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Preparation and characterisation of poly(vinyl alcohol)/cyclodextrin microspheres as matrix for inclusion and separation of drugs

Marieta Constantin[a,b], Gheorghe Fundueanu[a,b], Fabrizio Bortolotti[a], Rita Cortesi[a,*], Paolo Ascenzi[c], Enea Menegatti[a]

[a] *Department of Pharmaceutical Sciences, University of Ferrara, Via Fossato di Mortara, 19 44100 Ferrara, Italy*
[b] *Department of Bioactive and Biocompatible Polymers, "Petru Poni" Institute of Macromolecular Chemistry, 700487 Iassy, Romania*
[c] *Department of Biology and Interdepartmental Laboratory for Electron Microscopy, University "Roma Tre", I-00146 Rome, Italy*

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Abstract

Poly(vinyl alcohol) (PVA) microspheres containing cyclodextrin (CD) were obtained by chemical cross-linking with glutaraldehyde of an acidified mixture solution of PVA and α-, β- or γ-CD. The amount of linked CD in microspheres, estimated by tetrazolium blue method, decreases in the order β- > γ- > α-CD. The dimensions of PVA/γ-CD microspheres are much higher than those of PVA/α- and β-CD. The cross-linking density of microspheres was estimated by the amount of iodine retained by the polymer matrix. The pore size as well as the porous volume of PVA/CD microspheres decrease significantly on increasing the amount of glutaraldehyde, but are enough large to permit the access of drugs to the CD cavity. In order to test the PVA/CD microsphere inclusion ability, the microspheres were packed in a glass column and the liquid chromatographic behaviour by isocratic elution of different drugs or typical organic compounds, taken as model drugs, was investigated.

Keywords: Poly(vinyl alcohol)/Cyclodextrins; Microspheres; Inclusion complexes; Liquid chromatography

1. Introduction

Poly(vinyl alcohol) (PVA) is a hydrophilic polymer that is frequently used in biomedical applications such as implants (Juang et al., 1996), soft contact lenses (Hyon et al., 1994), artificial organs (Chen et al., 1994) and protein immobilization (Kobayashi and Ykada, 1991; Uhlich et al., 1999). The large applicability of PVA depends on its low price, easy

Abbreviations: BA, benzoic acid; CAB, cellulose acetate butyrate; CR, congo red; CD, cyclodextrin; EP, ethyl-paraben; GA, glutaraldehide; HPLC, high performance liquid chromatography; In, indol; ISEC, inverse size exclusion chromatography; MP, methyl-paraben; PE, propyl-paraben; PVA, poly(vinyl alcohol); S.D., standard deviation; SEM, scanning electron microscopy

* Corresponding author. Tel.: +39 0532 291259; fax: +39 0532 291296.
E-mail address: crt@unife.it (R. Cortesi).

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availability and chemical properties (i.e. the presence of hydroxyl groups capable of chemical modifications) (MüllerShulte and Brunner, 1995; Preininger and Chiarelli, 2001).

Beside its hydrophilic character, PVA forms hydrogels that are widely used in pharmaceutics as drug delivery matrix. Such hydrogels are biocompatible, nontoxic and are characterized by a high degree of swelling. However, PVA does not show selectivity for drugs or other biological active compounds (Yamauchi et al., 1979; Zentner et al., 1979; Thanoo et al., 1993; Li et al., 1998).

Cyclodextrins (CDs) are well known for their ability to form inclusion complexes (host-guest type) with several classes of compounds including drugs. α-, β- and γ-CD are cyclic oligomers of glucose consisting of six (α-), seven (β-) or eight (γ-) D-glucopyranose units linked by α-glucosidic bonds. CDs have a torus-shaped, apolar and electron-rich hydrophobic cavity with an internal diameter of 5.7, 7.8 and 9.5 Å, respectively. Due to this inherent property, CDs are used widely in pharmaceutical sciences to improve drug stability, dissolution rates and bioavailability (Irie and Uekama, 1995; Loftsson and Brewster, 1996). However, the broadest range of CD applications is in separation science. The basis of separations stems directly from the selectivity in binding that the different isomers and compounds have towards a particular CD present in the stationary phase. Those compounds that interact strongly with CD (i.e. have large values of association constant) will be retained longer and thus exhibit long retention times (or volumes). Compounds that weakly bind CD (i.e. small association constant values) will be eluted rapidly. Since the natural CDs are soluble in water and other polar or dipolar aprotic solvents, they must be converted to an insoluble matrix. The most part of authors study the retention properties of CDs linked on silica supports (Guillaume and Sebille, 1996; Guillaume et al., 2001). However, few authors use as a stationary phase other natural or synthetic polymer microspheres with potential biomedical applications (Tojima et al., 1999; Fundueanu et al., 2003).

Herein, the preparation and characterization of PVA/CD microspheres that put together the properties of both compounds, is reported. The ability of PVA/CD microspheres to include drugs or other organic compounds has been proved by HPLC. The method, here developed, on the basis of retention volumes, gives informations about appropriate drugs to be conditioned in PVA/CD microspheres, as a potential drug delivery system (Fundueanu et al., 2003).

2. Materials and methods

2.1. Materials

PVA (M_w = 18,000 g/mol; hydrolysis mole% = 98.4) was purchased from AIR Products and Chemicals, Inc. (Utrecht, The Netherlands). α-, β- and γ-CDs were provided from Roquette Frères (Lestrem, France). Cellulose acetate butyrate (CAB) was purchased from Eastman Inc. (Kingsport, Tennesse, USA). Glutaraldehyde (GA) (25% aqueous solution, v/v) was supplied by Fluka (Seelze, Germany). Drugs and model drugs used for chromatographic and release studies, were provided from different suppliers. The molecular weight standards, deuterated water (for determination of total volume of the column), D (+)-sucrose, and maltodextrins (with different degrees of polymerization = 3, 5, 7, 9 and 13) were obtained from CERMAV (Grenoble, France).

Dextrans and blue-dextran 2000 were provided from Pharmacia (Uppsala, Sweden). Proteins with standard molecular weights (i.e. lysozyme, M_w = 14,300 Da, β-lactoglobulin, M_w = 18,400 Da, trypsinogen, M_w = 24,000 Da, pepsin, M_w = 34,700 Da, albumin egg, M_w = 45,000 Da, albumin bovine, M_w = 66,000 Da, aldolase, M_w = 158,000 Da, catalase, M_w = 232,000 Da, thyroglobulin, M_w = 669,000 Da) were provided from Amersham-Pharmacia Biotech Europe GmbH (Freiburg, Germany). All chemicals were of the highest analytical grade.

2.2. Preparation of microspheres

PVA/CD microspheres were obtained using a cylindrical glass reactor, provided with an anchor type glass stirrer, and a reflux condenser. The reactor was maintained at 50 °C with a thermostatic water bath. Briefly, 2 g of PVA were dissolved in 10 ml hot water, then 0.5 g of α-, β- or γ-CD was solubilised under magnetic stirring. The solution was acidified with 2 ml H_2SO_4 solution (2%, v/v), and then poured in 50 ml of dispersion medium (1,2-dichloroethane) containing 0.5 g of CAB (as the dispersion agent). This

water/organic solvent emulsion was stirred for 15 min (stirring speed = 600 rpm), then 0.18–3.6 ml of GA were added, and the cross-linking reaction was carried out for 2 h at 50 °C. The cross-linked microspheres were recovered by filtration through a sintered glass filter, under vacuum. The removal of residuals was performed by washing the microspheres in the following order: 1,2-dichloroethane, acetone, hot water, cold water, methanol. Then, the microspheres were completely dried by overnight exposure to 60 °C, under vacuum. PVA microspheres without CD were also prepared following the same protocol.

2.3. Microspheres recovery

After drying at constant weight, microspheres were weighed. The weight was compared to the initial mass of PVA and CD plus GA:

$$\text{Recovery}(\%) = \frac{W_{\text{Ms}}}{W_{\text{PVA}} + W_{\text{CD}} + W_{\text{GA}}} \times 100 \qquad (1)$$

where W_{Ms}, W_{PVA}, W_{CD} and W_{GA}, represent the weight of the dried microspheres, PVA, CD and GA, respectively.

2.4. Cyclodextrin determination in microspheres

CD determination in microspheres was carried out by the tetrazolium blue method (Jue and Lipke, 1985), using glucose for the calibration curve. Briefly, 50 mg of PVA/CD microspheres were refluxed in 30 ml of 0.5 M H_2SO_4 at 100 °C, for 15 h. The solution was neutralized with 3 M NaOH, filtered and diluted to 100 ml. One millilitre sample was prelevated and added to 4 ml of the freshly mixed reagent containing 0.1% (w/v) tetrazolium blue. The mixture was heated in a boiling water bath for 3 min, then it was cooled for other 3 min in running water, and the absorbance was determined at 660 nm. PVA microspheres obtained in similar conditions but without CD were subjected to the same treatment in order to determine the unremoved stabilizer (CAB).

2.5. Morphological and dimensional analysis of microspheres

The morphology of microspheres was evaluated by observation at optical and scanning electron microscopy (SEM). Microsphere size and size distribution was determined by fractionation using standard test sieves.

2.6. Wrack density of microspheres

The wrack density was determined by weighting the volume of 1 ml of microspheres measured with a graduated cylinder (12 mm i.d.).

2.7. Swelling degree of microspheres

The volume expansion of microspheres was determined at equilibrium, after having placed the microspheres in water. The volume of the swollen beads (V_s) compared with the dried volume (V_d), measured by placing the microspheres in a graduated cylinder (12 mm i.d.), was defined as the swelling factor (q).

$$q = \frac{V_s}{V_d} \qquad (2)$$

2.8. Cross-linking degree

The degree of cross-linking was estimated by a novel approach evaluating the amount of iodine retained by the microspheres: briefly, 100 mg microspheres were soaked in 10 ml of 0.1 N solution of iodine in 10% (w/v) potassium iodide solution, and kept for 48 h under gentle stirring. After 48 h the equilibrium was reached and 5 ml of the iodine solution were taken up and assayed for the iodine content by titration with 0.1 N $Na_2S_2O_3$, in the presence of starch (1%, w/v) as the indicator.

2.9. Determination of microsphere porosity

The porosity of PVA/CD microspheres was determined by inverse size exclusion chromatography (ISEC) as described elsewhere (Fundueanu et al., 1999). The chromatographic system consisted of a HPLC pump provided with an injection valve (sample loop, 20 μl), a differential refractometer detector (model R 401, Waters, Milford, MA, USA) and a Spectra Physics Integrator (LKB-Produker AB, Bromma, Sweden). The glass column (10 cm × 0.55 cm i.d.) was filled, under pressure of the peristaltic pump, with an aqueous suspension of PVA/CD microspheres (50–160 μm), and then inserted between the loop and the detector. Experiments were carried out at room tem-

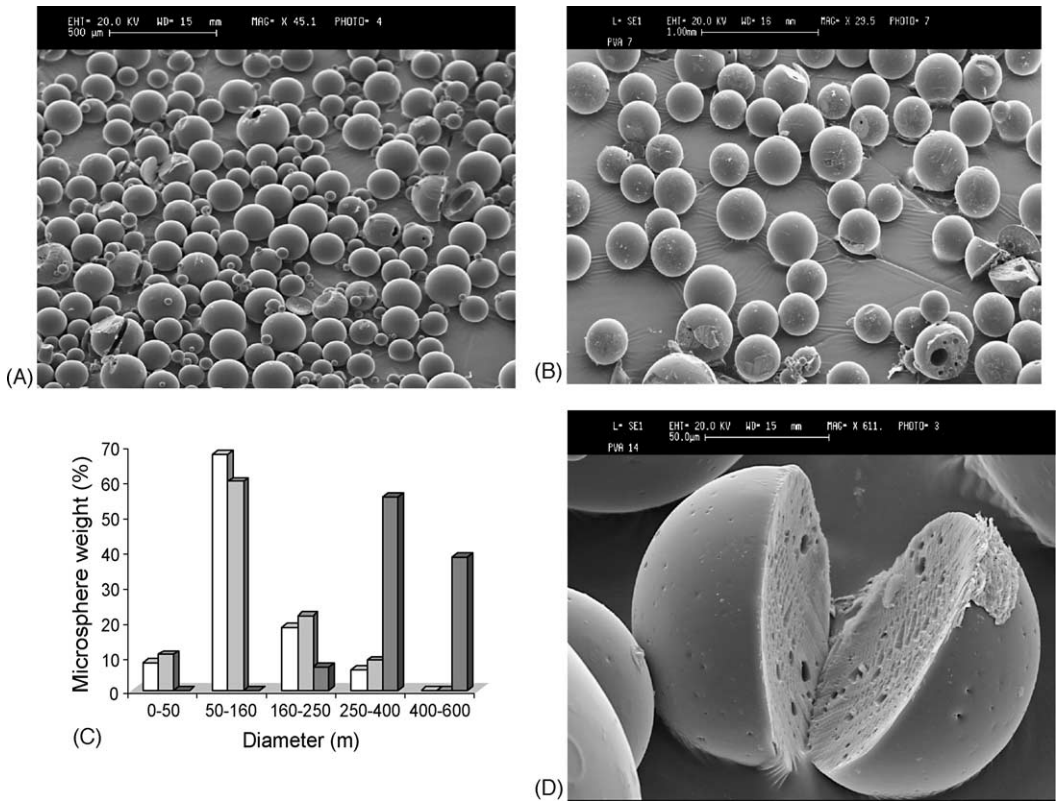

Fig. 1. SEM photograph (general view) of PVA/α- (A) and PVA/γ-CD (B) microspheres. Size distribution of PVA/α-CD (white), PVA/β-CD (grey) and PVA/γ-CD (dark grey) microspheres (C). SEM photograph (cross-section) of PVA/β-CD microspheres obtained at a PVA/GA ratio of 5/1 (D).

perature. The flow rate was 0.3 ml/min. The mobile phase used was phosphate buffer at pH 7.4 (0.019 M NaH_2PO_4 + 0.08 M Na_2HPO_4). The buffer was filtered through a 0.2 μm membrane filter and degassed before use.

2.10. Inclusion properties of microspheres

The inclusion properties of microspheres were determined by HPLC with a chromatographic equipment identical to that describes in the previous section. However, the differential refractometer detector was replaced with a variable UV–vis detector (Merk, Germany). Experiments were carried out at in similar conditions as previously described. The mobile phases used were phosphate buffer at pH 7.4 or mixture water/acetonitrile (70/30, v/v).

3. Results and discussions

3.1. Preparation and characterization of microspheres

Spherical microparticles were successfully synthesized by chemical cross-linking with GA of a PVA and CD mixture (Fig. 1A and B). The association of a hydrophilic polymer to a CD improves the swelling properties of the microspheres and therefore increases the accessibility of the guest molecule to the CD cavity. Moreover CDs could be linked in the network of microspheres by a single step procedure, in a uniform distributed manner. The hydrogel microspheres are chemically stable, non-toxic, biocompatible, and could be safety used in liquid chromatography or as drug delivery systems. The preparation conditions as well as the characterization of microspheres are

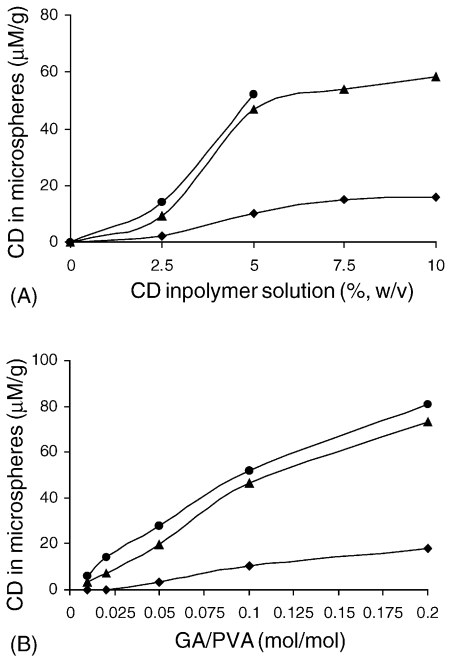

Fig. 2. Effects of CD concentration in the PVA solution (A) and of the GA/PVA molar ratio (B) on the percentage of CD linked in microspheres ((●), α-CD; (▲), β-CD; (■), γ-CD). Conditions: (A) PVA/GA = 10/1; (B) CD in polymer solution was 5% (w/v).

summarized in Table 1. The ability of β- and γ-CD to be linked in the microsphere is almost the same being higher than that of α-CD. Probably, a competition between the hydroxyl groups of the two components (PVA and CD) in their reaction with GA governs the CD-bounded efficiency. The low percentage of linked α-CD could be attributed one hand to the reduced number of the OH groups/molecule, therefore the probability of reaction with GA is diminished. On the other hand, α-CD possessing a small molecular weight could be more reactive than PVA in reaction with GA forming CD-olygomers that are removed by washing. In addition, the microsphere recovery is smaller for all type of microspheres obtained in the presence of α-, β- or γ-CD than those obtained from PVA itself because of their low molecular mass. A part of CD consumes the cross-linker giving rise a soluble linear olygomer that is removed by washing. As a consequence, a slightly increase of the swelling degree and porous volume is observed. However, these differences are not significant, if S.D. values are considered.

Table 1
Preparation conditions and the main characteristics of PVA and PVA/CD microspheres*

Sample code	PVA/GA molar ratio	CD in PVA solution (%, w/v)	Recovery (%)	CD in microspheres (μM/g)	Wrack density (g/ml)	Swelling factor (*q*)	Porous volume (ml)	R max (Å)	Mean diameter of microspheres (μm)
PVA	10/1	0	82 ± 7	0	0.66 ± 0.05	2.4 ± 0.2	1.45 ± 0.10	<18	128 ± 8
PVA/α-CD	10/1	5	73 ± 4	10.2 ± 1.2	0.54 ± 0.04	2.6 ± 0.2	1.38 ± 0.19	<18	137 ± 10
PVA/β-CD	10/1	5	76 ± 4	52.0 ± 4.3	0.61 ± 0.06	2.4 ± 0.1	1.28 ± 0.15	<18	140 ± 12
PVA/γ-CD	10/1	5	75 ± 5	46.75 ± 4.1	0.60 ± 0.08	2.3 ± 0.2	1.27 ± 0.18	<18	395 ± 22

Reaction time, 2 h; stirring speed, 600 rpm; T = 50 °C; The values are the results of three independent experiments ± S.D.

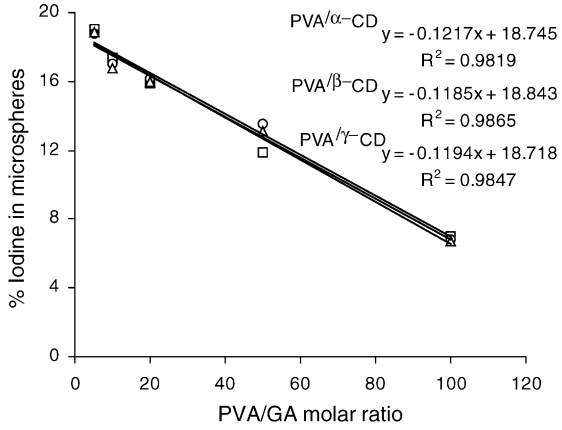

Fig. 3. Relation between the amount of the crosslinker (GA) and the percentage of iodine retained by PVA/CD microspheres. PVA/α-CD (◯), PVA/β-CD (△) and PVA/γ-CD (□).

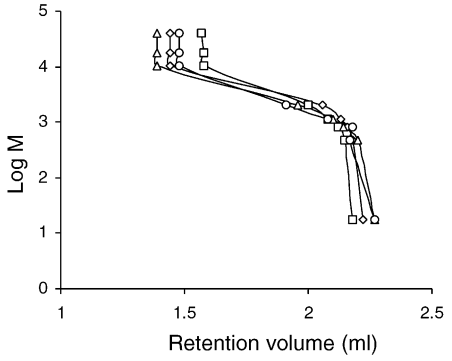

Fig. 4. Pore characterization of the PVA (◯), PVA/α-CD (△), PVA/β-CD (□) and PVA/γ-CD (◊) microparticulate stationary phase. Mobile phase: phosphate buffer, pH 7.4; flow rate: 0.3 ml/min, injected volume: 20 μl; solute concentration: 10 g/l (refractometric detection).

It must be noticed that the mean diameter of the PVA/γ-CD microspheres is much higher than that of PVA/α- and PVA/β-CD (Table 1). As it is well known the size of the microspheres is mainly related with the stirring speed and the viscosity of the two phases. The intrinsic viscosity of the acidified aqueous solution of CD-free PVA, PVA/α-, β- and γ-CD (25% CD, w/w) was determined at 50 °C, being 28, 29, 30 and 30 ml/g, respectively. Under conditions in where the stirring speed was unchanged, the increased diameter of the PVA/γ-CD microspheres may be tentatively assigned to the decrease of the stabilization capacity of CAB in the presence of γ-CD. Indeed, simulating the conditions of preparation of PVA/CD microspheres, a fine and stable dispersion of PVA/α- and PVA/β-CD aqueous solution was obtained, while PVA/γ-CD formed a larger dispersion quickly destabilized. As follows, the most part of PVA/α- and PVA/β-CD microspheres has dimensions ranging from 50 to 160 μm, while the size of PVA/γ-CD microspheres ranges between 250 and 600 μm (Fig. 1C). Particularly, the PVA/CD microsphere fraction characterized by dimensions comprised between 50 and 250 μm was used for next experiments.

3.2. Influence of CD concentration and PVA/GA ratio

With the aim to increase the amount of CD linked to the polymer hydrogel network, but keeping at the same time a good accessibility of the swellable matrix (low degree of cross-linking), we firstly augmented the CD concentration in polymer solution.

The results are presented in Fig. 2A. Even at a high CD concentration in the polymer solution the amount of bounded α-CD is still low. The amount of β- and γ-CD linked to the polymer matrix increases significantly at a concentration of CD ranging between 2.5 and 5% (w/v), thereafter the augmentation is moderate because the most part of GA was reacted. In a second attempt to increase the amount of bounded CD, we increased the amount of the cross-linker. A higher amount of the cross-linker (Fig. 2B) carries out to a higher percentage of linked CD, but the swelling properties of the microspheres are strongly diminished. At a 5/1 molecular ratio of PVA/GA, the microspheres are almost unswellable. No significant increasing of the linked α-CD to the polymer support was noticed at higher cross-linker amount (Fig. 2B).

3.3. Degree of cross-linking

The affinity of iodine for PVA in aqueous solutions is a well known phenomenon (Yakota and Kimura, 1989). The blue-coloured complex has been studied extensively (Yakota and Kimura, 1985, 1986), but the discrete structure of the chromofore has not yet been clarified. Moreover, in a cross-linked structure such as PVA hydrogel microspheres, the mechanism of iodine retention appears to be difficult to interpret. However, for a certain range of reticulation, we found a quite good

Table 2
Retention volume of solutes (drugs or typical organic compounds) eluted using different stationary phases*

Solutes	Stationary phase				
	PVA_0	PVA/α-CD	PVA/β-CD	PVA/γ-CD	
	V_R (ml)	V_R (ml)	V_R (ml)	V_R (ml)	
				Phosphate buffer	H_2O/CH_3CN
Benzoic acid	2.16	2.89	3.18	2.45	1.29
Indol	4.46	n.e.[a]	18.36	23.04	5.32
Congo red	n.e.	n.e.	n.e.	n.e.	1.44
Methyl-paraben	4.18	20.16	11.48	15.55	3.01
Ethyl-paraben	4.32	n.e.	17.22	19.58	3.11
Propyl-paraben	4.32	n.e.	22.90	n.e.	3.18
Diclofenac	2.30	4.54	6.25	2.50	–[b]
Indometacin	2.45	2.81	3.95	4.82	–
Metronidazol	3.16	3.45	9.75	–	–
Propranolol	2.32	3.85	3.16	4.20	–
Lysozyme	2.16	1.65	1.36	1.72	–
β-lactoglobulin	1.44	1.72	1.29	1.51	–
Trypsinogen	1.44	1.72	1.43	1.58	–
Pepsin	1.44	1.72	1.43	1.51	–
Albumin (egg)	1.44	1.72	1.29	1.51	–
Albumin (bovine serum)	1.44	1.72	1.36	1.55	–
Aldolase	1.38	1.80	1.43	1.51	–
Catalase	1.44	1.58	1.43	1.51	–
Ferritin	1.44	1.58	1.43	1.58	–
Thyroglobulin	1.44	1.58	n.e	1.51	–
Blue dextran	1.40	1.58	1.29	1.29	1.22

Chromatographic conditions: eluent, phosphate buffer (pH 7.4) or water/acetonitrile mixture (70/30, v/v); flow rate 0.3 ml/min; injected volume, 20 μl; detection, UV; Data are the results of three independent experiments.
[a] Not eluted.
[b] Not determined.

correlation between the percentage of iodine retained by the microspheres and the amount of cross-linker (Fig. 3). For these determinations we used PVA/CD, without taking into account that CDs themselves form stable inclusion complexes with iodine (Cramer and Henglein, 1957).

In fact, the amount of iodine retained by CDs is enough low in comparison with iodine retained by microsphere (1.5, 7.7 and 7%, for α-,β- and γ-CD, respectively, at a PVA/GA ratio of 10/1), and therefore the results are reliable and the method can be satisfactory used for practical applications.

3.4. Microsphere porosity

The porosity of PVA/CD microspheres was determined by ISEC. In this case, the mobile phase was used to characterise the stationary phase, thus obtaining data concerning the maximum pore size and the total pore volume of the microspheres.

Plotting the logarithm of the molecular mass of standard molecules (deuterated water, maltodextrins and dextrans) against the elution volume (Fig. 4) the maximum radius of the pores in the swollen state was determined.

At the PVA/GA ratio of 10/1, taken as optimal, the maximum radius of the pores does not overpass 28.7 Å (corresponding to the radium of gyration of dextran with an average molecular weight of 10,000 Da) (Fundueanu et al., 1999).

However, in chromatographic experiments using standard proteins (see next section) β-lactoglobulin was found as the protein with the inferior limit of molecular weight of exclusion (the Stöck radius is

18 Å). In any case, the pores are large enough to permit the access of usual drug molecules (M_w < 1000 g/mol) in the CD cavity.

The porous volume of PVA and PVA/CD microspheres is closely similar, but they are smaller than those of polysaccharide microspheres (Fundueanu et al., 2003).

At the 5/1 PVA/GA molar ratio, the microspheres are almost unswellable (q = 1.5, 1.4 and 1.4 for PVA/α-, PVA/β- and PVA/γ-CD microspheres, respectively) and are characterized by a porous volume of 0.65 ml/g, 0.63 ml/g, and 0.51 ml/g for PVA/α-, PVA/β- and PVA/γ-CD microspheres, respectively. Also, at this molar ratio, the SEM photomicrographs show a microsphere cross-section almost without pores in the dried state (Fig. 1D).

3.5. Inclusion properties of microspheres

The inclusion properties of PVA/CD hydrogel microspheres were studied by HPLC. The PVA/CD microspheres were packed in a glass column and the interaction with drugs and other organic compounds was estimated from the retention volume (V_R) measured by liquid chromatography. Chemicals that interact strongly with CD, such as methyl-paraben, have high values of the association equilibrium constants (K_a) and exhibit relatively large retention volumes. On the other hand compounds that weakly bind to PVA/CD such as β-lactoglobulin, (low K_a values), are rapidly eluted (Table 2, Fig. 5A).

With the aim to prove such correlations, benzoic acid (BA), indol (In) and congo red (CR) were chosen as typical organic compounds that form stable inclusion complexes with CD with well known values of their association equilibrium constants ($K_a = 10^3\ M^{-1}$ for BA binding to α-CD, $K_a = 6 \times 10^5\ M^{-1}$ for In binding to α-CD, and $K_a = 1.7 \times 10^4\ M^{-1}$ for CR binding to γ-CD) (Connors, 1995) (Table 2). Also, characteristic drugs such as indometacin or metronidazole were used for tests (Szejtli, 1982).

Indeed, the values of V_R for In and CR are much higher than those observed for other compounds (e.g. BA) whose K_a values are lower. Also, the V_R increase is accompanied by a broadening of the peaks (Fig. 5B).

In order to determine the factors that determine the inclusion properties of PVA/CD microspheres,

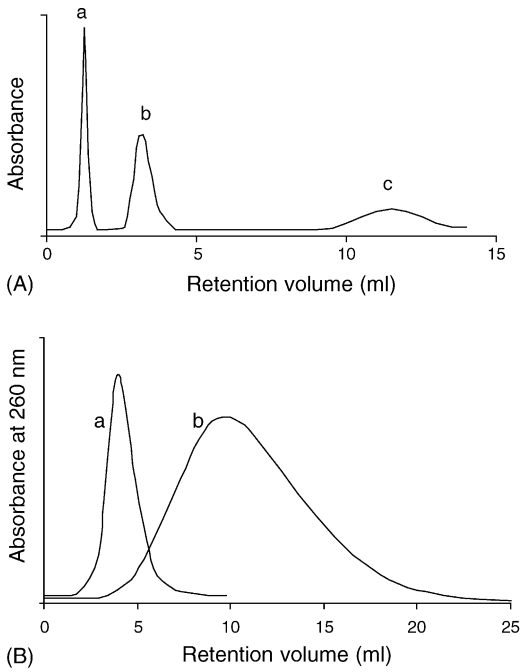

Fig. 5. (A) Liquid chromatographic profiles of β-lactoglobulin (a) and methyl-paraben (c) on PVA/β-CD stationary phase. BA was taken as the standard (b). Mobile phase: phosphate buffer, pH 7.4; flow rate: 0.3 ml/min, injected volume: 20 μl; solute concentration: β-lactoglobulin (a), 2 mg/ml; BA (b), 10 μg/ml; and MP (c), 100 μg/ml. (B) Liquid chromatographic profiles of metronidazol on PVA neutral stationary phase (a) and PVA/ β-CD active stationary phase (b). Mobile phase: phosphate buffer, pH 7.4; flow rate: 0.3 ml/min, injected volume: 20 μl; solute concentration: 0.4 g/l (a) and 1 g/l (b).

methyl-, ethyl- and propyl-paraben (MP, EP and PP, respectively) were used as standards solutes.

Also, organic eluents such as acetonitrile were added to the mobile phase (water/acetonitrile, 70/30, v/v). The V_R values of these parabens runned on PVA/α-, β- and γ-CD active stationary phase increased on increasing the length of the alkyl chain but are almost the same when are runned on the PVA neutral stationary phase. Therefore, the elution behaviour is not determined by a size exclusion mechanism, but by an adsorption mechanism based on size, geometry and hydrophobicity of the guest molecule. The V_R values of the tested solutes decrease when acetonitrile was added to the mobile phase, confirming that hydrophobic interactions are involved in the retention mechanism of these compounds. Even if the relative size

and geometry are the most important factors that rend thermodynamically favourable the formation of a given inclusion complex, other factors could affect the complex stability. The V_R of MP, EP and PP runned on the PVA neutral stationary phase are closely similar (4.18, 4.32 and 4.32, respectively) but these values are still high indicating that the PVA hydrogel matrix as such displays a certain affinity for the solutes. Therefore, the high V_R values of these compounds on the PVA/CD stationary phase are due to co-operative binding.

Finally, the inclusion ability of PVA/CD hydrogel microspheres for different drugs (diclofenac, indometacin, methronidazol and propranolol) and water-soluble proteins was investigated. All PVA/CD microspheres display a good affinity for the tested drugs. On the contrary, the water-soluble proteins are completely excluded from the pores and no inclusion complexes or surface adsorption was detected with the exception of thyroglobulin that is never eluted.

Last but not least, a low CD concentration in PVA microspheres (for α-CD is just 10.2 μM/g) is enough to allow a good separation of drugs or other organic compounds due to the accessibility of CD in the swellable microspheres and to cooperative binding of the PVA matrix.

4. Conclusions

Cyclodextrin-containing poly(vinyl alcohol) microspheres were successfully obtained in a single step procedure by chemical crosslinking with GA of an acidified aqueous solution of PVA and CD. β- and γ-CDs were found to be linked in microspheres in a higher proportion than α-CD. All PVA/CD microspheres display a microporous structure with pore dimensions ranging from few to 18 Å, depending to the amount of the crosslinker.

The stationary phase containing CD exhibits selectivity in HPLC separation of drugs or organic compounds with small molecules.

Acknowledgements

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