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Cross-linked β-cyclodextrin microcapsules: preparation and properties

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Abstract

Microcapsules were prepared by interfacial cross-linking of β-cyclodextrins (β-CD) with terephthaloyl chloride (TC). Batches were prepared from β-CD solutions in 1 M NaOH, using 5% TC and a 30 min reaction time. Microcapsules were studied with respect to morphology (microscopy), size (laser diffraction technique) and, for selected batches, IR spectroscopy, determination of β-CD content (polarimetry after alkaline dissolution of microcapsules) and complexing properties, evaluated using *p*-nitrophenol (pNP) as the guest molecule. Well-formed microcapsules were obtained from 5, 7.5, and 10% β-CD solutions. The mean size of all batches was in the 10–35 μm range. The IR spectrum showed bands at 1724, 1280 and 731 cm^{-1}, reflecting the formation of esters. The β-CD contents were 46, 56–58 or 60–66% for batches prepared from 5, 7.5 or 10% β-CD solutions, respectively. The experiments conducted with 1 mM pNP showed a rapid complexation reaching a maximum within 1 h. When incubating 50 mg lyophilized microcapsules in 10 ml pNP solution, the maximal fixation (97.8 μmol/g microcapsules) was observed for small-sized particles ($\approx$ 11 μm) prepared from a 7.5% β-CD solution. The method then appears as a simple and rapid procedure to provide stable microcapsules, having an interesting guest-binding ability.

Keywords: Microcapsules; Cyclodextrin; Interfacial cross-linking; Terephthaloyl chloride; p-nitrophenol

1. Introduction

Cyclodextrins (CD) represent a group of cyclic oligosaccharides known for their ability to form inclusion complexes with a variety of organic molecules (Saenger, 1980). Among CD derivatives, insoluble CD polymers have received increasing attention, due to their numerous potential applications (Fenyvesi, 1988; Friedman, 1991). For example, they can be used for separation of optical isomers, or for selective removal of compounds from mixtures, such as caffeine from tea, or phenylalanine from protein hydrolysates. Otherwise, insoluble CD polymers have been claimed to behave as controlled release agents for complexed drugs (Fenyvesi and Szejtli, 1990).

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Two different approaches have been described for the preparation of CD polymers, i.e. polymerization of the CD on a pre-existing polymer on the one hand, and cross-linking of the CD on the other hand (Friedman, 1991). Concerning the second approach, epichlorhydrin (ECH) is the most widely used bifunctional reagent. For example, spherical beads can be prepared by cross-linking the CD at 80°C with ECH in an emulsion system, the CD being dissolved in an aqueous solution of NaOH (dispersed phase), and ECH in an organic solvent (Wiedenhof, 1969). Buckler et al. (1969) obtained CD resins with sebacoyl chloride by performing the reaction homogeneously in dimethylsulfoxide, heating the mixture at 100°C for 6 h. A modification of this method was brought in by Zemel et al. (1990) in order to improve the porosity of the resin and to obtain a thin film of insolubilized CD. These authors performed the cross-linking with acid dichlorides once the CD had been absorbed on the surface of a porous inorganic oxide.

To our knowledge, no attempt has been made with CD using acid dichlorides in emulsion systems. In a continuation of our work, dealing with microencapsulation by interfacial cross-linking of biopolymers, i.e. polysaccharides (Lévy and Andry, 1992), proteins (Lévy et al., 1995; Andry et al., 1996), and plant polyphenols (Andry et al., 1998), we attempted to apply the method to β-cyclodextrins (β-CD), using terephthaloyl chloride (TC) as the bifunctional reagent. It seemed interesting to prepare a novel type of cross-linked CD particles since the complexation properties of CD polymers are known to depend on the structure of the polymer, which determines the accessibility to the CD rings (Frömming and Szejtli, 1994). In that respect, whereas the ECH method provides monolithic gel beads, the well-known interfacial polycondensation techniques using acid dichlorides and hydrophilic monomers bearing amino or hydroxy groups, can provide vesicular particles composed of a liquid core and a polymer membrane (Arshady, 1989). The expected resulting microcapsules with walls made of cross-linked CD appeared likely to favour complexation by allowing easier access of guest molecules to the CD cavities as compared with gel beads.

In the first part of the work, microcapsules were prepared varying the reaction conditions and compared with respect to morphology and size. Additional assays were performed on selected batches for determination of IR spectra and content in β-CD. The second part of the study was devoted to an evaluation of microcapsule complexing properties, choosing *p*-nitrophenol (pNP) as the guest molecule. The effect of variations in the preparation conditions of microcapsules on pNP complexation was investigated.

2. Materials and methods

2.1. Preparation of the microcapsules

β-CD was supplied by Sigma. TC was purchased from Janssen Chimica, France. Cyclohexane and chloroform (Osi, France) were of analytical grade. The surfactants were sorbitan trioleate and polysorbate (Seppic, France).

In the standard procedure, a 7.5% (w/v) β-CD solution in 1 M NaOH (6 ml) was emulsified (Heidolph RGL 500 stirring motor, Prolabo, France) for 5 min at room temperature in 30 ml of cyclohexane containing 5% (v/v) sorbitan trioleate, using a stirring rate of 2000 rpm. Then, 40 ml of a 5% (w/v) solution of TC in a chloroform: cyclohexane (1:4, v/v) mixture was added to the emulsion and stirring was continued for 30 min. The reaction was ended by dilution with 80 ml cyclohexane. Then, the resulting microcapsules were separated, and washed successively with cyclohexane, 95% ethanol containing 2% (v/v) polysorbate, 95% ethanol and distilled water.

Variations were introduced in the standard procedure, concerning the stirring speed, the TC concentration and the β-CD concentration. The reaction time was kept constant in the study, as a 30 min reaction time had been shown to result in an efficient acylation of hydroxy groups of polysaccharides (Lévy and Andry, 1992) and proteins (Lévy et al., 1995; Andry et al., 1996).

2.2. Microcapsule characterization

The microcapsule morphology was studied by optical and scanning electron microscopy (SEM). All particles were sized by a laser diffraction technique (Coulter Particle Sizer, type LS 100, Coultronics, France). Size distributions were displayed in terms of volume versus particle size.

2.3. Infrared spectroscopy

IR spectra were measured by the KBr method using a Bomem Fourier transform IR spectrometer (MB series).

2.4. Microcapsule β-CD content

Microcapsule β-CD content was evaluated by polarimetry. Due to the high optical rotation of the rigid oligosaccharide structure, polarimetry has proved to be an excellent technique for quantitative assay of β-CD (Goodall, 1993), providing good selectivity and sensitivity, allowing the measurement of trace amounts of β-CD to a precision of 0.01% (Liu et al., 1993). The method has been used for estimation of the extent of coupling of CD to Sepharose gels (Subbaramaiah and Sharma, 1984), and for determination of the CD contents of CD polymers after release of the CD through a hydrolysis reaction (Behar et al., 1987).

In this study, samples of lyophilized microcapsules were dissolved in 0.5 M NaOH (45 min contact at 20°C). The solution was neutralized with HCl and filtered on a 0.22 μm membrane. It was verified with the initial β-CD that this treatment did not change the results of the polarimetric measurements. The moisture content of the original β-CD and that of all microcapsule batches were determined using a halogen moisture analyzer (HR 73, Mettler Toledo), in order to express the results as a function of dry weight. Two samples were analyzed per batch of microcapsules, and two batches of microcapsules were examined for each value of the varied parameter. The determination was done with a Perkin–Elmer 241 polarimeter. Calibration graphs were constructed with the starting β-CD in the range 0.2–0.8 g/100 ml. The linearity was demonstrated by the high determination coefficients ($r^2 > 0.999$).

2.5. Microcapsule complexing properties

Microcapsule complexing properties were determined with pNP as the guest compound, using conditions inspired from Komiyama and Hirai (1987). Variable amounts of lyophilized microcapsules were incubated at 20°C in 10 ml of a 1 mM solution of pNP in an acetate buffer pH 4, with magnetic agitation. After centrifugation, the supernatant was assayed spectrophotometrically for the concentration of uncomplexed pNP. For a given sample, four determinations were performed (two batches and two determinations per batch).

3. Results and discussion

3.1. Influence of reaction parameters on the morphology and size of microcapsules

Table 1 displays the preparation conditions of all microcapsule batches.

The standard procedure of batch 1 provided a white sediment in water, which was made of spherical vesicles with a granulous content and a distinct membrane, as observed by light microscopy (Fig. 1). Microcapsules of batches 3, 4 and 5 also exhibited a spherical shape in water, whereas those of batches 2, 6 and 7 were not perfectly spherical (dented aspect). The microscopic aspect of all microcapsules was found to be unchanged after 1 year of storage in water at 45°C.

Although microcapsules could be obtained with 5% CD, this concentration was not found optimal considering the reduced volume of the sediment.

Lyophilization of all particles provided free-flowing powders. The microcapsules were intact and quickly recovered their initial shape after rehydration, as shown by light microscopy. Examination of all batches of lyophilized microcapsules by SEM mainly showed collapsed particules with a continuous and smooth membrane, a part of them remaining roughly spherical (Fig. 2a). Grinding the lyophilized microcapsule powders prior to SEM examination made it possible to observe the structure of broken particles which appeared as hollow spheres with a burst open

shell (Fig. 2b). This observation confirms that the polycondensation reaction occurred at the interface, forming a membrane around the dispersed droplets.

Microcapsule mean size was shown to depend on the reaction parameters, increasing with the CD concentration, decreasing when raising the stirring speed, and slighly increasing when decreasing the surfactant concentration (Table 1).

3.2. FT-IR spectra

The spectrum of microcapsules of batch 2 (Fig. 3b) was compared with the spectrum of original β-CD (Fig. 3a). Three bands appeared in microcapsule spectrum at 1724, 1280 and 731 cm^{-1}, respectively, which reflect the formation of esters from hydroxy groups of the β-CD. With this respect, and although the TC/β-CD ratio was much lower than in the present study, it is interesting to mention the work of Tabushi et al. (1976) who treated β-CD with 1 equivalent of TC in pyridine. This treatment resulted in an intramolecular cross-linking of the CD involving two primary hydroxy groups (Nair and Dismukes, 1983). 'Capped CD' were then obtained whose IR spectrum exhibited bands at 1710, 1280 and 730 cm^{-1}.

3.3. β-CD content of microcapsules

The microcapsule β-CD contents appear in Table 1. The results were expressed in % of dry weight, the moisture contents of all batches being in the 6–11% range. The β-CD content increased with the initial β-CD concentration, which might be accounted for by the lower CT/β-CD ratio during the polycondensation step, resulting in a decrease of the degree of cross-linking.

The amount of terephthalic acid involved in ester bonds was obtained by subtracting the β-CD content from microcapsule dry weight. The molar terephthalate/β-CD ratios were calculated for all batches, giving around 5 mol terephthalate for 1 mol β-CD for the first group of microcapsules prepared with 7.5% β-CD, around 4 mol terephthalate for 1 mol β-CD for the group prepared with 10% β-CD, and around 8 mol terephthalate for 1 mol β-CD for the batch prepared with 5% β-CD. The primary hydroxy groups of CD are supposed to be preferably involved in the cross-linked membrane since these groups are known to be more easily acylated than the secondary hydroxy groups. In the case of highly cross-linked microcapsules of batch 7, secondary hydroxy groups would also be concerned by acylation.

Table 1
Microcapsule mean size, β-CD content and pNP complexing properties as a function of the preparation parameters

Batch	1	2	3	4	5	6	7
β-CD%	7.5	7.5	7.5	7.5	10	10	5
TC%	5	5	2.5	5	5	5	5
Surfactant %	5	5	5	2	5	5	5
Stirring speed rpm	2000	5000	2000	2000	2000	5000	2000
Size μm (mean value ± SD)	27.4 ± 19.3	11.4 ± 10.0	30.4 ± 16.2	35.0 ± 14.7	34.3 ± 22.9	11.3 ± 6.0	20.7 ± 12.0
β-CD content (mean value ± SD)							
%	55.9 ± 0.2	57.9 ± 0.8	57.4 ± 0.6	–	60.6 ± 0.5	65.7 ± 1.2	45.9 ± 1.1
μmol/g	492.6 ± 1.6	509.9 ± 6.9	505.9 ± 5.6	–	533.9 ± 4.5	578.8 ± 10.65	404.2 ± 9.4
Molar ratio terephthalate/CD (mean value ± SD)	5.4 ± 0.0	5.0 ± 0.2	5.1 ± 0.1	–	4.4 ± 0.1	3.6 ± 0.2	8.1 ± 0.35
Complexed pNp[a](μmol/g)(mean value ± SD)	83.5 ± 0.7	97.75 ± 0.9	85.1 ± 2.25	–	76.7 ± 3.6	–	92.05 ± 1.7

[a] 50 mg microcapsules, 1 h incubation.

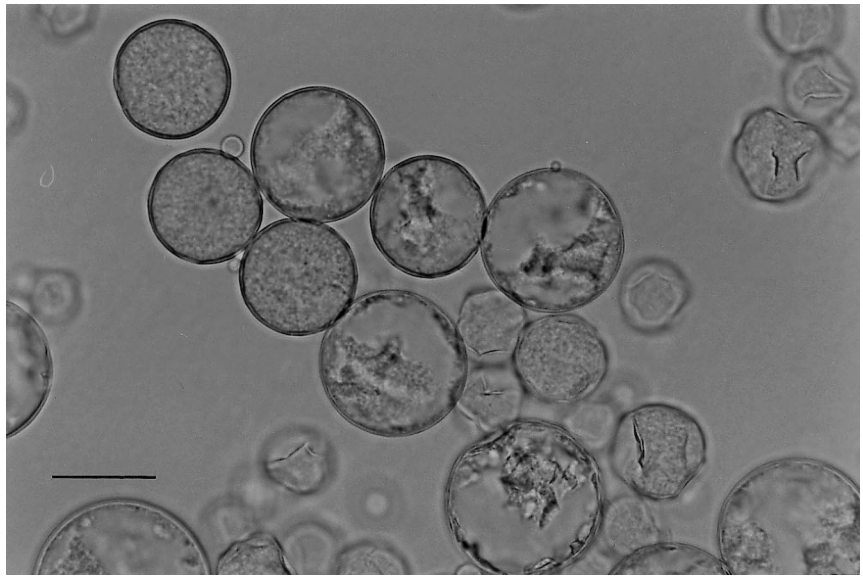

Fig. 1. Optical photomicrographs of cross-linked β-cyclodextrin microcapsules (batch 1), bar = 25 μm.

3.4. Microcapsule complexing properties towards p-nitrophenol

3.4.1. Preliminary assays: influence of sample weight and incubation time

A series of experiments conducted with increasing amounts (10–100 mg) of microcapsules of batch 1 for various incubation times (5 min–24 h) showed that, for each incubation time, an increase in sample weight resulted in a progressive increase in the amount of pNP fixed by the microcapsules when expressed in % of the initial pNP amount, and in a progressive decrease when the results were expressed in μmol pNP fixed per g lyophilized microcapsules, as expected.

Otherwise, comparison of the results obtained after various incubation times shows that the equilibrium was reached rapidly. Slight variations of the values were observed after 5 min, the plateau being reached after 1 h in most cases.

3.4.2. Influence of the parameters of microcapsule preparation

Table 1 presents the results of pNP complexation (with 50 mg lyophilized microcapsules and 1 h agitation) as a function of the preparation parameters of the microcapsules.

Concerning microcapsules prepared with 7.5% CD solutions, increasing the stirring speed to 5000 rpm (batch 2 as compared with batch 1) resulted in an increase in pNP complexation (significant difference, $P < 0.05$), while decreasing the TC concentration to 2.5% (batch 3) had no effect on complexation. In this group, the conditions of batch 2 were then found the most convenient for complexation. Presumably, pNP fixation was favoured by the reduced size of microcapsules, which resulted in an increase in interfacial area.

Surprisingly, raising the initial β-CD concentration to 10% (batch 5) did not result in an improvement of complexing properties, although the β-CD content of batch 5 was found higher than that of batch 1, also prepared at 2000 rpm. In fact, the amount of complexed pNP was lower (significant difference, $P < 0.05$). This decrease in complexation may be due to the slight increase in microcapsule size.

In the case of microcapsules prepared with 5% CD (batch 7), although the β-CD content of this batch was lower, the complexing properties of these microcapsules were found higher than those of batch 1 (significant difference, $P < 0.05$), suggesting that only the external CD cavities of the cross-linked membrane would be involved in pNP

complexation. The improvement of guest-binding properties would be due to the reduced size of microcapsules of batch 7 as compared with microcapsules of batch 1.

These results lead us to select the microcapsules prepared according to conditions of batch 2 as they proved to be the most efficient for pNP complexation. Further studies of complexation were then performed on new batches of these microcapsules.

3.4.3. Complexing properties of a selected batch as a function of sample weight

Table 2 displays the amounts of complexed pNP as a function of the sample weight of microcapsules prepared according to batch 2, after 1 h agitation.

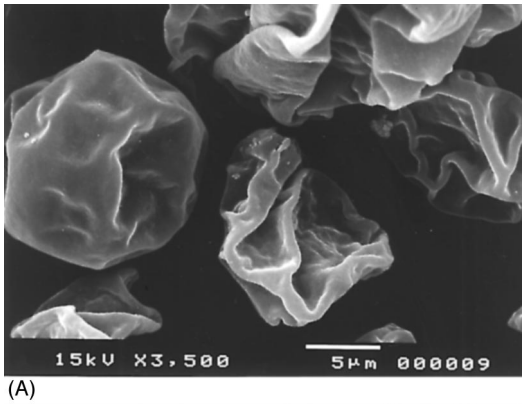

Fig. 2. Scanning electron micrographs of cross-linked β-cyclodextrin microcapsules (batch 1) (a) aspect before grinding; (b) burst open microcapsule.

As already observed with microcapsules of batch 1, increasing the sample weight resulted in an increase in the amount of pNP fixed by the microcapsules when expressed in % of the initial amount, and in a decrease, when the results were expressed in μmol pNP fixed per g lyophilized microcapsules. Except for one value (% of pNP fixed by 10 mg microcapsules), all values were found higher than the corresponding values obtained with microcapsules prepared at a lower stirring speed, confirming the effect of microcapsule size on pNP complexation (significant differences, $P < 0.05$).

With this respect, it was interesting to conduct a parallel assay with 100 mg of microcapsules of batch 6, prepared at 5000 rpm with a higher concentration of β-CD (10%), and whose mean size was equal to that of microcapsules batch 2. In this case, the mean value was 64.7 ± 2.6 μmol pNP fixed per g, which was not found significantly different from the result obtained with batch 2. In addition to underlining the importance of microcapsule size, these results again show that increasing the concentration of β-CD to 10% did not result in an improvement of the complexing properties.

Otherwise, as the β-CD content (57.9% of dry weight) of microcapsules in batch 2 had been determined by polarimetry, the amount of complexed pNP as a function of the sample weight of microcapsules allowed us to calculate the equilibrium constant K of the complex, using the following equation:

$$K_c = \frac{[\beta\text{-CD guest complex}]}{[\text{uncomplexing } \beta\text{-CD}][\text{uncomplexed guest}]} = \frac{([\text{Go}] - X)}{([\text{CDo}]\text{-}[\text{Go}] + X)X},$$

where Go = initial pNP concentration; X = final pNP concentration; and CDo = initial β-CD concentration.

Accordingly, the calculated values for 10, 20, 50 and 100 mg microcapsule powder were 431, 437, 463 and 444 M^{-1}, respectively. Although these values were approximate and somewhat underestimated, since the moisture content of these new lyophilized samples was not determined, the re-

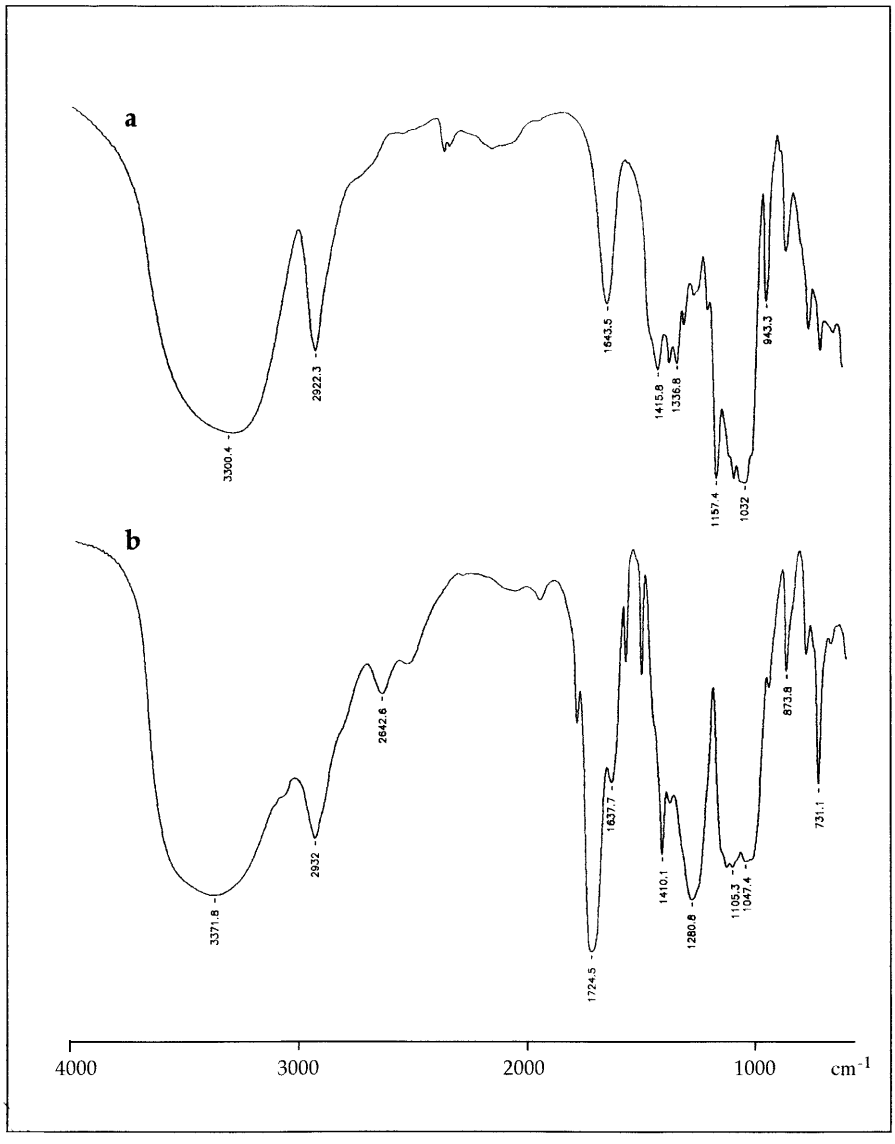

Fig. 3. FT-IR spectra: (a) original β-cyclodextrin; (b) cross-linked β-cyclodextrin microcapsules (batch 2).

sults were close to each other, suggesting a 1:1 complex formation.

Similar conclusions were given by Komiyama and Hirai (1987) concerning β-CD polymers in the form of beads prepared with several alcanediol diglycidyl ethers. The K values in binding of pNP, which did not exceed 313 M^{-1}, varied with the cross-linking agent and one of them was higher than that of ECH cross-linked beads prepared for comparison ($K = 263$ M^{-1}). The differences were attributed to variations in the participation of the cross-linking residues in guest binding. As a matter of fact, it is known that, in addition to interactions between the guest compound and the CD (inclusion mechanism), adsorption can take place in the secondary cavities originated by the cross-linking agent (Frömming and Szejtli, 1994). Accordingly, in the series of

Table 2
Complexation of pNP by β-CD microcapsules (50 mg) of batch 2 after 1 h incubation: effect of variations in sample weight

	Complexed pNP %/μmol/g of microcapsules, mean values ± SD sample weight (mg)			
	10	20	50	100
%	13.8 ± 0.8	25.15 ± 0.6	48.9 ± 0.4	66.3 ± 1.1
μmol/g	138.3 ± 8.2	125.8 ± 2.9	97.75 ± 0.9	66.5 ± 1.3

polymers of Komiyama and Hirai, variations in apolar interactions between the pNP and the cross-linking residues were thought to account for the observed differences in guest binding.

Likewise, in the present study, interactions with terephthalate groups might interfere and contribute to the enhancement of guest-binding ability, which was observed, as compared with the polymer beads of Komiyama and Hirai, including ECH cross-linked beads. However, the improved pNP complexation in this work might be mainly related to the structure of the microcapsules. In contrast with gel beads requiring penetration of the guest molecules for reaching the internal CD rings of the network, the external localization of the cross-linked CD in the form of a membrane is assumed to favour interactions with the substrate.

Otherwise, the K values of the microcapsules were in the range of the binding constants of 1:1 complexes reported for pNP (acid form) and the parent β-CD, which are listed in the supplementary material of Connors (1995). These constants, which were determined at 25 ± 5°C in aqueous solution using different methods, were comprised between 130 and 1150 M^{-1}, giving a mean value of 443 M^{-1}. It should be stressed that two K values in this list were obtained at pHs close to pH 4, i.e. 314 and 301 M^{-1}, for pH 3.5 (Sanemasa et al., 1984) and pH 4.3 (Korpela and Himanen, 1984), respectively. The higher K values obtained in this study suggest that the stability of the complexes formed with the microcapsules might be higher than that of complexes formed in homogeneous solution, as already observed with insoluble CD polymers (Frömming and Szejtli, 1994).

In conclusion, it was shown in this work that CD microcapsules can be easily prepared at room temperature by interfacial cross-linking with TC. The cross-linked CD maintained a guest-binding ability. Interestingly, the complexation was rapid, reaching a maximum within 1 h. Variations of the preparation parameters demonstrated the influence of reducing microcapsule mean size on guest binding. Complexation studies are currently in progress using other guest molecules. The results already confirm the microcapsule efficiency, which suggests various potential applications.

The method then appears as a simple and rapid procedure to prepare cross-linked β-CD microcapsules with interesting complexing properties.

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References

Andry, M.-C., Edwards-Lévy, F., Lévy, M.-C., 1996. Free amino group content of serum albumin microcapsules: III. A study at low pH values. Int. J. Pharm. 128, 197–202.

Andry, M.-C., Vezin, H., Dumistracel, I., Bernier, J.L., Lévy, M.-C., 1998. Proanthocyanidin microcapsules: preparation, properties and free radical scavenging activity. Int. J. Pharm. 171, 217–226.

Arshady, R., 1989. Review. Preparation of microspheres and microcapsules by interfacial polycondensation techniques. J. Microencapsulation 6, 13–28.

Behar, N., Richard, N, Thuaud, N., Sebille, B., Grousset, M., Raynal, S., 1987. Propriétés des polymères porteurs de β-cyclodextrine pour le relargage différé de médicaments. S.T.P. Pharma 3, 237–247.

Buckler S., Martel R.F., Moshy R.J., 1969, Insolubilized Schardinger dextrins. US Patent No. 3,472,835.

Connors, K.A., 1995. Population characteristics of cyclodex-

trin complex stabilities in aqueous solution. J. Pharm. Sci. 84, 843–848 Supplementary material, http: //www. pharmacy. wisc.edu./facstaff/sciences/CONNORS_supp.pdf.

Fenyvesi, E., 1988. Cyclodextrin polymers in the pharmaceutical industry. J. Incl. Phenom. 6, 537–545.

Fenyvesi, E., Szejtli, J., 1990. Complex formation of cyclodextrin polymers with iodine. In: Duchêne, D. (Ed.), Minutes of the Fifth International Symposium on Cyclodextrins. Editions de Santé, Paris, pp. 276–281.

Friedman, R.B., 1991. Cyclodextrin-containing polymers. In: Duchêne, D. (Ed.), New Trends in Cyclodextrins and Derivatives. Editions de Santé, Paris, pp. 158–177.

Frömming, K.-H., Szejtli, J., 1994. Cyclodextrins in Pharmacy, Topics in Inclusion Science, vol. 5. Kluwer Academic Publishers, Dordrecht, pp. 31–32.

Goodall, D.M., 1993. Chiral analysis based on polarimetric detection. Trends Anal. Chem. 12, 177–184.

Komiyama, M., Hirai, H., 1987. Preparation of immobilized β-cyclodextrins by use of alkanediol diglycydylethers as cross-linking agents and their guest binding abilities. Polymer J. 19, 773–775.

Korpela, T.K., Himanen, J.P., 1984. Determination of equilibrium constants by gel chromatography: binding of small molecules to cyclodextrins. J. Chromatogr. 290, 351–361.

Lévy, M.-C., Andry, M.-C., 1992. Microcapsules with walls made of cross-linked starch derivatives. In: Whateley, T.L. (Ed.), Microencapsulation of Drugs, Drug Delivery Series, vol. 1. Harwood Academic Publishers, Chur, pp. 7–16.

Lévy, M.-C., Lefèbvre, S., Andry, M.-C., Manfait, M., 1995. Fourier transform infrared spectroscopic studies of human serum albumin microcapsules. 3. Influence of terephthaloyl chloride concentration on spectra and correlation with microcapsule morphology and size. J. Pharm. Sci. 84, 161–165.

Liu, G., Goodall, D.M., Loran, J.S., 1993. Quantification of trace levels of β-cyclodextrin and substitution patterns in hydroxypropyl-β-cyclodextrin using high-performance liquid chromatography with polarimetric detection. Chirality 5, 220–223.

Nair, B.U., Dismukes, C., 1983. Models for the photosynthetic water oxidizing enzyme. 1. A binuclear manganese(III)-β-cyclodextrin complex. J. Am. Chem. Soc. 105, 124–125.

Saenger, W., 1980. Cyclodextrin inclusion compounds in research and industry. Angew. Chem. Int. Ed. Engl. 19, 344–362.

Sanemasa, I., Mizoguchi, T., Deguchi, T., 1984. Application of gel chromatography to determining formation constants of inclusion compounds of cyclodextrins. Bull. Chem. Soc. Jpn 57, 1358–1361.

Subbaramaiah, K., Sharma, R., 1984. Polarimetric determination of substitution of oxirane groups on epoxy-activated sepharose 6B gel by cyclodextrins. J. Biochem. Biophys. Methods 10, 65–68.

Tabushi, I., Shimokawa, K., Shimizu, N., Shirakata, H., Fujita, K., 1976. Capped cyclodextrin. J. Am. Chem. Soc. 98, 7855–7856.

Wiedenhof, N., 1969. Properties of cyclodextrins, Part III. Cyclodextrin–epichlorhydrin resins: preparation and analysis. Staerke 2, 119–123.

Zemel H., Koch M.B., Rohrbach R.P., 1990, Crosslinked cyclodextrins supported on porous refractory inorganic oxides. US Patent No. 4,902,788.