Chitosan-carboxymethylcellulose based microcapsules formulation for controlled release of active ingredients from cosmeto textile

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Abstract. Chitosan-based emulsions were prepared at pH from 4.0 to 6.0. The zeta potential and droplet size were monitored at different pH. Double emulsions (water-oil-water) were observed due to the stiff conformation of chitosan at pH 4.0. At pH 5.0, the emulsion droplets were the smallest (2.9 μm) of the experimental pH range. The emulsion droplets were well dispersed due to high surface charge of chitosan (for example, +50 mV at pH 5.5) in entire pH range. The emulsion was treated with carboxymethyl cellulose (CMC) for neutralizing the charged chitosan on the surface of emulsion droplets. Above 10×10⁻² mg/ml of CMC, no change in zeta potential was observed indicating no more free chitosan existed after neutralization with CMC. The emulsion was then crosslinked with different amount of glutaraldehyde. Upon increasing the amount of glutaraldehyde, the amount of core content inside the microcapsule and encapsulation efficiency of shell materials decreased gradually. The Dynamic Scanning Calorimetry data confirmed no interaction between core and shell material in the microencapsulation process. The thermal degradation of the microcapsules was examined by thermogravimetric analysis and a gradual decrease in the degradation temperature upon increasing glutaraldehyde concentration was found. The tuning of CMC concentration can provide valuable information regarding stable emulsion and efficient microcapsule formulation via coacervation.

1. Introduction
Encapsulations of active ingredients are increasingly being used to improve controlled delivery of cosmetic and pharmaceutical products. Encapsulated products are used in the development of medical and antibacterial textiles, and these products must comply with consumers safety, which have become an issue of great concern. Focussing on this issue, the project aims to deliver active ingredients in a controlled manner for therapeutic purpose and mitigate risk using natural biopolymers, particularly for textile industries. The research is focused on designing and formulating microcapsules using cost effective biopolymers and green processes. Two natural polymers, Chitosan (CH) and carboxymethylcellulose (CMC) were used as shell materials to deliver essential ingredients and, at the same time, increase the level of safety for consumers.

2. Materials and Methods
Chitosan (CH) of low molecular weight and Carboxymethyl cellulose (CMC) (low viscosity, Avg Mw ≈ 90000 Da) were purchased from Aldrich, France. CH degree of deacetylation (DD) was 75-85% and the molecular weight 50,000-190,000 Da. Blends of CH and CMC were used as shell materials. n-hexadecane, selected as core material, was purchased from SESOL Performance Company, Germany. Zeta potential (ZP) values were determined using the Zeta sizer 2000 (Malvern Instruments Ltd.,
Malvern, UK). Specific conductance values were achieved using a CMD210 conductometer. The microscopic images were taken using a CCD camera (uEye, GmbH) for the evaluation of droplets dispersion. Surface tension was measured using GBX 3S Tensiometer, France. The dynamic scanning calorimetry and thermogravimetric analysis were done by using simultaneous thermal analysis (DSC/TGA 3+, Mettler Toledo).

2.1 Emulsion preparation
The zeta potential of aqueous CH and CMC solutions were determined at different pH. Besides, the surface tension values were measured at room temperature. Then, the emulsions were prepared for the dispersion of core materials in the aqueous CH (1% wt) solution at different pH (4.0 – 6.0). The formulation consisted of 20 ml paraffin oil and 80 ml aqueous CH (1g/100ml) solution using homogenizer at 13500 rpm for 30 min. The microscopic images, droplet size and surface charges of the emulsions were evaluated after dilution 1: 200 in the buffer at pH range from 4.0 to 6.0. The emulsion at pH 5.5 was chosen for the further analysis for two reasons: first, to avoid double emulsion droplets and secondly, the CMC exhibited the highest negative charge at pH 5.5 to neutralize CH-based emulsion. Then, CMC solutions of different concentrations were added to the chitosan-based emulsions until constant zeta potential (ξ) value was achieved. These emulsions were called 2nd emulsion. During this period, the zeta potential, microscopic images, and dispersions were monitored for the evaluation of 1st layer on the chitosan-based emulsions. The emulsions were crosslinked with different concentration of glutaraldehyde. The crosslinked emulsions were dried at 50°C. The dried microcapsules were analyzed by DSC and TGA analysis for the evaluation of thermal stability.

3. Result and Discussion
Emulsion preparation is the primary step of microencapsulation via coacervation. Therefore, the evaluation of chitosan behaviour during emulsion formulation at different pH is considered as an important step. The chitosan-based emulsions were studied to observe the droplet size and dispersion as a function of pH.

![Figure 1. Droplets of CH-based emulsions after homogenization at different pH](image)

3.1. CH stabilized Emulsion
The microscopic images of chitosan-stabilized oil-in-water emulsion at pH between 4 and 6 are analysed (Figure 1). Besides, the zeta potential (ξ) and specific conductance values versus pH range and the corresponding droplet mean diameters are shown in Figure 2. No significance difference was observed in specific conductance indicating a limited ionization of chitosan in the tested pH range. ξ values decreased with pH increase, suggesting that the surface charges of the droplets
Figure 2. i) Zeta Potential and specific conductance ii) diameters (µm) of CH-based emulsion against different pH.

decreased at higher pH due to deprotonation of CH molecules on the surface of emulsion droplets. Besides, the emulsifying ability of CH was influenced by the pH of the medium in such a way that the average size of emulsion droplets diameters gradually decreased as the pH approached the highest value (from pH 4.0 to 5.0). The emulsion droplets at pH 4.0 were the largest compared pH, and small droplets inside each large droplet were clearly observed in the microscopic images (Figure 1). This was an indication of multiple or double emulsion (water-oil-water) formation that mainly occurred for having a rigid conformation of chitosan [1, 2]. The conformational changes of CH depend on the deacetylation degree (DD) and aqueous pH. In the present study, the DD of CH was in the range of 75-85%. Alternatively, N-acetyl-D-glucosamine units or degree of acetylation (DA) was around 15-25% which impacted on the stiffness of the CH chain. The stiffness, a result of intramolecular hydrogen bonds increased with the increase of acetamide groups present in the CH backbone. The conformation exhibited at this stage was coil-like and started expanding upon increasing the pH to higher values. Consequently, steric hindrance was developed due to the bulky acetamido groups that resist rotation around the glycosidic bonds [1]. Therefore, the number of individual CH molecules (D-glucosamine, hydrophilic) is higher in case of low DA, while it is low in case of high DA value. Thus, CH behaviour is more hydrophilic in case of low DA (<10%) and more hydrophobic in case of high DA (>10%). The low DA allowed to the formation of oil-in-water (o/w) emulsion as while higher DA of CH produced water-in-oil (w/o) emulsions. Therefore, w/o emulsions were trapped inside the oil-in-water emulsions at pH 4.0 [2]. Besides, the presence of D-glucosamine promoted the expansion and elongation of CH chains due to the repulsion and less share thinning rheological behaviour. The multiple emulsion system (w/o/w) disappeared upon increasing the pH to highest pH value until pH 6.0. The increasing of pH from 4.0 to 6.0 decreased the persistence length and increased the compactness of CH structure. This affected the mean diameter of the emulsion droplets, which decreased up to pH 6.0.

3.2 Interaction between CH-based emulsion and CMC
The influence of CMC on the zeta potential and dispersion behaviour of the emulsion droplets against the CMC concentration is illustrated in Figure 3. The net charge of pure chitosan-based emulsion droplets was highly positive (+51 mV) at pH 5.5. Moreover, chitosan displayed surface active property by adsorbing on the oil-water interface, but the CMC did not have any surface activity (Figure 4). Therefore, the chitosan-based emulsion was quite stable, and droplets were well dispersed (Figure 3). The incorporation of aqueous CMC into the chitosan-based emulsion drastically reduced the zeta potential at pH 5.5. The concentration of CMC highly influenced the net surface charges of the droplets. The addition of 2.5×10² mg/ml CMC decreased ξ value from +50 mV to +38 mV while the
Figure 3. Emulsion after First layer formation of CH-CMC blend at different zeta potential

Figure 4. Surface pressure of CH and CMC at room temperature in the oil/aqueous interface

Figure 5. Zeta Potential of two different concentrations CMC at various pH

Figure 6. The dependency of CMC concentration on the zeta potential of Chitosan-based emulsion

Figure 7. Thermal degradation (TGA) of pure paraffin oil and microcapsules
dispersion behaviour was quite high (Figure 3). Further increase of CMC concentration to $5 \times 10^{-2}$ mg/ml, the $\xi$ value decreased to +24 mV. CMC is a negatively charged polysaccharide and exhibits negative $\xi$ value in the studied pH range (Figure 5)[3]. Therefore, the negatively charged CMC neutralized the positively charged chitosan on the emulsion droplets surfaces. The reduction of the $\xi$ values was more pronounced at $7.5 \times 10^{-2}$ mg/ml CMC and negatively charged (-3.2 mV) surfaces of droplets were observed. The emulsion droplets started aggregating due to low surface charges (-3.2 mV) as the CMC concentration increased (Figure 3). With a further increase of CMC concentration to $10 \times 10^{-2}$ mg/ml, a negative $\xi$ value (~$-25$ mV) was observed again. The microscopic image showed well-dispersed droplets due to high negative surface charges. It is worth observing that further increase of CMC (at $15 \times 10^{-2}$ mg/ml) did not change the $\xi$ value. This results suggested the achievement of the saturation level of chitosan-CMC complexes onto the surface of droplets (Figure 6). Similar behaviours were claimed between anionic polysaccharide and positively charged protein in some previous study [3–5].

![Figure 8.](image1.png)  
**Figure 8.** Differential Thermograph analysis of paraffin oil and microcapsules

![Figure 9.](image2.png)  
**Figure 9.** Melting and Crystallization behavior of Paraffin oil by using Dynamic Scanning Calorimetry

| CH  | CMC | Glut | $\Delta H^d$ | $\Delta H^d$ | $T^*_{\text{melt}}$ | $T^*_{\text{cryst}}$ | $T^*_{\text{max, melt}}$ | $T^*_{\text{max, cryst}}$ | Microcapsules |
|-----|-----|------|------------|-------------|----------------|----------------|----------------|----------------|--------------|
| a   | b   | c/g  | (J/g)      | (J/g)       | (°C)          | (°C)          | (°C)           | (°C)           |              |
| 1   | 1   | 2    | 154.83     | 155.49      | 13.54         | 15.20         | 26.02          | 3.82           | 135          |
| 1   | 1   | 4    | 136.29     | 136.12      | 17.89         | 16.47         | 29.12          | 8.42           | 135          |
| 1   | 1   | 6    | 106.35     | 108.02      | 19.22         | 14.82         | 31.50          | 5.89           | 118          |
| 1   | 1   | 12   | 89.16      | 83.02       | 19.62         | 14.51         | 30.71          | 5.86           | 113          |

$^a$CH indicates chitosan  
$^b$CMC indicates Carboxymethyl Cellulose  
$^c$Glut indicates glutaraldehyde  
$^d$$\Delta H$ corresponds to enthalpy (J/g)  
$^e$T indicates Temperature (°C)

### 3.3 Microcapsule Preparation

The emulsion was then crosslinked using different amount of glutaraldehyde (2.0, 4.0, 6.0 and 12.0 g/g of total biopolymers) and solidification was obtained by drying the crosslinked emulsion. The
encapsulation efficiency was determined by measuring the mass of dry microcapsules while the amount of active content (%) inside the microcapsules was determined from the enthalpy measurement by dynamic scanning calorimeter (DSC) (Table 1). Besides, TGA and DTG data were used for the evaluation of thermal degradation rate and temperature (Figure 7 and Figure 8 respectively).

3.4. Encapsulation yield
The encapsulation yield decreased with increasing the amount of glutaraldehyde used in the crosslinking process. It might attribute to the covalent interaction between chitosan and glutaraldehyde by affecting the bond between chitosan-CMC. Competition between glutaraldehyde and CMC occurred that reduce the encapsulation upon increasing glutaraldehyde. Glutaraldehydes quickly reacted with free NH\textsubscript{2} groups of chitosan and weaken the interaction between chitosan and CMC. As a result, the encapsulation efficiency and amount of active content decreased with the increase of glutaraldehyde. The onset temperatures of melting and crystallization were almost same for microcapsules and pure paraffin oil (Figure 9) indicating that there was no interaction between shell and core materials (Table 1).

4. Conclusion and Future work
CH-based emulsions provided highly dispersed and stable dispersions that led to producing microcapsules via layer by layer technique. Double emulsion formation was observed at low pH 4.0, and the droplet size was found the lowest at pH 5.0. The layer formation using CMC was confirmed by changing the ZP value from positive to negative. The CMC concentration was the main influential factor for changing the zeta potential indicating oppositely charged interaction. The crosslinking of emulsions with glutaraldehyde led to the lower value of encapsulation yield and the amount of active content in the microcapsule. The useful layer-by-layer technique could tune the release of active material. In this study, further experiments will be undertaken to apply these microcapsules by padding and grafting processes on the fabric surface. The kinetics of release profile will be evaluated using Franz diffusion cell.

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