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Poly(acrylic acid) microspheres containing β-cyclodextrin: loading and in vitro release of two dyes

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Abstract

Microspheres containing poly(acrylic acid) and β-cyclodextrin or maltose were prepared by a w/o solvent evaporation technique. The dispersed aqueous phase contained poly(acrylic acid) (800 mg) and β-cyclodextrin or maltose (0, 200 or 800 mg). Food-grade olive oil was the continuous phase. Microsphere particle size was consistently between 15 and 25 μm, and carbohydrate content was in good agreement with that added to the dispersed phase in all cases. Two dyes, phenolphthalein and rhodamine B, having different solubility characteristics and strengths of association with β-cyclodextrin, were selected for loading and in vitro release studies. Microspheres were loaded by soaking in a saturated propan-2-ol solution of the appropriate dye (6 h). Microsphere dye content ranged between 2.8 and 4.8 mg/g microspheres for phenolphthalein and between 2.2 and 3.7 mg/g for rhodamine B. Release studies were performed in phosphate buffer (pH 7.4; 37°C). No difference in the release profile of either dye was observed between microspheres. The failure of microspheres containing β-cyclodextrin in particular, to alter the in vitro release kinetics of either dye may be due to a number of factors and include: (i) limited cross-linking giving rise to a the rapid hydration of the polymer matrix; (ii) perturbation of the dye–β-cyclodextrin complex by oil and/or organic solvent residues; and (iii) conformational changes/steric hindrance of the β-cyclodextrin cavity (due to its covalent binding with PAA) resulting in a reduction in its ability to form inclusion complexes. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Complexation; Controlled release; Cyclodextrin; Microspheres; Poly(acrylic acid)

1. Introduction

Poly(acrylic acid) (PAA) [$(-CH_2-CHCOOH-)_n$] is a hydrophilic polymer having a carboxylic acid content of between 56 and 68% (w/w) (USP XXII, 1990). These characteristics render it mucoadhesive (Park and Robinson, 1987; Anlar et al., 1993). Thus, PAA-based dosage forms often demonstrate prolonged retention on absorbing mucous membranes resulting in improved drug delivery. For example, Davies et al. (1991) demonstrated that a solution of PAA had an enhanced precorneal retention compared to an

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equiviscous non-mucoadhesive poly(vinyl alcohol) solution in rabbits. This enhanced retention, facilitated by the mucoadhesive PAA, in turn resulted in an improved bioavailability of the miotic, pilocarpine. Enhanced precorneal retention has also been reported for PAA microspheres (Durrani et al., 1995), and such microspheres have received attention for nasal drug delivery (El-Hameed and Kellaway, 1997).

A problem often encountered with PAA-based delivery systems, particularly those having a high surface area to volume ratio, such as microspheres, is a rapid drug release that is characterised by a 'burst' component (Leucuta et al., 1997). Some degree of control of drug release from polymeric matrices can be achieved by cross-linking (Chien, 1978). García-González et al. (1993) investigated the use of cyclodextrin as a cross-linking agent to control drug release from PAA hydrogels. Cyclodextrins are cyclic oligosaccharides composed of glucopyranose units linked by α-1,4-glycoside bonds. These agents have a torus structure characterised by a hydrophilic exterior and a hydrophobic cavity. The primary and secondary hydroxyl groups of the native cyclodextrins (α, β, γ) protrude outward from the structure and are available for esterification (Friedman, 1991). Furthermore, cyclodextrins are well known for their ability to complex suitably sized drugs within their hydrophobic cavities, thereby changing the physicochemical properties of the drug (Stella and Rajewski, 1997). Thus, in addition to their potential as cross-linking agents, their inclusion within hydrophilic polymeric matrices (physically incorporated or chemically bound) may alter the kinetics of drug release by changing drug solubility and diffusivity. Sreenivasan (1997), for example, demonstrated that the release of salicylic acid from a poly(vinyl alcohol) hydrogel could be reduced by physically incorporating β-cyclodextrin (β-CD).

Our aim was to investigate the potential of cyclodextrins to control drug release from PAA microspheres. The synthesis and characterisation of β-CD/PAA microspheres has been previously published (Bibby et al., 1998, 1999). In this paper microspheres were loaded with two dyes, phenolphthalein (PP) and rhodamine B (RB), both capable of forming complexes with β-CD. Microspheres containing maltose were also prepared and loaded with dye. The effect of the incorporated carbohydrate (β-CD or maltose) on the in vitro release of dye was then investigated.

2. Materials and methods

2.1. Materials

Maltose monohydrate, PP and RB were purchased from BDH (Poole, UK). β-CD was gifted by Cerestar USA (Hammond, IN). Poly(acrylic acid) ($\bar{M}_w$ 90 000; PAA 90) was purchased as a 25% (w/w) aqueous solution from Polysciences (Warrington, PA) and was used as received. Food-grade olive oil (Borges®) was purchased from Industrias Pont (Tárrega, Spain) and distilled, deionised water was obtained from a Milli-Q® reagent water system (Millipore, Bedford, MA). All other reagents including solvents were of at least AR grade.

2.2. Microsphere synthesis

Microspheres were synthesised according to a w/o solvent evaporation technique developed in our laboratory (Bibby et al., 1998). A series of microspheres containing PAA 90 and either β-CD or maltose at a concentration of 0, 20 or 50% (w/w) were prepared. The composition and nomenclature used for the different microsphere types are listed in Table 1.

Briefly, the dispersed aqueous phase consisted of β-CD or maltose (0, 200 or 800 mg) and PAA 90 (800 mg) dissolved in 50 ml water. The solution was then added to olive oil (150 ml) and the preparation homogenised using a Silverson laboratory mixer–emulsifier fitted with a No. 8 standard head (13 000 rpm/5 min; Silverson Machines, London, UK). This was then poured into a wide-mouthed, round-bottomed flask (500 ml), containing 100 ml olive oil preheated to 105–115°C. The system was subsequently stirred with a serrated impeller (ten teeth, 1 cm^2 each) at 2100 rpm for 3 h. The temperature of the system was maintained between 105–115°C for the duration. Product and

oil were then cooled to room temperature, centrifuged ($1000 \times g/20$ min) and the olive oil decanted. Diethyl ether (25 ml) was then added, the product shaken by hand and allowed to stand for 5 min, then centrifuged ($1000 \times g/2$ min) and the ether decanted. The washing process was repeated a total of five times to remove olive oil from the microsphere surface. Finally, the microspheres were dried (50°C/24 h) and stored in vacuo over phosphorous pentoxide until required.

Following synthesis, microspheres were sized (Martin's diameter, $n = 300$) by light microscopy (Nikon Optiphot, Nikon, Tokyo, Japan) and the volume number mean (d_{vn}) determined.

2.3. β-CD/maltose content

β-CD/maltose content (%, w/w) was determined using the phenol–sulphuric acid reaction for carbohydrates as previously reported (Bibby et al., 1998), and based on the method described by Koh and Tucker (1986).

2.4. Spectrophotometric assay for PP and RB

Solutions of each dye were prepared in triplicate in 27% (w/w) propan-2-ol in 0.01 M NaOH. PP standards ranged in concentration from 4×10^{-6} to 4×10^{-5} M and were assayed at 552 nm; RB standards ranged in concentration from 1×10^{-6} to 1×10^{-5} M and were assayed at 554 nm (Cary Series 1E UV-VIS spectrophotometer, Varian, Melbourne, Australia). For both dyes, absorbance was a linear function of concentration over the range studied. Within and between day (determined over 3 days) coefficients of variation at 2×10^{-5} M PP and 5×10^{-6} M RB concentration were $< 4\%$ in all cases. Calibration curves for each dye were also prepared in the presence of a 1:4 (by weight) physical mixture of β-CD:PAA 90. The physical mixture was added to the dye standards at a concentration of 0.25 and 1 mg/ml for PP and RB, respectively. Neither assay was affected by the presence of β-CD or PAA 90.

2.5. Solubility of dyes in selected organic solvents

Excess PP or RB was added in triplicate to 10 ml of acetone, diethyl ether or propan-2-ol. Each solution was mixed longitudinally (33 rpm) for 24 h at room temperature, then centrifuged ($1500 \times g/30$ min). A 200-μl aliquot of the supernatant was taken and subsequently evaporated to dryness under vacuum. Samples were then reconstituted in an appropriate volume of a 27% (w/w) propan-2-ol in 0.01 M NaOH solution and the dye concentration determined by spectrophotometry as described.

2.6. Uptake of organic solvents by BP-90 microspheres

The sorption capacity of BP-90 microspheres for acetone, diethyl ether and propan-2-ol was determined according to a method described by Mi et al. (1997). Microspheres were weighed (50 mg) in triplicate into capped poly(propylene) vials and 2 ml of the appropriate solvent added. Sam-

Table 1
Characterisation of microspheres

Microsphere type	Carbohydrate:PAA in aqueous phase (by weight)[a]	Particle size (d_{vn})	β-CD/maltose content (%, w/w) (mean ± SD, $n = 3$)	Volume of 100 mg of hydrated microspheres (ml)[b] (mean ± SD, $n = 4$)
HBP-90	β-CD:PAA 90 (1:1)	24	48.5 ± 1.1	0.39 ± 0.08
HMP-90	Maltose:PAA 90 (1:1)	24	46.6 ± 1.3	0.58 ± 0.16
BP-90	β-CD:PAA 90 (1:4)	18	18.6 ± 0.3	0.75 ± 0.13
MP-90	Maltose:PAA 90 (1:4)	23	17.8 ± 4.4	0.66 ± 0.16
P-90	PAA 90	23	–	0.83 ± 0.14

[a] In all cases the mass of PAA 90 added to the aqueous phase of the emulsion was 800 mg.
[b] Determined according to Bibby et al. (1999); initial volume was 0.14 ml in all cases.

ples were left to stand at room temperature for 6 h, the solvent decanted and the microspheres blotted with filter paper to remove any remaining surface liquid. The 'wet' weight of each microsphere sample was then recorded and the percentage uptake of solvent determined according to the formula:

$$\mathrm{Up}\% = \left(\frac{W_{\mathrm{sol}} - W_{\mathrm{dry}}}{W_{\mathrm{dry}}}\right) \times 100$$

where Up% is the percentage solvent uptake by the microspheres, W_{sol} the weight of microspheres after 6 h soaking in the appropriate solvent, and W_{dry} the initial weight of microspheres added.

2.7. Loading of microspheres with dye

A study to establish optimal dye loading conditions was performed using BP-90 microspheres. Microspheres were weighed (100 mg) in triplicate into capped test tubes and a 10-ml solution of PP or RB in either acetone, diethyl ether or propan-2-ol added. In each case the solution was saturated with the appropriate dye, with the exception of the PP/acetone solution which was prepared at a concentration of 5% (w/v). Each sample was then mixed longitudinally (33 rpm) for 6 h and the dye solution decanted. Microspheres soaked in the RB solution were washed with diethyl ether (2×1 ml), filtered and the filtrate rinsed with a further 1 ml of diethyl ether. Microspheres soaked in the PP solution were filtered without rinsing with diethyl ether. Loaded microspheres were dried (50°C/24 h) and stored in vacuo over phosphorous pentoxide until required.

HBP-90, BP-90, HMP-90, MP-90 or P-90 were then loaded in saturated solutions of dye in propan-2-ol. Samples (500 mg) were weighed into large screw-capped test tubes containing a saturated solution of PP or RB in propan-2-ol (50 ml). Microspheres were mixed longitudinally (33 rpm) for 6 h and the dye solution decanted. Microspheres were then treated as described above using 5-ml washes for those microspheres loaded with RB.

2.8. Determination of microsphere dye content

Samples of microspheres ($n = 3$) containing either dye were dispersed in a 27% (w/w) propan-2-ol in 0.01 M NaOH solution. Microspheres loaded with PP were assayed at a concentration of 0.25 mg/ml; microspheres loaded with RB were assayed at a concentration of 1 mg/ml. All systems were stirred for 24 h prior to analysis. The concentration of dye in each system was determined by spectrophotometry at the respective $\lambda_{\max}$ of each dye as described previously. Turbidity in each sample solution was taken into account by subtracting the baseline absorbance at 650 nm from the peak absorbance at $\lambda_{\max}$.

2.9. In vitro release of dye

In vitro release studies were performed in phosphate buffer (0.13 M; pH 7.4). PP-loaded microspheres (20 mg) or RB-loaded microspheres (40 mg) were weighed in triplicate into glass vials and 10 ml of buffer added. Vials were incubated at 37°C in a horizontally reciprocating water bath (50 oscillations/min). Sample aliquots (0.5 ml) were taken with replacement at 10, 20, 30, 45 min; 1, 1.5, 2, 3 and 4 h. To each 0.5-ml aliquot was added 0.5 ml of a 54% (w/w) propan-2-ol in 0.02 M NaOH solution. For samples containing PP, a further 50 μl of 1 M NaOH was added to adjust the pH to above 10. Dye concentrations were then determined by spectrophotometry as described previously.

3. Results and discussion

The conditions used for the preparation of microspheres produced smooth and spherical particles that were consistently between 15 and 25 μm in size (d_{vn}) when viewed by light microscopy. The particle size of the various microspheres produced and their carbohydrate content are listed in Table 1. In all cases, a high entrapment ($> 85\%$) of the carbohydrate was observed. This may be expected considering the hydrophilic nature of the carbohydrates, and hence a low partitioning tendency into the oil phase. We have previously

Table 2
Dye solubility, percentage solvent uptake and load of phenolphthalein (PP) and rhodamine B (RB) in BP-90 microspheres after 6 h soaking in various saturated solutions of the appropriate dye[a]

Solvent	Solubility (%, w/v)		Solvent uptake (Up%)	Dye load (mg/g microspheres)	
	PP	RB		PP	RB
Acetone	>5	0.16 ± 0.01	22 ± 2	3.5 ± 0.2	0.5 ± 0.0
Diethyl ether	0.25 ± 0.01	0.003 ± 0.000	107 ± 16	ND	0.3 ± 0.0
Propan-2-ol	4.50 ± 0.54	0.66 ± 0.01	15 ± 2	4.8 ± 0.5	2.8 ± 0.2

[a] Data are mean ± SD, $n = 3$. ND, not detected.

shown that two concomitant chemical reactions occur during microsphere synthesis: esterification of the carboxylic acid groups of the PAA with the hydroxyl group(s) of the carbohydrate (β-CD) and polymer acid anhydride formation. Furthermore, the release of β-CD from these microspheres in water indicated that ≈70% of the β-CD was physically entrapped within the matrix, with the remainder being chemically bound to the polymer (Bibby et al., 1999).

The aim of this study was to evaluate whether the addition of β-CD to the microsphere matrix could modify the release kinetics of encapsulated drug. Microspheres containing maltose (a disaccharide incapable of drug–host complex formation but containing hydroxyl groups capable of undergoing esterification with PAA) were also prepared for comparison. Two dyes capable of forming inclusion complexes with β-CD were chosen as model compounds, namely, PP and RB. Frijlink et al. (1989) determined the association constant (K_{assoc}) for the PP–β-CD complex to be 19 157 M^{-1} in carbonate buffer (pH 10.8; 37°C). The strength of association between RB and β-CD is lower, with the K_{assoc} determined by Lincoln et al. (1987) to be 5900 M^{-1} (pH 6.4; 25°C). Since both dyes exhibit decreased absorptivity (spectral shift) upon complexation, it is important that, prior to any spectral assay, complete dissociation of the complex is achieved. Further, PP demonstrates a pH-dependent absorption spectrum, only absorbing in the visible wavelength in the ionised form ($pK_a \approx 9$; Merck, 1983). Thus, dye was assayed in a propan-2-ol/0.01 M NaOH solution to disrupt complex formation and adjust pH. Under these conditions, the absorbance of both dyes was unaffected by the presence of either β-CD or PAA.

To prevent the possibility of drug being involved in any chemical reaction during synthesis, microspheres were loaded with dye post-synthesis by soaking in saturated organic solutions. Three organic solvents having different dielectric constants were used: acetone, diethyl ether and propan-2-ol. It was demonstrated by scanning electron microscopy (not shown) that the integrity of the microspheres was not affected by soaking in any of these solvents over the 6-h loading period.

A preliminary loading study involving only BP-90 was undertaken to determine optimal loading conditions. Dye load was expected to be a function of the concentration of dye in the loading solvent and solvent uptake by the microspheres (in the absence of any interaction between the dye and the microsphere matrix). The solubility of both dyes in each solvent and solvent uptake are listed in Table 2. (NB: microspheres loaded with PP were not rinsed with diethyl ether during filtration, as the dye was moderately soluble in this solvent.)

A significant relationship was observed between the loading of RB in BP-90 microspheres and the product of its solubility and percentage uptake of solvent, with a correlation coefficient (r) of 0.96 ($P < 0.05$; Fig. 1). However, no statistically significant correlation between these parameters was noted for PP ($r = 0.62$; $P > 0.05$). Comparison of the actual dye loading with its theoretical maximum (determined by multiplying dye solubility and solvent uptake into the microspheres) highlighted the difference in loading between dyes.

Mean RB content slightly exceeded theoretical following loading in all three solvents, which may be due to the basic nature of the dye. Mean PP content, however, was consistently less than theoretical, suggesting some degree of exclusion from the microsphere matrix. This effect is unlikely to be due to size exclusion, as RB has a higher molecular weight (479.0) than PP (318.3). Further investigations are required to elucidate the mechanism(s) involved.

For BP-90 microspheres, the highest loading was achieved for both dyes using propan-2-ol. Consequently, all microspheres types were loaded by soaking in saturated propan-2-ol solutions of the appropriate dye. The presence of carbohydrate was not expected to influence loading, as the formation of dye–β-CD complexes is unlikely to occur in organic solvents (Mulski and Connors, 1995). Mean microsphere dye content ranged between 2.8 and 4.8 mg/g microspheres for PP and

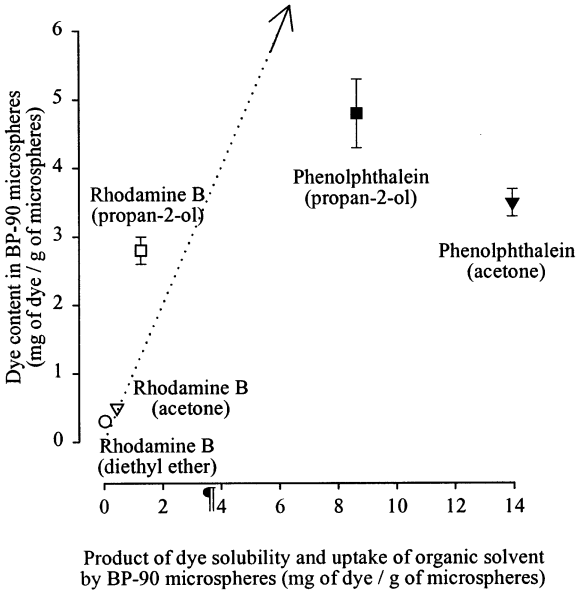

Fig. 1. Relationship between dye content of BP-90 microspheres following loading in three organic solvents and the product of dye solubility in solvent and percentage solvent uptake by BP-90. Dotted line represents theoretical load. Dye content in microspheres loaded using a phenolphthalein/diethyl ether solution was below limit of detection (< 1 mg/g microspheres). ¶Product of dye solubility of phenolphthalein and solvent uptake by microspheres in diethyl ether.

Table 3
Dye load in microspheres after 6 h soaking in a saturated propan-2-ol solution of the appropriate dye[a]

	PP load (mg/g microspheres)	RB load (mg/g microspheres)
HBP-90	4.7 ± 0.7	2.2 ± 0.1
HMP-90	3.2 ± 0.2	3.0 ± 0.4
BP-90	4.8 ± 0.5	2.8 ± 0.2
MP-90	3.2 ± 0.4	3.0 ± 0.1
P-90	2.8 ± 0.3	3.7 ± 0.1

[a] Data are mean ± SD, $n = 3$.

between 2.2 and 3.7 mg/g microspheres for RB (Table 3). For PP, the highest loading was indeed observed for those microspheres containing β-CD (BP-90 and HBP-90). In contrast, however, these microspheres were found to contain lower amounts of RB compared to the other microsphere types. Despite differences in K_{assoc}, this would suggest that loading did not involve complexation.

The in vitro release profiles of PP and RB from each microsphere type are shown in Figs. 2 and 3, respectively. In all cases initial release was rapid, with greater than 75% cumulative release into the

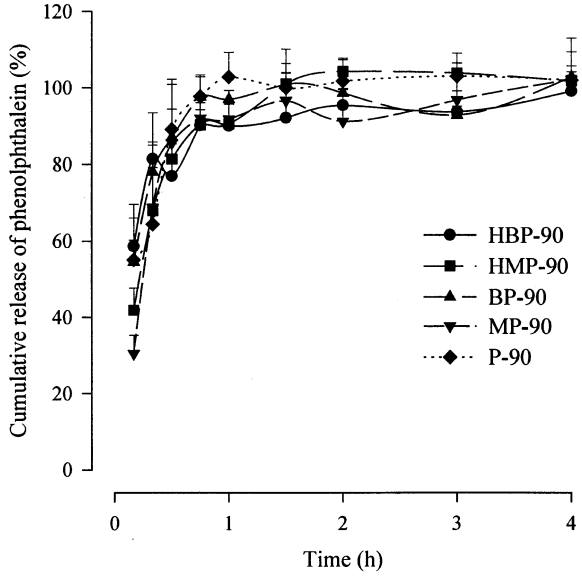

Fig. 2. Cumulative release (mean ± SD; $n = 3$) of phenolphthalein from microspheres in phosphate buffer (pH 7.4; 37°C).

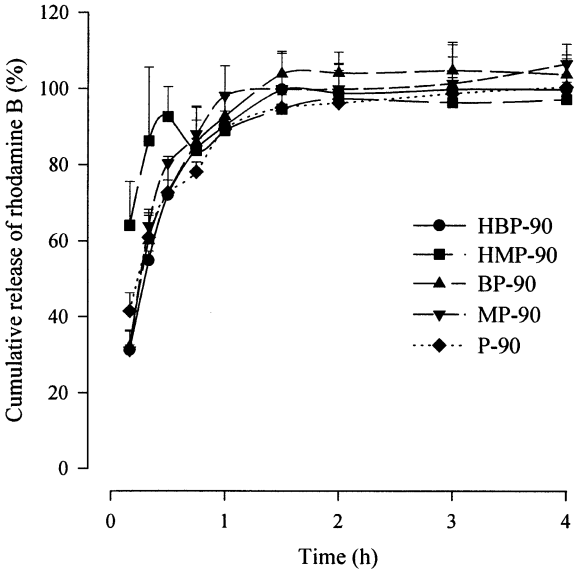

Fig. 3. Cumulative release (mean ± SD; $n = 3$) of rhodamine B from microspheres in phosphate buffer (pH 7.4; 37°C).

buffer medium after 30 min and a near complete release of dye being observed within 1 h. Importantly, no change in the release profiles was noted between microsphere types. That is, neither the incorporation of β-CD nor maltose at 0, 20 or 50% (w/w) concentration had any appreciable effect on dye release. The failure of the incorporated carbohydrate, and in particular β-CD, to modify the in vitro release of PP or RB from the microsphere formulation may be due to a number of factors.

Firstly, all types of microspheres hydrated rapidly upon dispersion in water forming highly swollen aggregates. This suggests some form of cross-linking, as the individual components (β-CD, maltose and PAA) are water-soluble. However, neither the degree of cross-linking of the carbohydrate (β-CD/maltose), nor the extent of cross-linking in the matrix as a whole was quantified. The equilibrium swelling volume of the different types of microspheres in aqueous solution are listed in Table 1. Reduced swelling volumes were observed for microspheres containing carbohydrate, but this reduction in swelling was found to be a result of the reduced polymer content of the microspheres, rather than an increased cross-linking density.

In the absence of significant differences in cross-linking, the presence of physically mixed β-CD or esterified β-CD–PAA conjugates within the matrix could still modify drug release by changing drug solubility and diffusivity. Importantly, such changes require complexation to occur between dye and cyclodextrin. However, this association may have been impaired, given the conditions present within the polymer matrix.

For reasons discussed, microspheres were loaded in an organic solvent. Such solvents are known to perturb the complexation process, even at relatively low concentrations (Mulski and Connors, 1995). Despite drying the product following loading, the presence of organic solvent residues within the microsphere cannot be discounted. Furthermore, the procedure used for the synthesis of microspheres used olive oil as the continuous phase and resulted in significant entrapment of the oil within the microsphere matrix ($\approx 10\%$, w/w, oil residues; Bibby et al., 1999). The components of olive oil, particularly the free fatty acids, are known to form strong complexes with a variety of cyclodextrins (Schlenk and Sand, 1961; Laurent et al., 1994; López-Nicolás et al., 1995). Considering that the maximum dye loading achieved was $< 0.5\%$ (w/w), it would be expected that the oil residues would strongly compete for the cyclodextrin cavity. Thus, the association of dye with β-CD may have been compromised by both the presence of residual solvent and oil.

Finally, any esterified β-CD may itself have an impaired ability to form inclusion complexes with dye as a result of changes in its conformational structure or obstruction of its cavity, upon binding with PAA. Szeman et al. (1987) for example, demonstrated a 70% reduction in the K_{assoc} of hydrocortisone for a β-CD–epichlorohydrin cross-linked polymer, compared to free β-CD. Such factors, or combinations thereof, may have prevented β-CD from complexing dye.

4. Conclusions

Microspheres containing PAA and β-CD or maltose in a range of concentrations can be prepared by a w/o solvent evaporation technique.

Loading of these microspheres with PP or RB was possible by soaking in saturated propan-2-ol solutions of the appropriate dye. The presence of carbohydrate (β-CD/maltose) within the microsphere matrix failed to modify the in vitro release kinetics of both dyes. The failure of those microspheres containing β-CD in particular, to alter the in vitro release of dye, may be due to a number of factors and include: (i) limited cross-linking giving rise to a the rapid hydration of the polymer matrix; (ii) perturbation of the dye–β-CD complex by oil and/or organic solvent residues; and (iii) conformational changes/steric hindrance of the β-CD cavity (due to its covalent binding with PAA) resulting in a reduction in its ability to form inclusion complexes.

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