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Application of molecularly imprinted polymers in supercritical fluid chromatography

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Abstract

Molecularly imprinted polymers (MIPs), for the templates free base racemic propranolol and the L-enantiomer of phenylalanine anilide (L-PA), were investigated as stationary phases in supercritical fluid chromatography (SFC). Large retention differences were observed on the propranolol MIP for both the template molecule and the structural analogue metoprolol compared to that observed on the corresponding blank polymer. Mobile phase composition and solute concentration were found to affect this retention behaviour. The phenylalanine anilide MIP (L-PA MIP) was found to be enantioselective in SFC with stronger retention observed for the template enantiomer. Throughout the study, characteristic imprinting peak shapes for the stronger retained template molecule were observed for both MIPs examined. After a number of days under supercritical fluid conditions, the performance of the photochemically initiated L-PA MIP was found to significantly deteriorate whereas the thermally initiated propranolol MIP revealed only small changes in its separation performance after a long term of operation. The separation behaviour of these two MIPs in SFC was compared with results obtained on the same columns in high-performance liquid chromatography (HPLC) both before and after their application in SFC.

Keywords: Chiral stationary phases, SFC; Molecularly imprinted polymers; Propranolol; Phenylalanine anilide

1. Introduction

Over the last decade, packed column SFC has emerged as an interesting alternative to normal-phase liquid chromatography for the analysis of pharmaceutical substances [1,2]. Advantages of SFC include faster equilibration times, fast and efficient separations as well as more environmentally acceptable carbon dioxide mobile phases, compared to those traditionally applied in normal-phase HPLC [1]. To obtain a successful and optimised separation, the selection of a suitable stationary phase is a crucial point. Stationary phases normally adopted in SFC have been predominantly silica gel based including silica itself, diol and aminopropyl derivatised silica for achiral separations or polysaccharide derivatised silica, Chiralcel OD and Chiralpak AD, for separat-

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ing enantiomers [1,3]. Since the user still becomes confronted with common chromatographic problems such as coeluting compounds or difficulties in improving the selectivity for structurally related molecules when employing the above phases, it is of interest to thoroughly investigate the potential of alternative separation materials in SFC.

Molecularly imprinted polymers (MIPs) [4–6] are impressive new materials that can potentially be an alternative for SFC. MIPs are very suitable as stationary phases in separation science and have already proven their capability in HPLC [7–12], thin layer chromatography (TLC) [13] capillary electrophoresis (CE) [14] and capillary electrochromatography (CEC) [15–17] for separating structural analogues but to a greater extent for separating enantiomers. Besides their evident potential as stationary phases in chromatographic techniques, the usefulness of MIPs has also been demonstrated in sample pretreatment by solid-phase extraction (SPE) [18–20] and in analyte recognition studies employed as affinity assays [15,21,22].

The technique of molecular imprinting is based on a simple principle, in which a polymer is assembled around an analyte of interest, called a template to create a polymer with potentially specific interaction sites for that template molecule. Synthetic parameters like the amount or kind of monomer employed, crosslinker or porogen as well as the temperature applied during the polymerisation process govern the properties of the resultant MIPs in terms of their overall stability, porosity and quality as well as accessibility of the generated recognition sites. MIPs are characterised as having special recognition sites with predetermined selectivity for the template. Furthermore, they are robust with a high mechanical strength, resistant to elevated temperatures or pressures and stable in the presence of extremes of acid, base, metal ion or organic solvent. These are attractive features and clearly indicate the continued potential of MIPs in the field of separation science. Nevertheless, important drawbacks limiting their use in analytical LC include the slow mass transfer kinetics leading to excessive band broadening [9,10,23–25]. Mass transfer limitations may be caused by slow interactions at the binding sites and/or slow diffusion through the polymer network [23]. Therefore, it is of interest to assess separation systems allowing potentially faster diffusion rates.

We have, therefore, assessed the combination of MIPs and SFC, which, to our knowledge, has not been investigated to date. It was decided to start this SFC investigation on MIPs whose imprinting properties have been well documented earlier. The L-PA MIP was chosen since it had been used as a model system to thoroughly investigate the chromatographic properties of MIPs in HPLC [9,10,25] including physico-chemical frontal analysis studies on the relation of mass-transfer kinetics and the quality of recognition sites [23,24]. Propranolol, however, is a substance of pharmaceutical interest since it belongs to the group of β-adrenoreceptor blocking agents, which are well known for the treatment of hypertension or the prevention of angina. The imprinting behaviour of propranolol MIPs has already been reported in affinity assay [15,21], SPE [18] and CEC studies [12,15,16].

In this paper we describe the application of L-PA and propranolol MIP stationary phases in SFC. The impact of parameters such as mobile phase composition, temperature, pressure and loadability on the recognition properties of the MIP compared to the corresponding blank polymer is investigated. The SFC separation behaviour of both MIPs was compared to that in HPLC on the same column before and after application in SFC. Furthermore, the limitations and problems of this first application of MIPs as stationary phases in SFC are addressed and possible measures for solving these outlined.

2. Experimental

2.1. Synthesis and materials

The propranolol MIP was thermally initiated at 60°C using toluene as porogen, whereas the L-PA MIP was photochemically initiated at 15°C using methylene chloride as porogen. The precise synthetic conditions for the propranolol MIP [21], L- and D-phenylalanine anilide (L,D-PA) [26] and the L-PA MIP [23] are described elsewhere. The corresponding blank polymer was synthesised analogously to the respective MIP but in the absence of template molecules.

Carbon dioxide (4.8 grade) was the primary component of the mobile phase and purchased from AGA (Lidingö, Sweden). Methanol, (MeOH/gra-

dient grade for HPLC) and acetic acid (HOAc) were obtained from Merck KGaA (Darmstadt, Germany). Chloroform and acetonitrile (ACN) (both of HPLC grade) were purchased from Rathburn Chemicals Ltd. (Walkerburn, UK). D,L-propranolol hydrochloride was purchased from Sigma Aldrich (Steinheim, Germany), metoprolol succinate was received from the Department of Medicinal Chemistry, AstraZeneca R&D Mölndal, Sweden.

2.2. Instrumentation

SFC separations were carried out using the HP G1205A SFC instrument (Hewlett-Packard, Wilmington, DE, USA), which was equipped with dual reciprocating pumps, a multiple-wavelength UV detector, programmable oven and an autosampler. Flow-rate, fluid composition, temperature, detection wavelength and column outlet pressure were independently controlled by the system software.

HPLC separations were performed with a L-7100 pump from Merck-Hitachi (Darmstadt, Germany), a 717 plus autosampler from Waters (Millipore Corporation, Milford, MA, USA) and a 206 PHD detector (Linear Instruments Corporation, Reno, NE, USA). The data was processed with the software AccessChrom (Perkin-Elmer Nelson Systems, Cupertino, CA, USA).

2.3. Chromatography

The propranolol MIP and the corresponding blank polymer were packed into 150×2.1 mm stainless steel tubes (Skandinaviska GeneTec AB, Kungsbacka, Sweden) using a high pressure slurry packing procedure with an air-driven pump (Shandon Southern Products Ltd., Astmoor, UK). The polymers which had particle sizes smaller than 25 μm were suspended in a chloroform–acetonitrile mixture (70:30, v/v), ultrasonicated for 5 min and the slurry filled into the reservoir attached just above the respective column. A packing pressure of 200 bar was applied and completed with acetonitrile as packing solvent. The packing of the L-PA MIP is described elsewhere [23].

The SFC conditions were as follows (except stated otherwise): flow-rate, 2 ml/min; oven temperature, 50°C; outlet pressure, 150 bar; mobile phase, CO_2, with variable modifier composition added (the exact modifier compositions are described in each Figure caption or text passage, respectively); UV-detection was performed at either 230, 260 or 292 nm. Stock solutions (1 m*M*, 5 m*M* and 10 m*M*) were prepared for PA in ACN and β-blockers in MeOH and 10-μl samples injected. The LC conditions were as follows (except stated otherwise): flow-rate, 1 ml/min; ambient temperature; mobile phase, ACN/HOAc/H_2O (92.5:5:2.5, v/v/v); UV-detection was performed at 260 or 292 nm and 10-μl samples of the above stock solutions injected.

3. Results and discussion

3.1. L-PA MIP

3.1.1. HPLC application prior to application in SFC

The material was first assessed in HPLC for obtaining information on the existence of recognition sites of the MIP investigated. The primary MIP evaluation was carried out using a mobile phase composition of ACN/HOAc/H_2O (92.5:5:2.5, v/v/v), since the usefulness of this method has already been demonstrated in several cases [5,23]. The separation properties of the L-PA MIP for PA solutes under the above MIP standard HPLC conditions are shown in Fig. 1A. The template effect is unequivocally demonstrated by the observed elution order of the PA enantiomers, their peak shape and the significant difference compared to the retention obtained on the blank polymer (Fig. 1C). It has to be noted that the illustrated chromatogram (Fig. 1A) does not represent an optimised HPLC separation, the optimisation of this PA enantiomeric separation employing mixtures of organic solvent and aqueous buffer as mobile phases is described elsewhere [9].

3.1.2. Application in SFC

Since recognition on this L-PA MIP was demonstrated in HPLC, the same column was then transferred to the SFC system. The chiral separation of PA solutes employing the L-PA MIP in SFC at 50 and 100°C is shown in Fig. 2A and C, respectively. Similar to the HPLC findings, the imprinting characteristics described above for the L-PA MIP are also evident in SFC. This is particularly the case when comparing the elution profile of PA solutes on the

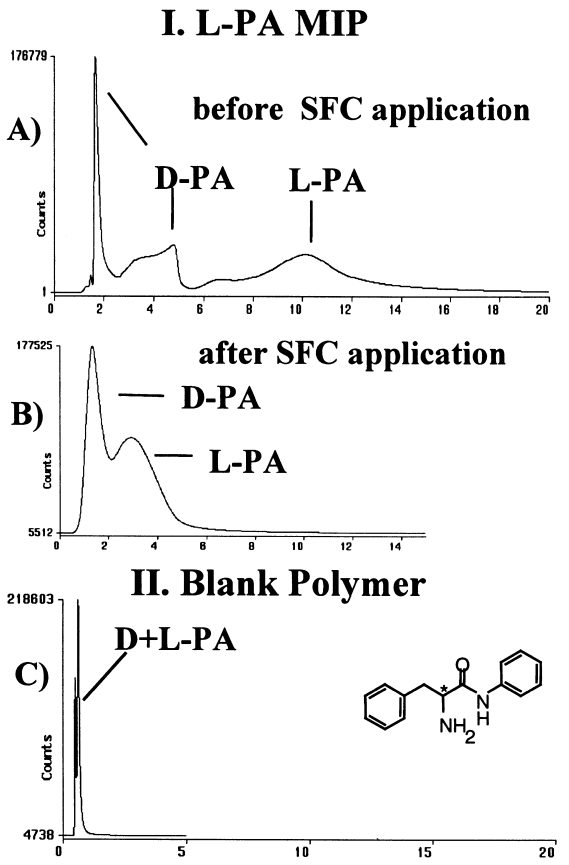

Fig. 1. Comparison of the HPLC separation performance of the L-PA MIP for racemic PA solutes before SFC operation (A) and (B) after. Retention behaviour of PA solute molecules on the blank polymer under identical conditions (C). Separation conditions were as follows: flow-rate, 1 ml/min; ambient temperature; mobile phase, ACN/HOAc/H_2O (92.5:5:2.5, v/v/v); detection, 260 nm; injection, 10 μl of a 5 m*M* stock solution.

L-PA MIP with that obtained on the corresponding blank polymer (Fig. 2D) under identical conditions. A rather high amount of modifier (40%, MeOH–HOAc 95:5, v/v) had to be added to CO_2, the main component of the mobile phase, before elution was obtained. In fact, mobile phases containing such high amounts of modifier cannot unconditionally be associated with supercritical conditions, since for methanol–CO_2 mixtures the critical temperature and pressure increase rapidly as the methanol concentration is increased [27]. However, under subcritical conditions there is no discontinuity of the fluidic properties and the mobile phase composition employed is still a one-phase system when the temperature is below the critical point [28]. For the sake of simplicity the conditions employed throughout this study will therefore be referred to as SFC-conditions [28]. Without adding any HOAc to the modifier or when using a lower percentage of modifier, the PA sample molecules elute with even broader signal widths and significantly longer retention times, at even higher flow-rates (3 ml/min). It was shown that neither higher temperatures (Fig. 2C, Table 1) nor elevated outlet pressures up to 200 bar (data not shown) had a favourable impact on either selectivity or peak shape of this enantiomeric separation. The imprinting properties of the L-PA MIP were additionally confirmed by investigating the retention of compounds such as acetone (void marker) or dihydropyridines as structurally nonrelated reference compounds. As expected, these compounds were eluted with the void time or just slightly retained indicating non-specific interactions with the polymeric backbone of the L-PA MIP. Similar to HPLC (Fig. 1C), peak splitting is observed for the PA injection on the blank polymer (Fig. 2D), presumably due to a heterogeneous distribution of ''recognition sites''. This finding is rather surprising and cannot be associated with poor packing of the stationary phase, since other solutes eluted as a single peak.

Unfortunately, the separation properties of the L-PA MIP significantly deteriorated after a couple of days of operation in SFC. This ageing process is illustrated in Fig. 2B by the significantly reduced enantioselective recognition of the L-PA MIP compared to the performance at an earlier date under identical conditions. This can be attributed to either time dependent changes in the swelling behaviour of the MIP due to an increased shielding of the recognition sites or to an extensive breakdown of the three-dimensional structure of the polymer under SFC conditions thus resulting in the destruction of recognition sites. The polymer has not totally collapsed since the L-enantiomer is detected as a shoulder on the D-enantiomer peak (confirmed by injection of single enantiomers). Since the gradual decline of the separation potential of the L-PA MIP was observed for the first time before the characterisation of this MIP in SFC was completely finished,

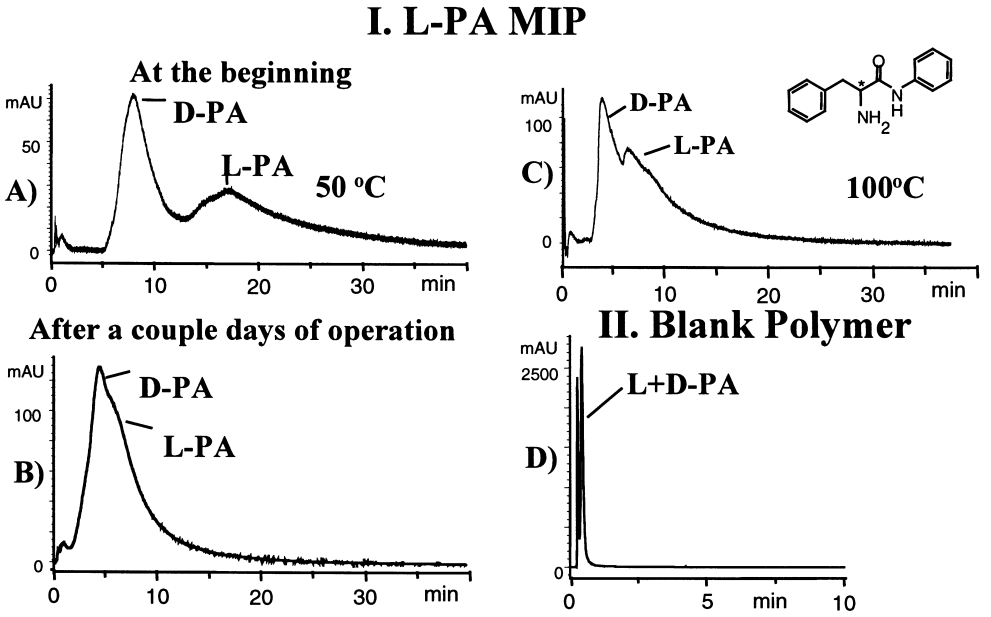

Fig. 2. Chiral SFC separation of PA solutes at 50°C (A) and 100°C (C) employing the L-PA MIP (A, C). Deterioration of the separation performance of the L-PA MIP after a couple days of operation in SFC (B). Retention behaviour of PA solute molecules on the blank polymer under identical conditions (D). Conditions for each case: flow-rate, 2 ml/min; oven temperature, 50°C (A, B, D) and 100°C (C); outlet pressure, 150 bar; mobile phase, CO_2, 40% modifier (MeOH/HOAc 95:5, v/v) added; detection, 260 nm; injection, 10 μl of a 5 m*M* stock solution.

the chiral separation depicted in Fig. 2A has not been optimised.

3.1.3. HPLC application after application in SFC

The L-PA MIP was subsequently examined in LC and it was found that the MIP selectivity had deteriorated compared to that prior to application in SFC (Fig. 1B, Table 1). Additionally, the back pressure was drastically increased and remarkably a better enantioselectivity was now observed in the HPLC mode. The performance of the L-PA MIP in HPLC was not stable, however. The column back pressure increased continuously and after a couple of hours the pressure limit of the pump was reached. This is a clear indication that the polymer had finally collapsed and thus, the possible creation of fines as a result of the destruction of recognition sites. It is known from previous studies that this type of polymer has a limited life time in processes containing repeated drying/swelling cycles [29].

3.2. Propranolol MIP

3.2.1. Application in SFC

In a second approach a propranolol MIP was tested for its imprinting properties in SFC. Typical MIP elution properties were observed for a modifier concentration of 30% of MeOH (Fig. 3, Table 1). It was remarkable to detect differences in retention for the free base propranolol and its salt analogue. Both substances were eluted as extremely broad peaks, but the HCl salt of propranolol was retained to a greater extent than the corresponding free base, which reflects the protonation state of the polymer relative to that of the solutes. Since the methacrylic acid fragments of the MIP are deprotonated to a considerable extent under the conditions employed, the protonated propranolol molecules interact stronger than free base propranolol solutes. No suitable explanation can be offered for the splitting of the propranolol salt peak (Fig. 3B) at this point. Approximately 20% of the injected propranolol sample

Table 1
Chromatographic evaluation of the propranolol MIP and the L-PA MIP in HPLC and SFC

HPLC	L-PA MIP		Blank	
mobile phase	k(L)	α	k	α*
ACN/H_2O/HOAc, 92.5:2.5:5	6.96	2.87 (24.3)	0.33 (0)	21.09
	1.21[a]	40.33	0.33 (0)	3.67
SFC	L-PA MIP		Blank	
modifier	k(L)	α	k	α*
40%(MeOH/HOAc 95:5), 50°C	41.5	2.19	0.75 (0.1)	55.4 (415)
total of 2% HOAc	11.9[b]	[c]	0.75 (0.1)	15.8 (119)
40%(MeOH/HOAc 95:5), 100°C	19.2	1.64		
HPLC		Prop. MIP	Blank	
mobile phase	Solute	k	k	α*
ACN/H_2O/HOAc, 92.5:2.5:5	FB Prop (10 mM)	1.77	0.22	8.05
	Prop salt (10 mM)	0.15	0.22	0.68
	Metop (10 mM)	1.1	0.22	5.00
SFC		Prop. MIP	Blank	
modifier	Solute	k	k	α*
30% MeOH	FB Prop (10 mM)	246	3.92	62.9
	Prop salt (10mM)	360 (1.75)	0.25	1441 (7.0)
	Metop (10 mM)	64.9	1.98	32.8
30%(MeOH/HOAc, 95:5) total of 1.5% HOAc	FB Prop (10 mM)[b]	114		
40%(MeOH/HOAc, 95:5) total of 2% HOAc	FB Prop (10 mM)[b]	36.1	1.04	34.7
30% (MeOH/HOAc, 90:10)	FB Prop (1 mM)	[d]	0.95	
total of 3% HOAc	FB Prop (2 mM)	87.5	0.95	92.1
	FB Prop (5 mM)	38.5	0.95	40.6
	FB Prop (10 mM)	31.5	0.95	33.2
	FB Prop (10 mM)[b]	49.9	0.95	52.6
	Prop salt (10 mM)	3.49	0.23	15.2
	Metop (10 mM)	10.0	0.56	17.9
40% (MeOH/HOAc, 90:10)	FB Prop (10 mM)[b]	14.1		

a: After SFC application; b: after a longer term of operation; c: not resolved; d: not detectable; FB: free base; Prop: propranolol; Metop: metoprolol; α: enantioselectivity k(L)/k(D); α*: MIP selectivity k(MIP)/k(Blank); k(L): capacity factor of L-DA; $k = (t_R - t_o)/t_o$; values in brackets correspond to the smaller part (peak area) of a splitted peak.

elutes very early as a sharp peak, whereas the remaining sample is strongly retained showing a very broad peak which is a typical imprinting shape.

The recognition capabilities of the propranolol MIP for structural analogues such as metoprolol is another typical feature of MIPs. In an previous affinity assay study, Andersson observed recognition for metoprolol on similar propranolol MIPs [21]. Hence, it was not surprising to detect strong retention of metoprolol on this propranolol MIP in SFC (Fig. 3C) due to the identical aminopropanol part of these two β-blockers. As a result of the presence of a considerable amount of apolar CO_2 within the mobile phase, polar ion–pair and hydrogen bonding interactions between the chemical functionalities of the aminopropandiol part of the β-

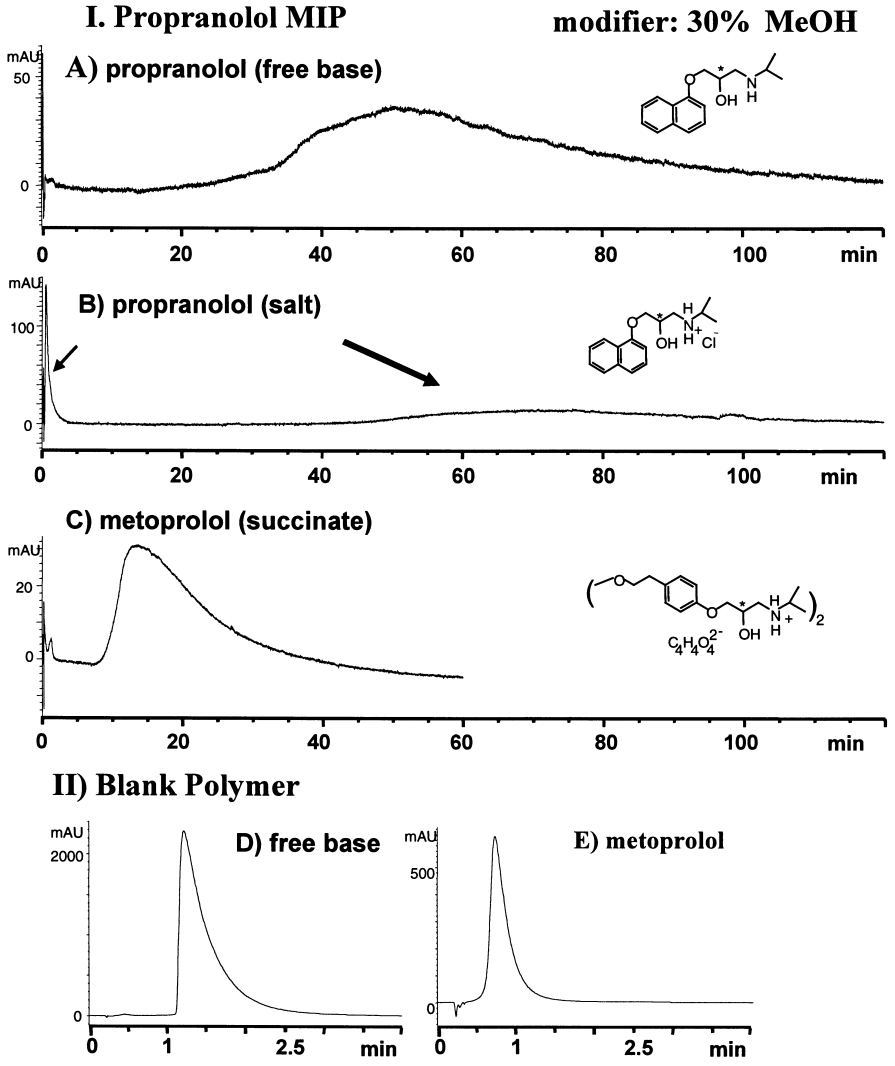

Fig. 3. Comparison of the SFC retention behaviour of β-blocker solutes on the propranolol MIP (A,B,C) and on the respective blank polymer (D,E). Conditions for each case: flow-rate, 2 ml/min; oven temperature, 50°C; outlet pressure, 150 bar; mobile phase, CO_2, 30% pure MeOH as modifier added; UV-detection, 230 nm; injection, 10 μl of a 10 m*M* stock solution.

blocker and the methacrylic acid fragments of the MIP are predominating. Differences in the residual parts of its structure are responsible for reduced interaction between metoprolol and the propranolol MIP which results in faster elution compared to the print molecule. No retention was found for acetone, the void marker or dihydropyridines (not shown) which is further proof for the observed imprinting effects.

The existence of imprinting properties is also confirmed by the retention behaviour of the investigated β-blockers on the blank polymer under the same conditions (Fig. 3D, E). Both free base propranolol and metoprolol were retained slightly stronger than acetone due to unspecific interaction with the polymeric backbone of the blank polymer and none of the peaks reveal any typical imprinting peak shape. These interactions are a little more pronounced for free base propranolol than for metoprolol or the salt of propranolol reflecting the

influence of the protonation state of the blank polymer versus the solutes.

The peak width and the retention times of the β-blocker solutes investigated were significantly reduced by adding acetic acid to the modifier (see Table 1). It is interesting to note that under these conditions more pronounced recognition is found for free base propranolol compared to the corresponding salt. This finding reflects, in a similar way to that observed above, the protonation state of the polymer relative to the solutes. The acetic acid added to the mobile phase shifts the association equilibrium of the MIP so that the methacrylic acid fragments are predominately protonated. As a result, the propranolol salt solutes, which are completely protonated due to the presence of the equimolar amount of strong acid (HCl), are considerably less recognised by the propranolol MIP compared to the recognition observed without adding acetic acid to the modifier. However, the amount of acetic acid added seemed not to be sufficient to completely protonate free base propranolol solutes, which are still considerably recognised by the propranolol MIP under these conditions. The acetic acid added to the mobile phase also provokes a strong decrease in the unspecific interactions on the blank polymer (Table 1, *k*-values for free base propranolol) and hardly any differences are observed in terms of retention between the β-blocker solutes employed.

The impact of the amount and type of modifier added to supercritical CO_2 on the retention behaviour of the print molecule free base propranolol is elucidated in Table 1 (lower part). The amount of acetic acid added to the modifier has a significant influence on the elution process, indicating a potential tool for controlling the retention behaviour of print solutes. This can be achieved by either increasing the percentage of modifier and keeping the amount of added HOAc at a constant level or vice versa. It is interesting to note that despite a higher total amount of HOAc (3%) the free base solutes are more retained compared to that using a total amount of 2% of HOAc due to differences in the overall polarity of the mobile phase. Therefore, it is conceivable to employ modifier gradients for obtaining faster elution and smaller peak widths for print solutes or structural analogues, respectively. It is also imaginable to investigate the impact of temperature or pressure gradients in SFC, since the influence of temperature and/or pressure on the separation performance has already been demonstrated in case of the L-PA MIP (Fig. 2). In future work, the exchange of HOAc with stronger acids has to be considered as a further possibility for obtaining better performance, since it was reported in two recent studies [3,30] that the overall separation performance in SFC strongly depends on the selection of a suitable acidic additive in the mobile phase. However, both studies were carried out on silica gel based stationary phases and it has to be clarified if the same will also apply to MIPs.

Retention dependence on the sample load is another feature of the propranolol MIP as illustrated in Fig. 4 and Table 1. A shift of the peak maximum towards shorter retention at increased sample loads indicates overloading of the high-energy binding sites. It is interesting to observe that under the conditions employed it was impossible to detect free base propranolol solutes at concentrations below 2 m*M*, since the height of this particular broad peak did not exceed that of the noise at low concentrations. A narrow, large and just slightly retained peak was detected on the other hand for the injection of propranolol at concentrations below 2 m*M* on the corresponding blank polymer, on which no retention dependency on the sample load was found.

In contrast to the L-PA MIP, no significant deterioration of the performance in SFC has been observed for the propranolol MIP to date despite a much longer operation time and several switches between SFC and HPLC. Ageing of the propranolol MIP has however occurred, as shown in Fig. 5 and Table 1. In both cases the same amount of free base propranolol was injected under identical conditions, but a different peak shape and increased retention is found for the sample injected at a later date. Although the observed changes were not as drastic as in the case of the L-PA MIP, since the propranolol MIP retains its recognition ability, it is clear that more stable material, providing long-term reproducibility, is required for routine applications of MIPs as stationary phases under SFC conditions. Therefore, thermal annealing of MIPs (i.e. thermal treatment subsequent to MIP polymerisation) should be included in the optimisation process, since the results of previous studies [24] indicate that this technique is a first step

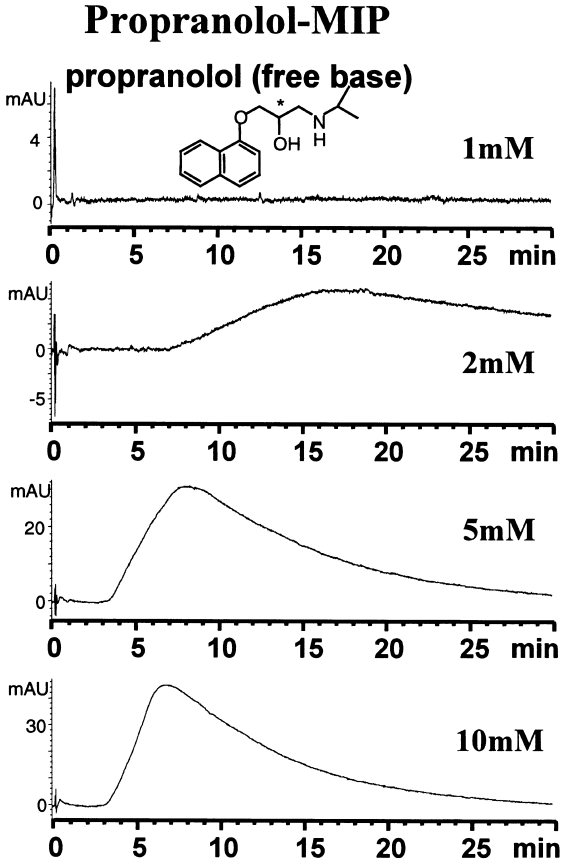

Fig. 4. Dependence of retention on the sample load of free base propranolol solutes employing the propranolol MIP in SFC. Conditions for each case: flow-rate, 2 ml/min; oven temperature, 50°C; outlet pressure, 150 bar; mobile phase, CO_2, 30% modifier (MeOH/HOAc 90:10, v/v) added; detection, 292 nm; injection, 10 μl of the stock solution concentration as indicated.

towards MIPs exhibiting improved long-term stability. Additionally, higher sample load capacity and accelerated mass transfer kinetics for enantioselective, high-energy recognition sites were other positive effects observed by thermal annealing [24].

3.2.2. Application in HPLC

The imprinting properties of the propranolol MIP were also evaluated in HPLC using the MIP standard HPLC conditions. No difference was found for the retention behaviour in HPLC before and after the application of this column in SFC. It was astonishing to find that the propranolol MIP hardly showed any imprinting behaviour under these conditions (Table 1). Broad, non-symmetric peaks with very small *k*-values were detected showing hardly any selectivity between metoprolol or propranolol solutes. This finding can still be attributed to a (slight) imprinting effect since the solutes were eluted with the void marker on the corresponding blank polymer, showing narrow gaussian peak shapes. It should be noted that whereas the L-PA MIP was synthesised by photochemical initiation, known to result in materials exhibiting superior chromatographic performance, the propranolol MIP was thermally initiated and results in material with poor performance at room temperature [9,25]. At elevated temperatures, however, the latter phases exhibit a comparable performance to that of the former phases at room temperature.

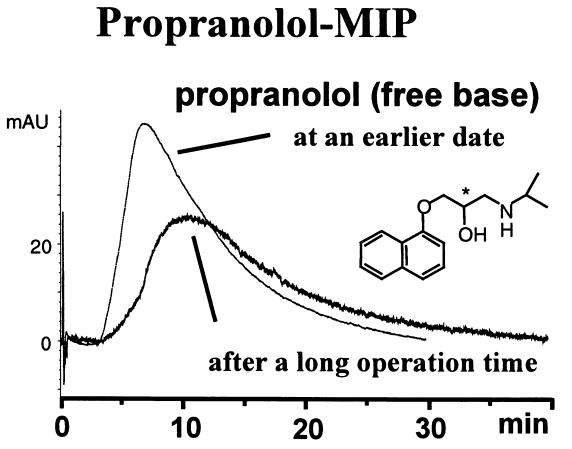

Fig. 5. Changes in peak profiles of free base propranolol solutes after long-term SFC operation on the propranolol MIP. Conditions for both cases: flow-rate, 2 ml/min; oven temperature, 50°C; outlet pressure, 150 bar; mobile phase, CO_2, 30% modifier (MeOH/HOAc 90:10, v/v) added; detection, 292 nm; injection, 10 μl of a 10 m*M* stock solution.

4. Conclusions

This work shows that in principle it is possible to employ MIPs as stationary phases in SFC. Typical imprinting properties were found such as the ability to differentiate between enantiomers or highest affinity for the print molecules together with characteristic imprinting peak profiles. Extremely broad peaks in combination with retention dependency on

the sample load remain a general problem with the application of MIPs in SFC. This implies, that mass transfer limitations due to slow interactions at the binding sites still prevail under the SFC conditions employed. In our studies, the impact of an acidic modifier additive (HOAc) on solute retardation was demonstrated. Retention times and peak widths can be considerably influenced by the HOAc concentration employing MeOH based modifiers. In the long run, peak performance has to be further improved which could possibly be achieved by employing gradients (modifier, temperature or pressure) and/or the exchange of HOAc with stronger acids.

Future efforts have to be directed towards the synthesis of MIPs exhibiting a higher long-term stability in SFC and a narrower site distribution. More detailed investigations on the effect of thermal treatment could be a reasonable starting point since in the present study a significantly increased long-term stability in SFC was found for the thermally initiated propranolol MIP in contrast to that of the photochemically initiated L-PA MIP. Thermal annealing of MIPs should be included in this optimisation process.

In a longer perspective, MIPs have a high potential as an attractive alternative to conventional stationary phases in SFC if it is possible to combine the following items: (i) highest affinity for the print molecules in combination with a possibly better accessibility of the recognition sites under SFC conditions, (ii) the improvements obtained by thermal annealing of MIPs and (iii) the realisation of long-term stabilized MIPs having a narrower site distribution of enantioselective, high-energy recognition sites.

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