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High Capacity Molecular Imprinted Mesomorphous Networks Usable as Antibody Mimics

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High Capacity Molecular Imprinted Mesomorphous Networks Usable as Antibody Mimics

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The preparation of high capacity molecularly imprinted polymers (MIP) that could be used as antibody analogues is reported. A functionalised mesomorphous polysiloxane network built up around theophylline was synthesized. After extraction of the guest molecule, the network preserved the mesomorphic organisation set up in the presence of the template. Batch rebinding analysis, performed in the presence of theophylline or structurally analogous caffeine, revealed that the imprinted polymer has a significant selectivity towards the template and a greater capacity than both an unimprinted mesogenic network and an imprinted non mesogenic network.

Keywords: caffeine; molecular imprinting; molecular recognition; side-chain liquid-crystal polymer networks; siloxane; theophylline

1. INTRODUCTION

The molecular imprinting technique is a valuable method for preparing synthetic materials able to mimic the molecular recognition phenomena present in living systems [1]. In a first step, a molecule, acting as a template, is associated with functional monomers to form a complex by means of covalent bonds or noncovalent interactions. A polymerisation-crosslinking reaction is then performed in a second step around this complex. Upon removal of the template species,

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functionalised cavities, that have memorized the special features and bonding preferences of the template, are left inside the polymer network. If the chosen template is of biological importance, this class materials can be assessed as antibody analogues. Thus, drugs like theophylline (bronchodilator) or diazepam (tranquillizer) have been quantified in human serum using MIPs with results comparable to those obtained by a well established immunoassay method [2,3]. Compared to alternative techniques (involving biomolecules, abzymes, etc.), molecular imprinting technique (MIT) presents several advantages: low cost, effectiveness, mechanical, thermal and chemical stability of the support, long lifetime. Nevertheless, this technique presents some drawbacks linked to the fact that a large amount of cross-linker is needed (usually around 80–90%) to restrict distortion of the polymer backbone [1]. Indeed, the resulting stiffness of the network hinders the extraction and reinsertion of the template in the imprinted cavities and drastically decreases the capacity of the material.

To improve this technique, the use of systems based on supramolecular organisation appeared to be an interesting alternative. The integrity of the structure could then be ensured by the so-called "weak interactions" between the components of the network. Some polymer gels [4] and 2-D films [5] have been developed. Recently we proposed an approach that relies on the synthesis of polymer networks that were at the same time liquid crystalline and molecularly imprinted. Previous studies have demonstrated the potentiality of this approach with two different templates: acetophenone and 1,8-diaminonaphtalene, the first being based on covalent linkages [6], the second on hydrogen bonding [7], between the template and the mesomorphous polymer. The imprinted networks exhibited a good specificity towards the template and a much higher capacity than the non-mesogenic materials [6]. The introduction of liquid crystalline groups appeared to be sufficient to ensure the molecular recognition properties even with low cross-linking ratios (5 to 10 mol%). Moreover the mesomorphic organisation set up in the presence of the guest molecule was shown to be preserved after extraction of the template [7].

Here, we report the study of an imprinted liquid crystalline material synthesized in the presence of theophylline as template. The mesomorphous properties of the resulting imprinted networks were analysed. Batch rebinding studies were performed with theophylline and structurally analogous caffeine in order to underline the role of hydrogen bonding in the molecular recognition properties. The results obtained were compared to those from previous studies with polyacrylate imprinted networks [2,8–11].

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2. EXPERIMENTAL

Apparatus

The nature of the mesophases and the corresponding transition temperatures were determined by polarised-light optical microscopy (Olympus microscope equipped with a Mettler FP82HT hot stage), Differential Scanning Calorimetry (DSC) using a Perkin Elmer PYRIS 1 calorimeter, and X-ray measurements were performed on a Brucker apparatus using MoKα radiation of a 20 kW anode generator. The scattered radiation was collected on a two-dimensional detector (Smart 1000). The transition temperatures recorded in Table 1 correspond to those determined from the position of the tops of DSC peaks as the temperature fell at 2°C/min; the glass transition temperatures (T_g) were obtained as the temperature increased at 10°C/min. Correlation lengths ζ were determined by the analysis of the radial intensity of the scattered peaks obtained in X-ray experiments [12]. ^{1}H NMR analysis was conducted with a Brucker ARX 400 MHz spectrometer using the HRMAS accessory for networks. Infrared measurements were performed on PERKIN-ELMER IR FT 1600. A Hewlett Packard UV diode array spectrophotometer (HP 8452A) was used to determine the concentration of the removed template in the extraction of the template and rebinding experiments.

Network Synthesis

All mesogenic compounds were synthesized as previously described [7]. Theophylline template was previously mixed with an excess (1/4 mol/mol) of 4-(3-butenyloxy)benzoic acid in toluene at 70°C for 5 hours. The carboxylic acid forms ionic interactions with amino groups and hydrogen bonds with polar functions of the template [2,8,10]. The formation of H bonding was demonstrated in ^{1}H NMR by the shift of δ_{COOH} in presence of theophylline. This complex and the mesogenic groups (Fig. 1) were end-fixed onto polysiloxane backbone (R_i). Crosslinker ratio was fixed to 5 mol%. Corresponding reference network (R_i^0) was prepared with exactly the same procedure except that no template was used. All the samples were cross-linked in their thermotropic nematic or smectic phase. At the end of the reaction, the solvent was slowly evaporated to avoid damage and theophylline was carefully extracted from the network by acetonitrile (for R_1 and R_2) or acetone (for R_3) until no absorbance at 274 nm appeared in supernatant. The imprinted networks and the reference material were obtained as circular membranes (diameter c.a. 2 cm, thickness c.a. 0.3 mm).

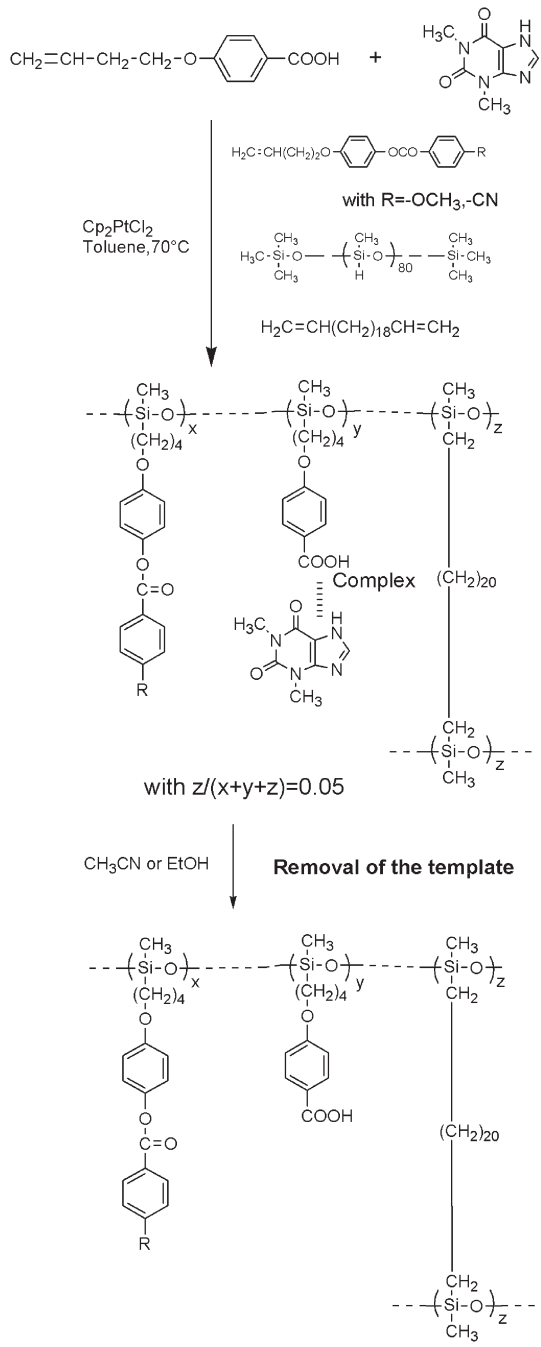

FIGURE 1 Chemical structure of the mesomorphous network imprinted around theophylline.

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Batch Rebinding Analysis

Batchwise adsorption tests were conducted using the imprinted (R_i) and the reference networks (R_i^0) in order to assess the effectiveness of the imprint technique. R_i or R_i^0 (single blocks of 200 mg) were incubated in a vial with 20 mL of a 15.10^{-6} M aqueous solution of theophylline or caffeine at room temperature for 36 h without stirring. Subsequently, the concentration of the molecule remaining in the supernatant was determined by UV measurements at 350 nm. The initial solution of template (control solution) was examined under identical conditions to check that no evolution of the concentration occurred during experiments.

3. RESULTS AND DISCUSSION

Polymorphism

The synthesised samples are reported on Table 1. Two different mesogenic groups are used (R=OCH_3 or CN) in order to obtain nematic or smectic A networks. Two proportions of template are introduced in the first samples. The mesomorphous properties of the imprinted networks and reference non-imprinted materials were analysed by DSC and polarised-light optical microscopy. The mesomorphic order

TABLE 1 Characteristics of the samples[a]

	Composition				Polymorphism			
Network	R	x[b] (% mol)	y[b] (% mol)	T_g (°C)	I→ M (°C)	ΔH_{I-M} (J/g)	(Å)[d]	ζ/ζ_0[c]
R_1^0	OCH_3	87	8	6.0	I 73.0 N	0.94	/	1
R_1	OCH_3	87	8	8.2	I 64.2 N	0.32	/	0.8
R_2^0	OCH_3	79	16	10.5	I 71.9 N	0.80	/	1
R_2	OCH_3	79	16	6.2	I 54.5 N	0.29	/	0.7
R_3^0	CN	87	8	27.7	I 129.8 S_A	0.95	36.4	1
R_3	CN	87	8	27.6	I 120.2 S_A	0.30	36.5	0.7

[a] T_g : glass transition temperature; M : Mesophase; I : isotropic phase; N : Nematic mesophase; S_A: Smectic A mesophase; ΔH : enthalpy variation for phase transition;

[b] %mol of substituents x or y linked to the polysiloxane chain. The crosslinker ratio was kept to z = 5% mol. x, y, z were as defined in Figure 1.

[c] ζ correlation length of imprinted network and ζ_0 correlation length of unimprinted network, determined by x-ray experiments.

[d] layer spacing of the smectic A phase; correlation length ratio of imprinted network against reference network.

remained in the imprinted R_i samples but it was destabilized compared to the non-imprinted R_i^0 networks: the transition temperature and the enthalpy variations were lowered. The differences were emphasized when the concentration of the template increased (compare R_1 and R_1^0 with R_2 and R_2^0). This fact is the manifestation of a significant memory effect of the template, imprinted inside the mesomorphic structure. It arises from the interactions between template and the other parts of the network which can induce conformational constraints inside the networks during cross-linking. It occurs even though the amount of cross-linker is low (5%) and is preserved after heating to the isotropic state. The lowering of correlation lengths between mesogenic units, when the network is synthesized around the template, is also in good agreement with the disturbance induced by theophylline on the mesogenic structure.

Molecular Recognition Properties

In Figure 2 are reported the results of the batch rebinding analysis performed with sample R_1 and R_1^0. As shown, the imprinted network exhibited a much higher affinity for theophylline than for caffeine.

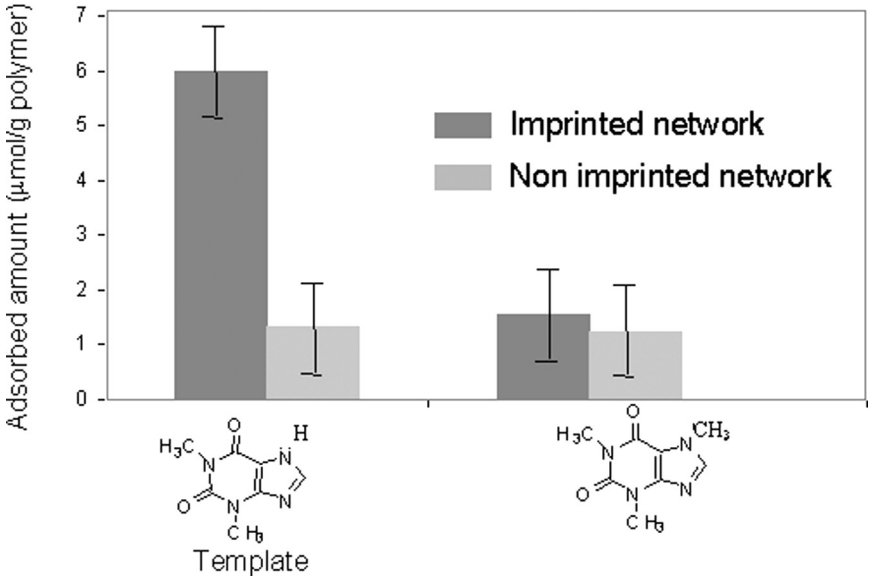

FIGURE 2 Molecular recognition properties of the network R_1 imprinted around theophylline compared to the non imprinted one R_1^0.

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Molecular separation by MIP materials occurs by selective transport through the template-specific cavities of the polymer barrier. This mechanism is provided by the conformational polymer micropores that are formed around templates during polymerization as well as by the ligand-functional groups interactions inside the cavities. In this sample, the importance of H-bonding for the recognition process is underlined. Moreover the imprinted network showed a higher affinity than the non-imprinted sample for theophylline. These results indicate that, in addition to the hydrogen bonding or electrostatic interactions between the functional groups of the polymers and the template, microcavities corresponding to the shape of the template are necessary for effective binding. Consequently the polymer gained affinity for the template through the molecular imprinting technique. On the other hand, the molecular trapping capacity of the networks (6 μmol/g of polymer) was shown to be much greater than that of the previously non mesomorphous studied systems (1 μmol/g of polymer studied by Wang and collaborators [8–10] and 0.057 μmol/g of polymer reported by Ye and collaborators [11]). Introduction of liquid crystalline moieties allowed the combination of the necessary stiffness of the network with an increase of its capacity.

4. CONCLUSION

Analysis of the mesomorphic order underlined a memory effect of the liquid-crystal structure set up in the presence of the theophylline template. The rebinding studies, performed in the mesogenic phase, showed a significantly higher affinity of the imprinted material towards the template compared to the non liquid crystalline materials. The liquid-crystal polymer networks kept the memory of the template while preserving the flexibility of the network that consequently increased the capacity of the material. Moreover, these materials exhibited a good selectivity towards the printed theophylline molecule compared to caffeine.

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