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Imprinted polymers as antibody mimetics and new affinity gels for selective separations in capillary electrophoresis

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Abstract

Methacrylate-based imprinted dispersion polymers could be prepared in situ in a fused-silica capillary as agglomerates (*ca.* 10 μm) of micrometer-sized globular particles, exhibiting antibody mimetic, molecular recognition properties. Thus, in one example, imprinted polymer particles selective for pentamidine (PAM), a drug used for the treatment of AIDS-related pneumonia, could be prepared in situ in the capillary. The retention could be varied predictably by changing the electrolyte pH. Thus, whereas no observable elution of PAM was achieved at near neutral pH, the PAM-selective capillary gave a retention time of 18 min for PAM and 7.8 min for benzamidine at pH 3.5, whereas the retention times were 6.6 and 6.1 min, respectively, with a reference capillary. Importantly, the electrolyte could by pumped hydrodynamically through the capillaries, allowing rapid phase changes and micro-chromatographic possibilities with high plate numbers.

1. Introduction

In capillary affinity gel electrophoresis a receptor molecule [1], e.g. a specific oligonucleotide sequence [2], or a protein [3] is bound to the gel or is dissolved in the electrolyte. The efficiency of this method together with the small sample amounts required are attractive for chiral drug bioanalysis. However, the acrylamide gels often suffer from poor stability, air bubble formation and limitations with respect to solvents. Moreover, gels or methods based on antibodies and other proteins suffer from poor stability and a complicated preparation scheme. Even if one can expect that some of these problems can be solved [4], the development of alternative polymeric phases for affinity capillary electrophoresis (CE) is worthwhile.

Previously, methacrylate-based polymers prepared by molecular imprinting around a template molecule [5] have been used as affinity stationary phases in HPLC for the successful and selective separation of several types of organic compounds, such as enantiomeric amino acid derivatives [6] and commercial drugs [7]. In general, this type of solid phase has shown high selectivity and good stability. We thought this type of technology would be useful in CE applications where high selectivity is required [8].

We now introduce imprinted polymer capillary electrophoresis (IMPCE) for analysis. In general, the gel material is prepared by template polymerization, which we found can be done directly in the capillary, whereby functional

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monomers, preorganized around a template molecule, are copolymerized with a cross-linking monomer. After washing, the gel capillary can be used for the separation of the template molecule and analogues thereof.

We tested the selectivity of different methacrylate-based polymers prepared by template-dispersion polymerization in various CE applications, the results of which are described below.

2. Experimental

2.1. Chemicals and equipment

The monomers ethylene glycol dimethacrylate (EDMA) and methacrylic acid (MAA) and the initiator azobisisobutyronitrile (AIBN) were obtained from Aldrich and purified before polymerization experiments according to standard procedures and D- and L-phenylalanine anilide (D- and L-PA) were synthesized as described previously [6]. Benzamidine (BAM) (Aldrich) and pentamidine (PAM) (Rhone-Poulenc Pharma, Helsingborg, Sweden) were used in their free-base form. The electrophoretic separations were carried out using a Beckman P/ACE System 2100 electrophoresis unit equipped with System Gold software. Capillaries (polyimido-coated fused silica; 25 cm × 100 μm I.D.) were obtained from Skandinaviska Genetech (Gothenburg, Sweden).

2.2. Preparation of imprinted polymer capillaries

Capillaries pretreated with trimethoxysilylpropyl methacrylate and blocked with hexamethyldisilazane were used in the polymerization procedure with L-PA as the template molecule [9]. AIBN (0.1 ml) was added to a mixture of monomers, EDMA (0.38 ml) and MAA (0.034 ml), and L-PA (18 mg) in 2.9 ml of cyclohexanol–dodecanol (4:1, v/v). The mixture was gently heated, purged with nitrogen and sonicated. One end of the silica capillary was allowed to dip into the solution and the other end was connected to an aspirator vacuum flask. After passage through the capillary of an appropriate amount (five drops) of the template polymerization mixture, both ends of the capillary were fixed in contact with the mixture and polymerization was carried out for 24 h at 60°C. The polymer remaining in the vials was washed with ethanol, dried and characterized.

The preparation of the capillary selective for PAM followed basically the same procedure, but was carried out in 2-propanol and employing capillaries that were used as supplied (see Section 2.1). Addition of MAA (0.05 mmol) to a solution of the free-base form of PAM (12.5 μmol) in a vial containing 2-propanol (0.28 ml) and EDMA (1.2 mmol) caused the formation of a precipitate that dissolved on addition of water (0.13 ml). The template polymerization was initiated by addition of AIBN (1.2 mg in 0.05 ml of 2-propanol), with polymerization in the capillary as above. The polymerization with BAM was carried out similarly.

2.3. Capillary electrophoresis

The polymer-containing capillaries were loaded into cassettes and acetonitrile–0.05 *M* potassium phosphate buffer (pH 2) (7:3, v/v) was pumped hydrodynamically through the capillary in order to wash out the template molecule and replace the 2-propanol used as solvent during the polymerization at a pressure of 1300 p.s.i. (1 p.s.i. = 6894.76 Pa). Both the current and UV baseline stabilized within a few hours. Injection of samples was carried out at 6 kV for 2 s (D- or L-PA, 0.33 mg/ml) or at 5 kV for 3 s (PAM, 1 m*M*, BAM, 5 m*M*). BAM was injected at a higher concentration owing to its low absorbance. Separations were carried out at ambient temperature with a separation voltage of 5 kV (unless stated otherwise). UV detection was used to record the electropherograms (254 nm; 280 nm for PAM).

3. Results and discussion

A facile strategy was used to prepare the new type of gel-filled capillaries: a mixture of meth-

acrylate monomers, initiator and template molecule was sucked into the capillary and after polymerization the template molecule was washed out of the column by hydrodynamic solvent replacement. No leakage of polymer was observed and only a relatively low pressure (1300 p.s.i. or lower) had to be applied. This constitutes an important advantage of the polymers, allowing facile removal of any air bubbles by hydrodynamic pumping of electrolyte, and also allows the use of the imprinted polymer capillaries for separations in the chromatographic mode.

Characterization, as described elsewhere [10], of the polymer formed outside the capillaries indicated the formation of agglomerates, *ca.* 10 μm in size, consisting of globular particles, which were in the size range 0.5–2 μm when L-PA was used as the template and 2–4 μm when BAM or PAM was used as the template.

In the first type of imprinted polymer capillary, L-PA was used as the template molecule. Here, the polymerization was carried out in cyclohexanol–dodecanol as the solvent and the ratios of cross-linker to methacrylate and of monomer to solvent (w/w) were relatively low (80% and 14%, respectively), compared with previous preparations of imprinted polymers used in HPLC applications [6,7]. This facilitated pumping of the electrolyte through the capillary and exchange of solvent. However, owing to the low concentration of monomers during polymerization, no enantiomeric selectivity towards L-PA amide over D-PA was observed (see Fig. 1). Higher selectivity might be expected using other polymerization conditions.

The runs could be repeated reproducibly with a stable current. The presence of polymer was indicated by the ca. four times higher current in the gel-filled capillary, whereas the migration times of the eluted compounds were *ca.* half those with an open pretreated capillary. No selectivity compared with blank polymer capillaries was observed in the above L-PA capillary. Photoinitiation of the polymerization may produce selective capillaries in analogy with previous studies [11].

The use of the drug PAM and of BAM as

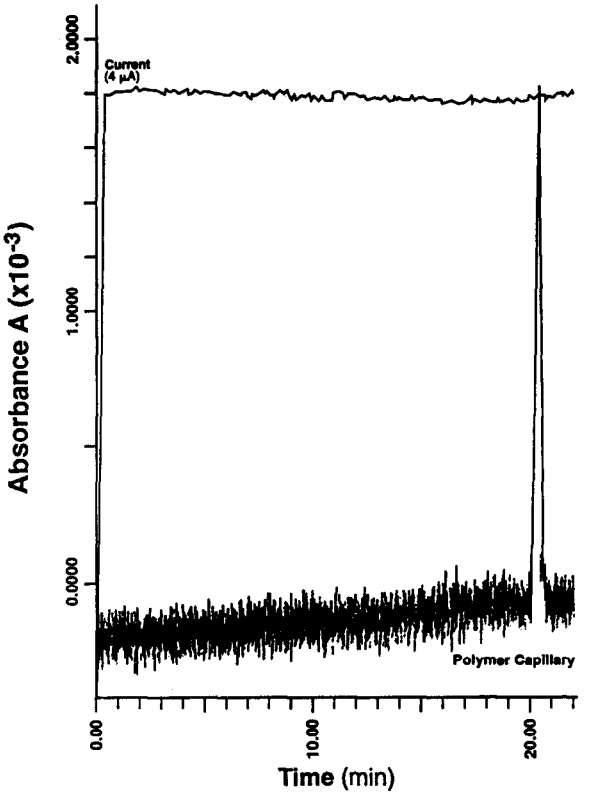

Fig. 1. Electropherogram of D,L-phenylalanine anilide (D,L-PA) injected on a polymer capillary prepared with L-PA as template.

template molecules was also investigated. Here, 2-propanol was used as the solvent during template polymerization, instead of cyclohexanol–dodecanol. Also, a higher concentration of monomers (52%, w/w solvent) and a higher ratio of cross-linker to methacrylate (96%, w/w) than in the L-PA polymerization were used.

In the CE application, the PAM-imprinted polymer capillary showed a very high selectivity towards PAM compared with BAM, which became much more pronounced at increased pH, whereas no such selectivity was observed with the BAM-imprinted or blank capillaries (see Table 1 and Fig. 2). Thus, whereas the BAM and blank capillaries showed similar retentions of PAM and BAM in the pH range 2–4, the PAM-imprinted capillary gave retention times of 6.8 and 6.1 min at pH 2 and 18 and 7.8 min at

Table 1
Retention times for benzamidine (BAM) and pentamidine (PAM) employing a PAM-imprinted polymer capillary or a BAM-imprinted capillary and different electrolyte pH

Capillary	Injected compound	Retention time (min)		
		pH 2	pH 3	pH 4
BAM-imprinted	PAM	5.8	6.1	6.5
	BAM	6.1	6.6	6.5
PAM-imprinted	BAM	6.1	7.5	7.1
	PAM	6.8	10.8	>20

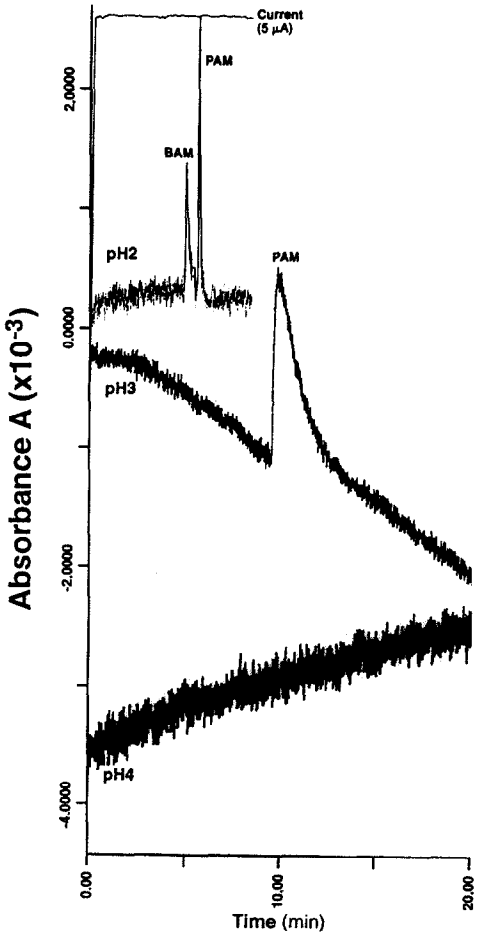

Fig. 2. Electropherograms of PAM and BAM injected onto a polymer capillary prepared with PAM as template and employing an electrolyte pH of 2, with superimposed electropherograms of PAM injected on to the same polymer capillary under the same conditions, but employing an electrolyte pH of 3 and 4, respectively.

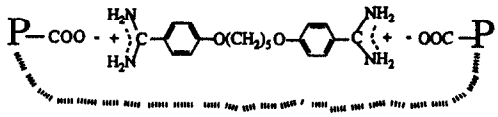

Fig. 3. Proposed interaction between PAM and the PAM-imprinted polymer.

pH 3.5 for PAM and BAM, respectively. At pH 4, PAM was apparently totally retained on the PAM-imprinted capillary. Also, considerable band broadening was observed for PAM on the PAM-imprinted capillary with increasing pH, whereas at low pH a fairly high plate number was observed ($N = 115\,000$/m at pH 2).

The results indicate that specific cavities for pentamidine might have been formed during the polymerization process and that the polymer might contain complementary carboxyl-groups containing binding sites to the two positively charged groups of PAM, as suggested in Fig. 3. BAM, which contains only one such group, does not interact specifically with the polymer.

In fact, the imprinted polymer capillaries behave similarly to antibody affinity columns, which show strong specific binding of antigen at near neutral pH and a dramatically decreased binding efficiency at lower pH, which, however, still allows specific elution of the bound material.

In conclusion, we have demonstrated the use of imprinted methacrylate polymers in CE. The polymer capillaries were prepared with straightforward methods and could be used repeatedly for several weeks. Moreover, solvents could easily be exchanged by hydrodynamic pumping. By using the proper conditions we could obtain capillaries with very high induced selectivity, which could be gradually modified from almost no selectivity to complete selective retention of the template molecule, by changing the electrolyte pH. We are further investigating the IMPCE technique for other template molecules and for chiral separation.

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