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Note

Design and characterization of protein-based microcapsules as a novel catamenial absorbent system

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Abstract

Hollow and flexible crosslinked microcapsules of soybean protein, 150–300 μm in diameter, were produced using a modified solvent evaporation method. A maximum plasma absorption of approximately 2000% was obtained. At this swelling rate though, microcapsules bursting was initiated. Thus, the effective absorption capacity may not exceed 1700%. The observed presence of residual unreacted protein inside the microcapsules, was believed to be the main osmotic driving force, to which the extensive swelling and eventual bursting of the microcapsules may be attributed. Thermal analysis of the microcapsules revealed their amorphous nature, as opposed to the significantly crystalline nature of the original protein.

Keywords: Protein microcapsules; Soybean protein; Superabsorbent; Catamenial device

1. Introduction

The most commonly used catamenial sanitary products today, are relatively hydrophilic fiber-based porous absorbent materials, known as tampons, with a maximum absorption capacity of up to 8–9 ml. Hence, there is a need for frequent replacement of these products. Therefore, efforts are being invested in an attempt to replace the currently used material for catamenial sanitary products, with new materials exhibiting higher absorption ability and, at the same time, improved confinement of the menstrual fluids.

An interesting approach to explore the potential of a new absorbent device consists of hollow and relatively hydrophilic protein-based microcapsules, with a crosslinked and relatively elastic wall structure.

Numerous studies have dealt with the formation and characterization of protein-based microcapsules, loaded with different active ingredients aimed at controlling their release and many examples of have been reported in the literature. Bovine serum albumin is one of the most commonly used proteins, crosslinked with tereph-

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thaloylchloride (Levi et al., 1994, 1995) or hardened by heat treatment (Ishizaka et al., 1985). Egg albumin microcapsules for drug delivery, were produced by heat coagulation (Ishizaka et al., 1981, 1985). Alternatively, human serum albumin microcapsules were prepared with terephthaloylchloride as crosslinking agent, for the release of 5-FU (Duc-Mauger et al., 1986) and doxorubicin (Sawaya et al., 1987).

The main objective of the present study was the design, identification and preliminary characterization of hydrogel-like soybean protein-based microcapsules for improved absorption and confinement of menstrual fluids.

Soybean protein was purchased from the Archer Daniels Midland (ADM) company, USA. A Baker analyzed NaOH 0.1 N solution was used. Phosphatidylcholine terephthaloyl-chloride and succinylchloride were purchased from the Sigma Co. The solvents used in this study were Cyclohexane (Baker), chloroform (Frutarom) and acetone (Frutarom), all of analytical grade.

1.1. Preparation of microcapsules

2.5 g of soybean protein were dissolved in 35 ml of NaOH 0.1 N solution, under magnetic stirring. The protein solution was emulsified in 75 ml of cyclohexane containing 0.5% of phosphatidylcholine under 350 rpm mechanical stirring. A solution of 168.2 mg of succinylchloride in 38 ml cyclohexane, was added dropwise to the emulsion for a period of 45-min. The microcapsules were then separated by decantation, washed three times with analytical acetone and air-dried.

1.2. Swelling and fluid absorption tests

The swelling process was followed by light microscopy, using an Olympus microscope fitted with a video camera, connected to a computer equipped with PHOTOSHOP software.

Absorption experiments were performed gravimetrically, by gradually adding either distilled water, plasma or blood to a weighed amount of dried microcapsules. The results were expressed as percent fluid uptake.

1.3. Morphological evaluation

The surface morphology of the different microcapsules obtained, was observed by scanning electron microscopy (SEM), using a Philips SEM 505 with accelerated voltage of 20–30 kV. Sections of the microcapsules were performed using a scalpel.

1.4. Thermal analysis

Thermal analysis of the microcapsules and original protein were performed on a Mettler Toledo TC15 thermoanalyzer. Heating and cooling rates were 10 and 2 °C/min, respectively. Nitrogen flow rate was 10 ml/min.

The main concept of the novel absorbent device is based on different variations of the experimental conditions of the well-known interfacial polycondensation method, which forms hollow microcapsules of denaturated proteins.

Different proteins, among which haemoglobin, egg albumin and soybean protein, were investigated. Several of the tested proteins resulted in microcapsules, which for our purposes seemed of acceptable properties. Nevertheless, the soybean protein was preferred due to its relative hydrophilicity, vegetarian origin and low cost.

The nature of the diacylchloride crosslinking agent proved to significantly affect the morphology and apparent physical properties of the resulting microcapsules. The first diacylchloride used in this study was terephthaloyl chloride (TC)—a relatively rigid, aromatic molecule containing two phenyl groups. The reaction stochiometry was determined by calculating the molar amount of amine groups in the protein, based on the amino acid content of the protein. Different molar ratios between the amine and acylchloride were used, but only a 1:1 amine: acylchloride ratio resulted in microcapsules of adequate properties. Significantly lower or higher ratios led to inefficient crosslinking processes.

Fig. 1a and b exhibits the SEM image of the microcapsules obtained by using TC as crosslinking agent. Compact, rigid microcapsules approximately 50 μm in diameter were obtained, with thick brittle walls.

The water absorption of these microcapsules was 500%, i.e. the material was able to absorb an amount of water equal to four times its weight and was significantly higher than the water absorption of the original protein, which was around 180%. This difference most probably stems both from the crosslinking process rendering the material a hydro gel-like nature and from the possible penetration of water into the inner cavity of the microcapsule.

For reasons of simplicity, fluid absorption experiments in this study were performed with distilled water, since in preliminary studies, similar results were obtained by using either distilled water or plasma.

The absorption capacity, although impressive, was not enough to allow the definition of superabsorbent materials.

In an attempt to obtain microcapsules of higher fluid absorption capacity, succininyl chloride, a more flexible aliphatic crosslinking agent, was used. The resulting microcapsules (150–300 μm in diameter), as observed by SEM (Fig. 1c and d), exhibited a different morphology than those obtained with TC, which are depicted in Fig. 1a and b. The apparently much softer membrane, though smooth, is very wrinkled due to shrinking folds which occurred during the drying process. Also, due to the decreased wall rigidity and the fact that they are hollow, there is an inward collapse in most of the microcapsules.

It is worth mentioning that by changing the nature of the organic solvent significant changes in the physical properties of the microcapsules were observed, e.g. the use of cyclohexane resulted in microcapsules of apparently higher flexibility than with chloroform. Also, the use of acetone in the washing procedure proved to be most efficient in preventing microcapsule coagulation and caking.

Water absorption of these new microcapsules reached a maximum value of approximately

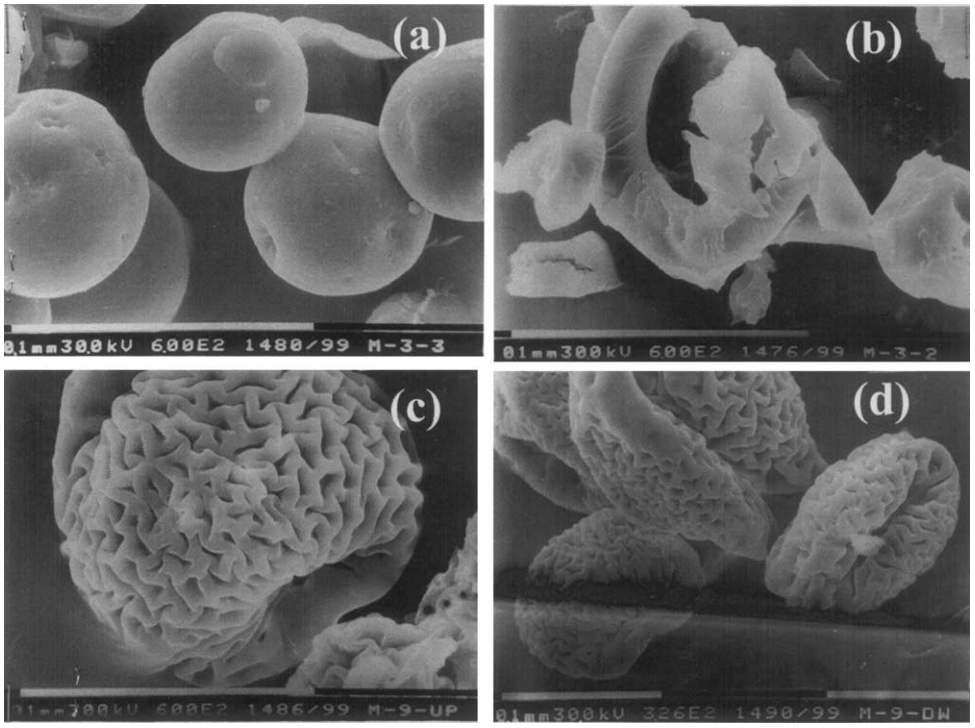

Fig. 1. SEM photographs of whole (a) and fractured (b) microcapsules crosslinked with TC, compared with the microcapsules crosslinked with SCC (c, d).

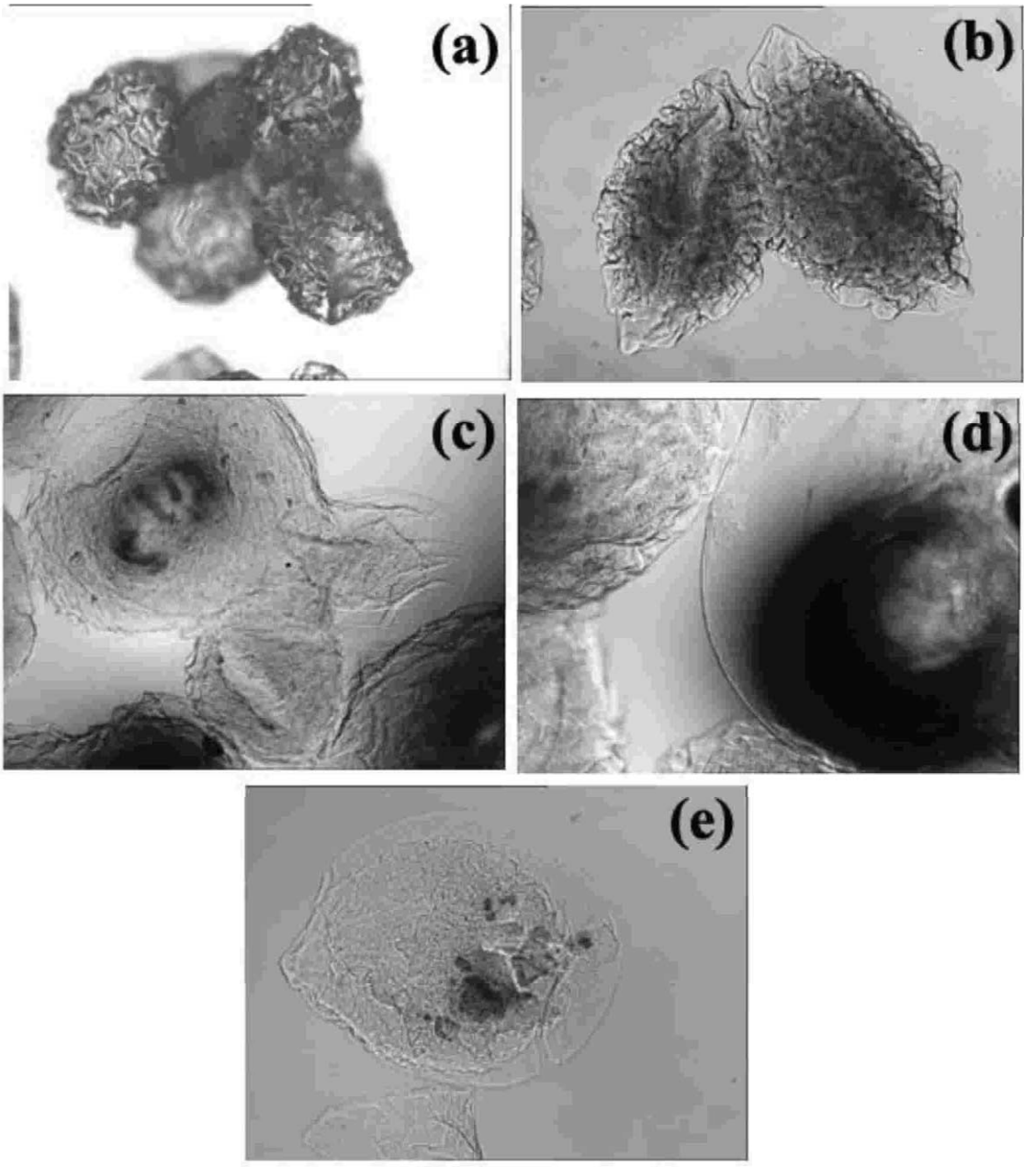

Fig. 2. Selected stages of the water absorption process of the soybean protein microcapsules crosslinked with SCC, observed by light microscopy. (a) dry microcapsules, (b) initial stage of swelling, (c) amoeboidal wall stretching pattern, (d) microcapsule with fully stretched memebrane.

2000%, which was much higher than that obtained with the microcapsules crosslinked with TC. As water absorption approached this value though, any agitation or less than subtle handling resulted in occasional microcapsules bursting and fluid release. Also, continuous water absorption

above 2000% eventually led to massive capsules bursting, finally resulting in a suspension of burst microcapsules ghosts in water. It was found that an absorption value of up to 1700% gave a fairly stable system.

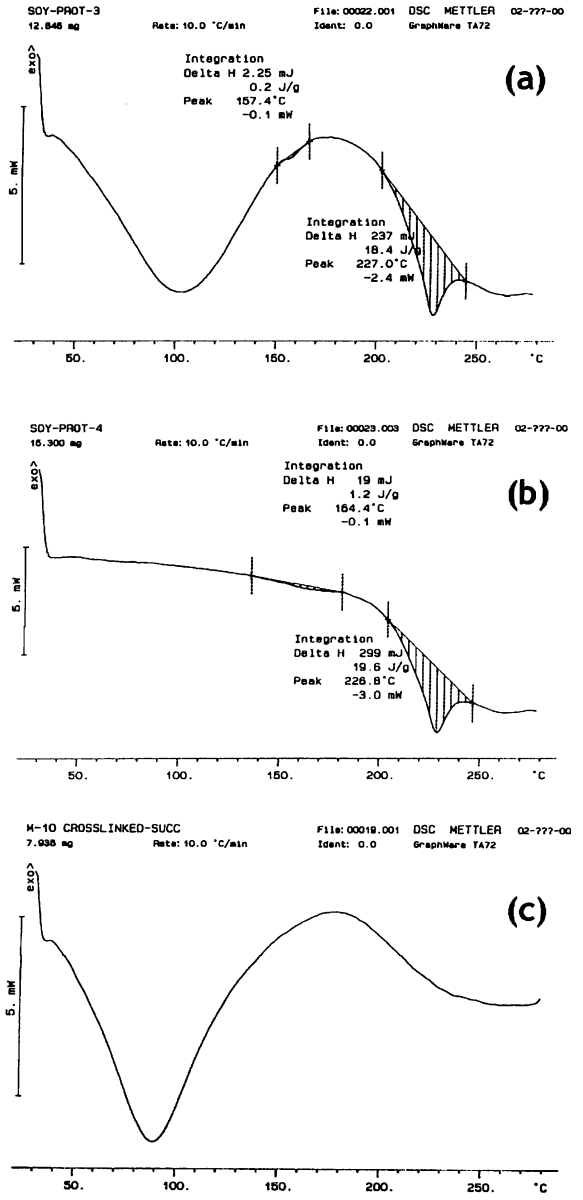

Fig. 3. DSC thermograms of the original (a) and thermally treated (b) soybean protein and of the SCC crosslinked microcapsules (c).

Observation of the swelling process by light microscopy, (Fig. 2a–e), confirmed the phenomenon described above. The swelling process starts with the gradual unfolding of the wrinkles (Fig. 2b), after which the membrane stretches non-uniformly in an amoeboid way (Fig. 2c), most probably due to uneven crosslinking and/or wall thickness. Eventually, a round balloon-like microcapsule is formed (Fig. 2d), which continues to swell until it finally bursts (Fig. 2e). Obviously, there is a significant osmotic driving force for the fluid to penetrate the microcapsule even after the membrane is fully extended, causing it to stretch and finally burst. This osmotic pressure is most likely due to the presence of residual unreacted protein inside the microcapsule. This residue can be clearly seen as darker granules inside the microcapsules in Fig. 2a–d.

Thermal analysis of the microcapsules as compared with that of the original protein is presented in Fig. 3a–c. In Fig. 3a, exhibiting the DSC thermogram of the original protein, a very large endotherm is seen at approximately 100 °C, followed by a second endotherm at 227 °C. The disappearance of the 100 °C endotherm following a thermal treatment (Fig. 3b) (which consisted of heating the sample to 110 °C, cooling it to room temperature and reheating at 10 °C/min), indicates that the origin of the endotherm was hygroscopic water evaporation. The second endotherm is related to the melting process of the protein crystalline phase. The absence of the 227 °C endotherm in the thermogram of the microcapsules Fig. 3c, reveals their amorphous nature. This is consistent with the fact that significantly crosslinked polymers are in most cases amorphous.

It can, therefore, be concluded, that hollow and flexible crosslinked microcapsules of soybean protein, 150–300 μm in diameter, were produced using a modified cross-linking method. Both plasma and water absorption values were up to 2000%. Nevertheless, since at this swelling rate microcapsules bursting was initiated, the effective absorption capacity should not exceed 1700%. The absorption process was observed by light microscopy, revealing the different changes, which occur in the membrane during the swelling process.

Thermal analysis revealed the amorphous nature of the microcapsules, as opposed to the crystalline nature of the original protein.

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