# Cyclodextrin-Anionic Polysaccharide Hydrogels: Synthesis, Characterization, and Interaction with Some Organic Molecules (Water Pollutants, Drugs, Proteins)

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**ABSTRACT:** Hydrogel microparticles have been prepared through cocrosslinking of cyclodextrin and of anionic carboxymethyl or sulfopropyl pullulan with a new bifunctional reagent: 3-(glycidoxypropyl)trimethoxysilane. This reagent forms crosslinking both through grafting with the epoxy end on the OH polysaccharide and through hydrolysis and condensation of the methoxy silane groups from the other end. Samples of cyclodextrin/anionic polysaccharides with various amounts of crosslinking agent were prepared. Characterization of the hydrogel microparticles considered the water swelling behavior, porosity measurements, retention of biologically active substances, which can occur by their inclusion in cyclodextrin inner cavities and in the pores of hydrogels, as well as through the electrostatic forces toward anionic polyelectrolyte charges. Their improved retention of various organic molecules as water pollutants, drugs, enzymes, recommend them as chromatographic supports, supports for the separation/immobilization of enzymes or for controlled release systems. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 1175–1183, 2009

**Key words:** cyclodextrins; anionic pullulan; microparticles; chromatographic supports

## INTRODUCTION

Cyclodextrins are cyclic oligosaccharides with 6 (α), 7 (β), and 8 (γ) glucopyranosic units linked through $\alpha^{1-4}$ linkages,[1] forming a torus-shaped ring structure. They are produced by enzymatic degradation of starch in the presence of bacteria. The most characteristic feature is the formation of inclusion complexes with various organic substances in their inner cavities through hydrophobic forces. This property occurs in both cyclodextrins and cyclodextrin derivatives, their polymers included. Between the multitude of substances included in cyclodextrins or in their polymeric gels, mention should be made of: drugs,[2–4] water pollutants such as β-naphthol,[5] phenolic derivatives,[6] aromatic compounds,[7,8] various dyes,[9–12] all providing a versatile method for the protection of the environment by waste water treatment.[13] Other CyD-based hydrogels were synthesized and tested for multidrug delivery[14] or/and controlled release systems.[15] The CyD hydrogels contain either polysaccharides[11–13] comonomers, which can improve their absorption performances through increasing the network hydrophilicity, or acrylic comonomers, which provide environmental stimuli response of the network during loading or release of the drug.[16,17] The cyclodextrin present in hydrogels can interact with hydrophobic drugs, control drug release and stabilize the amino acids, peptides and proteins,[18,19] thus improving the therapeutic values of these systems.

The aim of this article is to synthesize new cyclodextrin-based supports through their cocrosslinking with anionic pullulan derivatives, using a bifunctional agent: glycidoxypropyl trimethoxysilane (TMS); these supports were physicochemically characterized and their interaction with various molecules was studied, to appreciate their performances in the retention of organic pollutants or the retention/release of biologically active molecules. These supports can constitute an interesting alternative to that carboxymethyl cellulose-CyD based, synthesized and thoroughly studied by Crini et al.[10–12]

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## EXPERIMENTAL

### Materials

β-Cyclodextrin (CyD) (Fluka); stabilizer: Eastman cellulose acetate butyrate (Eastman Chemical Resins, USA) (CAB): 2 g% acetyl, 52 g% butyryl, average

$M_n \sim 30{,}000$; carboxymethyl pullulan (CMP) was synthesized in laboratory (DS: 0.80), as previously described[20]; sulfopropyl pullulan (SP) was obtained in laboratory (DS: 0.4), as described[21]; crosslinking agent: 3-(glycidoxypropyl) TMS (Aldrich); poly(ethylene glycol) (PEG) of various molecular weights (600; 1500; 2000; 13,500; 35,000) (Fluka); solvents: dichloroethane, methanol; biologically active substances: *m*-nitrophenol, *m*-chlorobenzoic acid, β-naphthol, salicylic acid (SA), indomethacin (indo), lysozyme (Fluka).

### Methods

*Crosslinking of CyD or of CyD-anionic polysaccharide with TMS*. To a 50 mL organic suspension medium (5 g CAB/100 mL dichloroethane), a CyD or CyD-anionic pullulan alkaline solution (15 mL, 20 g%) was added, under stirring at 400 rpm; after dispersion for 1 h at 50°C, the crosslinking agent (TMS) was added and the reaction was continued for 3 days at 50°C. The microparticles obtained were filtered, first washed with acetone to remove the stabilizer, then with water, dehydrated from methyl alcohol and finally dried in vacuum, at 40°C, for 24 h.

*The water regain* (g water/g dry microspheres) was determined through centrifugation of the swollen microspheres according to Pepper's method[22] (centrifuge: Janetzki T23, Poland). It was calculated with the formula:

$$\text{Water regain} = \frac{W_s - W_d}{W_d}$$

where $W_s$ is the weight of swollen microparticles (after centrifugation); $W_d$ is the weight of dry microparticles taken into study.

The water swelling represents the volume of microparticles in swollen state, reported to the amount of dry microparticles taken into study and is obtained by measuring the volume of swollen microparticles into graduated cylinders, at equilibrium.

*Retention of PEG* with various $M_w$ values was followed by an adapted method, as described[23] for fluorescein-labeled dextran (F*-dextran). The previously dried in vacuum and weighed microparticles were equilibrated in a 0.02% sodium azide solution, for 24 h, then placed in a PEG solution of 200 μg/mL concentration, which was monitored daily. After equilibration (about 3 days), the microparticles were filtered, the solution was replaced with water and the retained PEG was exhaustively released from the gels. The amount of released PEG has been monitored by measurements of the total carbon content, with a Shimadzu TOC-V CSN Total organic carbon analyzer apparatus. The molecular characteristics of PEG of various molecular weights were determined from HPSEC/MALLS/DRI, as previously described.[24]

*The specific densities*, *pore volumes*, and *porosities* of the microparticles were determined by cyclohexane retention, as described in literature.[25,26]

*The retention of organic molecules* was performed under "batch" conditions, in glass-stoppered flasks; 50 mL (2 g/L) solutions were added on the 50 mg support; aliquots from supernatant were withdrawn and the organic molecules concentration was determined spectrophotometrically (*p*-nitro phenol: 320 nm; *m*-chlorobenzoic acid: 290 nm; β-naphthol: 274 nm; SA: 298 nm; indomethacin: 320 nm); the equilibrium concentrations were attained in maximum 72 h. The sorbate concentrations are high enough (about 10 times higher than their retention capacity); in these conditions, the saturation point has been reached for all studied molecules; the retained substance is calculated as the difference versus its initial solution content. The lysozyme concentration was determined at 750 nm by the modified Folin method.[27]

*The in vitro* release of the biologically active molecules retained on supports was also performed under "batch" conditions, on a 50 mg support-organic molecule complex, in acidic (pH: 1.4) or buffered solution (pH: 7.4) on spectrophotometrically measuring the supernatant concentration at various time intervals.

*The enzymatic activity* was determined on a *Micrococcus Lysodeikticus* substrate (Sigma) at 450 nm,[28] on a 3 mg sample directly mixed in the spectrophotometric cell with a 3 mL solution of the substrate (0.3 mg/mL); a decrease in absorbance (A) was recorded every 30 s; 1/A is proportional to the enzymatic activity.[28]

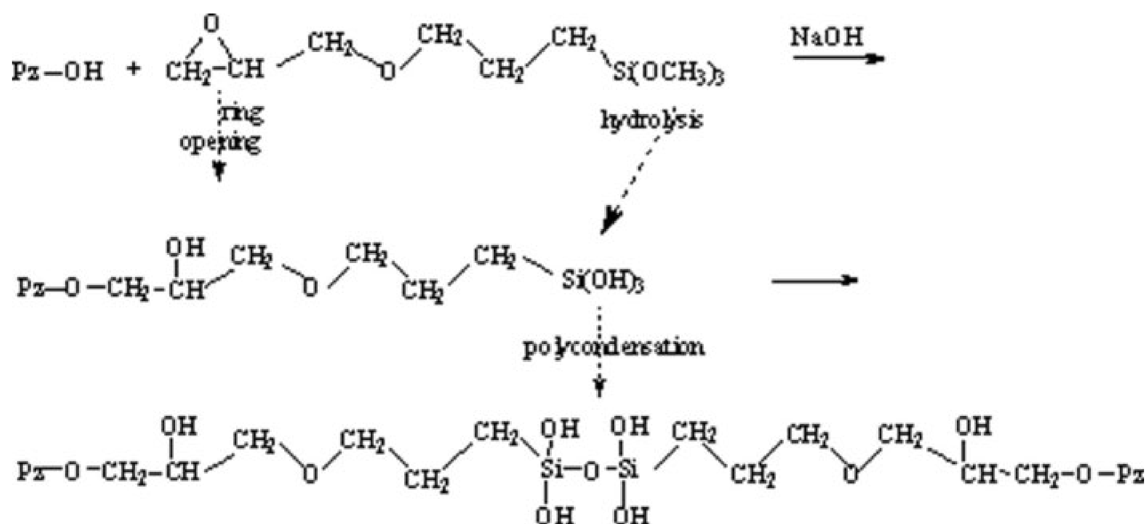
## RESULTS AND DISCUSSION

### Synthesis and characterization of microparticles

Polysaccharide microparticles were obtained by using 3-(glycidoxypropyl) TMS, which forms crosslinkings both through grafting with the epoxy end on the OH polysaccharide, and through hydrolysis and condensation of the methoxy silane groups at the other end (Scheme 1)[24]:

Polysaccharide microparticles with a mean diameter of 10–60 μm (>90% in weight) (Fig. 1) were obtained.

The microparticles obtained contain both types of cavities, namely: the ones produced by crosslinking with the siloxanic units and the CyD inner cavities. Because of the presence of glucopyranosic units in both the chain of the used polysaccharide and in the CyD moieties, it is difficult to determine exactly the amount of CyD incorporated in the gel; yet, taking




**Scheme 1** Crosslinking reaction with TMS; Pz: polysaccharide (anionic pullulan, CyD).

into account that the final yield in microparticles attained after cocrosslinking exceeds 90 g% of the theoretical value, one can appreciate that the anionic polysaccharide/CyD ratio in the gel is approximately the same as the initial ratio of the reaction.

The reaction conditions and the physicochemical characteristics of the CyD-anionic pullulan microparticles crosslinked with TMS are presented in Table I.

The water regain and water swelling of the microparticles decrease with the increase in the amount of TMS crosslinking agent used in the reaction. The CyD-TMS microparticles are less hydrophilic; the presence of anionic polysaccharides in the microparticle network improves the hydrophilicity of the supports and their porosities in swollen state; in this way, the accessibility of organic molecules inside the macromolecular network will be also improved.

The physicochemical characteristics of the CyD microparticles are presented in Table II.

As one may see (Table II), in dry state, the porosity and pores volume of the microparticles are insignificant, whereas in swollen state (Table I), their porosity (reflected by the water regain) becomes important. For CyD-TMS microparticles, the specific density value is higher and the porosity value is lower (Table II), which indicates a more compact network structure.

For better appreciation of the pore volume of the microparticles in swollen state, experiments on the

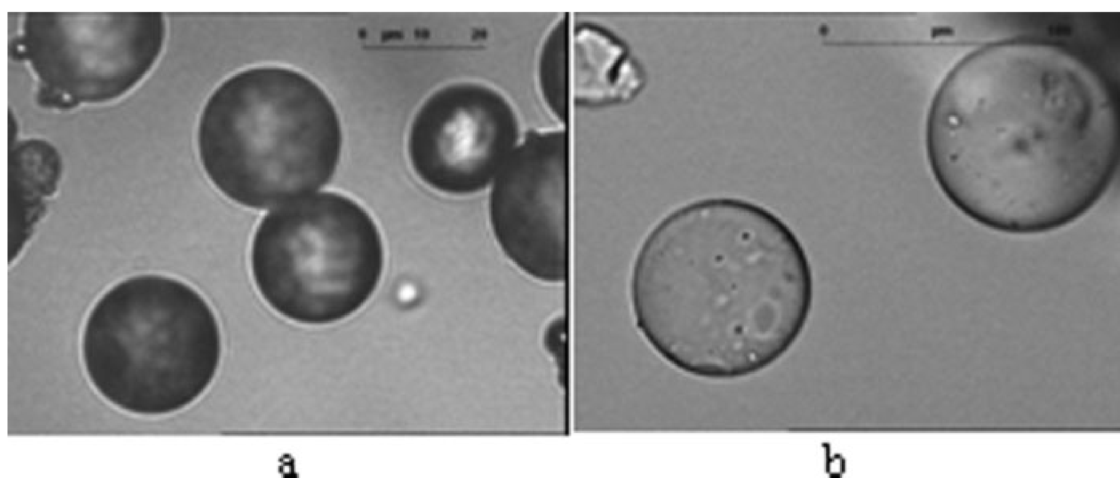



**Figure 1** Electronic photos of CyD microparticles in dry (a) and swollen state (b).

**TABLE I**
**Reaction Conditions and Water Swelling Characteristics of CyD Microparticles**

| Sample | Anionic pullulan (g) | β-CyD (g) | mmol CyD[a]/g micropart. | Molar ratio TMS/GU[b] unit | Water regain (g/g) | Water swelling (mL/g) |
|---|---|---|---|---|---|---|
| CMPCyD4 | Carboxymethyl, 1 | 1 | 0.38 | 0.15/1 | 9.08 | 15.1 |
| CMPCyD1 | Carboxymethyl, 1 | 1 | 0.35 | 0.31/1 | 5.07 | 8.40 |
| CMPCyD3 | Carboxymethyl, 1 | 1 | 0.31 | 0.39/1 | 3.20 | 5.30 |
| CMPCyD2 | Carboxymethyl | 1 | 0.29 | 0.48/1 | 2.23 | 3.40 |
| SPCyD | Sulfopropyl, 1 | 1 | 0.31 | 0.39/1 | 3.40 | 4.60 |
| CyDTMS | – | 1 | 0.88 | 0.27/1 | 1.40 | 2.20 |

[a] CyD content in initial reaction mixture.
[b] Glucopyranosic unit.

retention of various molecular weights PEG were performed. The results are presented in Figure 2.

The CMPCyD4 microparticles, possessing the lowest crosslinking degree, retain variable amounts of PEG 600–13,500, whereas PEG 35,000 is retained in very small amounts; consequently, PEG 35,000 can be considered as the exclusion limit for CMPCyD4 microparticles (hydrodynamic radius: 10 nm). CMPCyD3 retains very small amounts of PEG 13,500; hence, PEG 13,500 can be considered as the exclusion limit for CMPCyD3 microparticles (hydrodynamic radius: 6 nm). For the SPCyD microparticles, the exclusion limit can be considered PEG 2000 (hydrodynamic radius: 2 nm), whereas for the microparticles containing only cyclodextrin crosslinked with TMS (CyDTMS), the exclusion limit can be considered PEG 1500.

### Interaction with organic molecules

Polysaccharide gels have been studied to appreciate their performances in retention of water pollutants, both by formation of inclusion complexes in CyD cavities and by the presence of other interactions such as: physical adsorption, hydrogen and hydrophobic interactions induced either by the supports or by the crosslinking agent.[5]

The synthesized supports were tested from the view point of their interaction with various organic molecules; the organic molecules have been divided in three classes, namely: water pollutants (phenol and benzoic acid derivatives, β-naphthol), biologically active substances (SA, indomethacin), proteins (lysozyme). For comparative purposes, the study of the interaction of organic molecules with anionic-CyD microparticles was performed in distilled water, to avoid the influence of pH or ionic strength variation on the swelling characteristics of the supports or on protonation of the supports or sorbates; further studies are in development to establish the influence of above-mentioned parameters on the retention/release behavior of these systems.

### Interaction with water pollutants

The microparticles obtained contain the cyclodextrins moieties included in the macromolecular crosslinked network; even if the inclusion of organic molecules can be controlled by diffusion inside the network, the properties of the binding sites in crosslinked polymer do not differ from the properties of the parent β-CyD.[10] Indeed, as can be seen in Table III, the used organic molecules are retained in about 1 : 1 *M* ratio on CyDTMS microparticles, as reported in literature for β—CyD (1 : 1 for *m*-chlorobenzoic acid, Ref. 1, p 87; 1 : 1 for *p*-nitro phenol[29]). The *p*-nitro phenol, retained in very small amounts on the CMPTMS support without CyD, is retained on the anionic polysaccharide-CyD supports principally due to the presence of CyD moieties (the meq *p*-nitro phenol/meq CyD ratio is about the same as that for CyDTMS). The *m*-chlorobenzoic acid and the β-naphthol are retained both in the CyD cavities and in the macromolecular crosslinked network of

**TABLE II**
**Physico-Chemical Characteristics of CyD Microparticles**

| Sample | Specific density[a] ($d$), g/mL | $V_0 = W_0{}^{b}/d$ | Pores volume[a] ($V_p$), mL/g | Porosity[a], $V_p/V_0$ |
|---|---|---|---|---|
| CMPCyD3 | 1.1 | 0.91 | 0.032 | 0.035 |
| CMPCyD4 | 0.99 | 1.01 | 0.049 | 0.048 |
| SPCyD | 1.08 | 0.925 | 0.041 | 0.044 |
| CyDTMS | 1.17 | 0.85 | 0.022 | 0.026 |

[a] Each value is a mean of three measurements that deviated with ± 3%.
[b] $W_0$ = the weight of dry beads.[25,26]

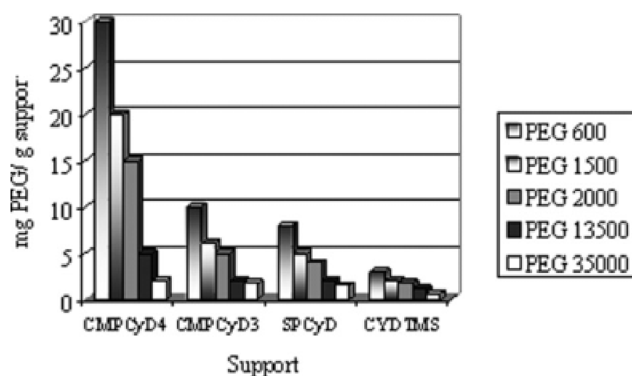
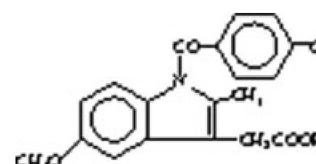



**Figure 2** Retention of various molecular weights PEG on CyD microparticles.

anionic pullulan-CyD microparticles; the fact that the CMPTMS microparticles retain very small amounts of these substances, whereas the CMP-CyD microparticles retain higher amounts that those corresponding to the CyD retention, can be probably explained by the synergistic effect between the retention due to the CyD cavities and the retention in the macromolecular ionic crosslinked network. Hence, through cocrosslinking of anionic pullulan and cyclodextrin, the microparticles obtained show improved retention properties of the organic molecules. Inclusion in the CyD cavities of CyDTMS microparticles is the main retention mechanism for organic molecules, controlled by diffusion in their hydrophobic network; the presence of hydrophilic anionic pullulan ensures a higher network swelling and an improved support-solute contact. This characteristic can be useful in further applications of the synthesized microparticles as chromatographic supports.

### Interaction with biologically active substances

SA and indomethacin (Scheme 2) are known as anti-inflammatory drugs; the first also evidences antiseptic and antifungal action; both of them have many secondary effects. By their inclusion in an appropriate macromolecular support, their performances may be improved, along with reduction of their side effects.



**Scheme 2** Indomethacin

The biologically active substances presented in Table IV are retained on CyDTMS crosslinked microparticles in about the same molar ratio as reported in literature for CyD itself (0.84 : 1 for SA, Ref. 1, p 231; 0.5 : 1 for indomethacin, Ref. 1, p 210; other references reported 1 : 1 complex stoichiometry for CyD-indomethacin[30]). Also, although the CMP-TMS microparticles retain very small amounts of SA and indomethacin, the microparticles containing anionic pullulan and CyD retain higher amounts than those corresponding to the inclusion in CyD cavities, probably by a synergistic effect caused by their inclusion in macromolecular crosslinked network. Hence, the presence of both anionic pullulan and CyD in the same crosslinked network improves the retention properties of the biologically active molecules.

The *in vitro* release of the indomethacin retained on the supports was followed, for its possible utilization as a controlled release drug system.

As shown in Figure 3, in acidic pH (1.4) the indomethacin is released in smaller amounts from its complexes with CMP-CyD gels than in basic pH (7.4), which can be explained by more collapsed structure of the weakly acidic carboxymethyl derivative in acidic pH that in a basic one[2]; the same indomethacin release behavior was reported for carboxylic (acrylic acid) pH-sensitive hydrogels.[31,32] Reversely, for strongly acidic SP-CyD gels, in a basic medium, the more collapsed structure releases the

**TABLE III**
**Retention of Various Organic Water Pollutants on the Synthesized Supports**

| Sample | *p*-nitro phenol | | | *m*-Clorobenzoic acid | | | β-naphtol | | |
|---|---|---|---|---|---|---|---|---|---|
| | g/g | meq/g | meq/meq[a] | g/g | meq/g | meq/meq[a] | g/g | meq/g | meq/meq[a] |
| CMPCyD3 | 0.05 | 0.36 | 1.15 | 0.1 | 0.64 | 2.06 | 0.13 | 0.94 | 3.02 |
| CMPCyD4 | 0.06 | 0.43 | 1.13 | 0.11 | 0.71 | 1.87 | 0.14 | 0.97 | 2.55 |
| SPCyD | 0.05 | 0.36 | 1.16 | 0.07 | 0.44 | 1.42 | 0.15 | 1.08 | 3.48 |
| CyDTMS | 0.11 | 0.79 | 0.90 | 0.12 | 0.74 | 0.84 | 0.14 | 1.01 | 1.14 |
| CMP-TMS[b] | 0.0074 | 0.05 | – | 0.03 | 0.22 | – | 0.01 | 0.1 | – |

[a] meq organic molecule/meq CyD of the support.
[b] microparticles of CMP crosslinked with TMS, without CyD. Each value is a mean of three measurements that deviated with ± 2%.

**TABLE IV**
**Retention of Salicylic Acid and Indomethacin on CyD Microparticles**

| Sample | Salicylic acid (SA) | | | Indomethacin (Indo) | | |
|---|---|---|---|---|---|---|
| | g/g | meq/g | meq/meq[a] | g/g | meq/g | meq/meq[a] |
| CMPCyD3 | 0.13 | 0.94 | 3.03 | 0.18 | 0.5 | 1.25 |
| CMPCyD4 | 0.15 | 1.09 | 2.87 | 0.23 | 0.64 | 1.68 |
| SPCyD | 0.13 | 0.92 | 2.97 | 0.20 | 0.56 | 1.33 |
| CyDTMS | 0.12 | 0.88 | 1.0 | 0.17 | 0.47 | 0.54 |
| CMP-TMS[b] | 0.04 | 0.28 | – | 0.014 | 0.04 | – |

[a] meq organic molecule/meq CyD of the support.
[b] microparticles of CMP crosslinked with TMS, without CyD.

drug more slowly than in an acidic one. The crosslinking degree of the microparticles also influences the drug release process (e.g., the less crosslinked CMPCyD4 versus the most crosslinked CMPCyD3). For the CyDTMS microparticles, in which the drug is hydrophobically included in the CyD moieties, the pH variation does not influence the release rate very much.

The release of drugs as a function of pH and duration is one of the main properties of the controlled release drug systems; further studies being carried out to establish their performances *in vivo*.

## Retention of lysozyme

Proteins and peptides contain aromatic amino acids, whose hydrophobic moieties can react with cyclodextrins.[33] Lysozyme, which is a small, basic, globular protein having 129 amino acids crosslinked with disulfide bridges, presents antibacterial activity in viral infections and skin diseases[34]; it has a $M_w \approx$ 14,600.

The lysozyme retention rate (the amount of lysozyme retained per time unit: $dc/dt$) on the studied supports is influenced by protein accessibility inside the microparticle; the CMPCyD4, less crosslinked, most hydrophilic microparticles present the highest rate of lysozyme retention. In the case of CyDTMS microparticles, the enzyme is probably retained, mainly on their surface, by hydrophobic interactions between the CyD moieties and the enzyme hydrophobic units, at a slow rate (Fig. 4).

The monomolecular adsorption of the adsorbate molecules from solution at constant temperature onto adsorbent may be described by the following Langmuir-type equation[35]:

$$C_{eq}/(x/m) = 1/(k_1 \times k_2) + C_{eq}/k_2$$

where $C_{eq}$ is the concentration of adsorbate remaining in solution at equilibrium (mmol/L); $x$ is the amount of adsorbate bound to the adsorbent and m the amount of adsorbent used. The constant $k_1$ may be defined as the adsorption coefficient or affinity constant and is related to the magnitude of the forces involved in the binding process. The Langmuir-capacity constant $k_2$ indicates the apparent maximum amount of adsorbate that can be adsorbed per unit weight of adsorbent. By plotting of $C_{eq}/(x/m)$ versus $C_{eq}$ on rectilinear coordinates, should yield a straight line from which one can obtain the constant $k_1$ and $k_2$.

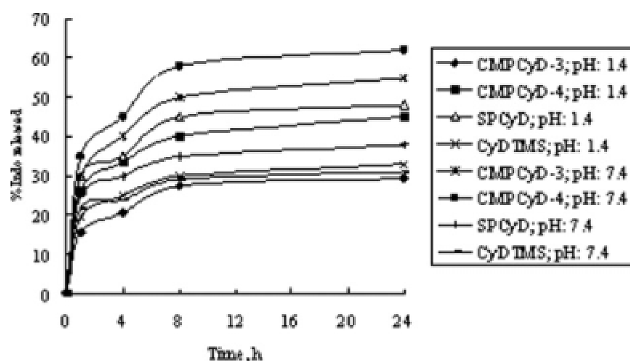



**Figure 3** *In vitro* release of indomethacin from its complexes with the CyD microparticles.

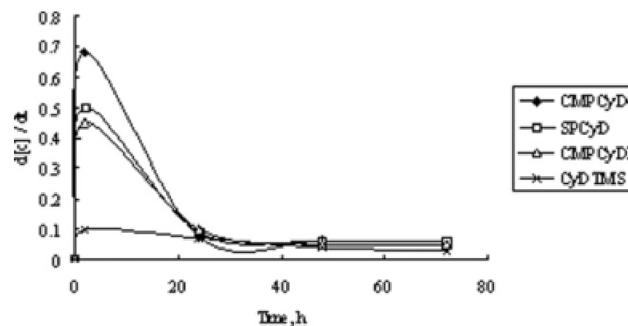



**Figure 4** Lysozyme retention rate ($dc/dt$) as time function on CyD microparticles.

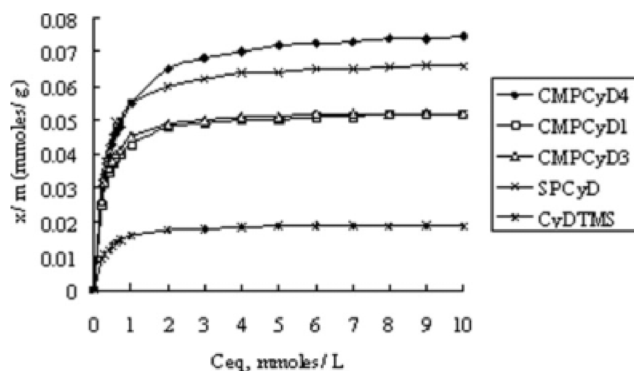



**Figure 5** Adsorption lysozyme isotherms on CyD microparticles.

**TABLE V**
**Langmuir' s Constants for Lysozyme Retention on CyD Microparticles**

| Sample | $k_1$, L/mmol[a] | $k_2$, mmol/g[a] |
|---|---|---|
| CMPCyD4 | 2.5 | 0.08 |
| CMPCyD1 | 3.7 | 0.06 |
| CMPCyD-3 | 4.8 | 0.05 |
| SPCyD | 5.2 | 0.06 |
| CyDTMS | 4.0 | 0.02 |

[a] The values represent the mean of three determinations whose deviation was 3–5%.

The experimental proteins adsorption data on various adsorbents (cholestyramine, methacrylamide grafted fibers, dye attached poly(hydroxypropyl) methacrylate beads, microporous polypropylene, phospholipid bilayers, amphoteric polymer particles, magnetic nanoparticles, etc.) fitted well to the Langmuir isotherm model.[36–42]

The data obtained for lysozyme adsorption on the synthesized CyD microparticles were well described by the Langmuir isotherm model, suggesting a protein monolayer adsorption on the studied supports. The adsorption isotherms show a tendency to reach a plateau at high $C_{eq}$ values; this plateau corresponds to the maximal amount of lysozyme retained on each support, in mmol/g (Fig. 5). By representing the data of lysozyme adsorption in Langmuir coordinates, straight lines were obtained (Fig. 6), allowing the calculation of constants $k_1$ and $k_2$ (Table V). The values $k_2$ correspond with the lysozyme amounts retained on the studied supports, in mmol/g; these follow the order: CMPCyD4 > SPCyD > CMPCyD1 ≅ CMPCyD3 > CyDTMS. An explanation for this order can be the higher hydrophilicity of CMPCyD4 support, which facilitates the more freely access of the lysozyme to the reactive sites; the greater acidity of sulfopropyl groups of SPCyD support which influence on the electrostatic interactions with the basic protein; CyDTMS less hydrophilic support, without ionic charges, adsorbed smaller amount of lysozyme, through hydrophobic forces with CyD moieties, at microparticles surface. The values $k_1$ increase in the order: CMPCyD4 < CMPCyD1 < CyDTMS < CMPCyD3 < SPCyD. These values indicate on the one part an enhanced affinity of strongly acidic sulfopropyl groups of SPCyD support for basic adsorbate, in addition with the affinity through hydrophobic forces towards CyD moieties (CyDTMS support). Hence, one can suppose that the affinity between the basic lysozyme and the supports is a resultant of the cooperative effects of electrostatic and hydrophobic interactions, but the proportion in which the electrostatic or hydrophobic forces influence on protein retention is less obvious.

*In vitro* release of lysozyme (Fig. 7) was faster in acidic pH for CMPCyD microparticles, despite their more collapsed network, to be explained by the higher solubility of the basic enzyme at this pH value, with a subsequent decrease in its affinity for the CyD cavities.[2]

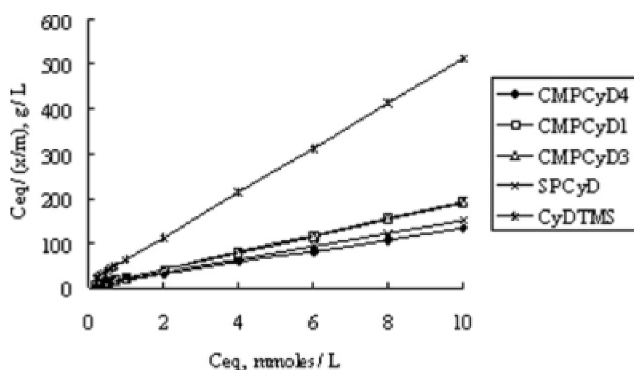



**Figure 6** Langmuir' s isotherms for lysozyme adsorption on the studied CyD microparticles.

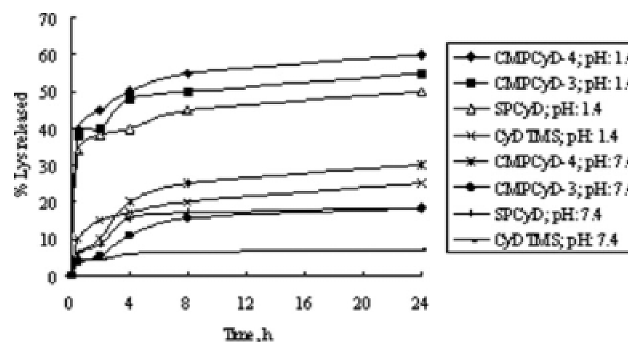



**Figure 7** *In vitro* lysozyme release from its complexes with CyD microparticles.

The lysozyme retained on the supports can be recovered with NaCl solutions of ionic strength over that 0.5*N* (Fig. 8), which means that the CyD synthesized microparticles can be used for lysozyme recovery from its crude solutions. The ionic strength of NaCl solution weakens the electrostatic interactions between support and sorbate and facilitates the release; even if some affinities of the protein with cyclodextrin cavities are still expected, these provide a weak control of the release process.

The lysozyme immobilized on the supports preserves its enzymatic activity, as proved by its lytic activity towards *Micrococcus Lysodeikticus* (Fig. 9). The rate of the enzymatic process (which is proportional with the decrease of the absorbance: 1/A) depends on the access of the substrate to the immobilized lysozyme; for less hydrophilic supports, which bind the enzyme only on the surface, the enzymatic process has a smaller rate than for the more hydrophilic ones, in which the substrate can also reach to the lysozyme immobilized inside microparticles, too.

Hence, by an appropriate choice of the support, the enzymatic process may occur at the desired rate.

## CONCLUSIONS

New cyclodextrin-anionic pullulan microparticles were synthesized and characterized. Their improved retention of various organic molecules as water pollutants, biologically active substances, enzymes, recommend them as chromatographic supports, supports for the separation/immobilization of enzymes or for controlled release systems.

Further studies on these new supports are in development to provide additional data concerning the conditions and the performances in retention/release of biologically active substances.

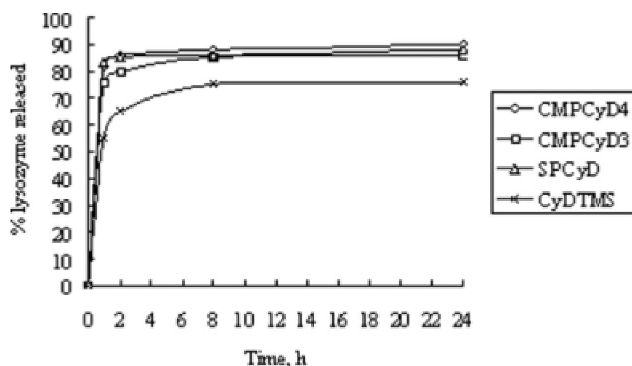



**Figure 8** Release of lysozyme in NaCl 0.5*N* solution.

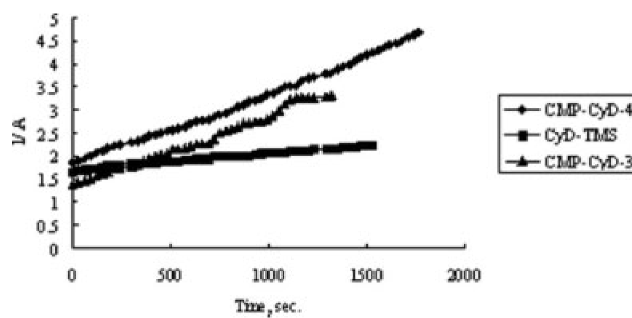



**Figure 9** Enzymatic activity (proportional with 1/A; A-absorbance) of the lysozyme immobilized on the CyD microparticles.

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