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Increasing the adsorption capacity and selectivity of poly(vinyl alcohol) hydrogels by an alternative imprinting technique

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ABSTRACT: In this work, we report an innovative alternative imprinting method for obtaining chemically crosslinked poly(vinyl alcohol) hydrogels with β-cyclodextrin as template. The materials present high affinity toward the template that imprinted them, revealed by the higher sorption yield of β-cyclodextrin and higher selectivity factors of the imprinted material, by comparing with the nonimprinted reference. The imprinting kinetic and mechanism has been demonstrated by adsorption studies, binding isotherms and Scatchard analysis and is in good correlation with the information regarding the morphology of the materials, determined by fluorescence microscopy and atomic force microscopy. By using a novel fluorescence spectroscopy method of the starting polymer solution, the optimum amount of imprinting template could be determined. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42024.

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INTRODUCTION

Molecular imprinting is an ecologic method for designing synthetic materials that possess molecular memory. They bear "active cavities" in their structure, which resemble the size and shape of a certain template molecule. These materials are capable of specifically recognizing and binding the template molecule, from a mixture of structurally related compounds. Molecularly imprinted materials are used in a large variety of applications, such as biosensor assays; stationary phases for chromatography, as well as in organic compounds separations from complex mixtures.[1,2]

β-cyclodextrin (BCD) is a cyclic oligosaccharide comprised of seven α-D-glucopyranoside units linked 1–4, similar to amylose from starch, from which it is obtained by means of enzymatic conversion. BCD and its derivatives are largely used in medicine, pharmaceutical and food industry.[3]

The complex mixture resulting after the enzymatic conversion of starch into β-cyclodextrin usually contains unreacted glucose, BCD ether derivatives as well as other types of other cyclodextrins (α and γ). BCD separation from this complex mixture is usually employed by differential solubility of the derivatives in several volatile organic solvents such as methanol or dichloromethane. This solvent extraction procedure leads to an increased overall price of the final product and trace impurification by solvent adsorption inside the cyclodextrin cavity.[4]

Several materials that possess good affinity for β-cyclodextrin, such as silica gel or poly(vinyl alcohol)[5] could not be used as such for the selective adsorption of BCD from complex mixtures, because their low selectivity when in contact with structurally related compounds.

The aim of the work is to increase the sorption capacity and selectivity of poly(vinyl alcohol) films for β-cyclodextrin by the formation of active cavities via the imprinting technique. Chemically crosslinked poly(vinyl alcohol) (PVA) hydrogel films imprinted with β-cyclodextrin (BCD) have been obtained by starting directly from the aqueous polymer solution in the presence of different amounts of BCD, thus eliminating the use of toxic monomers and organic solvents from the traditional imprinting approach. Glutaraldehyde in gaseous phase has been used as crosslinker for PVA, in order to minimize the unreacted residual crosslinker amount from the polymer matrix.

Poly(vinyl alcohol) is a hydrosoluble polymer that could generate interactions with many compounds, due to the hydrogen bonding promoted by the hydroxyl groups. Our previous studies have demonstrated that PVA could be a suitable matrix for molecular imprinted materials obtaining.[6,7] Supplementary, it is a nontoxic, noncarcinogenic, biocompatible, biodegradable, water-soluble polymer, in consequence easy to handle and friendly for environment.[8]

The imprinting of the material has been demonstrated by batch adsorption studies of BCD (capacity factor) as well as its ether

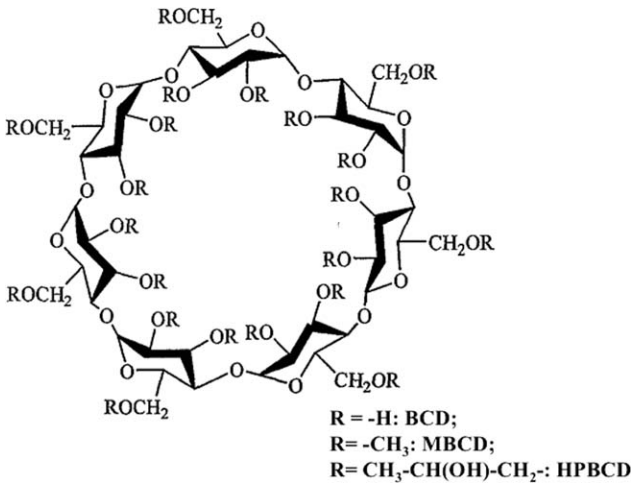

Figure 1. Cyclodextrin and its ether derivatives structure.

derivatives: hydroxypropyl-β-cyclodextrin (HPBCD) and methyl-β-cyclodextrin (MBCD; selectivity factor) into the imprinted polymer, binding isotherms and Scatchard analysis. The imprinting has also been confirmed by morphology studies, such as atomic force microscopy (AFM) and fluorescence microscopy.

The article presents an original analytical method for predicting the performance of the cyclodextrin-imprinted material by fluorescence spectroscopy analysis of the starting poly(vinyl alcohol) solution mixtures with different amounts of BCD template. The influence of the template structure and concentration on the active cavities uniformity and shape has also been studied and assessed by fluorescence microscopy studies.

EXPERIMENTAL

Materials

PVA 120-98 (1200 polymerization degree and 98% hydrolysis degree) was purchased from RomReRo S.A. Rasnov, Romania, and used without further purification.

Aqueous solution of glutaraldehyde (GA) 45% wt concentration was purchased from Sigma-Aldrich.

β-cyclodextryn (BCD) KLEPTOSE, methyl-β-cyclodextrin (MBCD), 2-hydroxypropyl-β-cyclodextrin HPBCD (Figure 1) were purchased from Roquette Ltd. The average M_n of MBCD is 1310, with 1.6–2.0 mol $-CH_3$ per unit anhydroglucose. The average M_w of HPBCD is 1460 Da, with a 0.8-molar substitution degree. All cyclodextrins have purity higher than 98%, and were used as provided.

Preparation of PVA Imprinted Material

The obtaining of the cyclodextrin-imprinted PVA comprises of three steps, described in the following paragraphs: firstly, the obtaining of the polymeric films with the template molecules in composition, secondly the crosslinking of the polymeric matrix to ensure the later formation of the active cavities and the stability of the material and in the third step the elimination of the template molecule, to form the active cavities.

The polymer solution has been prepared by adding PVA powder into a determined volume of distilled water, followed by heating the mixture to 85°C under magnetic stirring for 4 h. The obtained solution had a solid content of 11%.

The films have been prepared from 10 mL of 11% PVA aqueous solution which contains 5, 10, 15, 25, and 30 (%) wt CDs calculated by ratio to the dry polymer in the solution, casting in plastic Petri dishes (3 cm diameter) and solvent evaporation at 25°C and 55% relative humidity for 24 h. The obtained transparent films with an average thickness between 0.12 and 0.16 mm have been immersed for 5 s in a 1-*M* aqueous solution of sulfuric acid (crosslinking catalyst).

The obtained PVA films with CDs in composition with a solids content of 90–93%, have been introduced into a desiccator and placed over a recipient that contained 20 mL of 45% wt GA solution. The installation has been connected to a vacuum pump, so as the film to be in contact with GA vapors for 3 h. Blank samples, without CDs in composition, have been crosslinked following the same procedure.

By immersing the films into the crosslinking catalyst for 5 s, water: polymer ratios of 0.52–0.57 have been found. This ensures the swelling of the PVA films (relative volume modification of 1–3% reported to the initial volume of the film) leading to the catalyst and crosslinker diffusion inside the material, ensuring a statistically homogenous crosslinking throughout. The films maintained their swollen state in the crosslinking installation, where they are in contact with both water and glutaraldehyde vapors.

As crosslinking occurs, the water: polymer ratios decrease to 0.31–0.36. This could serve as additional proof that PVA crosslinking took place. According to the reference literature, the mechanism of PVA crosslinking with glutaraldehyde involves the formation of a hemiacetal between the carbonyl groups of GA and the –OH groups of two adjacent PVA macromolecules, with the elimination of water.[9] A concentration of GA reported to the dry polymer of 3.7–4.51% has been determined for the obtained films, by means of gravimetric analysis. The PVA matrix crosslinking duration has been chosen at 3 h based on our previous studies. This crosslinking duration ensures an optimal crosslinking degree, determining lower amounts of water uptake at equilibrium, sorption properties improvement at relatively high rates and selectivity to the PVA matrix.[10]

After crosslinking, in order to complete the imprinting process, the template molecules have been removed from the polymeric matrix by immersing the crosslinked films in distilled water at room temperature, for a determined time period. The distilled water has been replaced periodically with a fresh one, to maintain a convenient concentration gradient between the CDs from the film and the released CDs amount. The complete removal of CDs from the polymer has been determined by the polarimetric method at 545 nm, based on the CD and derivatives optical activity, using a Carl-Zeiss POLAMAT A polarimeter with standard glass cuvette of 10 cm optical path. For the sorption studies (BCD and BCD derivatives adsorption) the imprinted materials have been equilibrated in distilled water, and for microscopic analysis (AFM, fluorescence), the films have been dried between microscope glass slides by using a Christ Alpha 2-4 LD freeze dryer.

Tests and Analysis

Fluorescence Spectroscopy. Based on the photofluorescence of PVA in aqueous solution, the fluorescence intensity at three successive wavelengths, $\lambda_{em1} = 415$ nm, $\lambda_{em2} = 436$ nm, and $\lambda_{em3} = 465$ nm of the polymer solution with different amount of cyclodextrins (10%, 25%, and 30% reported to the PVA) has been obtained by using a TunerQuantech filter fluorimeter, at an excitation wavelength of 400 nm. The respective solutions have been further used to obtain the imprinted polymer material, as described in section "Preparation of the imprinted material."

The three fluorescence emission maxima correspond to $\pi^* \rightarrow$ n transitions of the nonbonded oxygen electrons from the –OH groups of syndiotactic (415 nm), atactic (437 nm), and isotactic (465 nm) PVA.[11]

For each aqueous PVA/CD mixture the fluorescence emission intensities have been determined (I_{415}, I_{436}, and I_{465}), and the fractions of syndiotactic (f_s), atactic (f_a), and isotactic (f_i) PVA from the solution has been calculated with the following equations:

$$f_s = \frac{I_{415}}{I_{415} + I_{436} + I_{465}} \cdot 100 \quad (1)$$

$$f_a = \frac{I_{436}}{I_{415} + I_{436} + I_{465}} \cdot 100 \quad (2)$$

$$f_i = \frac{I_{465}}{I_{415} + I_{436} + I_{465}} \cdot 100 \quad (3)$$

The best imprinting effect should be expected of the material obtained by using the PVA/CD mixture with the highest isotactic fraction of the polymer chains, as this steric arrangement favors the interactions between the polymer and the template molecule, leading to well defined and undeformed cavities.

The values of f_i, f_s, and f_a presented in the article represent the arithmetic mean of three measurements, performed on different PVA solutions, with the same polymer and cyclodextrin amount. The cyclodextrin template does not present photofluorescence emission at the used excitation wavelength.

Sorption Studies

Imprinting Testing. The imprinting testing has been performed by comparing the amounts of cyclodextrin adsorbed from aqueous solutions from weighed samples of imprinted and nonimprinted films, respectively. The sorption of BCD in the polymeric matrix has been determined by the difference from the initial amount of cyclodextrin present in the solution and the amount of cyclodextrin from the solution after adsorption at determined time intervals, by using the polarimetric method, as mentioned in section "Preparation of the PVA imprinted material." The amount of cyclodextrin adsorbed by 1 g of dry polymer (xerogel) at different time intervals has been calculated by taking into account the solids content of the films, and represents the capacity factor (K) of the imprinted polymer. The initial concentration of the aqueous CD solution used for sorption studies was 1 g/L, and for each time interval the sample has been immersed in a fresh volume of solution (10 mL), to maintain a convenient concentration gradient between CD from the solution and the CDs from the polymeric matrix.

Selectivity Testing. The selectivity of the BCD imprinted films has been determined by CD derivatives, namely MBCD and HPBCD sorption into the BCD imprinted polymer matrix. Aqueous solutions of MBCD and HPBCD with 1 g/L initial concentration have been used (10 mL). The MBCD and HPBCD amount adsorbed by 1g of xerogel has been determined using the polarimetric method. The selectivity factors of the BCD imprinted films against MBCD ($\alpha_{BCD/MBCD}$) and HPBCD ($\alpha_{BCD/HPBCD}$) have been calculated by using eqs. (4) and (5), as the mass of BCD template adsorbed at equilibrium by ratio to the MBCD or HPBCD amount adsorbed at equilibrium by 1g of dry polymer:

$$\alpha_{BCD/HPBCD} = \frac{m_{BCD\ adsorbed,\ eq}/\text{g xerogel}}{m_{MBCD\ adsorbed,\ eq}/\text{g xerogel}} \quad (4)$$

$$\alpha_{BCD/HPBCD} = \frac{m_{BCD\ adsorbed,\ eq}/\text{g xerogel}}{m_{HPBCD\ adsorbed,\ eq}/\text{g xerogel}} \quad (5)$$

where $m_{BCD\ adsorbed,\ eq}$/g xerogel represents the amount of BCD adsorbed by the BCD imprinted films at equilibrium and $m_{HPBCD,\ MBCD\ adsorbed,\ eq}$/g xerogel, the amount of HPBCD, respectively, MBCD adsorbed by the BCD imprinted films at equilibrium.

Saturation Binding Curves and Scatchard Analysis. To obtain the saturation binding curve (isotherm) for the BCD imprinted films, the following steps have been performed: (a) the immersion of determined amounts of imprinted polymer in 10 mL of BCD aqueous solutions with a concentration range between 1 and 10 g/L for a week, for sorption equilibrium attaining; (b) after a week, the amount of adsorbed template has been determined by using the polarimetric method; and (c) the amount of sorbed template by 1 g of xerogel and expressed in (B) has been plotted against the initial concentrations of the solutions in which the imprinted samples have been immersed (c_{in}).

Scatchard plots are obtained by linearization of the binding isotherms according to the following equation:[12,13]

$$B = \frac{B_{max} \cdot c_{free\ template}}{K_a + c_{free\ template}} \quad (6)$$

where $c_{free\ template}$ represents the concentration of the unbound (free) BCD template in solution, after the sorption equilibrium has been reached (expressed in g/L), B_{max} represents the theoretical maximum amount of template that could be ab/adsorbed into the imprinted polymer and K_a represents the association (binding) constant of the template to the polymer (expressed in g template/L).

By plotting $B/c_{free\ template}$ (expressed in L/g polymer) as a function of B, B_{max}, and K_a can be determined by linear fitting. The slope of the dependency represents $-1/K_a$ and the ordinate at null adsorbed template represents B_{max}/K_a.[13]

Data Representation

The experiments representing the sorption kinetic, the selectivity factors, fluorescence spectroscopy emission of different PVA/BCD solutions and saturation binding curves have been performed in triplicate. The arithmetic mean of the three determinations has been presented in the article. The error bars associated with the plots represent the confidence interval,

Table I. Fluorescence Microscopy Analysis of the Starting PVA-BCD Mixtures

Imprinted sample	f_a (%)	f_s (%)	f_i (%)
PVA	40.3	50	9.7
PVA + 5% BCD	37.6	47	15.4
PVA + 10% BCD	34.7	39.2	26.1
PVA + 15% BCD	30.2	42.4	27.4
PVA + 25% BCD	23.5	42	34.5
PVA + 30% BCD	24.7	46.6	28.7

whose limits represent the minimum and maximum values of the measured parameters.

Morphology Studies

Fluorescence Microscopy. The formation of the active cavities in the imprinted polymeric matrix was verified by fluorescence microscopy. The fluorescence microscope images were attained by a Motic AE31 inverted trinocular microscope equipped with a digital camera, using a violet excitation filter ($\lambda_{max} = 455$ nm), specific to fluorescein excitation and a 40x objective with phase contrast. The imprinted PVA films were stained with a fluorescein fluorophore, by immersing them in a 0.1 mg/L fluorescein aqueous solution for 15 min. Nonimprinted films have been stained following the same procedure.

AFM Measurements. The surface morphology and porosity were asessed by using an atomic force microscope (AFM-NT-MDT model NTGRA PRIMA EC). The topography and phase images were taken in semi-contact mode with a "GOLDEN" silicon cantilever (NCSG10, force constant 0.15 N/m, tip radius 10 nm). Scanning was conducted on a certain area of 10 μm × 10 μm, randomly chosen, at a scanning rate of 1 Hz. Aditionally, AFM images analysis was carried using the instrument's software, in order to evaluate the active cavities size distribution.

Determination of Active Cavities Distribution. The active cavities distribution has been determined by using the "Image analysis" module from ImageJ software. The photographic image has been imported into the program and the scale has been set to the corresponding value. The image has then been converted to monochrome 8 bit, adjusting the "window of brightness" threshold until all of the cavities are selected. The software then lists the surface of each cavity, from which the diameter can be computed.

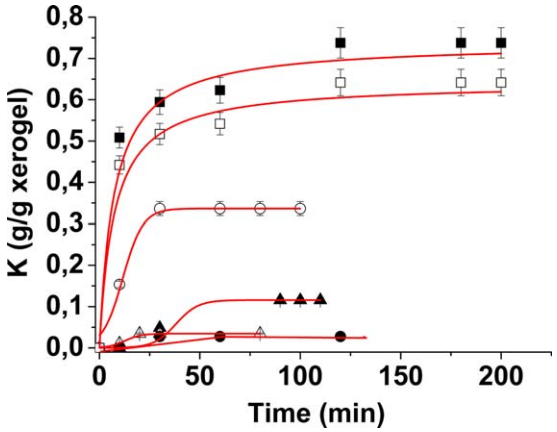

Figure 2. BCD sorption kinetic into the reference PVA (●) and imprinted PVA, with an initial BCD concentration of: Δ: 5%; ▲: 10%; ○: 15%; ■: 25%; and □: 30% reported to the polymer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS AND DISCUSSION

Fluorescence spectroscopy analysis of the aqueous PVA solutions, with different amounts of β-cyclodextrin in composition (5, 10, 15, 25, and 30% reported to the polymer) has revealed that BCD is able to determine the steric rearrangement of the –OH groups from the PVA macromolecule (Table I).

The blank PVA solution has the highest atactic fraction (random orientation of –OH groups), while the highest fraction of isotactic PVA is recorded for the PVA+25%BCD solution. At this concentration, BCD determines the steric rearrangement of the –OH groups of PVA in a form where the interactions between BCD and the polymer are at a maximum. Above this optimum BCD composition, the isotactic fraction decreases, probably due to the associations of the BCD molecules, thus promoting a weaker interaction with the polymer chains.

By analyzing the results from Table I it is expected that the highest sorption capacity to be recorded for the imprinted PVA film with an initial amount of 25% BCD reported to the polymer. The imprinted film preserves the original PVA conformational arrangement from the solution, as through solvent evaporation and crosslinking with glutaraldehyde, the relative movement of the polymer chains is impeded and the polymer-template complex arrangement is "frozen".

The sorption kinetic studies (Figure 2) are consistent with the results obtained from the fluorescence spectroscopy method.

Table II. Adsorption Kinetic Models Coefficients

	Pseudo-first-order kinetics			Pseudo-second-order kinetics		
Imprinted polymer	K_e (g/g polymer)	k_1 (min^{-1})	R^2	K_e (g /g polymer)	k_2 (min^{-1})	R^2
PVA + 5% BCD	0.081	0.437	0.811	0.036	0.507	0.988
PVA + 10% BCD	0.161	0.511	0.755	0.114	0.619	0.991
PVA + 15% BCD	0.253	0.664	0.766	0.333	0.771	0.981
PVA + 25% BCD	0.555	0.902	0.791	0.711	0.804	0.993
PVA + 30% BCD	0.48	0.455	0.899	0.617	0.689	0.996

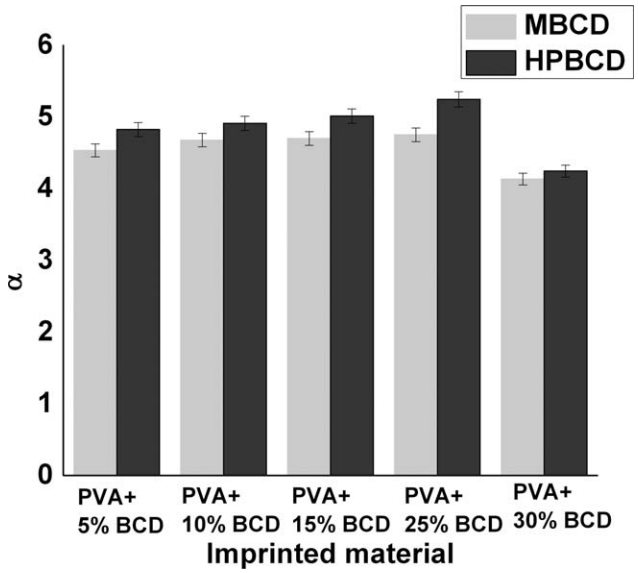

Figure 3. Selectivity factors of the imprinted PVA matrix for MBCD and HPBCD.

While all the imprinted samples adsorb a higher amount of BCD than the reference PVA, which confirms that the imprinting process is successful, the highest adsorbed amount could be recorded for the PVA+25% BCD sample, in which the template-polymer interactions are more favored.

At higher amounts of BCD (30%), the sorption capacity decreases, probably due to cyclodextrin molecules associations, which determine a nonspecific imprinting effect (larger and deformed cavities). Aiming to assess the kinetic mechanism that describes the solute uptake process, the pseudo first order and pseudo second order models were tested in order to understand the adsorption behavior of the imprinted polymers. The pseudo-first-order rate equation of Lagergren is one of the widely used for adsorption of solute form solution. The model has the following form:[14]

$$\log(K_e - K_t) = \log K_e - \frac{k_1}{2.303}t \quad (7)$$

and the pseudo-second-order equation can be written as:[15]

$$\frac{t}{K_t} = \frac{1}{k_2 K_e^2} + \frac{1}{K_e}t \quad (8)$$

where K_e and K_t represent the amount adsorbed (in g/g polymer) at equilibrium and time t, respectively. k_1 is the pseudo-first-order constant for adsorption process (min^{-1}), k_2 is the pseudo-second-order constant (min^{-1}). The straight-line plots of $\log(K_e - K_t)$ vs. t and t/K_t vs. t for pseudo-first- and second-order reactions, respectively, have been tested to evaluate the

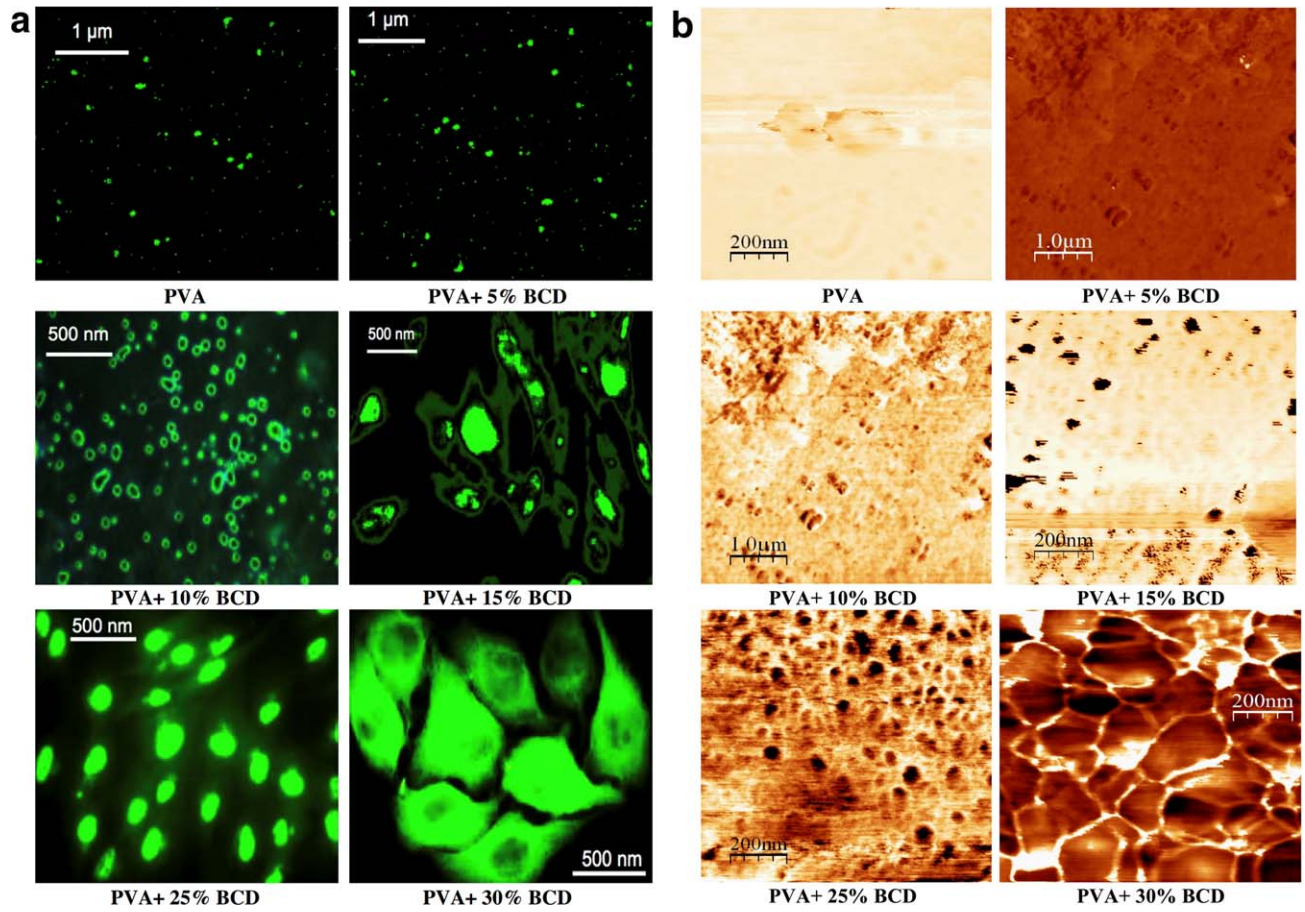

Figure 4. (a) Fluorescence microscopy images of imprinted PVA films and reference. (b) AFM phase images of imprinted PVA films and reference. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

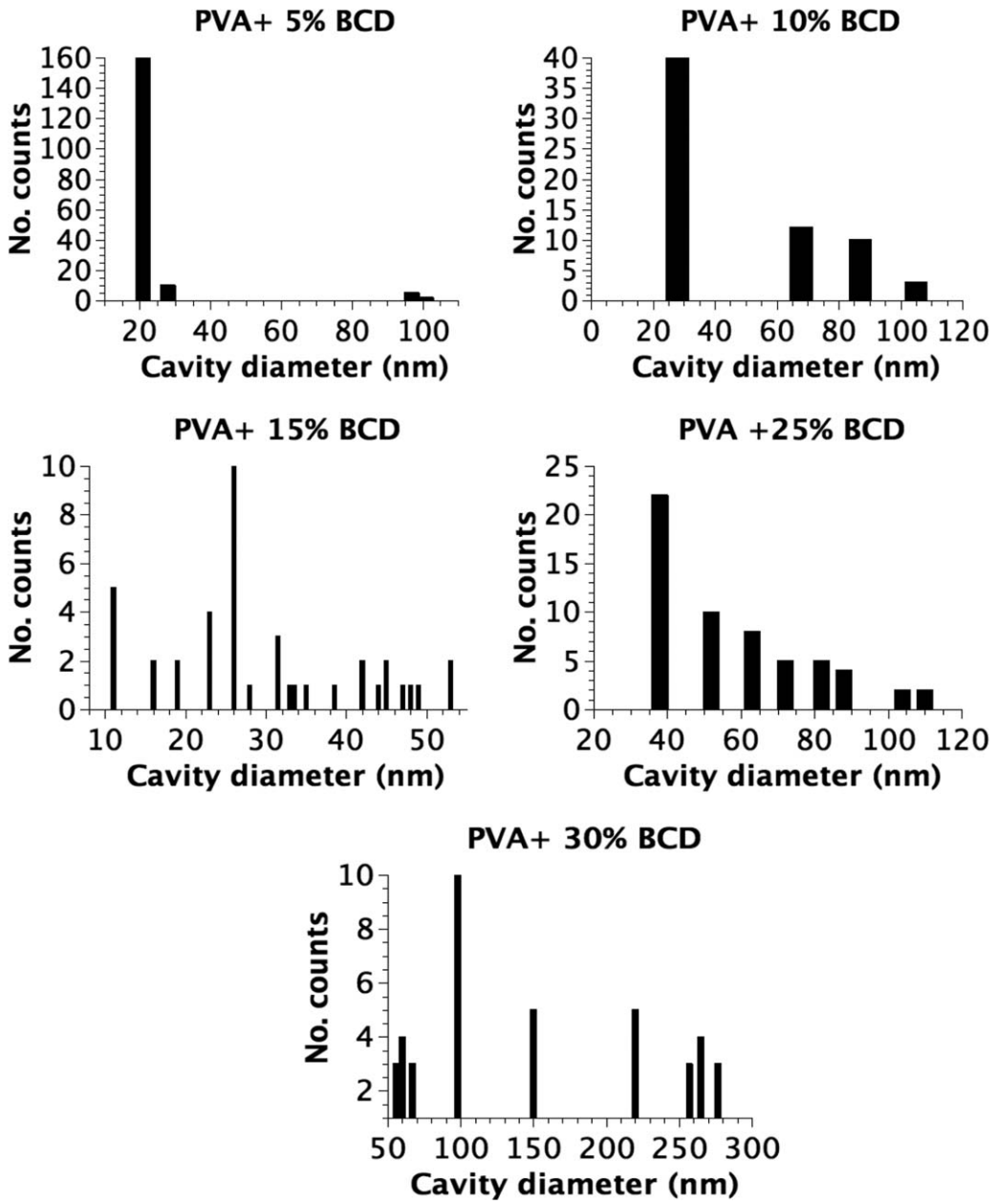

Figure 5. Active cavity diameter distribution.

rate parameters. k_1, k_2, K_e, and the correlation coefficient (R^2; Table II).

As recorded in Table II, the correlation coefficients for pseudo-second-order kinetic model were higher compared to pseudo-first-order kinetic model. Additionally, the calculated K_e values differed severely from the experimental K_e values for the pseudo-first-order kinetic model. It is therefore more probable that the adsorption kinetics follows the pseudo-second-order model.

Also, it could be noted that for the PVA+ 25% BCD sample, in which the active cavities size and distribution are the most favorable; the highest adsorption rate (k_2) of BCD from solution is recorded. For the polymer imprinted with a lower BCD amount, the active cavities may be present in an insufficient number and thus the sorption rates present also lower values. Also, for the PVA + 30% BCD sample the lower sorption rate could be the consequence of a nonspecific sorption into larger deformed cavities.

The selectivity factors of the imprinted PVA matrix against the cyclodextrin derivatives, namely MBCD and HPBCD also confirm that the imprinting of PVA with BCD is successful (Figure 3). All of the films preferentially adsorb BCD from solution, rather than its derivatives, with higher molar mass and volume, due to the presence of the active cavities in the structure of the material.

It can be observed that the selectivity factors of the BCD imprinted films against HPBCD are slightly higher than those corresponding to MBCD, due to the higher molar mass and bigger volume of the HPBCD derivative that is unable to fit the imprinted cavity corresponding to the diameter of BCD.

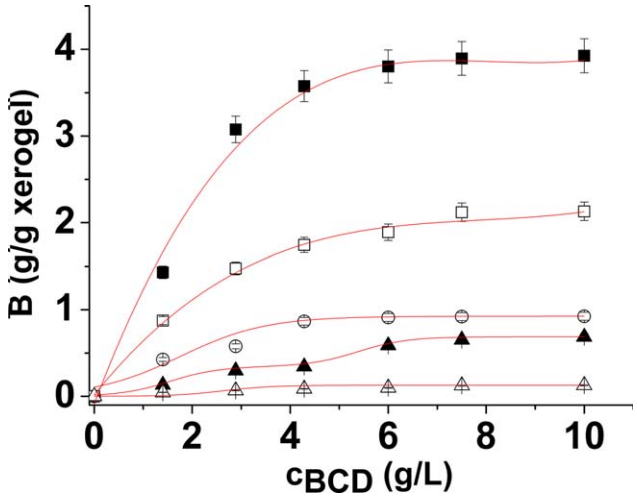

Figure 6. Saturation binding isotherms of BCD into the imprinted PVA matrix with an initial BCD concentration of: Δ: 5%; ▲: 10%; ○: 15%; ■: 25%; and □: 30% reported to the polymer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The fluorescence microscopy analysis [Figure 4(a)] confirms the presence of the active cavities in the structure of the imprinted PVA. It could be observed that for the blank PVA no specific accumulation of the staining fluorophore occurs, hence the lack of active cavities from its structure, which determines nonspecific BCD adsorption at the surface of the film.

For the imprinted PVA with 5–15% of BCD in the initial preparation step it could be observed that the number of fluorescent spots, resulted from the accumulation of the fluorophore in the active cavities is low, which can be correlated to an insufficient number of active cavities in the polymeric matrix that cause a low amount of cyclodextrin to be adsorbed. Also, it could be observed that in some cases (e.g., PVA + 10% BCD and PVA + 15% BCD) the active cavities are deformed, which may lead to insufficient sorption efficiency/selectivity. Also, the highest template concentration in the preparation step (30% BCD) leads to the formation of deformed active cavities, probably due to the lower amount of the polymer present in the material and its tendency to surround more than one agglomerate of template molecules or due to the inaccessibility of the crosslinker to the –OH groups of PVA.

The information obtained from fluorescence microscopy analysis is also confirmed by the AFM technique [Figure 4(b)]. It could be noted that the average diameter of the active cavities is larger in the case of fluorescence microscopy image analysis, than the corresponding value obtained from AFM, presumably due to the fact that the fluorophore molecules also have a tendency to associate, apparently generating fluorescent spots with a higher diameter than the corresponding active cavity, coupled with the more reduced resolution factor of the optical fluorescence microscopy technique, by comparing with AFM.[16,17] Furthermore, fluorescence imaging has been attained by immersing the imprinted films in the aqueous solution of the fluorophore, so they are in a swollen state. The films analyzed by AFM have been freeze-dried prior to the analysis. As it can be seen, by comparing Figure 4(a) with Figure 4(b), the cavities in the case of the swollen films (obtained by fluorescence microscopy) generally retain the pattern corresponding to their dry state (related to the AFM images). The cavities in the swollen films could be considered more representative, because the sorption process takes place in aqueous solutions, where swelling occurs. Usually the swelling phenomenon is unavoidable even in the case of very high crosslinking degrees. Nevertheless, such crosslinking degrees are generally to be avoided in the case of imprinted polymers, due to the slow solute uptake-release kinetic and because of the embrittlement of the imprinted material.[1]

Since the diameter of an individual BCD molecule is 4.5 nm,[18] it could be concluded that the imprinting of PVA is done not with individual cyclodextrin molecules, but with larger aggregates.

Generally, it could be observed from the AFM images that for all the imprinted samples, two main types of active centers occur: one type with average diameters between 10 and 50 nm, corresponding to the imprinting of the polymer with small BCD molecular clusters (3–10 molecules); and the other type, much larger, with diameters between 60 and 120 nm, corresponding to the imprinting with bigger molecular clusters (13–44 molecules).

The active cavities generated by the smaller sized molecular clusters seem to be more uniform in the case of the imprinted PVA with 25% BCD in the initial preparation step (average diameter of

Table III. Tested Binding Isotherms Models and Associated Parameters

	Isotherm type					
	Freundlich	Langmuir	Langmuir–Freundlich			
	$B=a\cdot c_{BCD}^{n}$	$B=\frac{a\cdot c_{BCD}}{1+a\cdot c_{BCD}}$	$B=\frac{a\cdot N_T\cdot c_{BCD}^{n}}{1+a\cdot c_{BCD}^{n}}$			
Samples	R^2	R^2	a	N_T	n	R^2
PVA + 5%BCD	0.980	0.982	0.24	153.3	0.23	0.998
PVA + 10%BCD	0.946	0.955	0.749	158.4	0.34	0.989
PVA + 15%BCD	0.901	0.921	0.876	160.23	0.54	0.988
PVA + 25%BCD	0.870	0.841	0.983	180.45	0.58	0.990
PVA + 30%BCD	0.847	0.829	0.998	170.22	0.80	0.985

Isotherm model parameters: a: coverage degree; N_T: number of binding sites; n: heterogeneity index; c_{BCD}: initial concentration of BCD in the adsorption solution.[13,20]

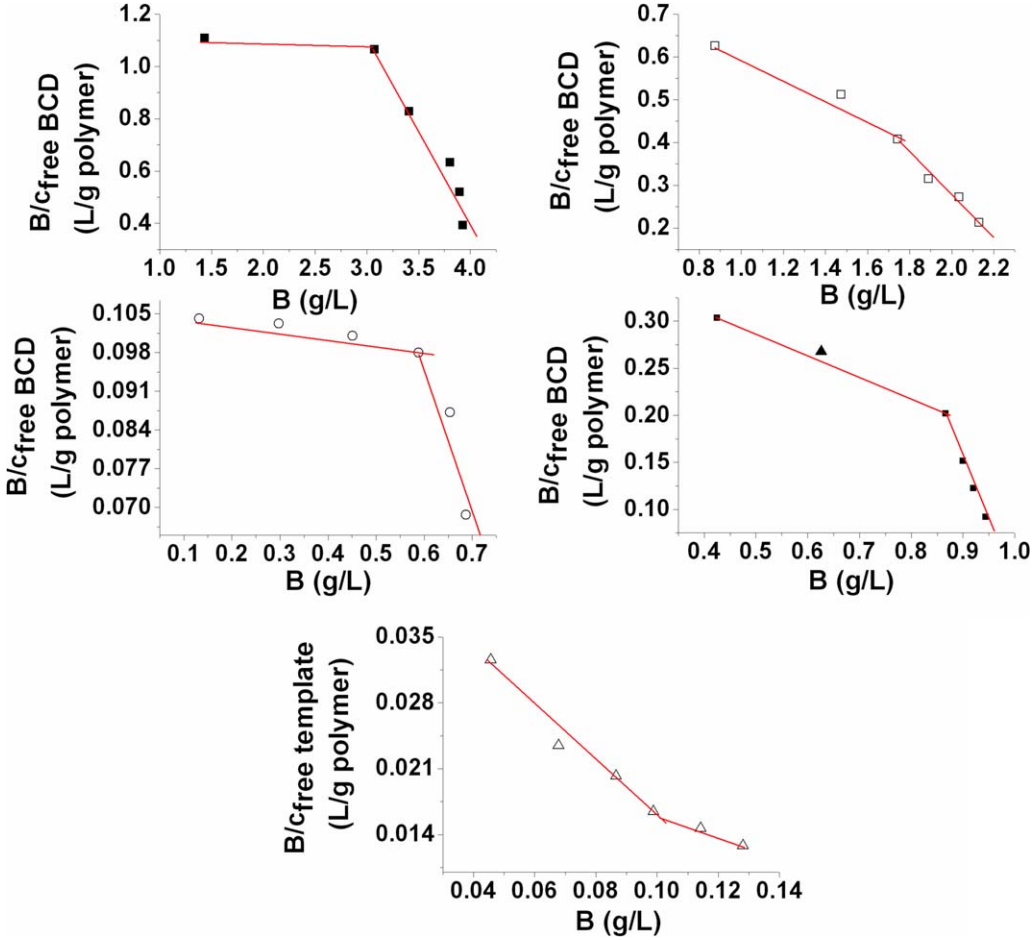

Figure 7. Scatchard plots corresponding to the sorption of BCD into the imprinted PVA matrix with an initial BCD concentration of: Δ: 5%; ▲: 10%; ○: 15%; ■: 25%; and □: 30% reported to the polymer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

37.6 nm, as it can be seen from Figure 5), percent which corresponds to the highest amount of the adsorbed β-cyclodextrins.

At 5–15% BCD concentration, the majoritarian active cavities have smaller dimensions (20–30 nm), due to the less pronounced tendency of the template to form molecular aggregates at small concentrations. Even so, the imprinting effect at this BCD concentration is unsatisfactory because of the low amount of BCD, which is unable to determine a favorable steric arrangement of the PVA macromolecules, as seen from Table I.

Table IV. Scatchard Analysis Parameters

Imprinted sample	Linear dependency	B_{max} (g/g polymer)	K_a (L/g polymer)	R
PVA + 5% BCD	1	0.142	1.856	0.991
	2	0.064	0.802	0.982
PVA + 10% BCD	1	0.708	2.150	0.992
	2	0.179	0.845	0.989
PVA + 15% BCD	1	1.013	2.638	0.989
	2	0.462	0.879	0.987
PVA + 25% BCD	1	4.085	0.942	0.999
	2	1.416	2.825	0.990
PVA + 30% BCD	1	2.747	2.804	0.988
	2	0.694	0.934	0.989

At the highest BCD concentration (30%), the majoritarian active cavities have an average diameter of 97 and 220 nm, which could be explained by taking into account the more pronounced agglomeration tendency of the cyclodextrin molecules.

The highest BCD sorption yield for the PVA+ 25% BCD imprinted film could be explained also by taking into account that the cyclodextrin molecules from the aqueous 1 g/L solution have a similar association degree with the BCD that imprinted the polymer, at that particular corresponding concentration. The high selectivity factors of the imprinted PVA for BCD, against MBCD and HPBCD could be explained also by the fact that those CD derivatives have different association modes in aqueous solution, different from those corresponding to BCD that imprinted the film, which owes for their adsorption in a lesser amount.

In order to characterize the mechanism of template binding into the active cavities from the polymer matrix, the saturation binding curves (Figure 6) have been fitted against several adsorption isotherms models (Table III). By analyzing the

correlation coefficient (R^2) of the fitted models it can be revealed that neither the Freundlich nor the Langmuir isotherm models are suitable for the description of the entire isotherm curve.[19]

At lower initial concentration of template from the adsorption solution, prior to saturation, the Freundlich model could be applied, while at higher template concentration values (adsorption equilibrium) the data is well modeled by the Langmuir isotherm. In order to characterize the whole saturation-binding curve, the Langmuir–Freundlich isotherm model could be used. This model has been used in fitting the experimental data points from Figure 6.

This model has already been successfully applied in modeling the sorption behavior of several imprinted polymers, obtained by noncovalent or alternative molecular imprinting,[19] and it also seems to be well suited for the adsorption behavior characterization of the PVA imprinted with BCD, as remarked from the high correlation coefficients (0.985–0.998).

From the values of the parameters associated with this model, it could be concluded that the highest number of binding sites (active cavities) is recorded for the PVA + 25% BCD imprinted polymer, which could explain its optimum sorption behavior, when comparing to other imprinted polymers from the series. A higher initial amount of β-cyclodextrin determines a higher heterogeneity index for the binding sites, due to the tendency of cyclodextrin molecules to associate, and also a higher coverage degree of the adsorption surface with cyclodextrin molecules.

In order to characterize the affinity of the cyclodextrin molecules to the binding sites in the polymer matrix, Scatchard plots have been obtained, as shown in Figure 7. As it can be seen from Figure 7, there seem to be different binding mechanisms of BCD sorption to the polymeric matrix. The value of the association constant and of the maximum theoretical amount of template adsorbed obtained by linear fitting of the two portions of the Scatchard plots are presented in Table IV.

As it can be seen from Table IV, for lower initial concentrations of the BCD solutions used for adsorption (Slope 1) the association constant of the template molecule with the PVA macromolecule is higher, probably due to the lower associations of the template molecules with themselves, which lead to a higher interaction with the polymeric matrix. For higher BCD concentrations (the second slope) it could be observed that the association with the polymer is lower, probably due to a higher degree of association of the template molecules between themselves in solution. Also, the highest association constant of the BCD template is recorded for the PVA + 25% BCD imprinted polymer, which corresponds to the maximum yield of sorption.

The information obtained from the Scatchard analysis is also sustained by the AFM microscopy technique (the presence of two types of active cavities).

CONCLUSIONS

New types of PVA hydrogels, with tailored morphology, have been successfully obtained by alternative imprinting technique, using β-cyclodextrin as template, aiming to increase the effectiveness and selectivity of its sorption. BCD molecular clusters generated the active cavities. 25% BCD in the initial preparation step ensure the highest effectiveness and selectivity of PVA hydrogel for BCD adsorption. Its sorption in the new designed PVA hydrogels is a multilayered complex process that could be described by Langmuir–Freundlich model, by a pseudo-second-order kinetic and by Scatchard analysis.

A simple fluorescence spectroscopy method has been proposed to predict the imprinting effectiveness of PVA with BCD, by their solution analysis and fluorescence microscopy has been proposed to characterize the imprinted materials. These methods could be useful in the field of separations and loading of poly(vinyl alcohol) hydrogels with pharmaceutically active compounds and they could be extended to a large variety of other imprinted materials.

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