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Microcapsules prepared through interfacial cross-linking of starch derivatives

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Summary

An interfacial cross-linking process was applied to hydrosoluble starch derivatives: hydroxyethylstarch (HES) and carboxymethylstarch (CMS). It resulted in stable microcapsules, which could be easily lyophilized and gave free-flowing powders. Sodium salicylate was encapsulated in HES microcapsules. In vitro dissolution studies indicated that these walls allow a prolonged release of the tracer. CMS microcapsules exhibited hydrophilic properties, as shown by water-induced swelling and gel formation. All cross-linked polysaccharide microcapsules were characterized by a total resistance to digestive media. However, when a protein, human serum albumin (HSA) or gelatin, was added to the starch derivatives in the aqueous phase, the process provided biodegradable microcapsules.

Introduction

Polysaccharides have been extensively studied as materials for the preparation of microparticles. Various processes have been proposed (Deasy, 1984) such as spray-drying, coacervation or gelation. Another group of methods involves chemical cross-linking of polysaccharides. For example, starch microspheres were obtained through cross-linking of a starch hydrolysate by means of epichlorhydrin (Rothman et al., 1976). These microspheres were shown to be degraded rapidly by serum-amylase. The same cross-linking agent was used for dextran, which yielded 'dextranomer' beads. Application to dextrins, followed by carboxymethylation and loading with iodine produced 'cadexomer' beads. Both dextranomer and cadexomer exhibited hydrophilic properties and gave a gel upon swelling in water. They were proposed in dermatology for the treatment of wounds and ulcers (Artursson et al., 1978; Skog et al., 1983). Goldberg et al. (1984) used commercially available microspheres made of epichlorhydrin cross-linked dextran (Sephadex): after carboxymethylation, they obtained particles which acted as cation-exchange drug carriers. Acrylic acid glycidyl ester was used to prepare biodegradable polyacrylstarch or polyacryldextran microspheres (Edman et al., 1980; Artursson et al., 1987). Lastly, a few patents deal with microencapsulation through interfacial cross-linking of

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polysaccharides. Most of them relate to oil-containing microcapsules to be used in pressure-sensitive copy systems, with isocyanates as cross-linking agents (Vassiliades and Chang, 1979).

In continuation of our studies related with microencapsulation through interfacial cross-linking of proteins (Lévy et al., 1982; Rambourg et al., 1982; Guérin et al., 1983; Desoize et al., 1986; Lévy and Andry, 1987; Lévy and Guérin, 1987), we attempted to apply the process to hydrosoluble polysaccharides with the aim of possible pharmaceutical uses. After encouraging initial assays (Gourdier et al., 1983), starch derivatives were chosen as materials: hydroxyethylstarch (HES) for its well-known biocompatibility, and carboxymethylstarch (CMS) because it seemed of interest to prepare anionic microcapsules in a one-step process, through direct cross-linking of a polycarboxylic polymer. The first part of this study is devoted to HES: manufacturing conditions were determined and the biodegradability of the microcapsules was investigated. Finally, dissolution studies were performed on sodium salicylate-loaded microcapsules. With CMS, hydrophilic microcapsules were obtained that were capable of considerable swelling in water. Therefore, the aim of the second part of this work was to set the manufacturing parameters allowing optimization of this special property. In addition, we investigated the effect of a protein, admixed with the polysaccharide, on the enzymatic lysis of the wall: as a matter of fact, previous studies of such mixed-walled microcapsules had shown an adjustable biodegradability (Lévy and Andry, 1986).

Materials and Methods

Materials

HES was obtained through lyophilisation of Plasmasteril saline solutions (Fresenius, F.R.G.) and CMS (Opagel CL, substitution degree = 0.3) was supplied by Doittau (France). The proteins used were lyophilized human serum albumin (HSA, Centre de transfusion sanguine, Lille, France) and gelatin (type B, bloom: 80, Méro-Rousselot-Satia, France). Terephthaloyl chloride was purchased from Aldrich-Chimie (France). The surfactants were sorbitan trioleate and polysorbate (Seppic-Montanoir, France). Chloroform, cyclohexane and ethanol, analytical grade, were used without further purification (Prolabo, France).

Methods

Preparation and evaluation of the microcapsules. A suitable amount of polysaccharide was dissolved in 6 ml of an alkaline aqueous phase consisting of phosphate buffer (pH 8), 0.45 M carbonate buffer, adjusted to pH 9.8 with HCl, or diluted NaOH. This solution was emulsified in 30 ml of chloroform : cyclohexane (1 : 4, v/v) containing 5% (v/v) sorbitan trioleate at a given stirring rate (Heidolph RZR II-stirring motor, Prolabo; or Virtis 23 homogenizer, Bioblock, France). Pure cyclohexane was used as organic phase, when gelatin was used in the process. A terephthaloyl chloride solution in the solvent mixture (40 ml) was then added and stirring was continued for 30 min. The interfacial cross-linking reaction was ended by dilution with 30 ml of cyclohexane and the microcapsules were separated by centrifugation. Several washes were performed, first with a 2% (v/v) polysorbate solution in 95% ethanol, then with 95% ethanol and finally with water. The microcapsules were usually resuspended in water and lyophilized. Variations were introduced in the composition of the aqueous phase (pH, polysaccharide concentration, possible addition of a protein), in the stirring speed, and in terephthaloyl chloride concentration.

All the batches were triplicated. The particle size range was assessed by conventional light microscopy. The surface characteristics of microcapsules were examined by scanning electron microscopy.

Effect of digestive media. Artificial digestive media were prepared according to U.S.P.XXI: pepsin (from porcine stomach mucosa, Sigma, U.S.A.), pH 1.2; and pancreatin (from porcine pancreas, Sigma), pH 7.5. The degradation properties of the microcapsules were characterized as follows: a sample of 50 mg lyophilized microcapsule powder was moistened in a test tube with 2 ml of distilled water and the suspension was further supplemented with 15 ml of enzymatic solu-

tion. After mixing, the tube was incubated at 37 °C. Lysis was evaluated by microscopic examination: the degradation time was defined as the time for disappearance of all microcapsules.

Incorporation of salicylate. Sodium salicylate (200 mg) was dissolved in 6 ml of buffer, pH 9.8, containing 10% HES (w/v). The microencapsulation process was then applied, with a 2.5% (w/v) solution of terephthaloyl chloride. After dilution with 50 ml of solvent mixture, the microcapsules were separated by centrifugation and then washed four times with cyclohexane. After elimination of residual solvent by vacuum evaporation, the microcapsules were congealed and lyophilized.

Evaluation of drug loss. Determination of salicylate was carried out in preparation and washing media, after evaporation and addition of a buffer, pH 7.4. A colorimetric method was used, involving reaction with Fe^{3+}.

In vitro release studies. A whole batch of lyophilized microcapsules (mean dry weight = 1.125 g) was dispersed in 500 ml of buffer, pH 7.4, at 37 °C (Erweka dissolution apparatus, agitation: 40 rpm). At appropriate intervals of time, samples of medium were withdrawn and filtered through a Millipore filter (0.22 μm) for salicylate determination.

Evaluation of hydrophilic properties. During the preparation of microcapsules, a significant increase in the sediment volume could be observed upon transfer from ethanol into water. It was further quantified by evaluation of the mean size of the microcapsules suspended in 95% ethanol and in water, respectively. Furthermore, a simple

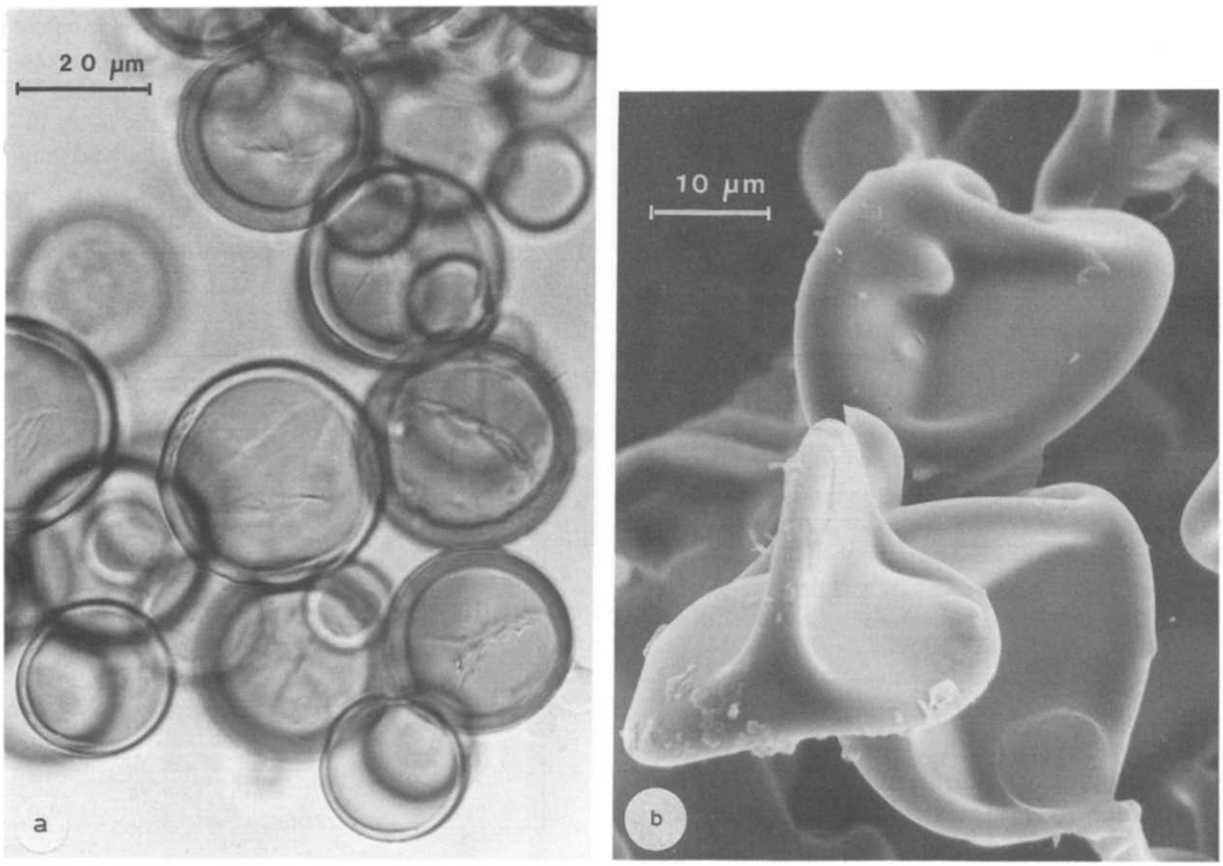

Fig. 1. Cross-linked HES microcapsules (HES concentration: 20% w/v in buffer pH 9.8; terephthaloyl chloride concentration: 5% w/v). (a) Optical photomicrograph (differential interference contrast); (b) scanning electron micrograph.

evaluation test was used for easy comparison between the different batches: two samples of 100 mg lyophilized microcapsules were introduced into graduated vessels, which were filled with absolute ethanol or water, respectively. After mixing, the microcapsules were allowed to sediment for 2 h. Sediment volumes were read and compared. The difference was considered to be the water intake corresponding to 100 mg microcapsule powder.

Results

Microcapsules prepared from HES

A series of experiments were conducted with HES solutions (10, 15 and 20%) in buffer, pH 8. Microcapsules were obtained from 20% HES solutions, with a terephthaloyl chloride concentration of 5% and a 30 min reaction time. However, they were obtained in small amounts, they formed aggregates and looked fragile: most microcapsules burst after washing. Increasing the reaction time to 60 min did not improve the results.

For a cross-linking pH of 9.8, microcapsules were obtained from 10, 15 and 20% HES solutions, with a terephthaloyl chloride concentration of 2.5% (w/v). They were all transparent and spherical in shape, as shown by optical microscopy. Increasing the cross-linking agent concentration to 5% (w/v) resulted in more distinct walls (Fig. 1a), and remarkably improved the stability of the microcapsules on storage: when suspended in water without any conservative agent added, they remained unaltered after a 1 year period at 4° C. The particle size could be adjusted by varying the stirring speed. For example, with a 10% HES solution and a 5% terephthaloyl chloride concentration, the size range was 10–45 μm for agitation at 2500 rpm and 15–100 μm when lowering the stirring speed to 1800 rpm. Lyophilization produced free-flowing powders and microcapsules recovered their initial shape by rehydration. Scanning electron microscopy showed collapsed particles with a smooth surface (Fig. 1b).

All HES microcapsules were shown to be unmodified after a 24 h incubation time in both artificial digestive media. However, when a protein (HSA) was added to the HES solution, the resulting microcapsules were dissolved by pancreatin and the degradation time was shown to be shortened with increasing amount of protein (Table 1). In comparison, microcapsules prepared from HSA alone under the same cross-linking conditions (pH, 9.8; concentration of terephthaloyl chloride, 2.5% (w/v)) were incubated in pancreatin: they exhibited the shortest lysis time.

TABLE 1

Degradation properties of microcapsules prepared [a] from HES and/or HSA

Concentrations (% w/v)		Degradation time in pancreatin
HES	HSA	
20	0	no lysis after 24 h
15	5	4 h 15 min
5	15	70 min
0	20	30 min

[a] Manufacturing conditions for all microcapsules: reaction pH = 9.8; terephthaloyl chloride concentration = 2.5% (w/v); reaction time = 30 min.

After encapsulation of sodium salicylate, determinations of the drug in reaction and washing media of microcapsules showed a very weak loss: 3% of the involved weight (200 mg), which gave 97% as the encapsulation percentage. The mean payload of microcapsules was 172 mg salicylate per g of dry powder.

The in vitro release profile in buffer, pH 7.4, is shown in Fig. 2. A slow and regular release was observed for 3 days, after an initial 1 h period of

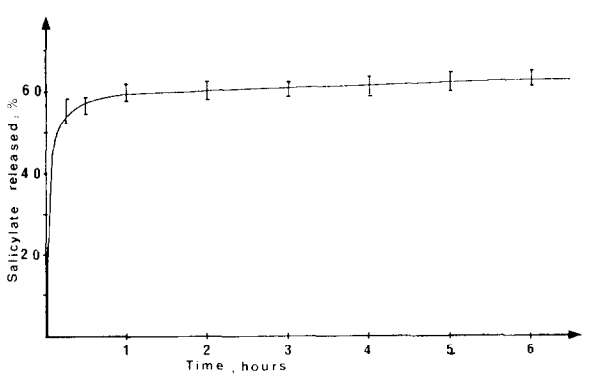

Fig. 2. Release of sodium salicylate from cross-linked HES microcapsules.

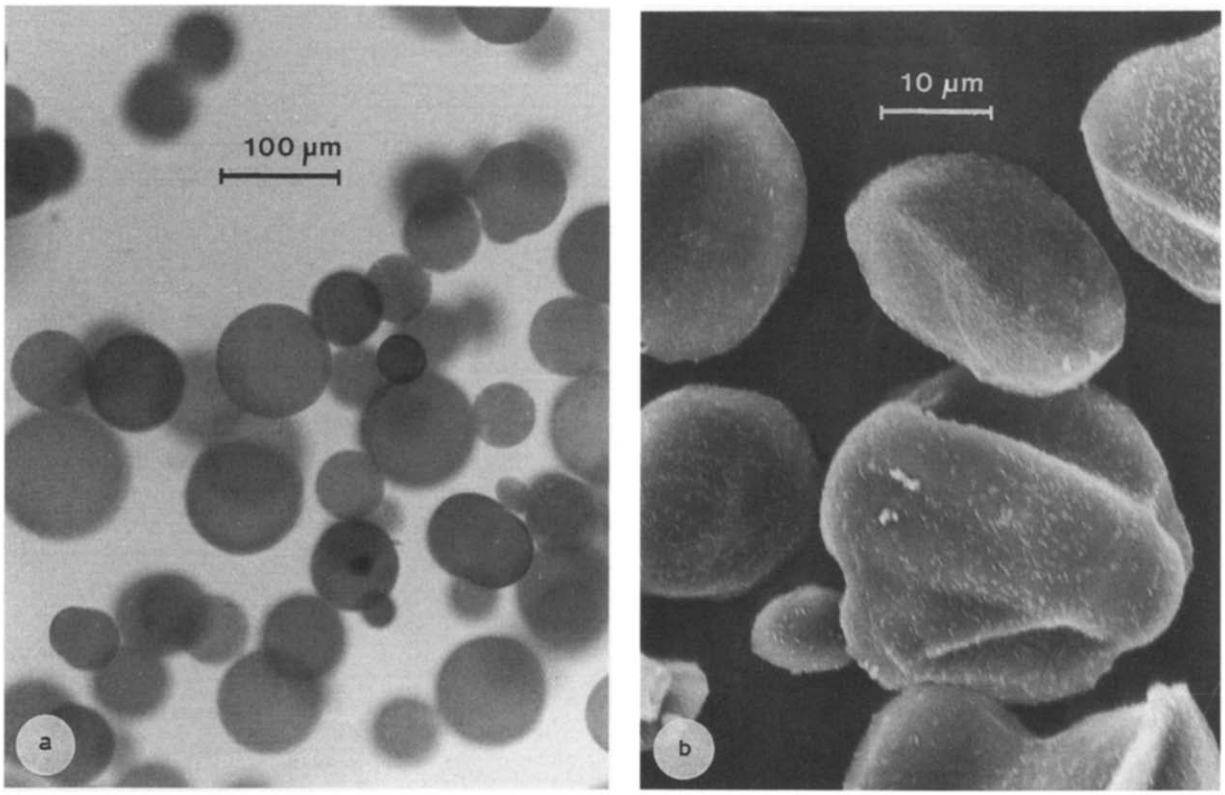

Fig. 3. Cross-linked CMS microcapsules (CMS concentration: 10% in buffer, pH 9.8). (a) Optical photomicrograph of microcapsules suspended in water and stained with methylene blue; (b) scanning electron micrograph.

faster release. However, it was not complete: a fraction of 15% remained entrapped in microcapsules.

Microcapsules prepared from CMS

CMS was first used alone, as an alkaline solution, and the general process was applied. Variations were introduced in the manufacturing parameters, concerning the pH of the aqueous phase, the concentrations of CMS and terephthaloyl chloride, and the stirring speed. Selection between experimental conditions was made with regards to two types of criteria: mechanical strength of the walls, which were expected to resist lyophilization and their ability to swell in water, as reflected by gel formation, increase in mean size and water intake from freeze-dried microcapsule powder.

Stable microcapsules were obtained from CMS solutions in buffer, pH 9.8. However, their characteristics varied, depending on the polymer concentration: for example, microcapsules formed aggregates in water when prepared from a 5% solution. Appropriate concentrations were 7.5 or 10%, for a 5% (w/v) terephthaloyl chloride solution and a reaction time of 30 min. Lowering the reaction pH to 8 or the terephthaloyl chloride concentration to 2.5%, or shortening the cross-linking duration to 15 min resulted in fragile microcapsules, a part of which appeared to be open on microscopic examination. The viscosity of the CMS solutions required efficient agitation; a Virtis homogenizer was used preferably, with a stirring speed of 7000 rpm. The best results were obtained with a CMS concentration of 10%: after washing with 95% ethanol, microcapsules formed a white sediment which swelled upon transfer into water, giving a transparent gel. Microscopic examination showed transparent and well-individualized spherical particles, which were stained with methylene blue for

TABLE 2

Hydrophilic properties of microcapsules prepared from CMS: Influence of formulation and cross-linking conditions

Concentrations (% w/v)			Aqueous phase	Reaction time (min)	Water intake of 100 mg microcapsule powder (ml)
CMS	Gelatin	Terephthaloyl chloride			
10	0	5	buffer, pH 9.8	30	7.5
7.5	0	5	buffer, pH 9.8	30	7.4
5	0	5	buffer, pH 9.8	30	7.4
5	0	5	0.5 M NaOH	30	5.0
5	7.5	5	buffer, pH 9.8	15	6.1
5	7.5	2.5	buffer, pH 9.8	15	11.2
5	7.5	1.25	buffer, pH 9.8	15	16.6

easier observation (Fig. 3a). The walls were smooth and continuous, as shown by scanning electron microscopy (Fig. 3b). The size range was 10–40 μm when suspended in 95% ethanol and 15–100 μm in water. Lyophilization provided a white powder with a light and fleecy texture; microcapsules recovered a spherical shape after rehydration.

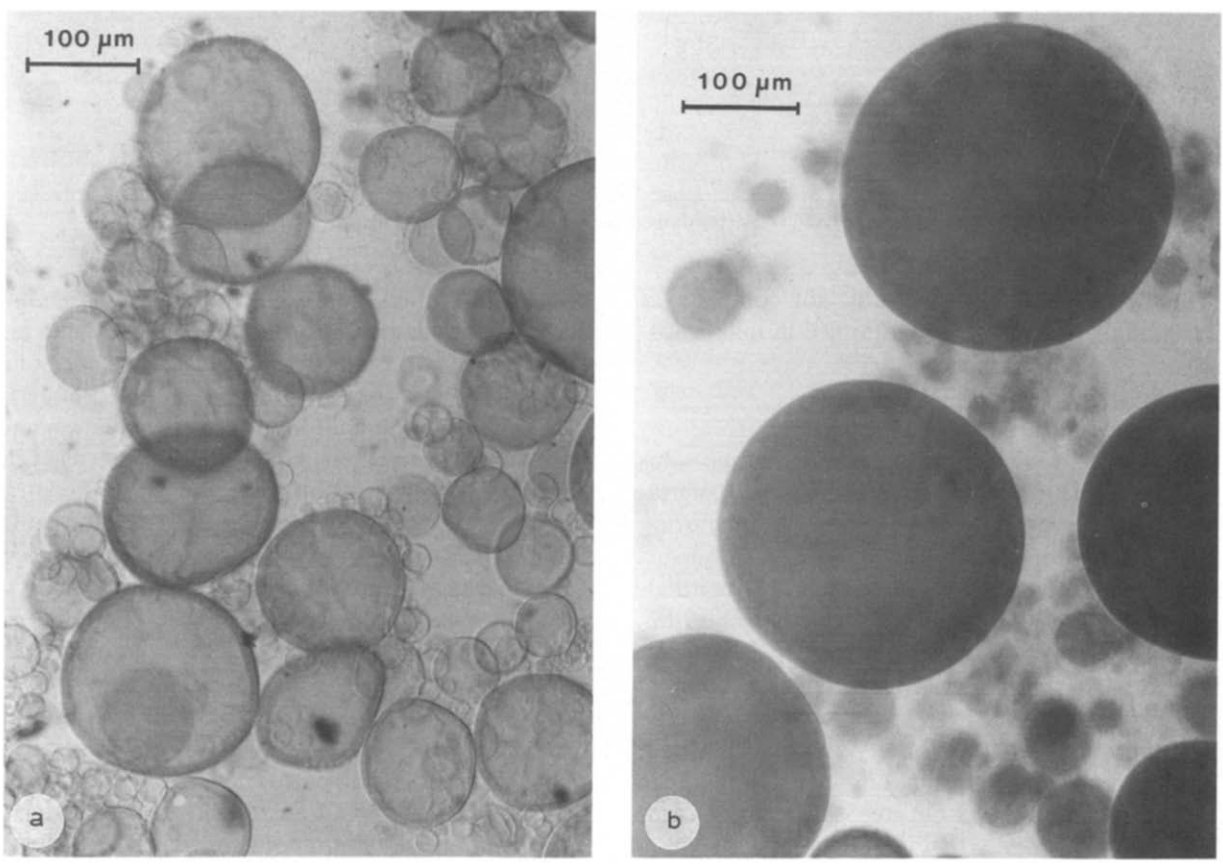

Fig. 4. Photomicrographs of microcapsules prepared from CMS and gelatin (concentrations: 5 and 7.5%, respectively, in buffer, pH 9.8). (a) Suspended in 95% ethanol; (b) as swollen in water (same magnification).

The water intake corresponding to 100 mg dry powder was 7.5 ml. Similar results were obtained with microcapsules prepared from 7.5 or 5% solutions in buffer, pH 9.8 (Table 2), while a lower value was observed when using a 5% CMS solution in 0.5 M NaOH.

All microcapsules remained unaltered after a 24 h incubation in gastric and intestinal media.

In another series of assays, CMS and gelatin were used simultaneously at constant concentrations of 5 and 7.5%, respectively, in buffer, pH 9.8. In order to determine minimal cross-linking conditions, reaction time was reduced to 15 min and assays were performed using decreasing concentrations of terephthaloyl chloride: 5, 2.5 and 1.25%. A constant stirring speed of 5000 rpm was used. Stable microcapsules were obtained under all the above conditions. The particles were shown to be well-individualized, spherical and gel-forming. In all cases, lyophilization yielded pale-yellow free-flowing powders. The water intake of 100 mg dry powder increased on lowering the terephthaloyl chloride concentration, with a maximal value of 16.6 ml observed for 1.25% (Table 2). Optical photomicrographs of the corresponding microcapcules are shown in Fig. 4, as suspended in 95% ethanol (Fig. 4a; size range: 10–180 μm) or in water (Fig. 4b; size range: 20–750 μm), respectively. Pleats of the membrane were observed in alcoholic medium, while they were absent from the surface of swollen microcapsules. Enzymatic lysis experiments showed that these microcapsules resisted a 24 h incubation in pepsin, but were destroyed within 90 min in pancreatin.

Discussion

We previously reported on microcapsules prepared through interfacial cross-linking of several hydrosoluble polysaccharides (hydroxypropyl-cellulose, gum arabic, dextran, soluble potato starch hydrolysate) with acyldichlorides (Gourdier et al., 1983). The present study demonstrates that the process also applies to starch derivatives of pharmaceutical interest. With HES and CMS, the resulting microcapsules could be lyophilized, giving free-flowing powders. In this process, the formation of the membrane results from establishment of ester bonds between hydroxy groups of the polysaccharides and the acyldichloride. As expected, the microcapsules were shown to be stable towards pepsin. The observed resistance to pancreatin demonstrates that the walls are not sensitive to pancreatic α-amylase. At first sight this could be attributed to an effect of the substituents on the polysaccharide chain: as a matter of fact, HES is known to be more resistant to amylase hydrolysis than the non-substituted polysaccharide. However, our previous studies (Gourdier et al., 1983) had shown that microcapsules prepared through interfacial cross-linking of soluble potato starch hydrolysate with terephthaloyl chloride were not degraded in pancreatin. It can therefore be concluded that it is the establishment of ester bonds which is determining in the resistance to enzymatic hydrolysis. In this respect, the microcapsules behave differently from epichlorhydrin cross-linked starch microspheres prepared by emulsion polymerization of a soluble potato starch hydrolysate (Rothman et al., 1976). In this last case, enzymatic hydrolysis is not prevented by glycerol ether cross-links.

As in the well-known interfacial polymerization procedure (Chang, 1964), microcapsule properties can be varied, depending on the manufacturing conditions. Size can be adjusted by changing the emulsification parameters, while the strength of the wall was shown to depend on the cross-linking parameters: mainly pH of polymer solutions, terephthaloyl chloride concentration and reaction time. Reaction pH is an important factor, due to its role in neutralization of released HCl, thereby allowing a higher degree of cross-linking. Actually, alkalinization of the aqueous phase results in improved feasibility. Moreover, microcapsule wall strength was enhanced by prolonging the reaction time from 15 to 30 min and using a high terephthaloyl chloride concentration (5% w/v).

Finally, results were different, depending on the substituent groups of the polymers: HES gave tougher membranes than CMS, due to easy acylation of primary alcohol functions in hydroxyethyl groups. The resulting microcapsules exhibited remarkable stability on storage, when suspended in

water, and were shown to entrap sodium salicylate with a high efficiency. Furthermore, the cross-linked polysaccharide behaved like a semi-permeable wall, allowing slow release in vitro, after a 1 h initial burst effect.

Concerning CMS, carboxylic groups were responsible for interesting hydrophilic properties, as shown by the considerable water intake of lyophilized microcapsule powder. Such particles may have numerous applications, such as absorption of exsudates in dermatology. Moreover, their anionic groups might allow retention of substances bearing charges of opposite signs. These swelling properties appeared to depend mainly on the reaction pH: optimal at pH 9.8, they decreased with 0.5 M NaOH. These results demonstrate that controlled, inframaximal cross-linking conditions should be used in order to retain the elasticity of the membrane. Further experiments performed with gelatin and CMS are consistent with these findings: the involvement of the protein in the wall together with its own buffer effect made it possible to use lower acylating agent concentrations and shorter reaction times. Under these conditions, and for a given amount of CMS, the hydrophilic properties of microcapsules were greatly improved. Otherwise, the resulting mixed-walled microcapsules were shown to be biodegradable. They might then find interesting medical applications, for example, in the field of chemoembolization: as a matter of fact, microparticles to be used as emboli are preferably hydrophilic, as pointed out for example by Chithambara Thanoo and Jayakrishnan (1989), who recently reported on hydrogel microspheres prepared from cross-linked poly(methylmethacrylate). Moreover, the swelling properties of the microcapsules might allow their immobilization at a definite location in the vessel to obstruct.

Finally, mixed-walled microcapsules were also prepared through interfacial cross-linking of HSA admixed with different amounts of HES. They were shown to degrade in pancreatin. In addition, increasing the relative amount of protein results in shorter degradation times. These results are in good agreement with our previous findings (Lévy and Andry, 1986) and this possibility of obtaining biodegradable microcapsules greatly improves the versatility of the process.

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