Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1100 (2005) 60–67
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Chromatographic behavior of silica–polymer composite molecularly imprinted materials

B. Tóth [a], K. László [b], G. Horvai [a,*]

[a] *Department of Chemical Information Technology, Budapest University of Technology and Economics, H-1111 Budapest, Szt. Gellért tér 4, Hungary*
[b] *Department of Physical Chemistry, Budapest University of Technology and Economics, H-1111 Budapest, Szt. Gellért tér 4, Hungary*

Received 13 May 2005; received in revised form 6 September 2005; accepted 9 September 2005
Available online 26 September 2005

Abstract

Molecularly imprinted polymers (MIP) have recently been prepared inside the pores of silica based HPLC packing materials. Detailed physical and chromatographic characterization of such a silica-MIP composite material is presented. The chromatographic peak shape obtained with the uniformly sized spherical silica–MIP composite is mainly determined by the nonlinear adsorption isotherm. Comparison of the composite with the conventional sieved and grinded bulk MIP is therefore based on the nonlinear isotherm and not on retention factors and plate numbers.

Keywords: Imprinted polymer; HPLC; Nonlinear; Composite

1. Introduction

Molecularly imprinted polymers (MIP) are selective sorbents with advantageous properties like tailored selectivity, physical and chemical robustness and reproducible preparation technologies [1]. Although they have been known for decades there has been an upsurge of interest about MIPs in recent years. A wide range of analytical applications for MIPs has been investigated, e.g., chromatography and electrochromatography, solid phase extraction (SPE), binding assays, sensors, etc. The range of analytes tested extends from small molecules through macromolecules to microorganisms [2,3].

Chromatographic utilisation of MIPs has often been demonstrated [4,5], particularly for the separation of enantiomers (chiral separations) in HPLC [6] and for sample cleanup using solid phase extraction cartridges [7]. These studies have provided ample proof that the preparation of MIP stationary phases with designed selectivity is feasible. Most studies report, however, chromatograms with severe tailing and low (apparent) plate numbers. These drawbacks have been mostly attributed to two factors:

(a) the large size (20–30 μm) and irregular shape of the packings (which have been prepared by the bulk method, i.e., by synthesising a solid block of MIP and then grinding it and sieving to the mentioned size fraction); and
(b) the inhomogeneity of binding sites (i.e., the existence of a variety of binding sites with different binding strengths on the MIP).

There have been many efforts to produce MIP packings for HPLC in the form of uniformly sized spherical, porous beads of a few microns diameter [8–12] The apparent driving force behind such work has been the hope to reduce peak tailing, to increase plate numbers and to find a more convenient way to manufacture MIP particles than that which involves grinding and sieveing.

A very notable work in this latter area has been published by Mosbach and co-workers recently [8]. They had started out with a commercially available reversed phase silica packing for HPLC, the Kromasil C4, 13 μm diameter spherical beads. They had filled the nominally 20 nm wide pores of this material with

* Corresponding author. Tel.: +36 14631480.
E-mail address: george.horvai@mail.bme.hu (G. Horvai).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.09.015

the MIP starting materials, dissolved in a suitable porogenic solvent. This was followed by heat polymerization. The resulting silica–MIP composite material was tested as HPLC packing. An alternative packing material was also prepared by leaching out the silica skeleton of the composite material. The spherical shape and the size of the original silica particles had been well maintained in both preparations. Other authors have presented interesting alternative ways of preparing MIPs in the pores of silica HPLC packings and other porous substrates [11–13] which are not in the focus of the present work.

The silica–MIP composite material made by the simple pore-filling procedure [8] appears very attractive. The procedure may be applied in principle to any known MIP without modification of the recipe. It may also be extended to using other porous solids as inert supports.

It is interesting, however, if the silica–MIP composite represents a solution to the long-standing problem of chromatography with MIP stationary phases, i.e., the wide and tailing peaks. The authors [8] claimed a twofold increase in the plate numbers of the silica–MIP column against the corresponding bulk MIP column of comparable particle size. The plate number had been determined, however, not from the template peak. The template in this case was one member of a pair of enantiomers and the plate number was determined from the peak of the other enantiomer. This peak was narrower and more symmetric than the template peak. Further on the plate numbers so obtained (2050/m for the composite) were still an order of magnitude less than might have been expected for a 13 μm spherical packing (note that a reduced plate number of 3 would give a plate count of about 25,000/m).

Clearly, there is a need to study further the peak shapes obtainable with the novel silica–MIP composite. This has been the purpose of the present work.

2. Theory

In elution chromatography, the separation of two substances depends on the difference in their retention and on the widths of the corresponding peaks. If both substances have linear adsorption isotherms in the given chromatographic system then the retention of each substance can be described with the corresponding distribution coefficient and the (common) phase ratio of the column. The width of the peaks depends on many factors (of kinetic origin) but for analytes with similar and not too low retention it does not depend appreciably on the analyte. Peak shapes are generally Gaussian. There is a well-developed formalism to express peak width with plate numbers.

Things become much more complex if the isotherms are not linear [14], which seems to be the case for many MIPs, at least with relation to their respective templates. In the case of pronounced nonlinearity, the chromatographic peak shapes are rather wide and tailing or fronting. The kinetic peak broadening effects become negligible. The actual peak shapes may be rather complex depending on the isotherm. Things become even more complex if the nonlinear effects are less pronounced, so that kinetics may also influence the peak shapes.

Guiochon and co-workers have repeatedly analyzed [15–17] the isotherms and peak shapes of a bulk MIP prepared for the separation of the enantiomers of an amino acid derivative. For our purposes one may summarize their results by saying that the isotherms of both enantiomers were nonlinear, upward convex and that nonlinear and kinetic effects have both influenced the peak shapes, the nonlinear effects being more important. Thus, to a rough first approximation, the peak shapes could be described by Eq. (1). This is a somewhat unusual way of describing an elution peak: instead of describing the eluted concentration as a function of time, the inverse function is shown, i.e., the time t elapsed from the injection until the concentration c is reached on the rear, tailing part of the peak (Fig. 1).

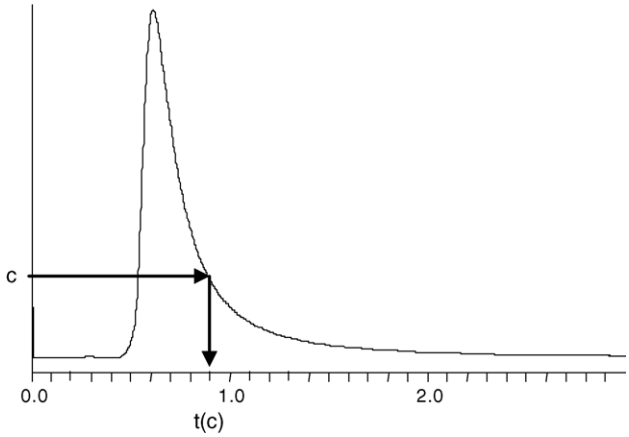

Fig. 1. A chromatographic peak measured with a MIP column. The arrows show a corresponding pair of c and $t(c)$ values, respectively, on the rear part of the chromatogram.

The frontal, increasing part of the chromatogram is not included because according to the theory of nonlinear chromatography the front is a vertical line when the kinetic effects can be neglected.

Thus, Eq. (1) reads:

$$t(c) = t_0 \left(1 + F\frac{\mathrm{d}q}{\mathrm{d}c}\right) \tag{1}$$

where t_0 is the column holdup time; F, the phase ratio in the column and the last term is the derivative of the isotherm at the concentration value c. This equation can be transformed into

$$t(c) = \frac{V_0}{W}\left(1 + F\frac{\mathrm{d}q}{\mathrm{d}c}\right) \tag{2}$$

with $t_0 = V_0/W$, where V_0 is the column holdup volume and W, the flow rate. Eq. (1) may also be transformed as

$$t(c) - t_0 = \frac{m}{W}\frac{\mathrm{d}q}{\mathrm{d}c} \tag{3}$$

since the phase ratio is $F = m/V_0$, where m is the mass of MIP in the column.

Eqs. (1)–(3) will be used below to check the applicability of the nonlinear chromatographic model to our experimental data. We shall investigate variations of the peak shape with the variation of the column length, the flow rate and the packing particle type (bulk or composite), respectively.

The holdup time in Eq. (1) is directly proportional to column length, while F and the isotherm derivative are unaffected

by it. Thus, by doubling the column length the tail of the chromatogram should be extended by a factor of two in the horizontal direction. One should note that if the whole chromatogram were extended twofold, then the area of the peak would double which is impossible, so the peak width has to be shorter.

The flow rate W appears explicitly in Eq. (2) and it does not influence any other term on the right hand side of the equation. Multiplying both sides of the equation by W we obtain a formula for the elution volume $V(c)$ pertaining to the concentration level c:

$$V(c) = Wt(c) = V_0\left(1 + F\frac{\mathrm{d}q}{\mathrm{d}c}\right) \tag{2a}$$

showing that the peak tails at different flow rates should coincide if peaks are presented as a function of elution volume.

Finally, the effect of packing type (bulk or composite) is most easily followed using Eq. (3). Here the holdup time, t_0, and the mass of polymer in the column, m, depend on the packing type. The flow rate is obviously independent of the packing. The isotherm of the two packings may be expected to be identical or at least proportional if the polymer in both packings is made from the same master batch. Thus, to check the validity of the model one measures the chromatograms on both packings at the same flow rate. One takes then a certain concentration (or detector signal, e.g., absorbance) value and measures the corresponding time value on each chromatogram. These values are then corrected by the respective t_0 values and the results are divided by each other. The result should be independent of the concentration value chosen if Eq. (3) is valid.

One may ask if it were not much simpler to check the validity of the nonlinear model by measuring the isotherm of the MIP and checking directly the fit of the chromatogram to Eq. (1) in each experiment. This approach has been indeed tried by Guiochon and co-workers [15] and we also made such attempts with our data. The method has proved to be much more difficult than the one proposed here. Apparently the complete fitting of the chromatograms is more influenced by kinetics than the here proposed tests are. Obviously, we have not investigated the effects of all possible variables. Yet, the good agreement of the studied effects with the model predictions and the clear disagreement of the results with the linear model (see below) give credit to the nonlinear model. Our results at least make possible to predict the effects of column length, flow rate and packing format.

3. Experimental

3.1. Materials

Kromasil C4, 13 μm diameter, 20 nm pore size HPLC packing material was a gift from EKA Chemicals AB (Bohus Sweden). Phenytoin (5,5-diphenyl-hydantoin) and MAAM (methacrylamide) were purchased from Sigma–Aldrich (St. Louis, MO, USA). AIBN (azo-bis-isobutyronitrile) and EDMA (ethylene glycol dimethacrylate) was a product of Fluka. For structures, see Fig. 2. HPLC grade acetonitrile was a product of Romil (Loughborough, UK), methanol was purchased from

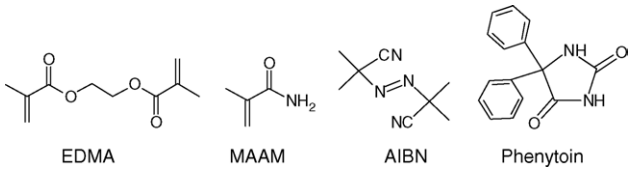

Fig. 2. Crosslinker EDMA, monomer MAAM, initiator AIBN and template phenytoin.

Carlo Erba (Milan, Italy), tetrahydrofurane (THF) was freshly distilled before use.

3.2. Molecularly imprinted polymer preparation

The phenytoin MIP and the non-imprinted control polymer (NIP) were prepared by the method of Lanza et al. [18]. The composition of the polymerization mixture was as follows: MIP: 1 mmol (252.3 mg) phenytoin as template, 4 mmol (340.4 mg) methacrylamide as functional monomer, 20 mmol (3.8 ml) EDMA as crosslinker, 4.16 ml acetonitrile and 1.44 ml THF as porogen, 50 mg AIBN as initiator. The mixture was deaerated with nitrogen for 10 min. An aliquot was taken out for making the composite material. The rest was sealed and polymerized at 60 °C for 24 h to obtain the bulk MIP. The NIP was prepared in the same way but without the template phenytoin. The ground and sieved bulk polymer particles (25–36 μm) were Soxhlet extracted with methanol containing 1% acetic acid for 10 h and packed (see supporting material) into HPLC columns.

3.3. Preparation of silica–MIP (and NIP) composite

0.65 ml of the polymerization mixture described in Section 3.2 was added to 1.00 g of the Kromasil material. This ratio is slightly less than the specific pore volume (0.7–0.8 ml/g) of the Kromasil packing material. The materials were mixed with a spatula, kept in an ultrasonic bath for 10 min and then deaerated by blowing nitrogen over the mixture for 2 min. The resulting free flowing powder was then polymerized at 60 °C for 24 h in the sealed container. In a separate experiment it was checked gravimetrically that the solvent was not evaporated measurably during the procedures which preceded the final sealing.

3.4. Physical characterization

Nitrogen adsorption/desorption isotherms were measured at 77 K, using a Quantachrome Autosorb-1 computer controlled apparatus (Quantachrome, Boynton Beach, Florida, USA). Samples were outgassed at ambient temperature. The apparent surface area (S_{BET}) was derived according to the BET model. Pore size analysis was performed by the Quantachrome software using the Saito-Foley (SF) [19] and Barrett, Joyner and Halenda (BJH) [20] methods.

The total pore volume (V_{tot}) was calculated from the amount of nitrogen vapor adsorbed at a relative pressure close to unity, on the assumption that the pores are then filled with liquid nitrogen. An average pore diameter $d_{\mathrm{ads}}(=4V_{\mathrm{tot}}/S_{\mathrm{BET}})$ was derived from the total pore volume and S_{BET}, assuming cylindrical geometry.

Electron microscopic observations were made on a JEOL 5500 apparatus.

3.5. Elution chromatography

Ten microliter samples of 1 M solutions of acetone (void marker, t_0) or solutions of either phenytoin or other compounds were injected into the acetonitrile eluent. The HPLC columns were 50 mm × 3 mm and 100 mm × 3 mm, respectively. The measurements were done with a Perkin-Elmer Series 200 chromatograph with the column thermostat set at 32.4 °C. The peaks were detected with the UV–vis detector set at 220 nm. All chromatograms were measured in triplicate and showed good reproducibility.

4. Results and discussion

Molecularly imprinted polymers for the anticonvulsive drug phenytoin (Fig. 2) have been prepared from the same polymerization mixture in two formats: as bulk MIP and as silica–MIP composite. The obtained materials have been characterized by electron microscopy and nitrogen adsorption isotherms. HPLC columns have been packed with both materials and compared. The comparison has been based on the nonlinear model of chromatography. To show the applicability of this model, its predictions were checked with respect to column length and flow rate. In these control experiments only the composite MIP was used.

We have used, in this study, the same hydrophobically modified Kromasil silica particles as Mosbach and co-workers [8]. This choice has been based on the careful selection principles of that work and on our goal to show that the method described there applies to different MIPs, just as predicted in that work.

4.1. Physical characterization of the silica–MIP composite

The physical characterization consisted of electron microscopic observation of the particles and nitrogen adsorption isotherm measurements.

4.1.1. Electron microscopy

The electron microscopic observations (data shown as supporting material) agree very well with those of Mosbach and co-workers [8]. They show that the composite particles are clean spheres without any excess polymer adhering to their surface. The size of the spheres is the same as that of the native Kromasil packing. We have also made experiments leaching out the silica base material from the composites by hydrofluoric acid and found that the remaining polymer still formed spheres of unchanged size. This proves that the polymer had formed a continuous phase when prepared in the pore network of the silica and not just a large number of independent plugs in different pores.

4.1.2. Nitrogen adsorption

Fig. 3 shows the nitrogen adsorption isotherms of the Kromasil reversed phase silica base material, the silica–MIP composite, the non-imprinted silica-NIP composite and the bulk MIP. Figs. 4 and 5 show the calculated differential pore distributions in the micropore and mesopore range, respectively. Table 1 summarizes the calculated parameters of the studied materials.

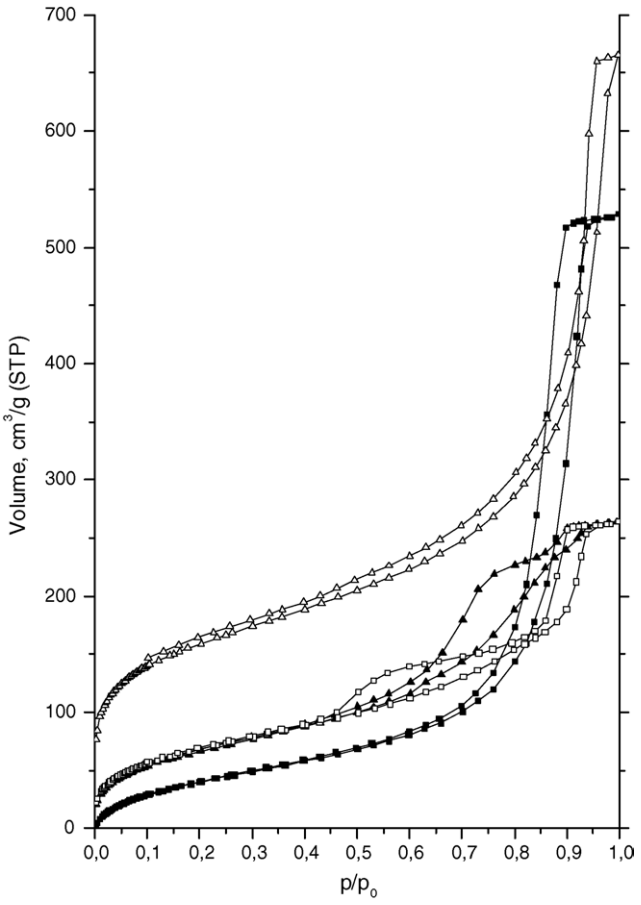

Fig. 3. Low temperature nitrogen adsorption isotherms of the samples: (■) Kromasil; (□) silica-NIP; (▲) silica–MIP; (△) bulk MIP.

When the data of Table 1 are compared with those of Mosbach and co-workers [8] one finds good agreement for all materials. A closer analysis of the isotherms reveals further properties of these packing materials.

All the samples exhibit Type IV [21] isotherms. The Kromasil and the bulk MIP sorbents have hysteresis loops of Type H1, which is often obtained with agglomerates or compacts of spheroidal particles of fairly uniform size and array. The hysteresis loop of type H2 shown by the two composite samples is typical when pore size and shape are not well defined, i.e., the desorption branch is dependent on network-percolation effect.

Table 1
Structural parameters derived from the nitrogen adsorption isotherms

Sample	S_{BET} (m^2/g)	V_{tot} (cm^3/g)	d_{ads} (Å)
Bulk MIP	540	1.0	76
Kromasil	168	0.8	194
Composite NIP	247	0.4	66
Composite MIP	244	0.4	67

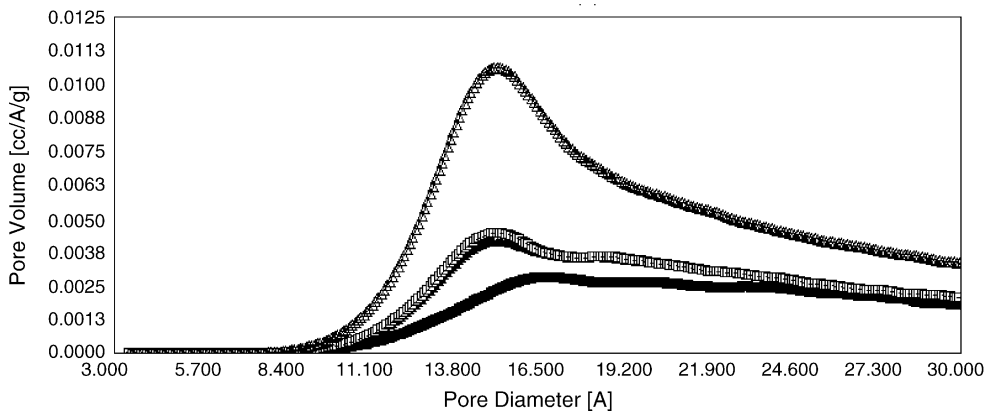

Fig. 4. Differential pore size distribution in the micropore range computed by the SF method. Symbols, see Fig. 3.

The Kromasil sample shows a more uniform pore size than the composite sorbents. The polymer in the composites modifies the shape of the isotherm. In the lower relative pressure range isotherms of the composites run above that of Kromasil as a sign of increased microporosity, while from the comparison in the higher relative pressure range it can be concluded that larger pores diminish when the polymer is deposited in Kromasil.

Data in Table 1 as well as the micropore size distribution obtained by the semi-empirical SF method (Fig. 4) show that the two composite samples are very similar and their surface area is larger than that of their silica templates. The reduction of the total pore volume stems from the mesopore region as it is reflected by the increased surface area and the narrowing of the mean porosity from 194 Å to 66–67 Å. The 17 Å peak recorded with the silica base material shifts to 15 Å with both composites.

In the mesopore range, the two composite samples exhibit different pore size distributions. The wide peak of the silica base material at 164 Å is separated into two peaks appearing at 39 and 163 Å in the silica-NIP composite while it is shifted to a single peak at 66 Å in the silica–MIP composite. There was no porosity detected in the composites above 220 Å.

The bulk MIP has a peak maximum in the SF distribution at 15 Å, i.e., identical with those of the composites. In the BJH distribution its maxima are at 35.5 and 327 Å.

These data show together that in the composite materials the original pores of the Kromasil base material are filled up partly with polymer. The polymer in the composites shows similar micropores to the bulk MIP. All this is in agreement with the expected structure of the composite.

4.2. Chromatographic behavior of the silica–MIP composite

4.2.1. Effect of column length

We have investigated the effect of the column length on peak shapes. The same amount of phenytoin was injected on two phenytoin imprinted silica–MIP composite columns of different length (50 and 100 mm, respectively) but identical i.d. (3 mm). The chromatograms are shown in Fig. 6. The dashed line shows the chromatogram on the 100 mm column on a twofold compressed horizontal scale. The fit to the tail of the chromatogram on the 50 mm column is nearly perfect. This observation is in agreement with the prediction of nonlinear chromatography based on Eq. (1) and contradicts the peak broadening model of linear chromatography. In linear chromatography both peaks should be symmetrical and their respective axes of symmetry should coincide after the twofold compression of the peak obtained with the longer col-

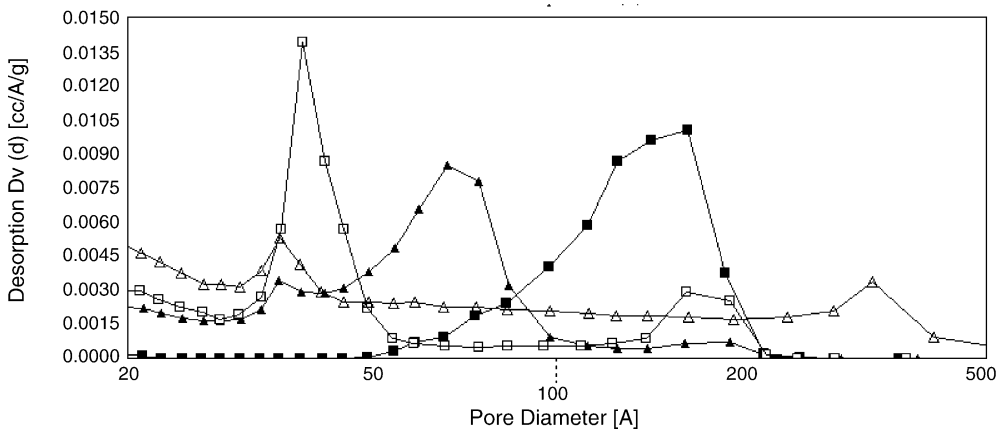

Fig. 5. Differential pore size distribution in the mesopore range computed from the desorption branch of the isotherm by the BJH method. Symbols, see Fig. 3.

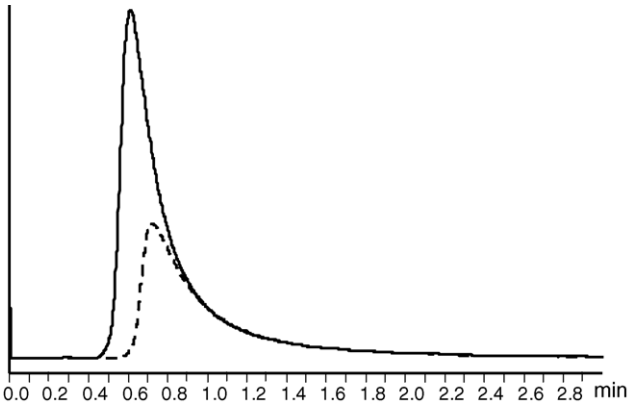

Fig. 6. The effect of column length. Silica–MIP composite columns: 50 mm (continuous line) and 100 mm (dashed line) length, respectively. The horizontal scale has been compressed twofold for the longer column. Acetonitrile eluent flow rate 1 ml/min; injected concentration: 1 mM phenytoin.

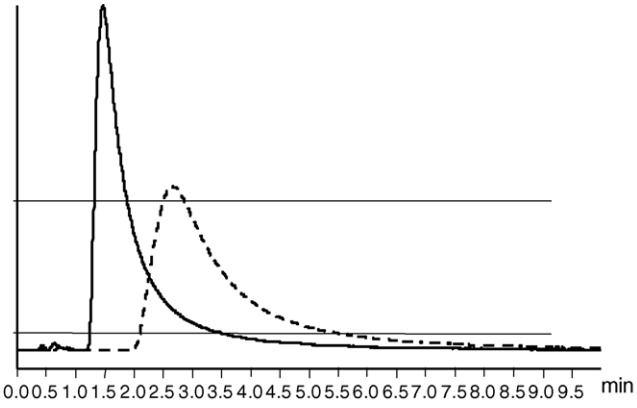

Fig. 8. Phenytoin chromatograms on a conventional (bulk) MIP column (dashed) and a silica–MIP composite column (continuous). The two horizontal lines in the figure show the range used for plotting Fig. 9. Flow rate 0.5 ml/min; injected concentration: 1 mM phenytoin.

umn. Clearly, these predictions of linear chromatography fail here.

4.2.2. Effect of flow rate

Fig. 7 shows the phenytoin peaks on the 50 mm composite column at different flow rates in the quite wide range of 0.05–1.0 ml/min. Note that the horizontal axis in this figure is not time, but elution volume as proposed in connection with Eq. (2a). The close fit of the tails of the chromatograms proves that the shape of the tail is almost independent of flow rate. This is again in good agreement with nonlinear theory and contradicts linear chromatographic theory. In linear chromatography, the peaks should be symmetrical and their respective axes of symmetry should coincide on this plot. The width of the peaks should be different due to the flow rate dependence of the plate height. These predictions of linear chromatography are not satisfied here.

The ascending part of the chromatograms shows a marked flow rate dependence. The front of the chromatogram is sharp at low flow rate and becomes more diffuse at higher flow rate. This is a well-known phenomenon in nonlinear chromatography and has been attributed to the usual (kinetic) band broadening effects. In analytical applications, the sharpness of the front is important in the separation of the template from other substances which are less retained on the MIP.

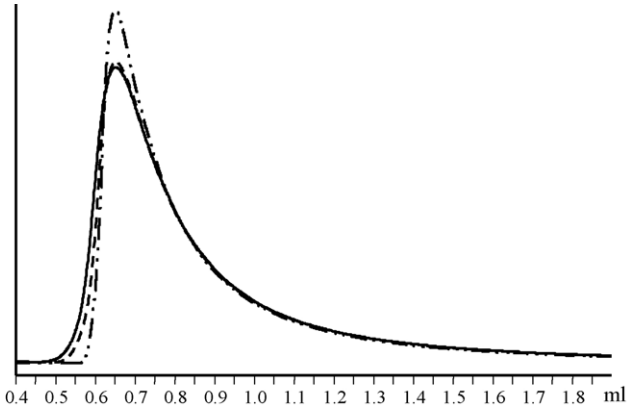

Fig. 7. The effect of flow rate. Silica–MIP composite column (50 mm × 3 mm). Flow rate: 0.05 (-···-); 0.5 (dashed) and 1 (continuous) ml/min. Injected concentration: 0.5 mM phenytoin.

4.2.3. Comparison with the bulk (conventional) MIP

Fig. 8 shows phenytoin chromatograms on the conventional bulk MIP column and the silica–MIP composite column. All other parameters except for the type of packing material are identical. The lower retention on the composite is obvious and the corresponding peak is also narrower (if measured at half height). It is interesting to compare these peaks on the assumption that the MIP in the pores of the silica is identical to the bulk MIP at least in terms of its adsorption isotherm. With this assumption there are still important differences between the two columns. In the composite column, the MIP is only present in the pores of the silica and it does not occupy even these pores fully. One has to consider also that a substantial fraction (about one third) of the composite column's volume is occupied by silica which behaves here as an inert, space-filling material. (It has been checked that phenytoin elutes at t_0 from the Kromasil base material with the acetonitrile eluent.)

To test the nonlinear chromatographic theory under the assumption of the identity of the isotherms of the bulk MIP with that in the silica matrix, we have replotted the tails of the chromatograms by subtracting their respective t_0 values and then dividing these reduced time values of the two chromatograms at all absorbance (or actually concentration) values with each other. According to Eq. (3) (derived from nonlinear chromatographic theory) this ratio should be constant. Fig. 9 shows that the ratio is indeed nearly constant in a substantial concentration range.

This result shows that both the relative position and the relative shape of the template peak on the composite column against the bulk MIP column is mainly determined by the quantities occurring in Eq. (3). This conclusion contradicts the expectation that the uniform size and spherical shape of the composite is mainly responsible for any of these changes (e.g., for the change of the ratio of peak width to retention time). It is also reasonable to expect that the same conclusion would be reached

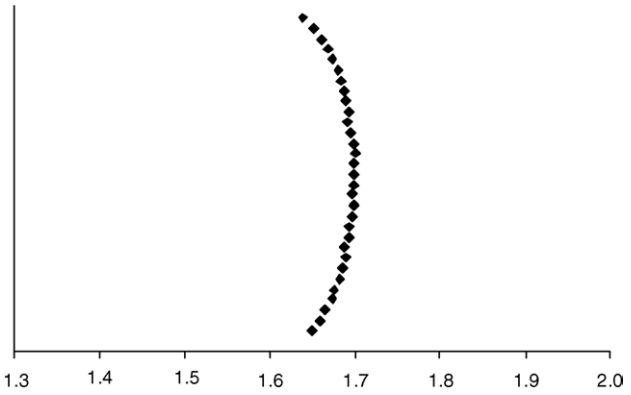

Fig. 9. Comparison of the rear (descending) parts of the chromatograms in Fig. 8 (between the two horizontal lines of that figure). The reduced retention times at different concentration levels have been divided by each other. The horizontal axis shows this ratio, the vertical scale is proportional to the absorbance.

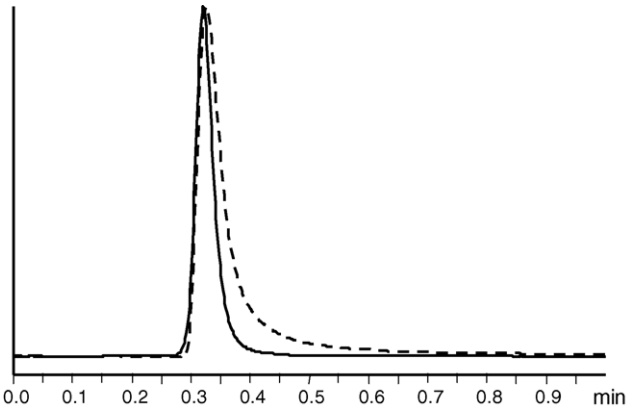

Fig. 10. Retention of ketoprophen (dashed line) on the Kromasil C4 column compared to acetone (continuous line). Flow rate of the acetonitrile eluent 1 ml/min; injected concentration: 1 mM ketoprophen. The peaks have been normalized to the same height.

not only for the template but also for other substances that are retained by the MIP columns and which are also having appreciable nonlinearity in their isotherms.

The result shown here is not saying, however, that making uniformly sized spherical MIP composites is useless chromatographically. The reduction of relative peak width is not an optimization goal for its own sake in chromatography. It is more important, for instance, to compare resolutions on different columns. One should consider that the kinetic dispersion is probably less on the composite column if for nothing else then for the smaller particle size. This may contribute to a sharper peak front and thus to better resolution. It is also important that the composites produce more stable packings and lower flow resistance than the irregular bulk MIP material.

4.2.4. Inertness of the support material of the composite

The MIP studied here differs very much in its scope of use from that investigated by Mosbach and co-workers [8]. That one was a MIP for the separation of two enantiomers. Chiral separations are unique in that they are often applied to pure solutions of the two enantiomers. The phenytoin MIP on the other hand exemplifies the application of MIPs to reasonably complex samples like blood plasma [22]. In such applications it is important to check the inertness of the base material used for supporting the MIP in the composite.

When making the MIP composites described here one does not attempt to make the surface of the support material unavailable for adsorption. Therefore, one has to check if the support material, the reversed phase silica in our case, may be considered sufficiently inert. One may think that this is not a problem with 100% acetonitrile as eluent. It turns out, however, that this is not the case. Fig. 10 shows, for example, that ketoprofen is slightly retained and shows substantial nonlinearity on the Kromasil C4 support material even in acetonitrile. Thus, the retention of ketoprofen on the composite would be due to both the support and the MIP. It had been proposed that the composites might be prepared in other porous supports than reversed phase silica [8]. When doing so one should be aware of similar or even bigger effects than shown in Fig. 10. For example, Fig. 11 shows the retention of ketoprofen on a polymer based HPLC packing (Asahipak ODP 50) in acetonitrile. The retention of ketoprofen is even higher on this packing than on the Kromasil material.

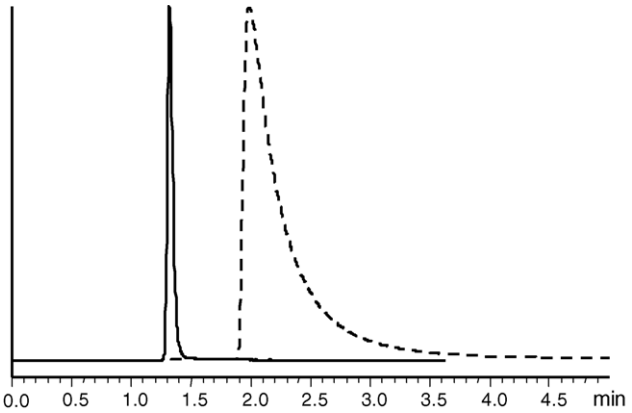

Fig. 11. Retention of ketoprophen (dashed line) on the Asahipak ODP 50 column compared to acetone (continuous line). Flow rate of the acetonitrile eluent 0.8 ml/min; injected concentration: 1 mM ketoprophen. The peaks have been normalized to the same height.

5. Conclusion

In this paper, we made a first attempt to systematically gather experimental data about the chromatographic behavior of silica–MIP composites. The dependences of the peak shapes on column length and flow rate contradict expectations based on the linear chromatographic model. Therefore, one should not use the linear chromatographic model to compare the chromatographic behavior of bulk MIPs and silica–MIP composites, respectively. The chromatographic properties of both types of MIP appear to be primarily determined by the nonlinear isotherm and not so much by the geometry of the particles. Thus, the main disadvantage of MIPs in chromatography, i.e., the strong tailing of the peaks and the consequent moderate peak capacity can hardly be improved by better particle geometry. This observation does not

deny, however, other advantages of the composites nor does it exclude other substantial improvements of MIP-s in chromatography, e.g., due to improved chemistry.

Useful chromatographic applications for the existing MIPs, like chiral separations, on-line solid phase extraction, etc. require guiding principles to prepare novel MIP stationary phases and to optimize separations. In recent years, a large amount of effort has been invested by several research groups in improving the chromatographic characteristics of MIPs. There has been, however, a lack of suitable methods for comparing the chromatographic behavior of different MIP stationary phases and for optimization of their use. Most researchers have compared the phases on the basis of linear chromatography, i.e., by using retention times determined from the peak maximum's position and theoretical plate numbers determined from the relative peak width (ratio of retention time to peak width at half height). The present work has shown that this procedure is inadequate. Another approach, represented only by a few groups, was to apply nonlinear chromatographic theory to MIP columns. This approach led, however, to serious complexities when a full agreement between theory and experiment was sought. The approach presented in this paper has been more relaxed: only certain consequences of nonlinear theory have been used for testing the experimental results. It has been found that this approach serves as a reasonable basis for comparing different MIP stationary phases and also for estimating the effects of varying column length and flow rate on the changes of peak shape.

Acknowledgements

The authors are indebted to EKA Chemicals (Sweden) for donation of the Kromasil material, Dr. K. Marthi for the electron microscopic measurements, Prof. R. Ohmacht for help with column packing, to Dr. T. Pap for help in polymer synthesis; to Institut de Recherches Internationales Servier (I.R.I.S.), France and to OTKA (T048912) and NKFP (3/043/2001) for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2005.09.015.

References

[1] B. Sellergren (Ed.), Molecularly Imprinted Polymers: Man-Made Mimics of Antibodies and their Application in Analytical Chemistry, Elsevier, Amsterdam, 2001.
[2] J.O. Mahony, K. Nolan, M.R. Smyth, B. Mizaikoff, Anal. Chim. Acta 534 (2005) 31.
[3] V.B. Kandimalla, H.X. Ju, Anal. Bioanal. Chem. 380 (2004) 587.
[4] B. Sellergren, K.J. Shea, J. Chromatogr. A 690 (1995) 29.
[5] E. Turiel, A. Martin-Esteban, Anal. Bioanal. Chem. 378 (2004) 1876.
[6] B. Sellergren, J. Chromatogr. A 906 (2001) 227.
[7] F. Chapuis, V. Pichon, M.C. Hennion, LC–GC Eur. 17 (2004) 408.
[8] E. Yilmaz, O. Ramstrom, P. Moller, D. Sanchez, K. Mosbach, J. Mater. Chem. 12 (2002) 1577.
[9] A.G. Mayes, K. Mosbach, Anal. Chem. 68 (1996) 3769.
[10] J.F. Wang, P.A.G. Cormack, D.C. Sherrington, E. Khoshdel, Angew. Chem. Int. Ed. 42 (2003) 5336.
[11] C. Sulitzky, B. Ruckert, A.J. Hall, F. Lanza, K. Unger, B. Sellergren, Macromolecules 35 (2002) 79.
[12] M.M. Titirici, A.J. Hall, B. Sellergren, Chem. Mater. 14 (2002) 21.
[13] F.A. El-Toufaili, A. Visnjevski, O. Bruggemann, J. Chromatogr. B 804 (2004) 135.
[14] G. Guiochon, S.G. Shirazi, A.M. Katti, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, Boston, 1994.
[15] P. Sajonz, M. Kele, G.M. Zhong, B. Sellergren, G. Guiochon, J. Chromatogr. A 810 (1998) 1.
[16] Y.B. Chen, M. Kele, P. Sajonz, B. Sellergren, G. Guiochon, Anal. Chem. 71 (1999) 928.
[17] K. Miyabe, G. Guiochon, Biotechnol. Progr. 16 (2000) 617.
[18] F. Lanza, A.J. Hall, B. Sellergren, A. Bereczki, G. Horvai, S. Bayoudh, P.A.G. Cormack, D.C. Sherrington, Anal. Chim. Acta 435 (2001) 91.
[19] A. Saito, H.C. Foley, AIChE J. 37 (1991) 429.
[20] E.P. Barrett, L.G. Joyner, P.P. Halenda, J. Am. Chem. Soc. 73 (1951) 373.
[21] S. Gregg, K. Sing, Adsorption, Surface Area and Porosity, Academic Press, London, 1982.
[22] A. Bereczki, A. Tolokan, G. Horvai, V. Horvath, F. Lanza, A.J. Hall, B. Sellergren, J. Chromatogr. A 930 (2001) 31.