MOLECULARLY IMPRINTED POLY (VINYL ALCOHOL) FOR THE SELECTIVE ABSORPTION OF GALLIC ACID FROM AQUEOUS SOLUTIONS

C. CROITORU¹  S. PAȚACHIA¹

Abstract: Molecular imprinting represents an innovative method for designing materials with molecular memory, consisting of cavities bearing the shape and size of a template molecule. The cavities are highly specific towards the molecule that imprints the polymer, making molecularly imprinted materials suitable for use in separations. In this work, a new ecological method of alternative molecular imprinting to design imprinted poly (vinyl alcohol) films has been proposed. Gallic acid has been used as template molecule. To demonstrate the formation of the active cavities in the polymeric matrix, fluorescence microscopy as well as studies of gallic acid sorption and desorption have been performed.

Key words: molecular imprinting, poly (vinyl alcohol), gallic acid, fluorescence microscopy, absorption kinetic.

1. Introduction

Molecular imprinting is a method to introduce molecular recognition sites into polymeric materials.

Traditional molecular imprinting involves the formation of a complex (ionic, hydrogen bonded or covalent) between the template molecule (the molecule which “imprints” the polymer) and the monomer. In the second step, the complex is polymerized and in the third step, the template is removed from the resulting polymer matrix, leaving specific binding sites that remember the dimensions and the structure of the template molecule [1-6], [12].

When the template molecule or other similar chemical species are in contact to the imprinted material, the recognition sites incorporate them preferentially, determining their separation from mixtures with other compounds [9], [10]. Among the most frequently used monomers we can mention carboxylic acids (e.g. acrylic, methacrylic and vinyl benzoic acids), sulphonic acids and heterocyclic bases (e.g. vinylpyridines, vinylimidazoles) [7-15].

Currently, great efforts are being made to broaden the range of templates that can be used and to improve the specificity of ‘non-covalent’ molecularly imprinted polymers [MIP]. The first systems developed were not very selective [1]. It was only once the experimental conditions had been optimized (conditions of synthesis, host/guest ratios, eluant etc.) that Mosbach et al. achieved materials with selectivities similar to those obtained with the covalent approach [13-25]. Owing to the weakness of the interactions occurring, a large excess of functional monomer must be added to shift the equilibrium towards

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formation of the complex (typical monomer: guest ratios are 4:1) [15-20]. This leads to the formation, inside the imprinted network, of sites with different affinities for the molecule to be recognized (polyclonality) and lowers the specificity of the MIP. In chromatographic applications, for instance, this causes broadening of the peaks and decreased resolution. To overcome this problem, new monomers interacting more specifically with the template at several points have been developed for addition in stoichiometric quantities [8-20].

In this work, a new technique of molecular imprinting has been used to prepare imprinted PVA [poly (vinyl alcohol)] towards the selective absorption of gallic acid [GAL] from aqueous solutions. Due to the hydrogen bondings generated by the -OH groups, PVA could generate interactions with many compounds, which means that it could be a suitable matrix for a wide range of template molecules [14-15].

Supplementary, it is a non-toxic, non-carcinogenic, biocompatible, biodegradable, water-soluble polymer, and consequently easy to handle and environment-friendly [11], [20].

The simplicity and the rapidity of the PVA imprinted matrix preparation, eliminating the polymerization step and the use of toxic volatile organic solvents for monomers, make our imprinting technique a promising trend line in the future of molecular imprint materials obtaining.

Gallic acid (Figure 1) is an organic acid, also known as 3,4,5-trihydroxybenzoic acid, found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants, as free or as part of tannins [7-10]. Gallic acid seems to have anti-fungal and anti-viral properties. It also acts as an antioxidant and helps to protect cells against oxidative damage. Gallic acid was found to show cytotoxicity against cancer cells, without harming healthy cells. In homeopathic medicine it is used as a remote astringent in cases of internal haemorrhage, albuminuria and diabetes.

In this study we have used glutaraldehyde [GA] as crosslinker for PVA. Nevertheless GA is a toxic compound and in the field of separations it is necessary to avoid the existence of toxic unreacted crosslinker traces in the polymeric matrix. Taking into account that a reaction in gaseous phase leads to minimizing the unreacted crosslinker amount in the polymer matrix, avoiding the crosslinker sorption, in this paper we present our results concerning the imprinting of the PVA, crosslinked with GA in gaseous phase [10], [23].

The obtained imprinted PVA has been characterized by fluorescence microscopy and sorption kinetics (absorption and desorption) of GAL from aqueous solutions. The selectivity of the imprinted PVA film has been tested by comparing the amount of gallic acid absorbed with the amount of other related compounds, such as glycyrrhizinic acid (Figure 2).
Also, the influence of the GAL: PVA ratio on the properties of the imprinted polymer has been studied, aiming a higher absorption yield.

2. Experimental

PVA 120-98 (1200 polymerization degree and 98% hydrolysis degree) was purchased from Chemical Enterprise Râşnov, Romania. Glutaraldehyde of 45% wt concentration was purchased from Sigma-Aldrich. Sulphuric acid 0.1N was of reagent grade. Gallic acid and glycyrrhizinic acid (Fluka) was of reagent grade.

The polymer solution was obtained by adding PVA powder into a determined volume of water and heating the mixture to 70 °C under magnetic stirring for 4 hours. The obtained solution had a solid content of 10%. Into 15 mL of PVA solution, at room temperature, certain amounts of gallic acid were added to obtain GAL: PVA ratios of 10%; 25%; 30% and 40% respectively. The solution was acidified with diluted sulphuric acid to pH = 4.

The films were prepared by PVA/GAL solution casting and solvent evaporation at room temperature.

The PVA films with GAL in composition, treated with the catalyst as mentioned above, were introduced into the crosslinking installation and placed over a recipient that contained a determined volume of 45% GA solution. The installation was connected to a vacuum pump, so that the film was in contact with GA vapours for 3 hours. Blank samples, without GAL in composition, were crosslinked following the same procedure.

The template molecule from the respective films was removed from the polymer by immersing them in a determined volume of distilled water for 5 days at room temperature. The immersing solution was replaced daily.

The imprinting testing was performed by comparing the amounts of gallic acid absorbed from aqueous solutions by the imprinted and non-imprinted films respectively.

The formation of the active cavities in the 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 (λ_{max} = 455 nm), 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.

The selectivity of the imprinted films was tested by comparing the amount of GAL absorbed in the polymeric matrix with the amount of glycyrrhizinic acid [GZ] absorbed. The sorption of GAL in the polymeric matrix was determined by the difference from the initial amount of GAL present in the solution and the amount of GAL from the solution at different time intervals, using the spectrophotometric method (GAL shows an absorption band at 262 nm, in the UV domain).

The absorbed GZ was determined following the same procedure, at 259 nm. A Carl-Zeiss UV-VIS spectrophotometer was used, with standard quartz cuvets of 1 cm optical path.

The gallic acid, respectively GZ solutions were of 0.001 g/L concentration and for each time interval the sample was immersed in a fresh solution, to maintain a convenient concentration gradient between the sterols from the solution and the sterols from the imprinted polymeric matrix.

Desorption of GAL from the polymeric matrix was studied by immersing the GAL loaded imprinted films in distilled water and by determining the amount of carboxylic acid desorbed at determined time intervals.
3. Results and Discussion

The absorption kinetic of GAL in the polymeric matrix is presented in Figure 3, for the imprinted films and reference, in terms of absorbed GAL (g), reported to 1 g of dry polymer [xerogel].

The experimental points have been fitted using a Weibull type equation (Eq. 1):

\[ y = a - (a - b) \cdot e^{-(kt)} \]

where \(a\) is the equilibrium value of the kinetic, \(b\) is the starting point of the kinetic (in this case 0); \(k\) and \(d\) are coefficients characteristic to the sorption/desorption kinetic (Tables 1 and 2).

As it can be seen from Figure 3, the impregnation has been successful. The imprinted films absorb a higher amount of GAL than the non-imprinted reference film.

The highest amount of GAL absorbed is recorded for the film with 10% GAL: PVA ratio. This could be due to the aggregation of the template molecules at higher template: polymer ratios and the deformation of the active cavities. Also, at higher template: polymer ratios insufficient crosslinking of the polymeric matrix could occur, due to a lower amount of present polymer.

The kinetics of GAL desorption from the imprinted films is presented in Figure 4, in terms of desorbed amount of GAL (g) reported to 1 g of xerogel. The experimental points have been fitted using a Weibull type equation.

### Table 1: Sorption kinetic fitting parameters

<table>
<thead>
<tr>
<th>GAL: PVA [%]</th>
<th>(a)</th>
<th>(b)</th>
<th>(k)</th>
<th>(d)</th>
<th>(R^2)</th>
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</thead>
<tbody>
<tr>
<td>10</td>
<td>0.066</td>
<td>0</td>
<td>0.018</td>
<td>1.39</td>
<td>0.995</td>
</tr>
<tr>
<td>25</td>
<td>0.053</td>
<td>0</td>
<td>0.018</td>
<td>1.39</td>
<td>0.983</td>
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<tr>
<td>30</td>
<td>0.038</td>
<td>0</td>
<td>1.185</td>
<td>0.04</td>
<td>0.913</td>
</tr>
<tr>
<td>40</td>
<td>0.034</td>
<td>0</td>
<td>1.185</td>
<td>0.04</td>
<td>0.953</td>
</tr>
</tbody>
</table>

### Table 2: Desorption kinetic fitting parameters

<table>
<thead>
<tr>
<th>GAL: PVA [%]</th>
<th>(a)</th>
<th>(B)</th>
<th>(k)</th>
<th>(d)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.065</td>
<td>0</td>
<td>0.02</td>
<td>1.14</td>
<td>0.993</td>
</tr>
<tr>
<td>25</td>
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<tr>
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<td>1.185</td>
<td>0.04</td>
<td>0.907</td>
</tr>
<tr>
<td>40</td>
<td>0.032</td>
<td>0</td>
<td>0.029</td>
<td>0.27</td>
<td>0.908</td>
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</table>
Fig. 5. Percentage GAL release from the PVA imprinted films: (●) - GAL: PVA = 10%; (■) - GAL: PVA = 25%; (▲) - GAL: PVA = 30%; (□) - GAL: PVA = 40%

As can be seen from Figures 4 and 5, the entire amount of GAL is desorbed from the polymeric matrix.

The average absorption/desorption rate in/from the imprinted polymeric matrix is calculated as the slope of the linear portion of the fitted kinetic, prior to equilibrium. Results are presented in Table 3:

<table>
<thead>
<tr>
<th>Sample</th>
<th>K [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-imprinted PVA</td>
<td>0</td>
</tr>
<tr>
<td>GAL: PVA = 10%</td>
<td>99.68</td>
</tr>
</tbody>
</table>

The selectivity factors presented in Table 4 show that the obtained imprinted material is suitable for use in sterols solid phase extraction (SPE) assays.

Fluorescence microscopy images of the four imprinted films with gallic acid as the template molecule are shown in Figures 7-11. As it can be seen the distribution of the fluorophore on the surface of the polymeric film shows that the ideal GAL: PVA ratio is 10%.
Above 10% percent, GAL molecules seem to associate, leading to the formation of non-uniform active cavities.

4. Conclusions

Imprinted films with gallic acid as the template molecules have been obtained and characterized. Sorption and desorption analyses have been performed. The results indicated that the impregnation of poly (vinyl alcohol) with gallic acid was successful. All the imprinted films absorb a higher amount of GAL than the non-imprinted PVA. The imprinted films also show selectivity to GAL. Glycyrrhizinic acid amount absorbed into the GAL-PVA imprinted
films is lower than that of GAL.
The imprinted films are transparent, homogenous and have good resistance to solvent action, owing to crosslinking.
Taking into account the non-toxicity, biocompatibility, biodegradability of PVA and the possibility of obtaining molecular imprinted materials based on PVA/GAL, new applications in special fields (medicine, pharmacy, separation processes) could be developed.

Acknowledgements

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References

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