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Brief/Technical Note

Influence of the Lipophilic External Phase Composition on the Preparation and Characterization of Xylan Microcapsules—A Technical Note

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INTRODUCTION

Scientific studies on new drug delivery systems have significantly increased in the past few years and this growth is expected to continue in the near future (1). Such systems are of great interest because of their ability to improve drug performance in terms of efficacy, safety, and patient compliance (1). In many cases, conventional drug delivery provides an increase of drug concentration at potentially toxic levels (2). Additionally, the need for delivering drugs with fewer side effects has prompted the development of new drug delivery systems (1).

Xylan is the second most abundant polymer found in hardwoods and annual plants (3), particularly in agricultural residues such as grain hulls, corn cobs, and corn stalks (4). Depending on the botanical source, the backbone chemical structure may vary. However, the majority of xylans present side chains of different sugars such as 4-*O*-methyl-D-glucoronic acid, *O*-acetyl-L-arabinose, L-arabinose, and D-glucoronic acid bond by a glycosidic linkage to the backbone (3). Because of its complex structure, the complete degradation of xylan requires the activity of several enzymes which are specifically produced by human colonic microflora (5). Therefore, xylan microcapsules may represent a novel and promising colon-specific drug delivery system.

Microcapsules based on natural polymers may be produced by means of a variety of methods. Emulsion solvent extraction, emulsion solvent evaporation and interfacial cross-linking polymerization are the most commonly employed processes for the production of microcapsules (4). One of the critical parameters in the microencapsulation process is the external phase used in the emulsification step (6). In fact, the external phase can influence the microcapsules morphology, their aggregation state, and mainly the release of the microencapsulated active compound (7). Because the production of xylan-based microcapsules is a subject barely studied by scientists worldwide, the aim of this work was to evaluate the influence of the lipophilic external phase composition on the production and mean particle size of xylan microcapsules produced by interfacial cross-linking polymerization.

MATERIALS AND METHODS

Materials

Terephthaloyl chloride and sorbitan triesterate were purchased from Sigma chemical, USA. Isopropanol, chloroform, cyclohexane, ethanol, Polysorbate® 20, and Polysorbate® 80 were obtained from Vetec chemical, Brazil. Medium Chain Triglycerides (MCT), Miglyol 810N, was obtained from Sasol, Germany, and Soybean oil was purchased from Lipoid, Germany. All the chemicals were used as received from manufacturers. Xylan samples were obtained after extraction from corn cobs in our laboratory (8).

Xylan Extraction

The polymer was isolated from corn cobs (8). Briefly, after grinding, the dried corn cobs were dispersed in water under stirring for 24 h. The sample was then treated with

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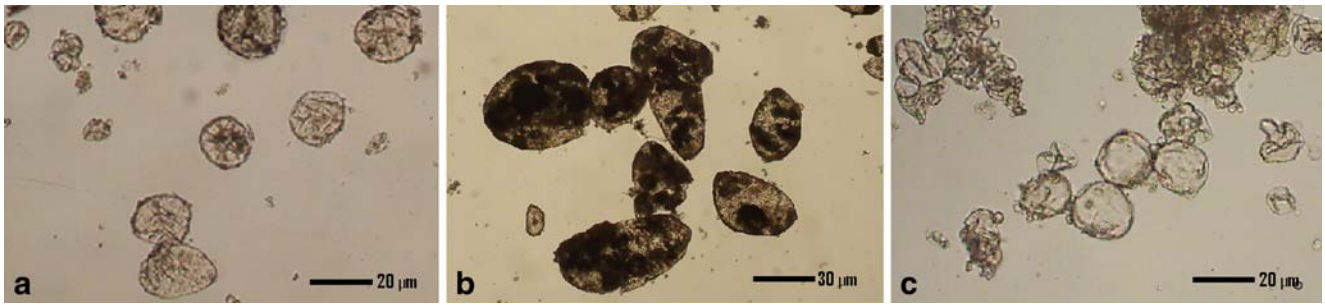

Fig. 1. Optical microscopy of CCM (**a**), SBM (**b**) and MCTM (**c**) at 40×, 40× and 10× magnification, respectively

1.3% (*v*/*v*) sodium hypochlorite solution in order to remove possible impurities. Then, an alkaline extraction was carried out by using 4% (*v*/*v*) sodium hydroxide solution. The bulk was neutralized with acetic acid, and xylan was extracted by settling down after methanol addition. Afterwards, several washing steps were performed by using methanol and isopropanol. Finally, the sample was filtered and dried at 50 °C.

Preparation of Microcapsules

Xylan microcapsules were produced by means of interfacial cross-linking polymerization (8). This method comprises a w/o emulsification step followed by a polymer cross-linking reaction (6). They were produced employing three different lipophilic phases in each experimental setup: 1:4 (*v*/*v*) chloroform/cyclohexane, MCT and soybean oil. First, 6 ml of an alkaline solution containing xylan and sodium hydroxide was prepared and, then, emulsified in 30 ml of the lipophilic phase also containing 5% (*w*/*v*) sorbitan triesterate. After 10 min, under stirring, the interfacial cross-linking reaction was triggered by adding 40 ml of a 5% (*w*/*v*) terephthaloyl chloride. Stirring was maintained for 30 min at room temperature. The reaction was ended by dilution with 30 ml of cyclohexane. Afterwards, the microcapsules were separated by centrifugation and washed several times: first with a 2% (*v*/*v*) polysorbate (HLB=15.85) solution in ethanol, then with ethanol, and finally with water.

Characterization of the Microcapsules

Macroscopic analysis

The macroscopic characteristics of the samples of microcapsules were observed by naked eye visualization.

Microscopic analysis

Homogeneity and morphological examination of the microcapsules were performed using an optical microscope (Leica, Germany). The samples were observed at 10× and 40× magnification.

pH evaluation

The pH value of the samples was measured at 25 °C by a pH meter (model pH 303I, WTW Inc., Germany). The measurement was made by the direct insertion of the electrode probe into the aqueous suspensions containing the microparticles.

Particle size analysis

The microcapsules were subjected to particle analysis under optical microscopy (Leica microscopic). The samples were placed on glass slides and size measurements of 1,500 microcapsules of each formulation sample were performed according to Ferret's diameter principle using an optical microscope calibrated with a stage micrometer scale (9). The mean particle size was estimated by statistical analysis assuming a normal distribution (graphic method; 10), non-normal distribution (RRSB-net; 11) and considering diameter values in terms of an equivalent sphere (geometric method). For each sample analysis, 500 particles were counted in triplicate.

Stability Studies

The microcapsules produced with different lipophilic phases were stored at room temperature (25±2 °C) for 3 months. Meanwhile, homogeneity and mean particle size of the microcapsules were evaluated.

Table I. Characterization of the Microparticulate Systems

	CCM			MCTM			SBM		
	M1	M2 (R^2)	M3	M1	M2 (R^2)	M3	M1	M2 (R^2)	M3
Mean diameter (µm)	21.2±8.0	21.7±1.8 (0.992)	23.7±8.2	61.3±2.3	71.7±2.9 (0.964)	74.7±4.1	9.6±1.4	13.3±2.1 (0.952)	16.3±1.5
Kurtosis		0.62			3.19			19.52	
Amplitude		46.8			198.0			89.0	
Microscopic aspect		Good			Good			Poor	
pH		4.5			2.8			4.1	

RESULTS AND DISCUSSION

Regarding the macroscopic aspect, all the systems presented distinct visual features. The samples obtained by using chloroform/cyclohexane (CCM) and MCT (MCTM) as lipophilic phases were shown to be white suspensions whereas soybean oil (SBM) as a lipophilic phase yielded a system presenting a white flocculated aspect. Furthermore, while CCM and MCTM were relatively homogeneous, SBM showed a higher agglomeration tendency (Fig. 1). Both CCM and SBM systems presented pH values of nearly 4.0 (4.5 and 4.1, respectively), while MCTM system showed a pH value of 2.8 (Table I).

Microscopy analysis showed that both CCM and SBM (Fig. 1) microcapsules were quite spherical in shape while MCTM microcapsules were observed to be larger and oblong in shape (Fig. 1). Also, according to macroscopic aspects, CCM and MCTM showed to remain very stable after storage. However, the SBM presented a high agglomeration with the visual aspect of a cream.

On the other hand, the microcapsules morphology was found to vary depending on the oil phase used for each experimental setup. The MCTM presented to be large and oblong in shape when compared with the other formulations probably due to the phenomenon of coalescence in the emulsion. This phenomenon leads to the formation of large oil droplets which increases the size of the xylan microcapsules (12). Comas *et al.* (13) studied this phenomenon and the influence of pH on the diameter value of different systems. When pH value underwent a decrease, an increase in the diameter was observed as a consequence of a diminution in their surface activity or due to the fact that the interfacial film formed was less resistant to avoid the coalescence during the homogenization and the cross-linking reaction.

Concerning particle size analysis, the graphic method (M1), RRSB grid (M2), and the geometric method (M3) indicated the following values as mean particle sizes of CCM, MCTM, and SBM, respectively: (1) 21.2±8.0, 21.7±1.8, 23.7±8.2 μm; (2) 61.3±2.3, 71.7±2.9, 74.7±4.1 μm; and (3) 9.6±1.4, 13.3±2.1, 16.3±1.5 μm. In addition, kurtosis and amplitude were determined by the software Statistica 6.0 (Statsoft Inc., USA; Table I).

As presented above, SBM showed the smallest diameter regardless of the particle size analysis method applied (Table I). For samples of the same formulation, however, the results differed according to the calculation method, disregarding the morphology characteristics presented by the samples (Fig. 1). This profile was imputed to the asymmetrical particle size distribution (non-normal), which was corroborated by the kurtosis parameter determined for CCM, MCTM, and SBM (Table I). In order to describe this type of distribution, the RRSB-net (Rosin–Rammler–Sperling–Bennet) is widely applied (13). Thus, the diameter values obtained by this methodology for CCM, MCTM and SBM, respectively, 21.7±1.8-, 71.7±2.9-, and 13.3±2.1 μm, were considered more accurate (11,14). The CCM distribution curve amplitude (46.8) was narrower if compared with those of SBM (89) and MCTM (198). These results agreed with the CCM, MCTM, and SBM kurtosis values of 0.62, 3.19, and 19.5, respectively, and indicated that the particle size distributions show a higher probability of extreme values.

After the same period of storage (3 months), SBM systems showed deterioration evidence, probably as a consequence of oxidative reactions of lipids present in the soybean oil. Furthermore, the system containing soybean oil appeared to have low dispersity properties and high tendency to agglomeration. This fact can be imputed to the washing process, which was inefficient due to the high viscosity presented by this oil. Therefore, the residual amount of soybean oil was responsible for the physicochemical changes of the product. On the other hand, CCM and MCTM remained very stable after storage for 3 months, suggesting that the polymeric cross-linking reaction may be carried out with a suitable efficiency in such lipophilic phases. The hypothesis for such approach is based on the characteristic of the diffusibility of these lipophilic phases, which can allow a suitable diffusion of the cross-linking agent on the interfacial surface of the emulsion. Additionally, the viscosity of these oils can contribute to the characteristics obtained in the microcapsules. Soybean oil, MCT, and chloroform/cyclohexane have the viscosity values of 52, 24, and <1 cP, respectively (15,16). The physicochemical properties of the microparticles followed the gradient of viscosity of the oils. The higher the viscosity value, the worse the product characteristics.

SUMMARY AND CONCLUSIONS

This work demonstrates the influence of three different lipophilic phases on the production of xylan microcapsules for medical purposes by means of interfacial cross-linking polymerization with different lipophilic phases. Stability data have indicated the feasibility of the method to produce xylan microcapsules with a suitable stability using MCT or chloroform/cyclohexane as lipophilic phases. However, MCT may be more advantageous than chloroform/cyclohexane due to its well-known use with reduced toxicity in the pharmaceutical industry (17).

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