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Radiation induced synthesis of molecularly imprinted polymers

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A new synthetic procedure for preparation of molecularly imprinted polymers (MIPs) was developed. The polymerization process was initiated by γ-radiation, in the model system with (±)-menthol as a template molecule. The thermal stability of MIP was investigated by thermogravimetric analysis and differential scanning calorimetry, while the specific surface area was determined from Brumauer–Emmett–Teller isotherm. Adsorption properties and recognition capabilities of MIPs were examined by comparison of the adsorption isotherms of menthol and camphor (test molecule) from *n*-hexane. The adsorption isotherms corresponded to Langmuir type adsorption, and the difference between adsorbate–adsorbent interaction energies was clarified. © 1997 Elsevier Science Ltd.

(Keywords: molecularly imprinted polymers; γ-radiation; (±)-menthol)

Introduction

Molecularly imprinted polymer (MIP) materials are nowadays commonly used for separation of organic materials of a similar structure, even enantiomers, as custom made chromatographic supports[1–13]. Additionally, MIPs can be used in drug assay, taking advantage of their antibody mimics[3,14,15], to control the directions of various reactions as designed catalysts[1,4,16,17], or as substrate selective sensors, simulating enzyme/antibody binding sites[2]. Since the pioneering work of Wulff and Sarhan[18], a great deal of research activity has been made in order to improve synthetic procedures and to obtain polymer-based adsorption materials with high molecular recognition capabilities.

In principle, synthesis of MIPs proceeds in three steps: (1) spatial arrangement of the monomer molecules around the print molecule; (2) polymerization; (3) extraction of the print molecule from the polymer. The first step can be achieved by either covalent or non-covalent interactions between the print molecule and the monomer. Both approaches have been well established and approved by using polymers based on styrene, acrylates, and silica[1].

The polymerization process can be triggered photochemically (ultraviolet, u.v., light) or thermally (elevated temperature at 35–80°C) in the presence of special polymerization-initiators. Both of the common initiation techniques led to imprinted polymers of satisfactory qualities, although the selectivity of the polymers strongly depends on the polymerization temperature: the lower the temperature of the mixture during the polymerization, the higher the separation factors that can be achieved[1,11,19,20]. This effect can be ascribed to the thermal destabilization of the complexes between the print molecules and the functional monomers.

In this paper we present the preliminary results of our efforts to prepare MIPs in an alternative way, inducing γ-radiolytically the polymerization process, and using (±)-menthol as a template molecule. The possible advantages of this new method could be: no need for initiators since radiation chemical processes provide a continuous supply of free radicals in the system; conduction of the polymerization process at desired temperature.

Experimental

Material and methods. The following chemicals were used in the experiments: ethylene glycoldimethacrylate (EDMA) and methacrylic acid (MAA) (p.a. grade, Merck); chloroform, *n*-hexane and methanol (p.a. grade, Fluka); (±)-menthol (Aldrich); (±)-camphor (Sigma). All chemicals were used as received, without further purification.

The thermogravimetric analysis (t.g.a, Perkin Elmer model TGS-2) and the differential scanning calorimetry (d.s.c., Perkin Elmer model DSC-2) of the polymer samples (7–8 mg) were carried out under an argon atmosphere in the temperature ranges 25–400°C, and 25–230°C, respectively. The heating rate was 10°C min^{-1}.

A HP5890/II gas chromatograph (g.c.) with a HP 5971 mass spectral (m.s.) detector (Hewlett Packard) equipped with a HP-5 (Hewlett Packard) capillary column (50 m, 0.32 mm i.d., 0.17 μm film), using He as carrier gas, was used for chromatographic measurements. The injector was operated at 250°C, the temperature program was started at 80°C, immediately ramped to 200°C at 10°C min^{-1}, then ramped to 300°C at 5°C min^{-1}. Samples of 1 μl were injected in split mode, with a 1/85 split ratio. The identification of the compounds was based on mass spectra (library match) and retention time, when pure compounds were available. Calibration standards were used for quantitative measurements, the linearity of the detector response was checked for at least five different concentrations in the expected range of concentrations.

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Radiation induced preparation of MIPs. Menthol (the print molecule, 0.788 g), MAA (the functional monomer 0.869 g), EDMA (the cross-linking monomer, 20.000 g) and chloroform (15 ml) as solvent were sonificated in a sealed glass vessel at room temperature for 15 min. The above mixture resulted in a 1/2/20 mol ratio between the print molecule, the functional monomer and the crosslinking monomer, respectively. This solution was sparged through the stopper by oxygen-free argon saturated by chloroform vapours, for 30 min, in order to remove dissolved oxygen. Finally, the solution was cooled to 15°C (the temperature of the irradiation chamber) and irradiated by γ-radiation (approximately point source of ^{60}Co) at a dose rate of 15.9 Gy min^{-1} for 120 min, resulting in a total absorbed dose of 1.9 kGy. A blank sample was prepared in the same manner, except that the print molecule was not added. After the polymerization, the glass vessel was crushed, and the compact block of the polymer was ground. This powder was filled in a glass column (15 mm i.d.), washed with five 30 ml portion of chloroform and five 30 ml portions of methanol until no peaks could be detected in the eluate by g.c.-m.s. All the eluates were collected and the total amount of recovered menthol and non-reacted EDMA were determined. The powdered polymer was dried at 80°C overnight, sieved, and only the fraction between 100 and 320 μm was further used.

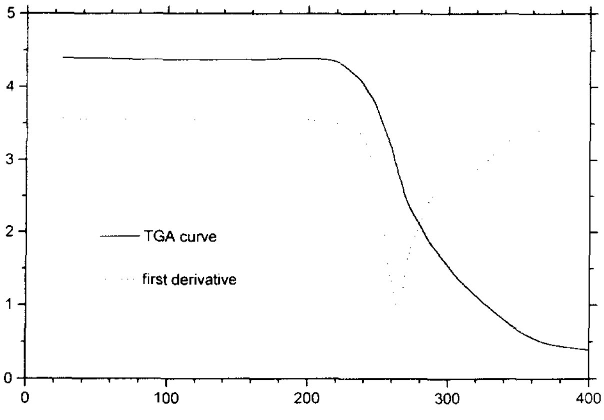

Figure 1 Thermogram (solid line) and its first derivative (dotted line) of MIP, obtained under the atmosphere of argon

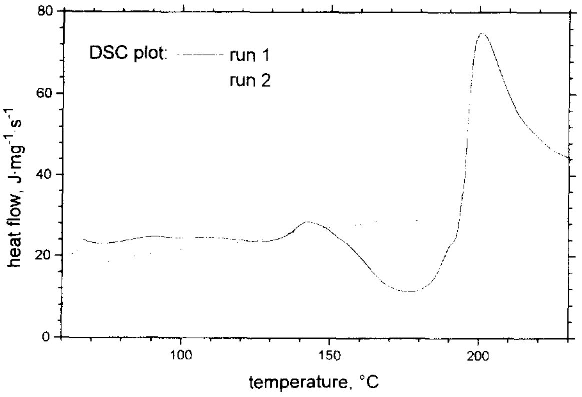

Figure 2 D.s.c. plots of MIP obtained during the first heating (solid line), and during the repeated runs of the same sample (dotted line)

Results and discussion

Initial characterization of MIPs. The mass balance of starting materials was determined by g.c.-m.s. analysis of the wash-out of the freshly synthesized MIPs. G.c.-m.s. analysis revealed that only 8.6% of the print molecule (menthol) was either permanently incorporated into the bulk polymer or degraded during polymerization. In the same manner, it was found that 7.2% of EDMA did not polymerize. Also, negligible amounts of various isomers of $(EDMA)_2$ and EDMA–MAA were noticed.

The specific surface areas of the MIP and blank polymer samples were determined by single point BET method using liquid nitrogen. The difference between the obtained specific surface areas (15.6 and 23.3 m^2 g^{-1} for MIP and blank polymer, respectively) indicated that the print molecules have a certain influence on the propagation of polymerization in the space.

The thermal stability of the MIP and blank polymer samples was investigated by t.g.a. and d.s.c. Identical t.g.a. results, within the limits of experimental error, were obtained for both polymer materials (MIP and blank). These results indicated that the print molecule has no influence on the thermal properties of MIP. The thermal decomposition of both materials started at 230°C and the polymers were almost completely decomposed at 400°C (see *Figure 1*).

D.s.c. measurements of the MIP and blank polymer samples revealed that the thermal treatment in the temperature range 25–230°C, in the same manner, lead to some irreversible processes in both materials. The small exothermic peak at 175°C (see *Figure 2*) can be ascribed to the thermal rearrangement of the polymer. Most likely, this effect is a result of the additional thermal-induced crosslinking of the polymer. The more pronounced endothermic peak at 200°C can be related to the process of melting. In order to see the effect of cyclic changes of the temperature, the d.s.c. runs were repeated four times. Both peaks were obtained only during the first heating of the polymers, underlying once again irreversibility of these processes.

Adsorption properties of MIPs. Adsorption properties of γ-radiolytically synthesized MIP materials with menthol as a template molecule was examined by comparison of the adsorption isotherms of menthol from *n*-hexane on MIP and blank polymer samples. Menthol was purposely chosen as a template molecule, since it is a simple organic molecule with only one functional group and a weak association tendency towards MAA. The recognition capabilities of γ-radiolytically synthesized MIP materials with menthol as a template molecule were checked in a cross-experiment, by using camphor as a test molecule. Camphor was selected as a test molecule, because its structure is quite similar to the structure of menthol.

Adsorption isotherms were determined in the following way. A certain amount of the dry polymer material (0.1 to 2 g) was precisely weighed in a glass vial, and 5 ml of a solution of menthol or camphor in *n*-hexane was added. The vial was closed and shaken for 30 min. After separation of the phases, the concentration of menthol or camphor in the supernatant was determined by g.c., giving the equilibrium concentration on the adsorption isotherm. The adsorbed amount of the adsorbate was determined from the differences between the initial and

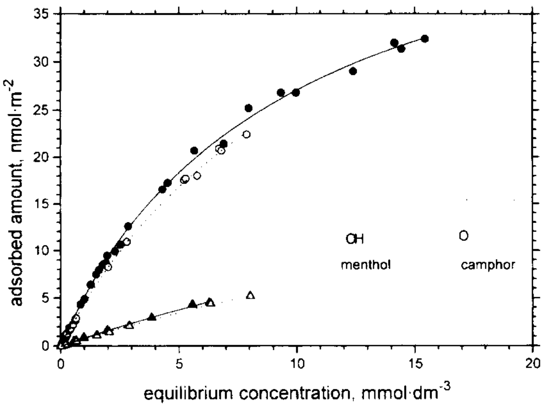

Figure 3 Adsorption isotherms from *n*-hexane of: ●, menthol on MIP imprinted by menthol; ▲, camphor on MIP imprinted by menthol; ○, menthol; ▲, camphor on blank polymer material

Table 1 Best fit coefficients of Langmuir equation obtained by a linear least square algorithm from the experimental data, presented in *Figure 3*

	a_m [nmol m^{-2}]	K [m × 10^8]
Menthol/MIP	51.5 ± 0.8	11.1 ± 0.4
Menthol/blank	56 ± 2	8.6 ± 0.4
Camphor/MIP	33 ± 6	2.5 ± 0.5
Camphor/blank	34 ± 3	2.3 ± 0.2

equilibrium concentration of the supernatant. It was found that the Langmuir equation, in the form

$$a(C) = \frac{a_m \cdot K \cdot C}{1 + K \cdot C} \qquad (1)$$

fits the experimental data quite satisfactory (solid and dashed lines on *Figure 3*). The calculated coefficients of Langmuir equation are collated in *Table 1*.

It should be noticed that the isotherms of camphor on MIP materials with menthol as a template molecule and blank polymer practically coincide (see *Figure 3*). These results indicated that the molecules of camphor cannot distinguish MIP material from blank polymer. On the other hand, there is a difference between the isotherms of menthol on MIP and blank polymer: MIP is more attractive to menthol than the blank polymer. This difference can be seen more clearly comparing the parameters of the Langmuir adsorption isotherm (see *Table 1*). Langmuir's parameter K (Henry's constant) is directly proportional to the adsorbate–adsorbent interaction energy[21]. This value is identical for camphor on MIP and blank polymer (within the limits of the error), while there is a 22.5% difference in the case of menthol, clearly underlying that the adsorption of menthol on MIP is more favourable than on the blank polymer. At first, the difference between adsorption energies of template molecules on MIP and blank polymer samples was determined. It is reasonable to expect that this difference might be more substantial for molecules with stronger association tendency towards MAA than menthol. This effect is a result of specific adsorption of menthol on the imprinted adsorption sites on the surface of MIP: molecules of menthol attached to these sites are more strongly bounded to the surface than to other, nonspecific, adsorption sites.

Conclusion

The preliminary results on the model system with menthol as a template molecule clearly showed that MIPs can be synthesized by radiation induced polymerization, under the reported conditions. At this moment, the obtained materials are far from being ideal (low thermal stability and relatively low specific surface area), and optimization of parameters of synthesis (temperature, dose rate, total absorbed dose) will be a matter of our further investigation, as well as comparison between radiatively and photochemically or thermally prepared MIPs.

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