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Investigations into the structure and composition of β-cyclodextrin/poly(acrylic acid) microspheres

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Abstract

Microspheres composed of the hydrophilic polymer poly(acrylic acid) (PAA), with and without β-cyclodextrin (β-CD), were prepared by a water-in-oil (w/o) solvent evaporation technique. Microspheres were characterised for particle size, β-CD and residual oil content. The type of matrix formed during microsphere synthesis was investigated by solid state carbon ^{13}C NMR, in vitro release of β-CD and swelling measurements. A high encapsulation efficiency of the β-CD was observed ($>90\%$). The in vitro release of β-CD in water over 24 h was initially rapid ($\approx 70\%$ in 3 h) with no further loss thereafter, suggesting potential covalent binding of the residual β-CD. NMR indicated that in the presence of β-CD, two concomitant chemical processes occur during microsphere synthesis: (i) esterification of the hydroxyl group(s) of the β-CD with the carboxylic acid groups of the PAA; and (ii) the formation of intra-/inter-polymer acid anhydrides.

Keywords: β-Cyclodextrin; Poly(acrylic acid); Microspheres; Hydrophilic polymer

1. Introduction

Microspheres, spherical polymeric matrices having a particle size in the micron range, have received interest as drug delivery systems. The small size of microspheres enables them to be used as injectable drug carriers and thus, have application in drug targeting (Joshi, 1994; Yoshikawa and Seebach, 1996). Microspheres have also received attention for use in controlled drug delivery (Okada and Toguchi, 1995; Zeng et al., 1995). Achieving a controlled and sustained drug release from microspheres composed of hydrophilic materials can, however, prove difficult. Frequently, a rapid 'burst' release is observed which may, in part, be attributed to rapid dissolution or swelling of the polymer matrix (Pongpaibul et al., 1988; Leucuta et al., 1997).

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Various strategies have been employed to modify drug release from hydrophilic polymeric matrices, including the use of cyclodextrins. Cyclodextrins are a group of cyclic oligosaccharides derived from the enzymatic degradation of starch. The most common, naturally occurring cyclodextrins, α-, β- and γ-, are composed respectively of six, seven or eight glucopyranose units and possess a torus structure in which their primary and secondary hydroxyl groups are orientated outward. Consequently, cyclodextrins have a hydrophilic exterior and a hydrophobic internal cavity. This cavity enables cyclodextrins to complex suitable drug substrates and in so doing, alter the physicochemical properties of the drug. Cyclodextrins have been incorporated into polymeric matrices as physical mixtures, to retard drug release by changing drug solubility and/or diffusion through the matrix (Filipović-Grčić et al., 1996; Sreenivasan, 1997). In addition these agents have been covalently bound to polymers. The primary and secondary hydroxyl groups on native cyclodextrins are potential sites for chemical reaction and consequently, cyclodextrins have been utilised as cross-linking agents within hydrophilic polymer matrices (Friedman, 1991; Crescenzi et al., 1997).

The aim of this work was to prepare microspheres composed of β-CD and the hydrophilic, mucoadhesive polymer PAA with potential for sustained release. Microspheres were prepared by a w/o solvent evaporation method, previously described by our group (Bibby et al., 1998). Identification of the chemical structure and composition of these microspheres is considered a necessary precursor to any investigation into their use as drug delivery systems.

2. Materials and methods

2.1. Materials

β-CD was a gift from Cerestar (Hammond, IN). Poly(acrylic acid) (PAA 90; $\bar{M}$w 90 000) was purchased as an aqueous solution (25% w/w) from Polysciences (Warrington, PA). Food grade olive oil (Borges®) was obtained from Industrias Pont (Tárrega, Spain) and contained $< 0.5\%$ free acid. Oleic acid (99%) and pentadecanoic acid methyl ester (99%) were obtained from Sigma (St. Louis, MO). Liquefied phenol (80% w/w) was purchased from Pharmaceutical Sales and Marketing (Auckland, New Zealand). All other reagents including solvents were of at least AR grade. Distilled, deionised water was obtained from a Milli-Q® reagent water system (Millipore, Bedford, MA).

2.2. Microsphere synthesis

Microspheres were synthesised according to a method described previously (Bibby et al., 1998). Three types of microspheres were produced using 0, 20 or 50% w/w β-CD and are designated P-90, BP-90 and HBP-90, respectively.

Briefly, β-CD (0, 200 or 800 mg) and PAA 90 (800 mg) were dissolved in 50 ml water. The aqueous solution was 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). The contents were then poured into a wide-mouthed, round-bottomed flask (500 ml), containing 100 ml olive oil, preheated to 105–115°C. This was subsequently stirred 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 *g* for 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 *g* for 2 min) and the ether decanted. The washing process was repeated a total of five times to remove residual olive oil. Finally, the microspheres were dried (50°C/24 h) and stored in vacuo over phosphorous pentoxide until required.

2.3. Particle size determination

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

2.4. β-Cyclodextrin content

β-CD content (% w/w) was determined using the phenol-sulphuric acid reaction for carbohydrates, as described by Koh and Tucker (1986). The resulting absorbance at 486 nm was a linear function of β-CD concentration over the range 1×10^{-5} M to 1×10^{-4} M. Within day and between day (determined over 3 days) coefficients of variation at 5×10^{-5} M β-CD concentration were 5.9 and 2.2% respectively. P-90 microspheres, composed of PAA alone, were also assayed and their absorbance was found to be negligible. Thus, neither polymer nor oil contaminant (present in all microspheres) affected the phenol-sulphuric acid assay at the levels tested.

2.5. Analysis of oleic acid content

The oleic acid content of microspheres was determined by gas chromatography (GC) using a Shimadzu 6L gas chromatograph interfaced with a RPR-G1 processor (Shimadzu, Kyoto, Japan). Dried microspheres (50 mg, $n = 2$) were dissolved by incubating in aqueous 1 M sodium hydroxide (4 ml) overnight at 50°C. Concentrated hydrochloric acid (1 ml) was then added and the fatty acids were extracted with *n*-hexane (4×5 ml). An internal standard (0.25 of a 4 mg/ml pentadecanoic acid methyl ester solution) was added to the first aliquot of *n*-hexane prior to mixing. Following each addition of *n*-hexane, samples were mixed using a longitudinal rotary stirrer (33 rpm) for 45 min. Aliquots were then combined and evaporated to dryness under reduced pressure.

Fatty acid methylation was performed as described by Bibby et al. (1998) and based on the method reported by Christie (1989). Analysis of oleic acid standards demonstrated the ratio of the peak height of methyl oleate to internal standard was a linear function of oleic acid mass from 0.2 to 10 mg. Within day and between day (determined over 3 days) coefficients of variation for a 2-mg mass of oleic acid were 8.4 and 11.6%, respectively.

2.6. Swelling studies

Microspheres (100 mg, $n = 4$) were placed into graduated flat-bottomed glass tubes (6 mm i.d.) and 2 ml of distilled, deionised water added. Samples were then allowed to hydrate for 24 h at room temperature. Each tube was periodically stirred to remove trapped air bubbles. After 24 h, water was decanted and 0.25 M di-sodium hydrogen orthophosphate added, sufficient to adjust the pH to 7–8. Samples were then incubated at 37°C for 2 h and the apparent volume of the hydrated microsphere aggregates determined.

2.7. Release of β-cyclodextrin from microspheres in water

BP-90 and HBP-90 microspheres were weighed separately (12.5 mg, $n = 3$) into capped test tubes and 15 ml of distilled, deionised water added. Samples were left to stand at room temperature and aliquots (1 ml) were taken with replacement at 0.25, 0.5, 1, 3, 6 and 24 h. Each aliquot was then centrifuged (15 000 *g* for 30 min) and 0.5 ml of the supernatant removed, diluted to 2 ml with water and analysed for β-CD content by the phenol-sulphuric acid assay as described in Section 2.4.

2.8. NMR analysis

P-90 and HBP-90 microspheres were analysed by solid state ^{13}C NMR. Samples were packed in 7 mm diameter zirconia rotors and spun at 5 kHz in a magic-angle spinning probe for ^{13}C NMR analysis (Doty Scientific, Columbia SC) at 50.3 MHz using an Inova-200 spectrometer (Varian, Palo Alto, CA). Cross-polarisation (CP) NMR spectra were obtained with a 5.5 μs proton preparation pulse, a 1-ms cross-polarisation contact time, 30-ms of data acquisition and a 3-s recovery delay. Transient signals from 1000 contacts were averaged. A single-pulse excitation (SPE) NMR spectrum was obtained with a 5.5 μs ^{13}C 90° pulse followed by 30 ms of data acquisition and a 3-s recovery delay. Transient signals from 1000 contacts were again averaged. Values of the proton rotating-frame spin relaxation time constant

Table 1
Characterisation of microspheres for particle size, β-CD and oleic acid content and swelling in aqueous solution

Microsphere type	Particle size (μm) ($d_{vn} \pm$ S.D.)	β-CD content (% w/w) (mean ± S.D., $n = 3$)	Oleic acid content (% w/w) (mean, $n = 2$)	Apparent volume of 100 mg hydrated microspheres (ml)[a] (mean ± S.D., $n = 4$)
P-90	23 ± 9	–	8.9	0.83 ± 0.14
BP-90	18 ± 6	18.6 ± 0.3	7.8	0.75 ± 0.13
HBP-90	24 ± 8	48.5 ± 1.1	9.3	0.39 ± 0.08

[a] Initial volume was 0.14 ml in all cases.

($T_{1\rho}$(H)) for HBP-90 microspheres were measured by inserting a 6-ms spin-locking pulse between the proton preparation pulse and commencement of cross-polarisation in the CP NMR sequence (Alla and Lippmaa, 1976) and comparing signal strengths with those observed without the spin-locking pulse.

3. Results and discussion

Microsphere synthesis involved heating the various components between 105–115°C for 3 h. During this time, water was evaporated leaving the hydroxyl group(s) of the β-CD and the carboxylic acid groups of PAA to possibly undergo a condensation reaction, resulting in the formation of esters. The involvement of the exterior hydroxyl groups of the native (α-, β- or γ-) cyclodextrin ring in chemical reactions with polymers has previously been documented and has been reviewed by Friedman (1991). Interestingly, microspheres were also formed in the absence of β-CD (P-90), demonstrating that esterification of the polymer matrix by β-CD was not necessary for microsphere synthesis.

Particle size, β-CD and oleic acid content and the extent of swelling for a 100-mg mass for the three microsphere types are listed in Table 1. Microspheres were smooth and spherical when viewed by light microscopy (× 400 magnification) and were < 25 μm (d_{vn}) in all cases. The encapsulation efficiency of β-CD in BP-90 and HBP-90 microspheres was high, being 18.6 and 48.5% w/w respectively, for a 20 and 50% w/w loading. Olive oil was used as the continuous phase in the syntheses. The oil is composed of mixed glycerides of oleic acid (83.5%) and other fatty acids (Merck Index, 1983). Its detection by GC involved a saponification step prior to methylation and subsequent analysis. Thus, oleic acid was used as the marker for residual oil contaminant. Oleic acid content in the microspheres ranged between 7.8 and 9.3% w/w.

All three microsphere types hydrated rapidly and formed aggregates when dispersed in water (in comparison with physical mixtures of the unreacted microsphere components which readily dissolved). Microsphere hydration was determined at pH 7–8, more than 2 pH units above the pK_a of PAA (Greenwald, 1980). Microspheres (100 mg) swelled from an initial volume of 0.14 ml to 0.83 ± 0.14 ml for P-90, 0.75 ± 0.13 ml for BP-90 and 0.39 ± 0.08 ml for HBP-90. A decrease in the apparent volume of hydrated microspheres as a function of increasing β-CD content would suggest cross-linking of the polymer matrix by the carbohydrate. However, the observed decrease in swelling only reflected differences in polymer content between microsphere types. After accounting for β-CD and oleic acid content, the mean volume of swelling per milligram of polymer was 9.1×10^{-3}, 1.0×10^{-2} and 9.2×10^{-3} ml for P-90, BP-90 and HBP-90, respectively.

The release profiles of β-CD from BP-90 and HBP-90 microspheres in water over a 24 h period are shown in Fig. 1. The release of β-CD from both BP-90 and HBP-90 in water was initially rapid (60% release in 1 h), however, between 3 and 24 h cumulative release remained constant at $\approx 70\%$. This rapid initial release may be associated with physically entrapped, unbound β-CD. The fact that complete release of β-CD was not observed for BP-90 or HBP-90 may be attributed

to the association of β-CD with the microsphere components or to the covalent bonding of β-CD with PAA.

Studies by García-González et al. (1993a,b; 1994) have highlighted the potential for β-CD as well as other carbohydrates to undergo esterification with polymers such as PAA. However, Blanco-Fuente et al. (1996) proposed that anhydride formation within and between the PAA polymer chains and not β-CD-PAA esterification, was the predominant chemical reaction at temperatures between 90 and 130°C. The formation of carboxylic acid anhydrides would explain the synthesis of P-90 (polymer only) microspheres.

Thus, there exist a number of potential reactions that may occur during microsphere synthesis:

1. the formation of an ester link between the hydroxyl groups of β-CD and the carboxylic acid groups of PAA;
2. the formation of carboxylic anhydrides within and between the PAA polymer chains; and
3. the esterification of the hydroxyl groups of β-CD or the carboxylic acid groups of PAA with the fatty acid and glyceride components of the olive oil, respectively.

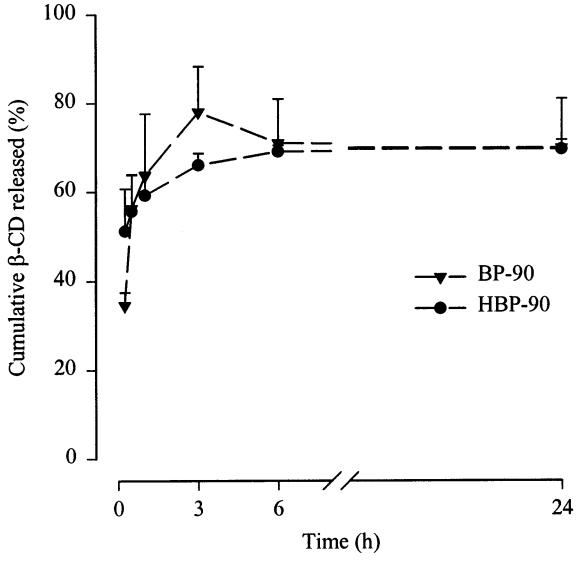

Fig. 1. Release of β-CD from BP-90 and HBP-90 microspheres in water at room temperature (mean ± S.D., $n = 3$).

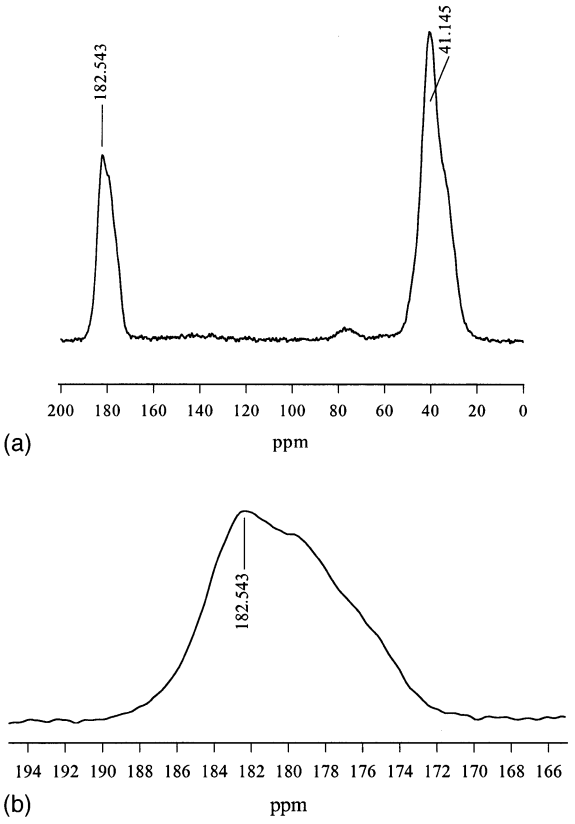

Fig. 2. (a) Solid state ^{13}C NMR spectrum of P-90 microspheres. (b) Expansion of carboxylic acid region of solid state ^{13}C NMR spectrum of P-90 microspheres.

Attempts by infrared spectrophotometry to identify ester or acid anhydride groups within the microsphere matrix proved unsuccessful, with broad carbonyl stretches possibly attributable to oil contaminant, obscuring the region of interest (1720–1760 cm^{-1}). Further, the determination of the free acid group content of these microspheres as described in the US Pharmacopeia (1990) was limited by the accuracy of the assay and would not differentiate between ester and anhydride forms.

The CP ^{13}C NMR spectra of P-90 and HBP-90 are shown in Fig. 2(a) and Fig. 3(a), respectively. The spectrum of HBP-90 is essentially a combination of the spectrum of P-90 and signals similar to those published for cyclodextrins (Veregin et al., 1987). For both HBP-90 and P-90 the peak with a

maximum at 41 ppm is assigned to the CH and CH_2 carbons of PAA, while the peak at ≈ 180 ppm is assigned to the carboxylic acid carbon (Shogren et al., 1991). Closer inspection of the latter peak however, shows a distinct difference between the two spectra, i.e. the peak maximum appears at 182.5 ppm in the spectrum of P-90 (Fig. 2b) and 179.0 ppm for HBP-90 (Fig. 3b). Formation of esters or anhydrides usually displaces the carboxylic signal to lower chemical shifts by a few ppm. For example, the chemical shift of propanoic acid is 179.6 ppm, while that of the methyl ester is 173.0 ppm and the propanoic anhydride is 169.8 ppm (Stothers, 1972). The peak maximum at 182.5 ppm in the spectrum of P-90 can therefore be attributed to unreacted carboxylic acid groups, while the shoulder on the low-chemical-shift side can be attributed to carboxylic anhydrides. In the case of HBP-90, the signal at 182.5 ppm (unreacted carboxylic acid) is no more than a shoulder on the broad peak assigned to anhydrides and/or esters. Given that anhydride formation and/or esterification of PAA with oil components should occur to an equivalent degree in both P-90 and HBP-90, the observed difference in peak profile of the carboxylic carbon can be explained by the esterification of β-CD with PAA.

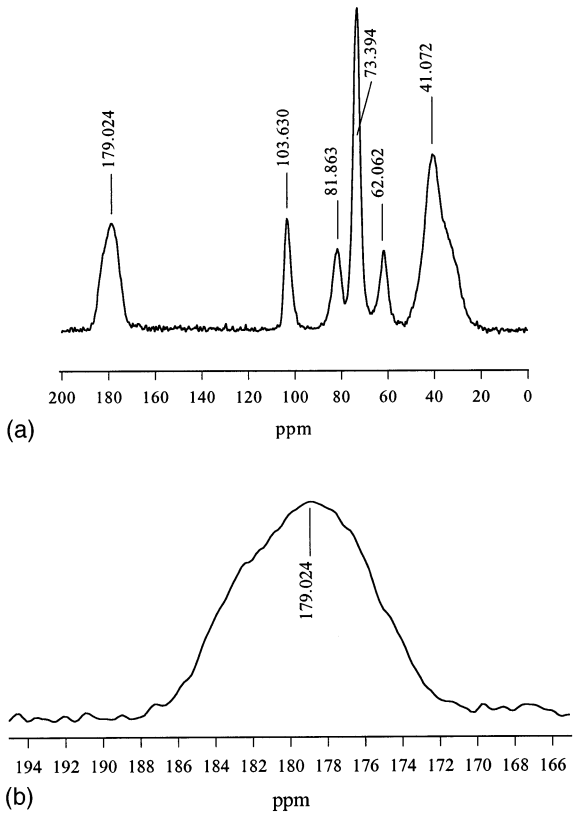

Fig. 3. (a) Solid state ^{13}C NMR spectrum of HBP-90 microspheres. (b) Expansion of carboxylic acid region of solid state ^{13}C NMR spectrum of HBP-90 microspheres.

Table 2
Relaxation time constants for HBP-90 microspheres

Peak resonance (ppm)	$T_{1\rho}$ (H) (ms)
β-CD component	
62	5.9
73	5.8
82	6.3
104	5.9
PAA component	
41	7.2
179	6.9

Values of $T_{1\rho}$(H) can provide a test for matrix heterogeneity, with distinct values indicating heterogeneity on a scale greater than nanometres (Zumbulyadis, 1983). More intimate mixing results in spin diffusion between domains, so that only a single averaged value of $T_{1\rho}$(H) can be observed. This approach has been used to test for miscibility of synthetic polymer blends (Chu et al., 1988; Jong et al., 1993). For HBP-90 microspheres (Table 2), values of 7.2 and 6.9 ms were measured for peaks at 41 and 179 ppm, giving a mean $T_{1\rho}(H) = 7.1$ ms for protons associated with the PAA. Values of 5.9, 5.8, 6.3 and 5.9 ms were measured for peaks at 62, 73, 82 and 104 ppm and thus, the mean $T_{1\rho}(H) = 6.0$ ms for protons associated with the β-CD. The values for the two components (β-CD and PAA) are not identical. Thus, the distribution of β-CD within the polymer matrix is not completely random, so domains rich in one component may exist.

4. Conclusion

The synthesis of microspheres containing the hydrophilic polymer PAA, with and without β-CD, is possible by a w/o solvent evaporation method. It appears likely that in the presence of β-CD, two concomitant chemical processes occur: (i) esterification of the hydroxyl group(s) of the β-CD and the carboxylic acid groups of the PAA; and (ii) the formation of acid anhydrides within or between the PAA chains.

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