Imprinted Poly (vinyl alcohol) as a Promising Tool for Xanthine Derivatives Separation

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Received 24 September 2010; accepted 6 February 2011
DOI 10.1002/app.34305
Published online 13 June 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: In this work, we report the obtaining of an ecological imprinted polymer for the selective sorption of xanthine derivatives (caffeine and theobromine) from aqueous solutions, through an innovative approach. Poly (vinyl alcohol) [PVA]-xanthine films have been obtained by aqueous solution casting. PVA crosslinking has been performed by using glutaraldehyde in gaseous phase. Template elimination has been achieved by "washing" of crosslinked films with water. To check the polymer imprentation and to determine the imprinted material effectiveness in xanthines separation, sorption studies have been performed. Freundlich, Langmuir, BET, Extended Langmuir models, and Scatchard analysis have been applied on the experimental data to better characterize the sorption mechanism. Fluorescence microscopy has been proposed as suitable method to check the imprentation level of the polymer. The information obtained by this method is in good agreement with the values for the efficiency of the imprinted polymer and could serve as an easier tool for prediction of the imprinted polymers performance. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 2081–2089, 2011

Key words: molecular imprinting; fluorescence microscopy; poly (vinyl alcohol); sorption; xanthine derivatives; separation

INTRODUCTION

Molecular imprinting is a novel method for designing materials with molecular memory, which consists of cavities that bear the shape and dimensions of a template molecule. The cavities are highly specific towards the molecule that imprints the polymer, making molecularly imprinted materials suitable for use in thermal sensitive isomer separations, catalysis, biosensor assays, or in controlled drug delivery.[1,2] These materials could be considered advanced and intelligent materials, and constitute an alternative to classical methods of separation, less specific and energy consuming.

Traditional molecular imprinting is a three step process. The first step involves the formation of a complex (hydrogen bonded or covalent) between the template molecule (the molecule that "imprints" the polymer) and a functional monomer. In the second step, the complex is polymerized by addition of a suitable crosslinking agent, and in the third step, the template is removed from the polymeric matrix, leaving specific binding sites (active cavities) that remember the dimensions and the structure of the template molecule.[1–4]

In general, the conventional imprinting technique is associated with some drawbacks, such as the use of toxic monomers and organic solvents, low binding efficiency, slow binding process (as the imprinted sites are embedded in bulk polymer matrices, the access of the template molecules to the imprinted binding sites being often suppressed) and difficult fabrication (time consuming and energy intensive).[3]

In our work we proposed a novel alternative imprinting approach, which employs the formation of imprinted materials starting directly from polymer solution, thus eliminating the polymerization step.

By using this approach we have obtained imprinted poly (vinyl alcohol) [PVA] towards the selective absorption of xanthine derivatives (caffeine and theobromine) from aqueous solutions.

Xanthine derivatives are widely used in pharmacy for the preparation of bronchodilatant/antidepressant receipts. Their separation from natural sources is a tedious task to be achieved by the alternative classical methods. The imprinted polymers designed for xanthine derivatives up to this date are obtained by classical approaches of imprinting, which use toxic monomers (methylmetacrylates; substituted vinylbenzenes or vinylpiridynes), with low efficiencies, and selectivity.[1] The separation of xanthine derivatives with imprinted PVA by our proposed method avoids their thermal degradation and uses ecologic materials.[5,6]

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Contract grant sponsor: The National University Council from Romania (CNCSIS); contract grant number: IDEI 839/2008.

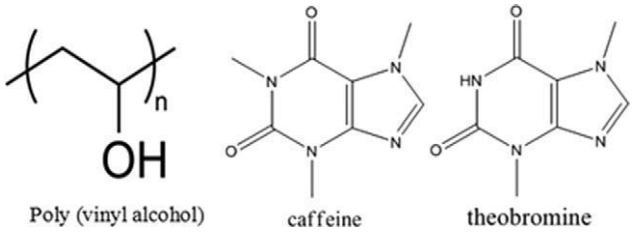

Figure 1 Structural formulas of PVA and xanthinic derivatives.

PVA is an ecologic and easy to handle polymer with many applications in the field of organic compound separations (diffusion, pervaporation membranes).[5]

To ensure the active cavities formation in the PVA and their swelling stability, the polymer has been chemically crosslinked using gaseous glutaraldehyde [GA], with sulfuric acid as catalyst. This crosslinking technique minimizes the amount of unreacted crosslinker from the polymeric matrix.

The imprinting effect has been proved by sorption studies of the template molecule in the polymeric matrix and the selectivity has been verified by sorption studies of structurally related compounds with the template molecule in the polymeric matrix. Also, to determine the association constant of the template with the polymer, the saturation binding curves (isotherms) for caffeine and theobromine have been obtained, and Scatchard plots have been performed. Several adsorption model isotherms (Freundlich, Langmuir, BET and extended-Langmuir) have been fitted against the experimental data to characterize the adsorption mechanism.

The imprinting has been demonstrated also by fluorescence microscopy. This method of analysis is time efficient, less expensive and thus more accessible than other variants of microscopy such as atomic force microscopy [AFM] and scanning electron microscopy [SEM][7–9] and provides complementary information.

Several fluorescence images of imprinted PVA films with xanthine derivatives (caffeine and theobromine) as template molecules have been attained using fluorescein as staining fluorophore. The influence of the template molecule's structure and concentration on the active cavities uniformity and shape has been studied. The results presented in this study are innovative and original, as fluorescence imaging of imprinted polymers has been not reported in the literature, as our knowledge.[1,7]

EXPERIMENTAL

Materials

PVA 120-98 (1200 polymerization degree and 98% hydrolysis degree) was purchased from RomReRo S.A. Rasnov, Romania. Glutaraldehyde (GA) of 45 wt% concentration was purchased from "Reactivul" S.A. Bucharest, Romania. Caffeine [CAF], theobromine [TBR] and fluorescein were purchased from ς-Aldrich, and were of reagent grade.

PVA, CAF, and TBR structural formulas are illustrated in Figure 1.

Methods

The obtaining of the xanthine derivatives 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.

PVA solution preparation

The polymer solution has been prepared by adding PVA powder into a determined volume of Milli-Q 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%.

PVA/xanthine films preparation

The films have been prepared from 10 mL of 11% PVA aqueous solution which contains 10, 20, 30, 40, and 50 $(\%)_{wt}$ xanthine derivative, calculated by ratio to the dry polymer in the solution, by PVA/xanthines mixture casting in plastic Petri dishes (3 cm diameter) and solvent evaporation at room temperature for 24 h. The homogenous and transparent films obtained have been treated at the surface with 1 mL of 1*N* sulfuric acid (crosslinking catalyst)

PVA/xanthine films crosslinking

The obtained PVA films with xanthine derivatives in composition have been introduced into the crosslinking installation (Fig. 2) and placed over a recipient that contained 20 mL of $45(\%)_{wt}$ GA solution.

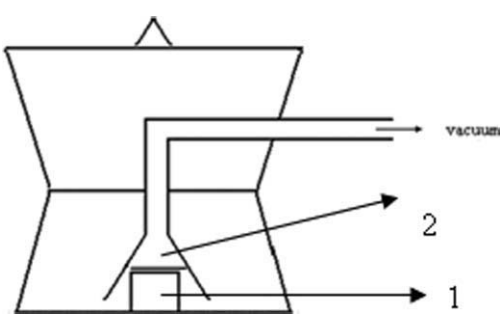

Figure 2 Installation for crosslinking in gaseous phase: 1, recipient with GA solution; 2, polymeric film.

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 xanthines in composition, have been crosslinked following the same procedure.

Template molecule removal

To complete the imprinting process, the template molecules have been removed from the polymeric matrix by immersing the crosslinked films in distilled water for 7 days at room temperature. The distilled water has been replaced daily with a fresh one, to maintain a convenient concentration gradient between the xanthines from the film and the released xanthines amount. The complete removal of xanthines from the polymer has been determined by UV spectrophotometry, based on their absorption maximum at 256 nm for CAF and 257 nm for TBR, using a Carl-Zeiss UV-VIS spectrophotometer with standard quartz cuvets of 1 cm optical path.

Imprinting testing

The imprinting testing has been performed by comparing the amounts of xanthine derivatives absorbed from aqueous solutions by the imprinted and non-imprinted films respectively.

The sorption of CAF or TBR in the polymeric matrix has been determined by the difference from the initial amount of CAF or TBR present in the solution and the amount of CAF/TBR from the solution after absorption at different (determined) time intervals, using the spectrophotometric method, based on their absorption maximum at 256 nm for CAF and 257 nm for TBR, using a Carl-Zeiss UV-VIS spectrophotometer with standard quartz cuvets of 1 cm optical path. The amount of xanthinic derivative absorbed by 1 g of dry polymer (xerogel) has been calculated.

The dry polymer amount from each sample has been calculated taking into account the solid content (CS) of the films. The CAF/TBR aqueous solution used for sorption studies was of 1.5 10^{-3} $(\%)_{vol}$ concentration 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 CAF/TBR from the solution and the CAF/TBR from the imprinted polymeric matrix.

Selectivity testing

The imprinted films used for selectivity tests were those with the optimum template concentration in the polymer, for which the maximum absorption yield has been attained.

The selectivity of the CAF imprinted film was determined by sorption of TBR from a 1.5 10^{-3}(%)$_{vol}$ aqueous solution (10 mL) using the analysis conditions mentioned above. Similar, the selectivity of TBR imprinted films has been determined by CAF sorption from a 1.5 10^{-3} $(\%)_{vol}$ aqueous solution following the same procedure.

The selectivity factor of the imprinted film (K) can be calculated as the mass of xanthine derivatives absorbed at equilibrium in the imprinted film (g) by ratio to the amount of xanthine derivative that imprinted the polymer absorbed at equilibrium (g), according to eq.1:

$$K = \left(\frac{m_{\text{xanthine der abs, eq}}(\text{g})}{m_{\text{xanthine template abs, eq}}(\text{g})} \right) \cdot 100 \qquad (1)$$

Saturation binding curves obtaining and Scatchard analysis

To obtain the saturation binding curve (isotherm) for the CAF and TBR imprinted films, the following steps have been performed: (a) the immersion of determined amounts of imprinted polymer in 10 mL of caffeine and theobromine aqueous solutions with a concentration range between 0.5 10^{-3} $(\%)_{wt}$ and 2.5 10^{-3} $(\%)_{wt}$ for a week, for sorption equilibrium attaining; (b) after a week, the amount of absorbed template has been determined by using the UV spectrophotometric method; (c) the amount of sorbed template by 1 g of xerogel and expressed in μmol (B) has been plotted against the initial concentrations of the solutions in which the imprinted samples have been immersed (c_{in}). The samples studied correspond to that ones which presented maximum absorption efficiency at 1.5 10^{-3} $(\%)_{wt}$ template concentration (with 40% CAF and 20% TBR by ratio to the dry polymer).

Scatchard plots are obtained by liniarization of the binding isotherms according to eq. (2)[10,11]:

$$B = \frac{B_{\max} \cdot c_{\text{free template}}}{K_a + c_{\text{free template}}} \qquad (2)$$

Where $c_{\text{free template}}$ represents the concentration of the unbound (free) template in solution, after the sorption equilibrium has reached (expressed for convenience as μmol/L), $B_{\max}$ represents the theoretical maximum amount of template that could be ad/absorbed into the matrix and K_a represents the association (binding constant) of the template to the polymer.

By plotting $B/c_{\text{free template}}$ 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 $B_{\max}/K_a$).

Fluorescence microscopy imaging

The formation of the active cavities in the imprinted polymeric matrix was verified by fluorescence

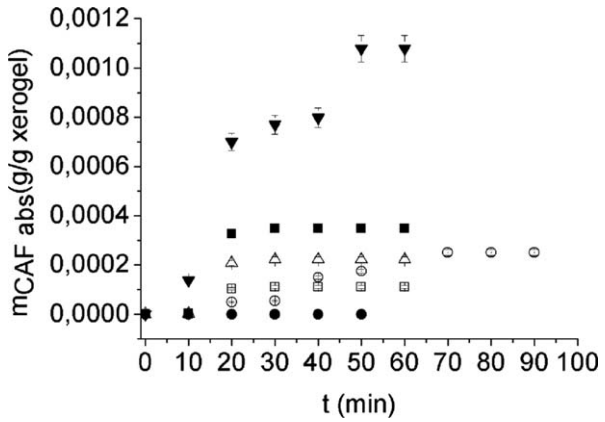

Figure 3 CAF sorption kinetic into the imprinted polymer for: (■)10%; (○)20%; (Δ)30%; (▼) 40%; (□) 50% CAF concentrations by ratio to PVA; (●): blank PVA.

microscopy. The fluorescence microscope images were attained by a Motic AE31 inverted trinocular microscope equipped with a digital camera, using a blue excitation filter ($\lambda_{max} = 455$ nm), and a ×40 objective with phase contrast. The imprinted PVA films were stained with a fluorescein fluorophore, by immersing them in a 0.01 g/L fluorescein aqueous solution for 15 min. Blank PVA films have been stained following the same procedure.

RESULTS AND DISCUSSIONS

By plotting the amount of xanthine derivative absorbed into the polymeric matrix corresponding to 1 g of xerogel versus time the sorption kinetic into the imprinted matrix can be obtained, as indicated in Figure 3 for CAF and Figure 4 for TBR.

As it can be seen from Figures 3 and 4 the imprinting of the PVA with caffeine and theobromine was successful. All the imprinted films absorb the template molecule that imprinted them, while blank PVA samples do not absorb any xanthine derivative.

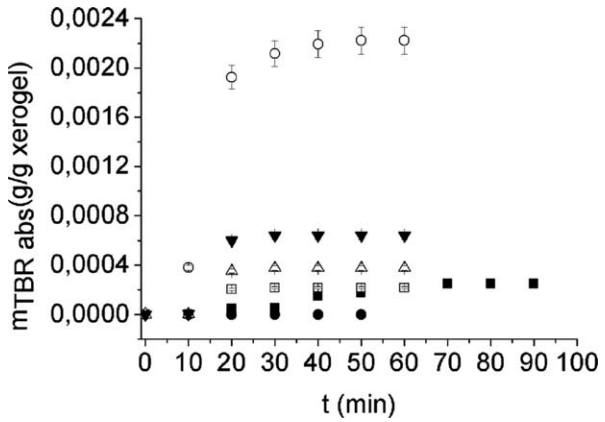

Figure 4 TBR sorption kinetic into the imprinted polymer for (■)10%; (○)20%; (Δ)30%; (▼) 40%; (□) 50% TBR concentrations by ratio to PVA; (●): blank PVA.

Maximum sorption efficiency could be observed only for certain template concentrations, 40% CAF in PVA and 20% TBR in PVA respectively. This behavior could be related to the concentration and dimensions of the active cavities from the polymeric matrix

Concerning our proposed imprinting protocol, it could be possible for the polymer to be imprinted not at molecular level (cavities with the exact dimensions of a single xanthine molecule) but with xanthine aggregates, which are formed in the polymer matrix as the water evaporates from the casting PVA/xanthine mixtures in the preparation step. The imprinting of the polymer "works" because xanthines tend to associate in aqueous solutions, their aggregates being stabilized by van-der-Waals, electrostatic and hydrophobic interactions and also due to formation of intermolecular hydrogen bonds.[12] Further studies about the mechanism of PVA imprinting are in development, and they will be published in a following article.

The results concerning the selectivity of the caffeine and theobromine imprinted films that presented the highest sorption efficiency are presented in Table I.

It is well known that a good selectivity for the imprinted polymers is achieved by a low value of the selectivity factor for compounds structurally related with the template molecule. The imprinted films absorb preferentially the type of molecule that imprinted them.

The binding isotherms corresponding to the sorption of CAF and TBR, respectively, in the polymeric matrix are presented in Figure 5.

The corresponding Scatchard plots for CAF and TBR are presented in Figures 6 and 7.

If the binding sites in the polymer are identical and independent, usually a straight line is obtained. If there is interaction between the binding sites or different associations between sorbed molecules at different concentrations or there are several classes of independent sites in the imprinted polymer, the plot is not linear.

TABLE I
Selectivity Factor of the Xanthine Derivatives Imprinted Films

Template molecule	Sorbed xanthinic derivative	$m_{\text{template abs,eq}}$ (mg/g xerogel)	K (%)
CAF	CAF	1.084	–
	TBR	0.409	37.73
TBR	TBR	2.222	–
	CAF	0.662	29.79

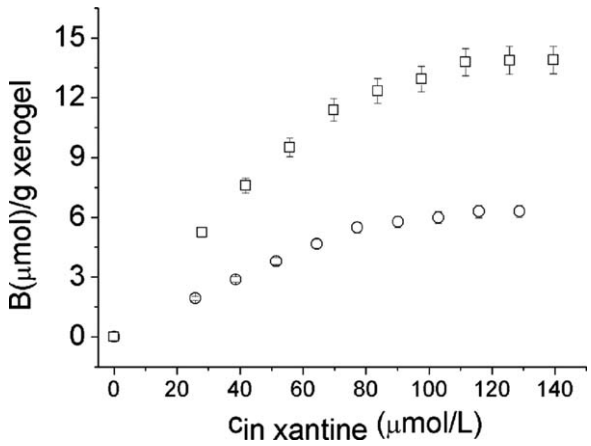

Figure 5 Binding isotherms of xanthine derivatives: (○): CAF; (□): TBR into the PVA imprinted matrix.

As it can be seen from Figures 6 and 7, there seem to exist different binding mechanisms of CAF and TBR to the polymeric matrix.

The value of the association constant and of the maximum theoretical amount of template absorbed obtained by linear fitting of the two portions of the Scatchard plots are presented in Table II:

As it can be seen from Table II, for lower concentrations of the CAF solutions of immersion (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 CAF 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.

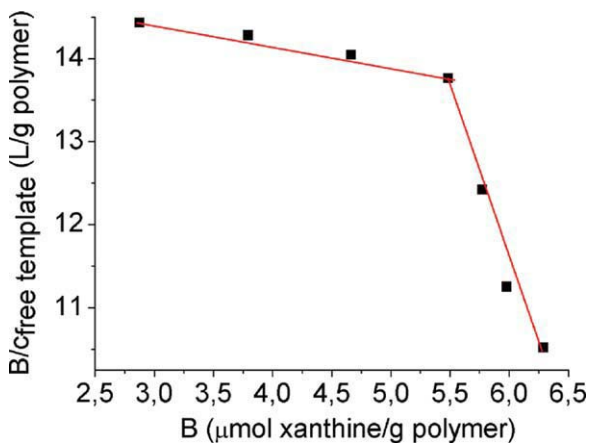

Figure 6 Scatchard plot corresponding to the CAF sorption into the imprinted matrix. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

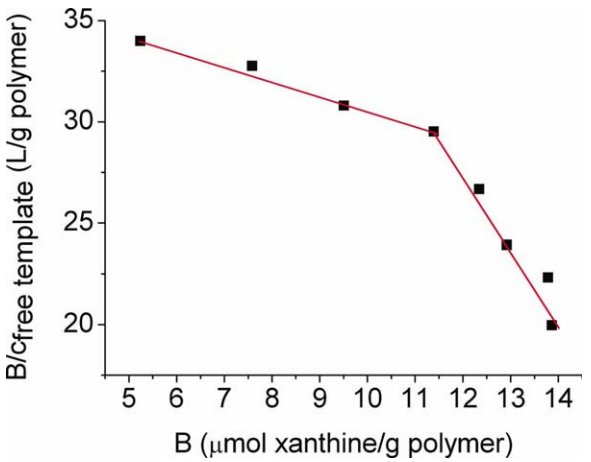

Figure 7 Scatchard plot corresponding to the TBR sorption into the imprinted matrix. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The values corresponding to the association constant for TBR is lower than the one for CAF, for the first part of the Scatchard plot, due to a higher degree of aggregation of the TBR molecules between themselves, in comparison with CAF. While for low concentrations of the aqueous solutions used for binding studies, the binding probability of the template with PVA is high, at higher concentrations the CAF/TBR molecules are more associated, which leads to a lower probability of binding to the polymer.

Also, related to the binding isotherms plotted in Figure 5, several adsorption models have been tested to determine the type of adsorption in the polymer. The equations expressed in Table III have been fitted against the obtained experimental data and the correlation coefficients (R) have been determined for each case.

As it can be seen from Table III, neither of the studied adsorption models fits the binding isotherms in a satisfactory matter, as the Langmuir and Freundlich models usually characterize monolayer adsorption.[10] This can offer some additional information regarding the imprinting of the polymer and the sorption mechanism of the template molecule

TABLE II
Scatchard Analysis Parameters

Template that imprined the polymer	Linear dependency	B_{max} (μmol/g polymer)	K_a (L/μmol)	R
CAF	1	59.0159	0.7525	0.9882
	2	6.2927	0.0470	0.9855
TBR	1	50.7533	0.2398	0.9921
	2	14.6111	0.0512	0.9779

TABLE III
Adsorption Isotherm Models Tested

Adsorption isotherm model	Mathematical equation of the model[10]	Imprinting template	R
Freundlich	$B = K \cdot c^P$ K, P = constants	CAF	0.744
		TBR	0.736
Langmuir	$\frac{1}{B} = \frac{1}{K \cdot b \cdot c} + \frac{1}{b}$ B, K = constants	CAF	0.789
		TBR	0.771
BET	$\frac{1}{B(\varphi - 1)} = \frac{b-1}{nb} \cdot \frac{1}{\varphi} + \frac{1}{nb}$ $\varphi = \frac{c}{c_{eq,ads}}$ n, b = constants	CAF	0.829
		TBR	0.806

that does not occur at single-molecule level, but at molecular-aggregate level.

The BET model is an extension of the Langmuir model for multilayer adsorption, providing that there is no interaction between the individual adsorbed multilayers. It can be observed that this model does not fit well with the experimental data, providing that there are interactions between the adsorbed molecules.

Supplementary, the recently proposed model named Extended Langmuir (EL) isotherm (eq. 3),[13] suitable in describing multilayer adsorption has been fitted against the experimental data (dependences from Fig. 5 until equilibrium reaching).

$$B = B_{\max} \cdot \frac{b \cdot c}{1 + b \cdot c} \cdot e^{n \cdot c / c_{\text{eq}}} \tag{3}$$

Where $B_{\max}$ represents the maximum sorbed amount of adsorbate, b represents a binding equilibrium constant; c is the template solution concentration, c_{eq} represents the template solution concentration at saturation (equilibrium), and n the multilayer parameter. Their corresponding values for CAF and TBR sorption are presented in Table IV.

As it can be observed from Tables IV and II, the parameters of the EL model are in good agreement with those obtained from the Scatchard plot.

Compared with the BET model (multilayer adsorption with no interlayer interaction), the EL model generated higher correlation coefficients, which could indicate EL as a possible adsorption model for the description of CAF and TBR sorption into the imprinted PVA.

The fluorescence images of the caffeine imprinted PVA are presented in Figures 8 and 9 are displayed the images for theobromine imprinted samples.

The fluorescence images from Figures 8 and 9, can confirm that fluorescence microscopy is a method which can characterize the imprinting effect in the poly (vinyl alcohol) based materials. Figure 8 shows that for the blank PVA no specific accumulation of the staining fluorophore occurs, hence the lack of active cavities from its structure. This could be well correlated with the sorption test results which conclude the blank PVA incapacity to absorb xanthinic derivatives. For the imprinted PVA with 10 to 30% of CAF and 10% of TBR, respectively, 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 xanthines to be sorbed. Also, it could be observed that in some cases (e.g., Fig. 8) the active cavities are deformed, which may lead to insufficient sorption efficiency/selectivity.

The shape and dimensions of the active cavities seem to be more uniform in the case of the imprinted PVA with 40% CAF and 20% TBR in the initial preparation step, percents which correspond to the highest amount of the sorbed xanthines (Figs. 8 and 9) that characterizes the best imprinting effect. Also, the higher diameter of the cavities from the PVA imprinted with 20% TBR ($\sim$ 5 μm) by comparing to that imprinted with 40% CAF ($\sim$ 2.5 μm) shows that TBR has a higher capacity to agglomerate, which has also been demonstrated by Scatchard analysis This could be the cause of the higher absorption of TBR (0.22 g TBR/g xerogel) by comparing to CAF (0.11 g CAF/g xerogel) in the most effective imprinted PVA films obtained.

A higher template concentration in the preparation step 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

TABLE IV
Extended Langmuir Model Fitting Parameters

Imprinting template	$B_{\max}$ (μmol/g polymer)	B (L/μmol)	c_{eq} (μmol/L)	n	R
CAF	6.0656	0.0147	110.7	0.7714	0.999
TBR	14.0318	0.0191	110.7	0.5046	0.996

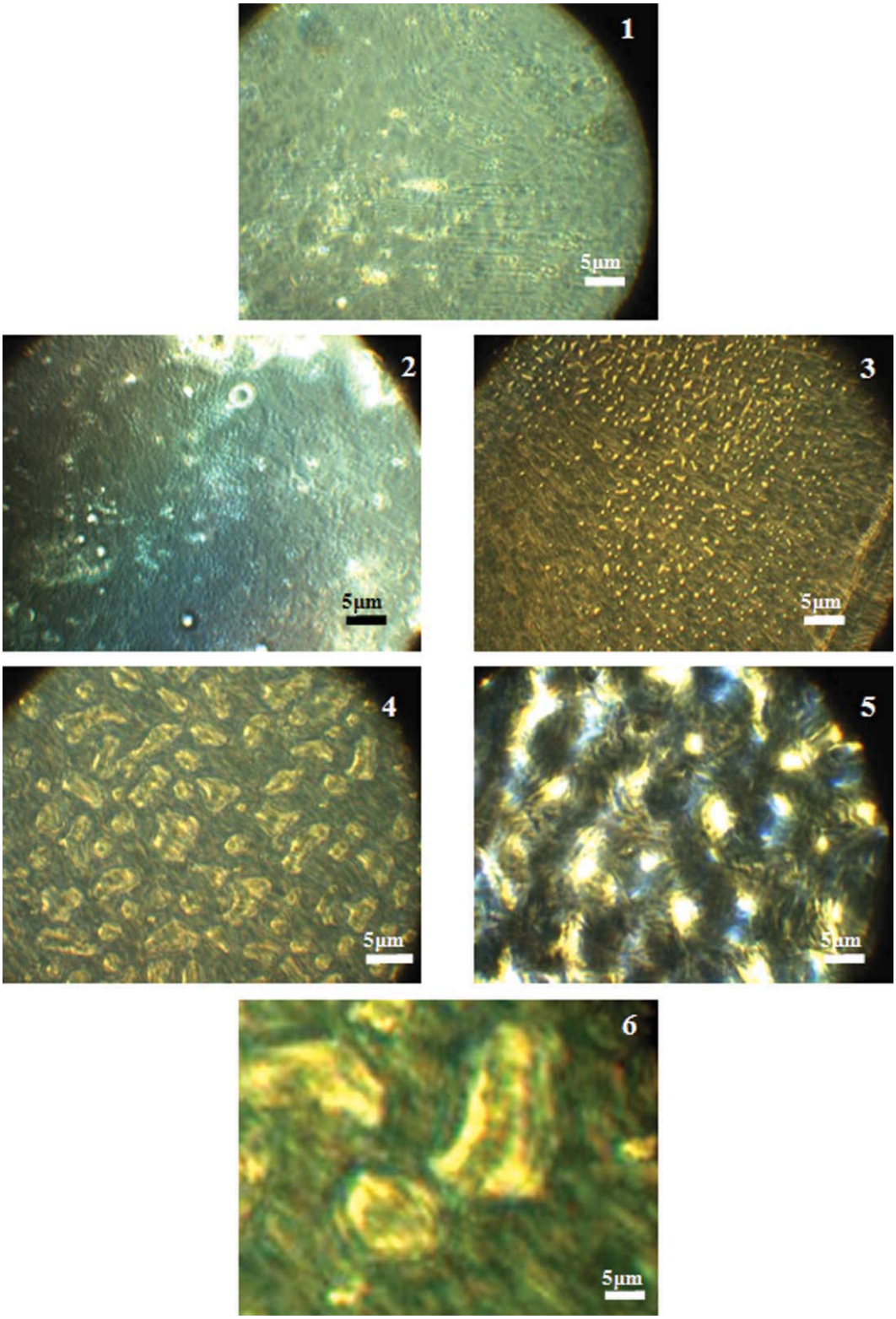

Figure 8 Fluorescence images of blank PVA (1) and caffeine imprinted PVA with: 10% CAF concentration (2); 20% CAF concentration (3); 30% CAF concentration (4); 40% CAF concentration (5); 50% CAF concentration (6). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

crosslinker to the hydroxyl groups of the polymer. The information obtained in this case is also in good correlation with the sorption tests.

Concerning the dimensions of the active cavities obtained from the fluorescence microscopy images, it is to be noted that they are much larger than the

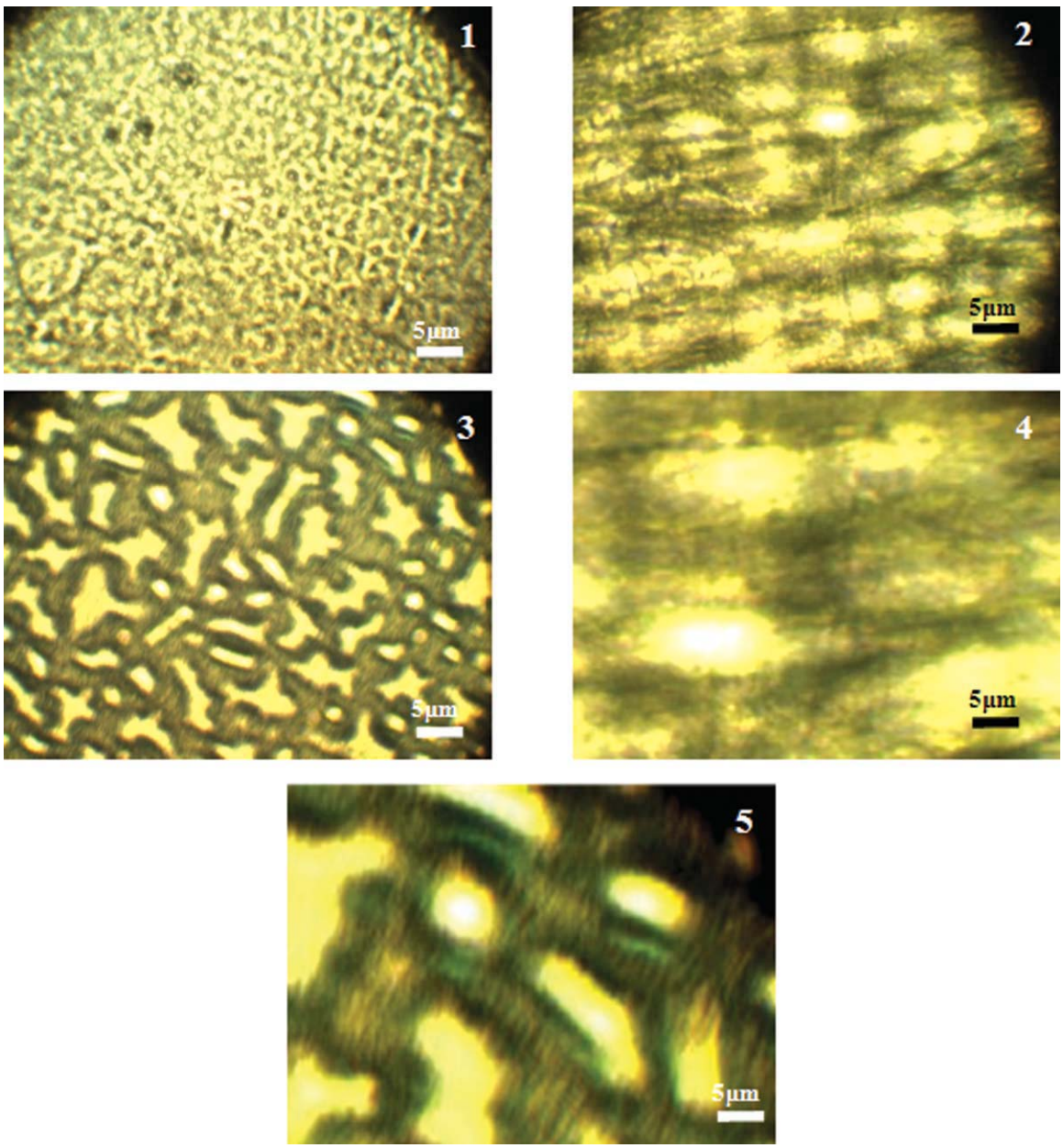

Figure 9 Fluorescence images of theobromine imprinted PVA with: 10% TBR concentration (1); 20% TBR concentration (2); 30% TBR concentration (3); 40% TBR concentration (4); 50% TBR concentration (5). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

caffeine or the theobromine molecules diameter (11.5 Å and 9.85 Å, respectively,[12]). The larger diameter of the determined active cavities could serve as a confirmation of the "template aggregate-imprinting" mechanism stated.

The fluorescein molecule is much smaller then that of the template molecules agglomerates, so in this case the fluorophore could fill the active cavities.

The fluorescence microscopy images are also in good correlation with AFM topology and phase images, which will be presented in a following paper.

CONCLUSIONS

This article presents innovative results in the field of poly (vinyl alcohol) imprinted materials obtaining, characterization, and application. Caffeine and theobromine have been used as template molecules. The obtained chemically crosslinked hydrogel is capable to selectively absorb the molecular aggregate that imprinted it. The imprinting mechanism is not clearly understood up to this date, but further studies are being performed. It seems that the xanthines aggregates formed in aqueous solutions show characteristic shape and dimensions as a function of their nature. The obtained PVA films, imprinted at

the aggregate level, have to be more effective in xanthine separation due to their aggregate state in aqueous solution by comparing with a molecular imprinted material.

It also has been demonstrated that fluorescence microscopy is a useful method for characterizing imprinted materials. The information obtained by this method is in good correlation with sorption test results, thus being able to substitute more sophisticated and expensive methods of analysis such as AFM or SEM. This characterization method could be successfully extended to a large variety of imprinted materials, aiming to predict the efficiency of the imprinting effect and to offer a better understanding of the imprinting phenomenon.

The extended Langmuir model (EL), recently proposed by Peikun Zhang and Li Wang,[13] describes better the xanthines sorption processes into PVA matrix, by comparing to Freundlich, Langmuir and BET models. This could be correlated to the multilayer sorption process, to the xanthines agglomeration state in aqueous solution and to the results of Scatchard analysis showing a complex sorption process.

References

1. Komyama, M. Molecular Imprinting, Wiley-VCH Verlag GmbH: Weinheim, Germany, 2003.
2. Kandimalla, V. B.; Ju, H. Anal Bioanal Chem 2004, 380, 587.
3. Yoshikawa, M. Bioseparation,2001, 10, 277–286.
4. Kubo, T. Anal Bioanal Chem 2005, 382, 1698.
5. Patachia, S. In Handbook of Polymer Blends and Composites; Vasile, C.; Kulshreshtha, A. K., Eds.; RAPRA Technology Ltd: UK, 2003.
6. Patachia, S.; Florea, C.; Friedrich C.; Thomann, Y. Express Polym Lett 2009, 3, 320.
7. Karadaglic, D.; Wilson, T. Micron 2008, 39, 808.
8. Patterson, G. H. Sem Cell Dev Biol 2009, 20, 886.
9. Smith, P. M. R. Organic Geochem 2010, 6, 839.
10. Roque-Malherbe, M.; Rolando, M. A. In Adsorption by Powders and Porous Solids; Rouquerol, F.; Rouquerol, J.; Sing, K., Eds.; Taylor & Francis: New York, USA, 1999.
11. Longo, L.; Vasapollo, G. Met Based Drugs 2008, 82, 28.
12. Shestopalova, A. V. J Mol Liquids 2006, 127, 113.
13. Zhang, P. K.; Wang, L. Separation Purif Technol 2010, 70, 367.