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Cross-linked β-cyclodextrin microcapsules. II. Retarding effect on drug release through semi-permeable membranes

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Abstract

Microcapsules were prepared by interfacial cross-linking of β-cyclodextrins (β-CD) with terephthaloylchloride (TC) as described previously. Complexation assays were conducted with propranolol HCl. After 1 h incubation of 50 mg lyophilized microcapsules in 10 ml propranolol solution, the amounts of fixed drug were 507.5 ± 8.6 μmol and 811.2 ± 16.0 μmol per g lyophilized microcapsules with 1 mM and 2 mM solutions, respectively. A dialysis experiment was then performed. After 1 h incubation of microcapsules (10 or 50 mg) in 10 ml of 2 mM propranolol solution, the suspension was dialysed against a phosphate buffer pH 7.4 at 37 °C. The drug diffusion was all the more retarded that the amount of added β-CD microcapsules was higher. Finally, double microcapsules were prepared using a suspension of β-CD microcapsules (10–100 mg) in a solution of methylene blue in an acetate buffer pH 7.4. After adding human serum albumin (HSA), the suspension was emulsified in cyclohexane and double microcapsules were obtained by cross-linking the HSA with TC. In vitro release studies showed that the incorporation of β-CD microcapsules resulted in a decrease in release rate of methylene blue, the decrease being related to the amount of encapsulated β-CD microcapsules. The study then suggests interesting applications of β-CD microcapsules for modulating the release rate of drugs through semi-permeable membranes.

Keywords: Microcapsules; Cyclodextrin; Terephthaloyl chloride; Propranolol; Methylene blue; Controlled release

1. Introduction

Cyclodextrins (CD) and their derivatives have been frequently incorporated into polymeric drug delivery systems with the aim of controlling the drug release (Hirayama and Uekama, 1999; Bibby et al., 2000). Depending on the system, the drug and the type of CD, the incorporation of CD can result either in an increase in release rate or in a decrease, numerous mechanisms being likely to interfere (Bibby et al., 2000). For controlling the release of soluble drugs across a membrane, water-soluble CD polymers have been shown to present a particular interest when their high molecular weight prevents the leakage out of the system. For example, Behar (Behar et al., 1987)

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reported on the retarded release of propranolol through a dialysis membrane when the drug was complexed with soluble CD polymers. Likewise, microencapsulation of a drug complexed with a soluble polymer that cannot cross the microcapsule membrane has been shown to result in a decrease in release rate (Fenyvesi, 1988a; Friedman, 1991). In addition, it has been observed that the retarding effect of CD polymers on diffusion was increased by making the polymer insoluble (Behar et al., 1987).

In this context, it seemed interesting to investigate the effect of cross-linked CD microcapsules on the diffusion of soluble drugs across semi-permeable membranes, insoluble particles being expected to remain entrapped in the system whatever the molecular weight cut-off of the membrane. This is the purpose of the present study. In a recent paper, we reported on β-CD microcapsules prepared by interfacial cross-linking with terephthaloylchloride (TC) (Pariot et al., 2000). These particles (size range: 10–35 μm) were shown to rapidly complex *p*-nitrophenol (pNP), the amount of fixed pNP increasing with decreasing microcapsule size.

The initial aim of the present work was to assess the complexing properties of the β-CD microcapsules towards propranolol and then to study the effect of these microcapsules on the release of the drug through a dialysis membrane. Propranolol was chosen both for its high water solubility and for its affinity for β-CD. The results prompted us to carry out a second series of experiments in which the dialysis membrane was replaced by a microcapsule membrane. This was achieved using a double encapsulation process. In order to make the determinations easier, methylene blue was used as the guest molecule, this dye being capable of forming a complex with β-CD. Methylene blue was encapsulated with variable amounts of β-CD microcapsules inside a membrane made of cross-linked human serum albumin (HSA). The choice of HSA was justified by earlier results of ours showing that stable microcapsules could be obtained at low pH values by interfacial cross-linking of the protein with TC (Andry et al., 1996). The encapsulation process with HSA probably did not affect the ester bonds of the β-CD microcapsules. The resulting 'double microcapsules' were characterized and then in vitro release assays were performed in order to evaluate whether the incorporation of β-CD microcapsules could modify the release kinetics of the encapsulated drug through the cross-linked HSA membrane.

2. Materials and methods

2.1. Materials

β-CD and DL-propranolol HCl were supplied by Sigma. TC was purchased from Janssen Chimica, and methylene blue from Fluka. HSA was obtained from CTS Strasbourg. Cyclohexane and chloroform (Osi) were of analytical grade. The surfactants were sorbitan trioleate and polysorbate 20 (Seppic).

2.2. Preparation of the β-CD microcapsules

Microcapsules were prepared by interfacial cross-linking with TC as described elsewhere (Pariot et al., 2000). We used the conditions that provided small-sized (≈ 11 μm) microcapsules exhibiting the highest complexing properties towards pNP in our previous work.

Briefly, a 7.5% (w/v) β-CD solution in 1 M NaOH (6 ml) was emulsified for 5 min at room temperature in 30 ml of cyclohexane containing 5% (v/v) sorbitan trioleate, using a stirring rate of 5000 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 cyclohexane. The resulting microcapsules were then separated, washed and lyophilized.

2.3. Complexation assays with propranolol

Lyophilized β-CD microcapsules (10 mg) were incubated at 20 °C in 10 ml of a solution of propranolol in a phosphate buffer pH 7.4, with magnetic agitation. The vessel was protected from light. After 1 h, the suspension was centrifuged

and the supernatant was assayed spectrophotometrically at 290 nm for the concentration of uncomplexed drug. Two concentrations of propranolol were studied i.e. 1 and 2 mM. For each concentration, four determinations were performed (two batches and two determinations per batch).

2.4. Dialysis experiments

A sample of lyophilized β-CD microcapsules (10 or 50 mg) was dispersed in 10 ml of a 2 mM solution of propranolol in a phosphate buffer pH 7.4 at 20 °C. Magnetic agitation was maintained for 1 h in a vessel protected from light. The microcapsule suspension was then introduced in a dialysis bag (Spectra/Por, molecular weight cut-off 5000) equipped with a magnetic clip for agitation. The suspension was then dialysed against a phosphate buffer pH 7.4 (140 ml) at 37 °C with magnetic agitation. Aliquots were removed at intervals for determination of propranolol and reintroduced in the release bath. Control assays were conducted omitting the addition of CD microcapsules. The series of experiments were triplicated.

2.5. Double encapsulation process

The principle of the process is illustrated on Fig. 1.

First, β-CD microcapsules were prepared as described above. Then, a variable amount of lyophilized microcapsules (20–100 mg) was dispersed in 10 ml of a 0.1% methylene blue solution in an acetate buffer pH 7.4. Magnetic agitation was maintained for 1 h at 20 °C in a vessel protected from light.

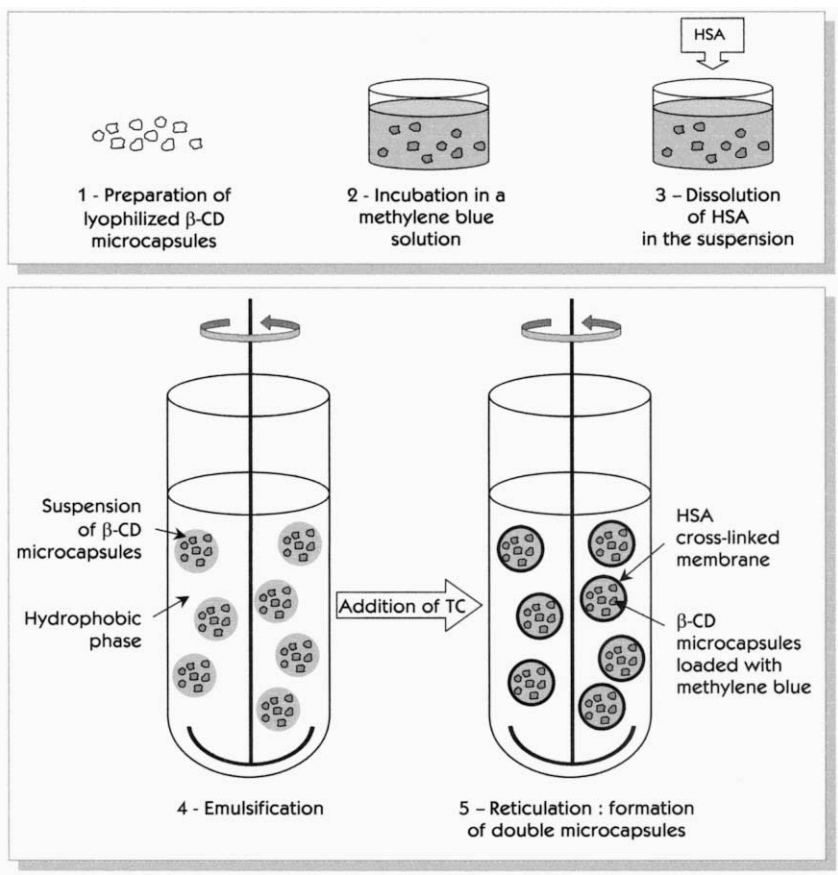

Fig. 1. Principle of the double encapsulation process.

Then 1 g HSA was dissolved in the microcapsule suspension. The resulting aqueous phase was emusified in 50 ml cyclohexane containing 2% (v/v) sorbitan trioleate (5 min agitation; stirring rate: 2000 rpm). The cross-linking step was started by addition of a 2.5% (w/v) CT solution in cyclohexane. Agitation was continued for 30 min. Then the double microcapsules were separated by centrifugation and washed four times with cyclohexane. After elimination of the solvent by vacuum evaporation, the microcapsules were congealed and lyophilized.

Control batches of HSA microcapsules containing methylene blue were also prepared omitting the addition of β-CD microcapsules. In addition, batches of microcapsules were prepared adding 50 mg of the parent β-CD instead of CD microcapsules. In this case too, the β-CD was incubated for 1 h in the drug solution prior to the addition of HSA preceding the encapsulation step.

2.6. Characterization of double microcapsules

The microcapsule morphology was studied by optical and scanning electron microscopy (SEM). Particles were sized by a laser diffraction technique (Coulter Particle Sizer, type LS 200, Coultronics, France). Size distributions were displayed in terms of volume versus particle size.

2.7. Double microcapsules: in vitro release study

A whole batch of lyophilized double microcapsules (mean dry weight between 2.01 and 2.14 g) was dispersed in 500 ml of phosphate buffer pH 7.4 at 37 °C (Erweka dissolution apparatus, agitation: 50 rpm). Samples were withdrawn at intervals, filtered through a Millipore filter (0.22 μm) for spectrophotometric methylene blue determination (668 nm), and reintroduced in the release bath. All assays were triplicated.

3. Results and discussion

3.1. Assays conducted with propranolol

3.1.1. Microcapsule complexing properties

With a 1 mM propranolol solution, the mean values of complexed propranolol obtained from the two batches were not found significantly different. The general means calculated from all values was 507.5 ± 8.6 μmol per g of lyophilized microcapsules.

It is interesting to mention that no further fixation was found when prolonging the incubation time to 2 h and even 3 h, as previously observed with pNP (Pariot et al., 2000). This rapid fixation was attributed to the structure of the microcapsules. These hollow spheres with walls made of cross-linked CD were thought to favour complexation by allowing an easy access to the CD cavities.

In addition, we observed that after the drug had been completely released by incubating the microcapsules three times in 50 ml buffer pH 7.4 for 15 min with magnetic agitation, the microcapsules could be reused for another complexation under the same conditions, giving comparable results for the second complexation (no significant difference, as compared with the results of the first incubation).

With a 2 mM propranolol solution, the mean values of complexed propranolol obtained from the two batches were not found significantly different. The grand mean calculated from all values was 811.2 ± 16.0 μmol per g of lyophilized microcapsules.

These experiments show important complexation properties of the β-CD microcapsules towards propranolol under the applied experimental conditions. With 1 mM propranolol, the amount of fixed propranolol was practically equal to the β-CD content of the microcapsules (509.9 ± 6.9 μmol β-CD per g microcapsule dry weight, as determined in our previous work (Pariot et al., 2000), while with 2 mM propranolol the amount of adsorbed drug exceeded the β-CD content of the microcapsules when considering the drug/β-CD content on a molar basis.

Such findings have been already reported for insoluble CD polymers. For example, Fenyvesi (1988b) observed that the values of equilibrium concentrations of several azo-dyes were higher than the CD content of the polymer, suggesting that the dye molecule not only fills the CD cavities but also the cavities inside the cross-linked structure and can adsorb on the surface of the beads, too. Likewise, Friedman et al. (1989) reported that CD-containing

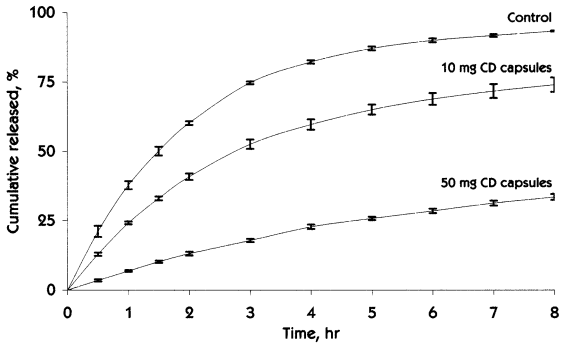

Fig. 2. Dialysis experiment: effect of the addition of β-CD microcapsules on the diffusion rate of propranolol. The error bar shows S.D.

polymers enhance binding with some aromatic compounds and that it was not clear if this involved interaction with the cross-linked regions of the polymer outside the CD or if the rigidity allowed stacking of the guest molecules within the CD.

3.1.2. Dialysis experiments

Fig. 2 presents the release profiles obtained with 10 and 50 mg microcapsules in comparison with that of the control assay conducted without microcapsules. The results clearly show that the addition of β-CD microcapsules resulted in a decrease in release rate which was all the more pronounced as the amount of microcapsules was higher. For example, while 93.3% of propranolol was released after 8 h in the control assay, only 74 and 33.5% were released when adding 10 and 50 mg microcapsules, respectively.

This effect was expected. As a matter of fact, whereas diffusion was the sole mechanism involved in the release kinetics of propranolol through the dialysis membrane, when β-CD microcapsules were added, the release kinetics not only depended on diffusion of the drug through the dialysis membrane but also on the equilibrium between the complexed and the uncomplexed fraction of the drug. Dissociation of the complex with CD progressively occurred as the drug diffused through the dialysis membrane.

3.2. Assays conducted with methylene blue

3.2.1. Characteristics of the double microcapsules

The double encapsulation process provided a blue sediment in the reaction medium which was easily separated by centrifugation. Examination by optical microscopy showed spherical particles containing strongly stained β-CD microcapsules (Fig. 3a). Whatever the amount of added β-CD microcapsules, the total amount was encapsulated. Moreover, the β-CD microcapsules appeared intact, suggesting that the ester bonds of the cross-linked β-CD membrane survived the

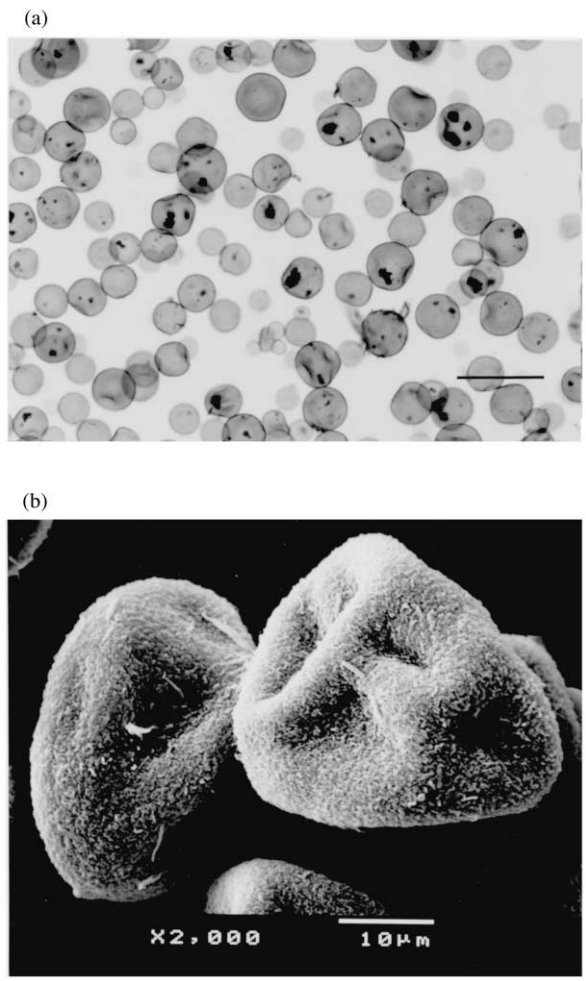

Fig. 3. Double microcapsules prepared with 50 mg β-CD microcapsules; (a) optical photomicrograph (bar = 150 μm); (b) SEM.

Table 1
Influence of the incorporation of β-CD microcapsules or parent β-CD on the mean size of HSA microcapsules

	Control batches		Double microcapsules		
β-CD microcapsules (mg)	–	–	20	50	100
Parent β-CD (mg)	–	50	–	–	–
HSA microcapsule mean size, μm ± SD	46.3 ± 12.4	52.7 ± 24.0	62.6 ± 28.7	60.0 ± 26.5	70.1 ± 30.2

double encapsulation process, due to the mild pH conditions.

After lyophilization, free-flowing powders were obtained, that could be rapidly dispersed in an aqueous medium. Examination of lyophilized microcapsules by SEM showed roughly spherical particles with a rough surface (Fig. 3b).

Table 1 shows the mean diameters of double microcapsules. All batches were in the 60–70 μm range, while a lower size was observed with HSA microcapsules prepared without CD, and an intermediate value was found for HSA microcapsules prepared with 50 mg of the parent CD.

3.2.2. In vitro release study

The release profiles of methylene blue obtained with the various batches of microcapsules are presented on Fig. 4. As observed with propranolol in the dialysis experiment, the release of methylene blue through the cross-linked HSA membrane was retarded by addition of β-CD microcapsules and this effect was all the more marked as the amount of added microcapsules was higher. For example, after 8 h, the percentage of released drug from the control batch prepared without CD was about two times higher than that released from microcapsules containing 50 mg β-CD microcapsules and about three times higher than that released from microcapsules containing 100 mg microcapsules.

The lower mean size of the control HSA microcapsules might be involved in the higher release rate, since the release rates are known to increase with decreasing microcapsule size. However, the fact that increasing the amount of encapsulated CD microcapsules, especially from 20 to 50 mg (comparable mean sizes), resulted in a decrease of the release rate, unambiguously demonstrates the retarding role of the β-CD microcapsules. Like in the dialysis experiments, the dissociation of the complex formed with the β-CD microcapsules was involved as an additional control mechanism in the release kinetics.

It should be stressed that an increase in release rate was observed with the HSA microcapsules containing 50 mg of the parent β-CD as compared with the control batches prepared without CD, despite a slightly higher mean size. In case the β-CD-methylene blue complex could cross the membrane, the release rate would be lower than that of the uncomplexed drug due to the increase in molecular weight, as it has been reported for the diffusion of β-CD complexes through a semipermeable membrane in an homogeneous solution (Frömming and Szejtli, 1994). The increase in release rate might be due to the ability of CD to act as hydrating agents promoting diffusion of water into the microcapsules. This mechanism has been proposed to account for the enhanced release of drugs in microcapsules containing CD compared to those containing drug alone (Bibby

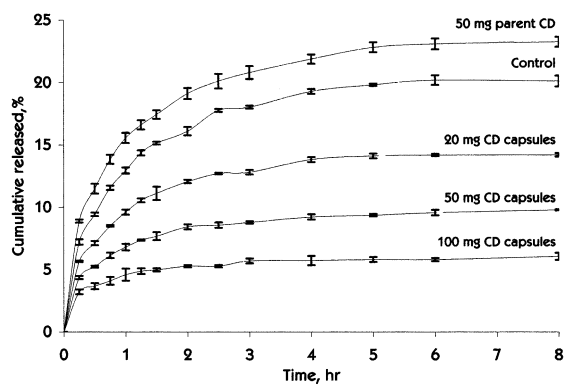

Fig. 4. Influence of the incorporation of growing amounts of β-CD microcapsules and of parent β-CD on in vitro release of methylene blue from HSA microcapsules. The error bar shows S.D.

et al., 2000). In the case of β-CD microcapsules, the numerous hydrophobic groups introduced by cross-linking are assumed to suppress the hydrating properties of the CD. Accordingly, there would be no enhanced diffusion of water into the double microcapsules that could impair the drug complexation and thereby the controlling effect of the β-CD microcapsules on the release rate.

In conclusion, it has been shown in this study that the cross-linked β-CD microcapsules could behave as an efficient controlled release agent capable of retarding the release of water-soluble drugs through semi-permeable membranes. The series of experiments conducted with methylene blue shows that double microcapsules containing variable amounts of β-CD microcapsules allow a versatile modulation of the release rate, which suggests various interesting applications.

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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.

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.

Bibby, D.C., Davies, N.M., Tucker, I.G., 2000. Mechanisms by which cyclodextrins modify drug release from polymeric drug delivery systems. Int. J. Pharm. 197, 1–11.

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

Fenyvesi, E., 1988b. Complexes of insoluble cyclodextrin polymers. In: Huber, O., Szejtli, J. (Eds.), Proc. 4th Int. Symposium on Cyclodextrins. Kluwer Academic Publishers, Dordrecht, pp. 227–335.

Friedman, R.B., Hedges, A.R., Black, F.L., Gottneid, D.J., 1989. Complexation of aromatic compounds with, and their release from, cyclomaltoheptaose-containing polymers, hydroxyethylcyclomaltoheptaose, and cyclomaltoheptaose. Carbohydrate Res. 192, 283–289.

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

Frömming, K.-H., Szejtli, J., 1994. Cyclodextrins in Pharmacy, Topics in Inclusion Science, vol. 5. Kluwer Academic Publishers, Dordrecht, p. 61.

Hirayama, F., Uekama, K., 1999. Cyclodextrin-based controlled drug release system. Adv. Drug Delivery Rev. 36, 125–141.

Pariot, N., Edwards-Lévy, F., Andry, M.-C., Lévy, M.-C., 2000. Cross-linked β-cyclodextrin microcapsules: preparation and properties. Int. J. Pharm. 211, 19–27.