Molecular Imprinting for Chiral Separations and Drug Screening Purposes Using Monolithic Stationary Phases in CEC

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Introduction

Molecularly Imprinted Polymers as Stationary Phases for Capillary Chromatography

Molecular imprinting technology [1, 2] has, in the last few years, become a well-established technique for preparing selective affinity media of predetermined selectivity. Molecularly imprinted polymers (MIPs) have been used in applications such as binding assays [3], solid phase extraction [4] and chromatography [1, 5] including capillary electrochromatography (CEC) [6].

Experimental

Adaptation of MIPs to Capillary Format and Applications in CEC

The adaptation of MIPs to CEC has recently been considered and different strategies for the preparation of capillary columns containing MIPs have been developed [6]. The preparation of super porous MIP monoliths (Figure 1) prepared *in situ* have shown interesting potential. The MIP is synthesised inside the capillary by photo- or heat-initiated polymerisation. To achieve good flow properties, the polymerisation is either interrupted prior to completion [7] or carried out using a porogenic agent compatible with the imprinting process [8]. Enantiomer separations of predetermined selectivity have been demonstrated in the CEC mode for *rac*-propranolol and *rac*-metoprolol using the (R)-propranolol and (S)-metoprolol enantiomers for the imprinting [7]. Also, enantiomer separation of the local anaesthetic drug ropivacaine was shown on columns imprinted with the (S)-enantiomer [8]. Another useful approach to adapt MIPs to capillary format where the MIP is held by an acrylamide gel support has been demonstrated [9]. Enantiomer separations of amino acids and amino acid derivatives have been obtained in this way. A third successful approach was recently reported in which the MIP was prepared as a coating on the capillary wall. Such columns were used to separate the enantiomers of dansyl phenylalanine in open tubular LC and CEC modes [10].

One great advantage of capillary based separation techniques is obviously the micro format. Since the concentration of imprint species in the pre-polymerisation mixture is high, the large scale preparation of MIP-based columns are more easily realised compared to the conventional LC columns frequently used. Typically, 10–100 mmol of imprint species are used for a MIP the size of a capillary column, which is about 1–10 µL. However, to be a true complement to the selectors commonly used for enantiomer separations, MIP-based stationary phases have to show improved separation efficiency.

Although, MIPs show a high degree of selectivity towards the imprint species, it has been shown that enantiomer separation of structural analogues to the imprint species are possible. In CEC, enantiomer separation of several β-adrenergic drugs using a MIP-based column prepared with (R)-propranolol as imprint molecule has been achieved [11], and enantiomer separation of structural analogues of the local anaesthetic ropivacaine was demonstrated using a MIP-column imprinted with (S)-ropivacaine [8]. We have noticed, however, that a very close structural resemblance to the imprint molecule is required in order to be able to separate the enantiomers of the analogues (Figure 2).

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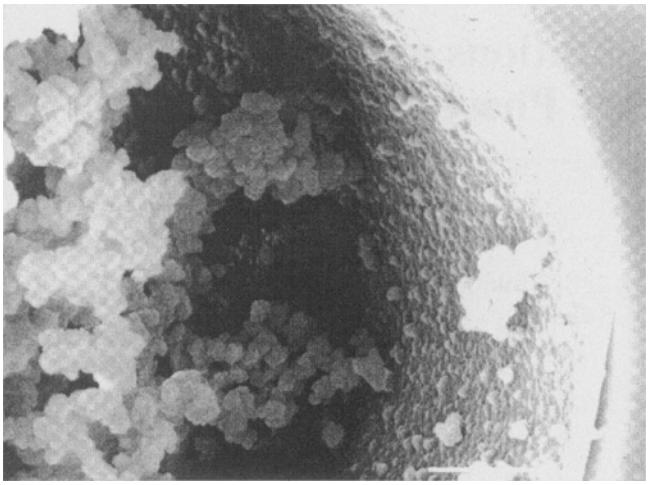

Figure 1

Scanning electron micrograph of a crossection of a super porous MIP filled capillary column. The MIP monolith is built by micron-sized globules surrounded by interconnecting super pores. Connections of the MIP to the capillary wall can also be seen.

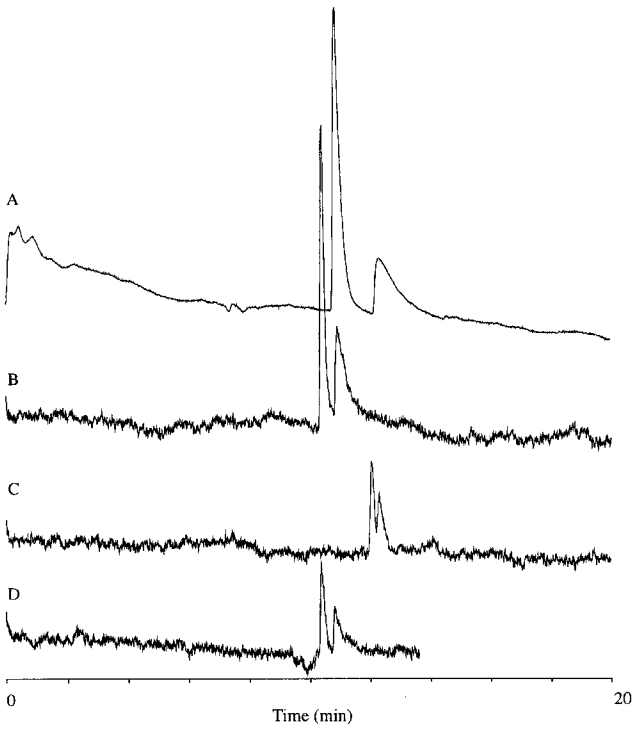

Figure 2

Example of the cross-reactivity which can be observed with MIP-based CEC systems. Enantiomer separations of (A) rac-propranolol, (B) rac-pindolol, (C) rac-prenalterol, and (D) rac-atenolol on a capillary column (length 90 cm, 75 μm inner diameter) with imprints of (R)-propranolol.

Discussion

Potential Screening Tool?

An MIP system might be useful for screening of chemical libraries. The MIP can be synthesised to selectively remove the chemical structure of choice or very close structural analogues. The capillary format for such an approach is beneficial not only in the possibility of preparing a great number of capillary columns of differing affinity and selectivity without excessive consumption of chemicals and imprint species but in the very small amount of sample needed.

Recently, the use of a MIP in LC to screen a combinatorial steroid library was reported [12]. In a similar preliminary study in our laboratory, MIP-based capillary columns were prepared using different β-adrenergic substances as imprint species and thus as target models for the screening. A target hit was determined either if an enantiomer separation was achieved, or by measuring of relative retention of the compounds on an imprinted column relative to that on a non-imprinted reference column. Using these approaches a chemical library of 11 compounds (10 amino alcohols (acebutolol, alprenolol, atenolol, metoprolol, pindolol, prenalterol, artenolol, labetalol, terbutaline, timolol) and tryptophane) were screened using an (R)-propranolol MIP as a target model. All compounds having the 2-hydroxy-3-(isopropylamino)-propoxy entity were determined as target hits. It should be mentioned that the (R)-propranolol, as expected, was determined as the strongest hit.

These preliminary results show the potential use of MIP-based capillary systems as a tool for screening of chemical libraries, although further research in this area is warranted and under way.

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