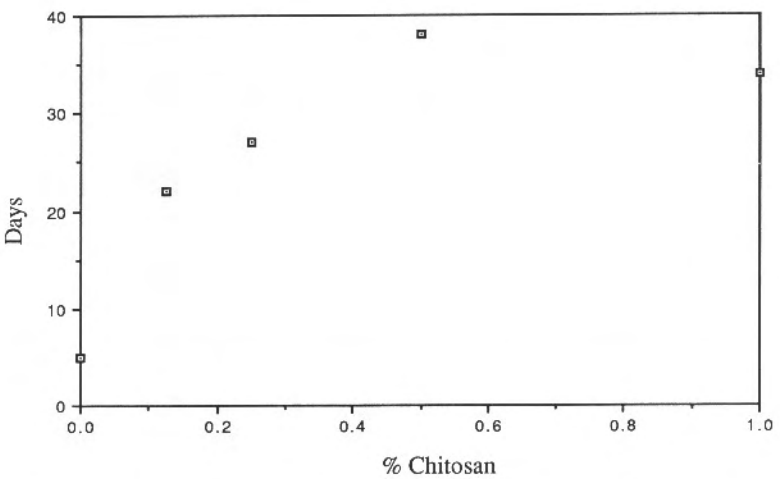

Figure 1. Stability of emulsions at pH = 2·5 and 0·35% w/w lecithin, at various chitosan concentrations, expressed by the time required for initial visual oil separation during storage at 45°C.

Figure 2. Scanning electron micrograph (×1000) of microcapsule dispersion having the composition: 15% oil, 1% chitosan, 0·5% lecithin at pH = 3·5.

to the systems at lower pH. The microcapsules, which were formed at pH = 3·5 were observed by scanning electron microscope, as shown in figure 2.

In order to determine whether the chitosan is indeed adsorbed onto the oil droplets and not simply increases the emulsion stability by increasing the viscosity of the continuous phase, the zeta potential of systems which contain chitosan at various concentrations, was measured. As clearly seen in figure 3, at pH = 3·5, a negatively-charged emulsion is obtained, when no chitosan is present, as expected due to the presence of the negatively charged surfactants. When adding chitosan at concentrations as low as 0·005% w/w, the droplets become slightly positively-charged. At higher chitosan concentrations, above 0·125% w/w, the zeta potential is about +40 mv. These results clearly indicate that the chitosan molecules are indeed bound to the oil droplets. The change in Zeta potential from negative to positive values was also observed by Bergenstahl *et al.* (1995), after addition of chitosan (1 ppm) to an emulsion stabilized by phospholipid and bile salt.

The form in which chitosan is present at the interface (precipitate or individual absorbed molecules) was evaluated by a separate experiment, while mixing the chitosan solutions with lecithin solutions at various weight ratios. In general, it was found that at a specific range of lecithin/chitosan weight ratio, a precipitate is formed. Turbidity measurements (figure 4) showed that above a weight ratio of 4, the turbidity increases, indicating formation of insoluble particles, while the maximal turbidity is obtained at a weight ratio of 5·75. It should be noted that the

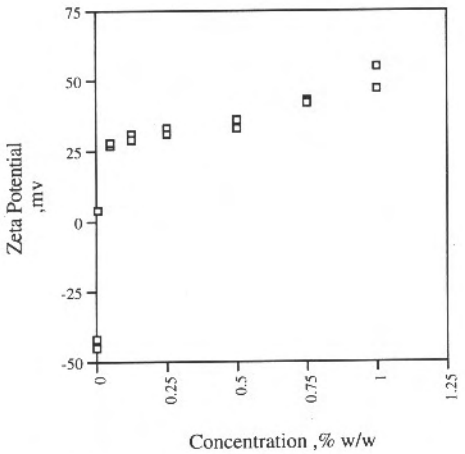

Figure 3. Zeta potential of emulsion (without chitosan) and microcapsules having various chitosan concentrations, and constant lecithin concentration (0·35%) at pH = 3·5.

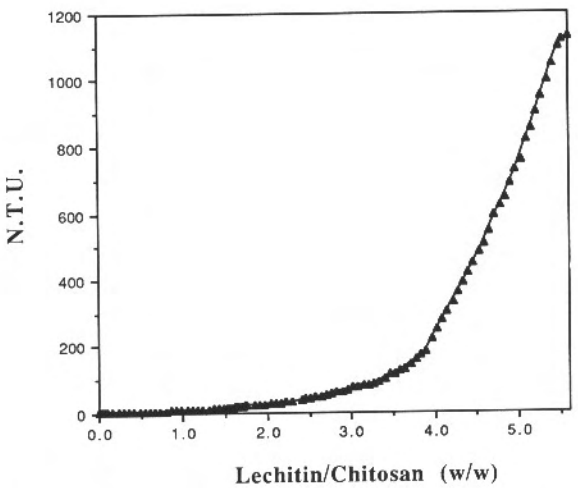

Figure 4. Turbidity (Mephelometric Turbidity Units, NTU) of chitosan solutions at various lecithin-chitosan weight ratios.

turbidity was measured one minute after mixing, indicating that the precipitation process is very fast and probably also occurs in the emulsion systems.

The lecithin used in this study was mixture of several phospholipids. As found by elementary analysis, the content of nitrogen and phosphorous in the lecithin mixture was 1·3 and 3·5% w/w, respectively. Since the molecular weight of chitosan monomeric unit is 162, and since only 72% of the monomers are deacetylated, we calculated that the formation of 1 : 1 complex of lecithin-chitosan is expected to occur at a weight ratio of 5·08, which is very close to the ratios in which the turbidity rises sharply.

The composition of the precipitate was also evaluated by analysing the content of nitrogen and phosphorous. Lecithin, at various concentrations, was added to a 0·5% chitosan solution and the amount of the two elements was measured in the

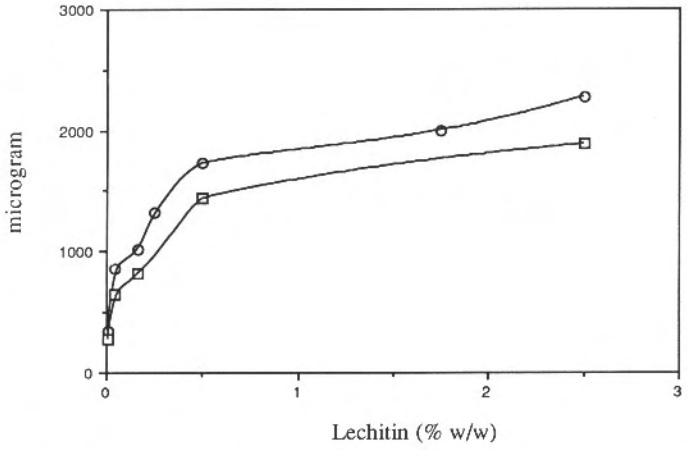

Figure 5. Weight of phosphorous (○) and nitrogen (□) in the precipitate formed by addition of various amounts of 1% w/w lecithin solutions to a chitosan solution (1% final concentration) as a function of the final concentration of lecithin.

Table 1. Weight ratio of phosphorous to nitrogen (P/N) in the precipitate formed at various initial lecithin/chitosan weight ratios.

P/N	Lecithin/chitosan
1·23	0·01
1·32	0·08
1·24	0·32
1·20	1
1·21	5

precipitate (figure 5). It is clear that the precipitate contains both nitrogen and phosphorous, even at very low lecithin concentrations. As presented in table 1, the weight ratio P/N in the precipitate is almost constant, 1·2–1·3 w/w, indicating that the lecithin and chitosan are co-precipitated at a constant molar ratio. Taking into consideration that: (a) the source of nitrogen in the complex is both the chitosan and the phospholipids; (b) the degree of deacetylation of the chitosan is 72%; and (c) the lecithin in this study contains 3·5% w/w phosphorous and 1·3% w/w nitrogen, it is possible to calculate the theoretical P/N weight ratio at any lecithin-chitosan binding ratio.

It was found that if a 1 : 1 molar complex is formed (and precipitated) the expected P/N ratio is 1·3, which is very close to what was obtained in the precipitation experiments.

Once the conditions for obtaining the precipitate were defined, the findings can be applied to the systems which also contain the oil droplets (figure 2): it is clear that at chitosan concentrations above 0·1% w/w (0·35% lecithin) we should have a precipitated complex at the oil-water interface. It is also possible to interpret the results as a two step process for the formation of microcapsules: (1) the formation of oil droplets (in emulsion form), which have lecithin molecules anchored on

their surface through their hydrophobic groups in the oil phase, providing negatively-charged adsorption sites for the chitosan molecules; (2) the formation of a rigid layer around the oil droplets, which results from the formation of a 1 : 1 insoluble complex at a suitable pH and lecithin-chitosan weight ratio. Since in the initial emulsion system the weight ratio of lecithin to chitosan is below 5·08, it can be assumed that in addition to the formation of the insoluble lecithin-chitosan complex, more chitosan molecules are attached to the surface, and can precipitate due to the pH effect. It was found (data not shown) that in the presence of citric acid the chitosan begins to precipitate at a pH of about 3·5.

The effect of pH and the addition of a cross-linking electrolyte, sodium tripolyphosphate on the microencapsulation process, capsule wall and slow-release properties, are currently under investigation.

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