An innovative approach to molecularly imprinted capillaries for polar templates by grafting polymerization.

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AN INNOVATIVE APPROACH TO MOLECULARLY IMPRINTED CAPILLARIES FOR POLAR TEMPLATES BY GRAFTING POLYMERIZATION

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ABBREVIATIONS: AIBN, N,N'-bisazoisobutyronitrile; AMP, ampicillin; AMPSA, 2-acrylamido-2-methyl-1-propansulfonic acid; AMX, amoxicillin; BA, benzoic acid; DIC, N,N'-diisopropylcarbodiimide; DIX, dicloxacillin; DMAP, 4-(dimethylamino)pyridine; DMSO, dimethylsulfoxide; HEMA, 2-hydroxyethylmethacrilate; MAA, methacrylic acid; MAn, methacrylic anhydride; γ-MAPS, 3-(trimethoxysilyl)propylmethacrylate; MIP, molecularly imprinted polymer; NIP, non-imprinted polymer; PG, penicillin G; PV, penicillin V.

ABSTRACT

Molecularly imprinted polymers have been successfully used as selective stationary phases in capillary electrophoresis. Notwithstanding, this technique suffers from several drawbacks as the loss of molecular recognition properties in aqueous media and the lack of feasibility for imprinted systems directed towards highly polar templates soluble in aqueous environments only. Thus, the preparation of imprinted polymers for highly polar, water-soluble analytes, represents a challenge.

In this work we present an innovative approach to overcome these drawbacks. It is based on a surface molecular imprinting technique which uses preformed macromonomers as both functional recognition elements and cross-linking agents. A poly-2-hydroxyethyl-*co*-methacrylic acid linear polymer was grafted from the surface of silica capillaries. The grafted polymer was exhaustively esterified with methacrylic anhydride to obtain polyethylendimethacrylate-*co*-methacrylic acid linear chains. Then, as a proof of concept, an adequate amount of a very polar template like penicillin V was added in a hydro-organic mixture and a thin layer of imprinted polymer was obtained by cross-linking the polymer linear chains.

The binding behaviour of the imprinted and non-imprinted capillaries was evaluated in different separation conditions in order to assess the presence of template selectivity and molecular recognition effects. The experimental results clearly show that this innovative kind of imprinted material can be easily obtained in very polar polymerization environments and that it is characterized by enhanced molecular recognition properties in aqueous buffers and good selectivity towards the template and strictly related molecules.

INTRODUCTION

Molecularly Imprinted Polymers (MIPs) are tailor-made polymeric materials obtained by polymerization of functional and cross-linkable monomers in the presence of a template molecule. The subsequent removal of the template leads to the formation of binding sites with selective binding properties towards the template itself or strictly related structures [Komiyama M. 2002].

Affinity capillary electrophoresis based on molecularly imprinted polymers has recently received attention in separation science due to the possibility of combining the predetermined selectivity of the MIPs with the high separation efficiency and the minimization of reagent and sample consumption proper of capillary electrophoresis [Giovannoli *et al.*, 2008]. Advantages and drawbacks of several different experimental approaches to the preparation of imprinted capillaries have been reported in literature: capillaries packed with micrometer-sized imprinted particulate or regular beads [Lin *et al.*, 1997a, 1998; Chirica and Remcho, 1999; Quaglia *et al.*, 2001, 2003]; monolithic macroporous stationary phases prepared by bulk polymerization [Lin *et al.*, 1997b, 1997c; Schweitz *et al.*, 1997; Schweitz *et al.*, 2001; Schweitz *et al.*, 2002]; open tubular capillaries with a thin imprinted layer synthesized *in situ* on the inner silica surface [Brueggemann *et al.*, 1997; Nilsson et al., 1997; Schweitz L. 2002; Huang *et al.*, 2004], and sub-micrometer imprinted beads used as pseudostationary phases [Schweitz *et al.*, 2000; Spegel *et al.*, 2001]. Among different approaches to the preparation of imprinted polymeric materials in capillary electrophoresis, the surface grafting of thin layers shows clear advantages of simple preparation and working procedures by eliminating the back pressure and minimizing the troubles related to clogging and bubble formation inside the capillary. Furthermore, the lack of a bulk stationary phase brings to a reduced dispersive contribution to peak widening, to faster association/dissociation kinetics and to lower non-specific binding as well.

Anyway, despite its potential benefits, the synthesis of imprinted thin layers is not a simple goal to be achieved, as capillary performances and reproducibility are deeply affected by the difficulties to get control of the layer thickness. It is well-known that the grafting of a polymer to a surface provides a versatile tool to get a surface modification and functionalization [Zhao and Brittain, 2000; Tsujii *et al.*, 2006]. Generally speaking, end-functionalized monomers or polymeric chains may be inserted on a surface by using the “grafting to” or the “grafting from” manner. The main

difference between the two approaches implies that in the “grafting to” techniques the reactive functionalities allow the linkage of the growing polymeric structure to the surface, while in the “grafting from” techniques the surface polymerization can proceed from the surface itself because of the preliminary introduction of reactive groups. In both cases a thin polymeric layer is formed but the differences are not negligible because different polymeric strategies led to different morphologies and properties. The presence of a reactive surface in the “grafting from” approach allows a greater control of the polymerization process above all in terms of length and density of surface polymer chains and for these reasons its use has been recently increased.

On this basis, in order to efficiently develop thin controlled polymeric layers, in recent years various surface molecular imprinting techniques based on “iniferter” [Ruckert et al., 2002; Perez-Moral and Mayes, 2007; Barahona *et al.*, 2010], “reversible addition-fragmentation chain transfer (RAFT)” [Titirici and Sellergren, 2006; Pan *et al.*, 2009; Chang *et al.*, 2010] and “atom transfer radical polymerization” (ATRP) [Wei et al., 2005; Zu et al., 2009; Sasaki *et al.*, 2010] have been developed.

Besides these methods to prepare controlled imprinted thin layers, a non-conventional approach to surface molecular imprinting has been reported [Matsui et al., 2002; Li et al., 2005]. This innovative approach is based on the synthesis of preformed polymeric chains and their subsequent use as functional macromonomers in an imprinting process. The presence of preformed polymeric chains makes easier the molecular imprinting process in polar solvents by circumventing a fundamental limitation of the classical approach to the imprinting process: the need for hydrophobic porogenic solvents (i.e. toluene, chloroform) that are partially or totally unsuitable to prepare molecularly imprinted materials directed towards highly polar template molecules. Some interesting examples have been recently described where these functional linear macromolecules are grafted from silica surfaces and subsequently cross-linked in the presence of a template protein [Guo *et al.*, 2006], small molecules such as pirimicarb [Gao *et al.*, 2008], cholic acid [Gao *et al.*, 2009], uric acid [Gao *et al.*, 2010a], creatinine [Gao *et al.*, 2010b] and the alkaloid cytisine [Gao *et al.*, 2010c].

In this paper we report an innovative approach to prepare molecularly imprinted open tubular silica capillaries. In this approach, depicted in figure 1, poly-2-hydroxyethyl-*co*-methacrylic acid (poly-HEMA-*co*-MAA) linear macromonomers able to act as functional recognition elements were synthesized by grafting from the inner surface of silica

capillaries, then chemically modified with methacrylic anhydride to add cross-linking functionalities and finally polymerized in the presence of the template molecule. As proof of concept a penicillin V-imprinted capillary was prepared and its electrophoretic behaviour towards the template molecule and some analogues was evaluated in different separation conditions in order to demonstrate the presence of template selectivity and molecular recognition effects.

MATERIALS & METHODS

Materials. Sodium hydroxide, hydrochloric acid, glacial acetic acid, sodium monohydrogen phosphate dihydrate, sodium dihydrogen phosphate monohydrate, phosphoric acid, HPLC grade methanol, ethanol 96% (v/v), toluene, acetone, dimethylsulfoxide (DMSO), pyridine, N,N'-bisazoisobutyronitrile (AIBN), N,N'-diisopropylcarbodiimide (DIC), 3-(trimethoxysilyl)propylmethacrylate (γ-MAPS), methacrylic acid (MAA), 2-hydroxyethylmethacrilate (HEMA), 2-acrylamido-2-methyl-1-propansulfonic acid (AMPSA), 4-(dimethylamino)pyridine (DMAP), methacrylic anhydride (MAn) and benzoic acid (BA) were from Sigma-Aldrich (Milan, Italy). Penicillin V (PV), penicillin G (PG), amoxicillin (AMX), ampicillin (AMP) and dicloxacillin (DIX) were from Apollo Scientific Ltd (Stockport, Cheshire, UK).

Organic solvents were dried by overnight treatment with molecular traps previously desiccated in an oven at 110 °C. Ultrapure water was obtained by reverse osmosis with a Purelab Prima System from Elga (Bucks, UK). All the buffers were prepared by dissolving the sodium dihydrogenphosphate dihydrate in ultrapure water; pH values were adjusted to 4 with phosphoric acid and to 5 with sodium monohydrogen phosphate monohydrate and the solutions filtered through a 0.45 μm filter before use.

Stock solutions of penicillin V, penicillin G, dicloxacillin, ampicillin, amoxicillin were prepared in ultrapure water every five days and kept in the dark at 4°C until use.

All separations were performed using an Agilent CE system (Agilent Technologies, CA, USA). Data acquisition and signal processing were performed by using the Agilent ChemStation, Agilent Technologies). The fused silica capillaries from Polymicro Technologies, Optronis GmbH, Kehl, Germany were 48.5 cm in length (40 cm to the detector) with an I.D. of 50 μm. The capillary washing reservoir CWR-10 (Supelco, Milan, Italy) and the syringe pump (model 11, Harvard Apparatus, Kent, UK) were used during the capillary modification procedures.

Synthesis of grafted capillaries. The imprinted polymeric layer onto the silica surface was synthesized in subsequent steps as depicted in Figure 1. The capillary was etched by flushing with 1 M sodium hydroxide for 30 minutes, filled with 0.1 M hydrochloric acid, left to react for 2 hours at 70 °C, washed with water and acetone and dried under a stream of nitrogen for 5 minutes. Then, it was filled with a 10% (v/v) DIC solution in anhydrous toluene, left to react overnight at room temperature and washed with toluene and acetone. Silanization was performed overnight at room temperature with a 10% (v/v) γ-MAPS solution prepared in 1+1 (v/v) acetone-acetic acid. The capillary was then washed with acetone and dried with nitrogen.

A poly-HEMA-*co*-MAA linear polymer (molar ratio HEMA:MAA 9+1) was synthesized by thermopolymerization at 60 °C for two days as showed in figure 1, step 2, by dissolving 15 μl (0.178 mmoles) of MAA, 198 μl (1.60 mmoles) of HEMA, 3.7 mg (17.8 μmoles) of AMPSA and 3 mg of AIBN in 1 ml of oxigen-free ethanol. The grafted macromonomer was exhaustively esterified to transform it to poly-ethylenedimethacrylate-*co*-methacrylic acid, poly-EDMA-*co*-MAA, (figure 1, step 3) by flushing into the washed and dried capillary a 10% (v/v) methacrylic anhydride solution in pyridine containing 1% (w/v) DMAP as catalyst and reacting for two days at room temperature. Finally, the grafted linear chains of poly-EDMA-*co*-MAA were cross-linked and imprinted (figure 1, step 4) in a nitrogen-saturated 1+3 (v/v) water-ethanol mixture in the presence of about 4 mg of AIBN as catalyst and 20 mg of penicillin V as template molecule. The capillary was dried, filled up, sealed and the thermopolymerized for two days at 60°C. At the end of the process, the capillary was washed with methanol, 9+1 (v/v) methanol-acetic acid and water and stored at room temperature. A non-imprinted capillary was prepared in the same manner, but without the introduction of the template molecule in the final cross-linking mixture.

Capillary electrophoresis. Electrophoretic separations were carried out at 25 kV both in normal and reverse polarity at 25 °C, by applying an external pressure of 2 bar at each vial. Hydrodynamic injections were performed by applying a pressure of 50 mbar for 20 seconds. Each run included a rinse step with water for 4 minutes, followed by methanol for 2 minutes, water for 2 minutes and run buffer for 4 minutes. Samples were diluted at 50 mg/l in ultrapure water and the detection was performed at 205 nm. All separations were performed at least three times to evaluate the repeatability of the measurements.

RESULTS AND DISCUSSION

Synthesis of grafted capillaries. The achievement of a molecular imprinted thin layer grafted from the inner capillary silica surface requires a series of subsequent steps of synthesis, as depicted in figure 1. First of all, the bare silica surface has to be very carefully treated before any chemical modifications [Nawrocki, 1997; Horvath and Dolnik, 2001]. In fact, in order to make its behaviour reproducible, the capillary was etched sequentially with 1 M sodium hydroxide and 0.1 M hydrochloric acid solutions. Then, after a preliminary drying step with acetone and a stream of nitrogen, the capillary was filled with a 10% (v/v) DIC solution in anhydrous toluene for 15 minutes and the reaction was left to continue overnight at room temperature. DIC is a well-known dehydrating reagent which is commonly used whenever is necessary to remove water; its ability to dehydrate is due to the reaction with water that leads to the formation of diisopropylurea [Williams and Ibrahim, 1981].

The silanization step of the synthesis, reported in figure 1, step 1, lead to the functionalization of the silica surface with vinyl groups on which a poly-HEMA-*co*-MAA linear copolymer was grafted from. Thus, these functionalities are the starting point to build up a polymeric structure through a radical thermopolymerization by using methacrylic acid, 2-hydroxyethylmethacrilate and a small relative amount (respect to MAA and HEMA) of acrylamido-2-methyl-1-propansulfonic acid as functional monomers, as shown in figure 1, step 2. The use of AMPSA during this grafting process was due to the will of introducing charged groups on the capillary surface that may lead to the presence of electroosmotic flow in the cathodic direction. This flow can accelerate the electrophoretic run and produce migration of neutral compounds as well.

At this point, the grafted poly-HEMA-*co*-MAA linear polymer has to be modified in order to add further insaturations which may promote next surface polymerization in the "grafted from" approach. This was achieved by reaction with methacrylic anhydride as depicted in figure 1, step 3. The novelty of this approach is represented by this surface modification which leads to the contemporary presence – with a presumed molar ratio 1÷9 - of both functional groups (i.e. carboxylic functions from copolymerized MAA) and cross-linking vinyl groups (methacryloyl groups from exhaustive esterification of HEMA) in the grafted chains of the resulting poly-EDMA-*co*-MAA. Then, the macromonomer structure was subsequently polymerized by only adding the radical initiator and the template molecules dissolved in a proper solvent, as depicted in figure 1, step 4. It is

worthy to be emphasized that a very significant advantage of this approach is that strongly polar solvents and therefore very polar templates may be used during the molecular imprinting process because troubles related to the solubilisation of organic monomers and polar templates in more or less hydrophobic porogens no longer exist. Anticipating the results obtained in the capillary electrophoresis results, it is possible to tentatively explain the efficient imprinting in these experimental conditions as an effect of the presence of stabilized polymeric chain – template complexes due to a more or less tight "coils" of the preformed chains around the template molecules, with a a substantial gain in complex stability due to the local very high molecular crowding of polymeric functional groups around the template molecules. So, as previously reported in literature [Matsui et al., 2002], this method can be considered to pave the way to a more general approach, useful whenever the chosen template molecule is soluble only in very polar organic solvent.

Capillary electrophoresis. The chosen template molecule was the β-lactam antibiotic penicillin V, which shows the main structural refrain of the class of penicillins without being commonly used as a drug. Due to its very polar chemical structure and to its ability to migrate in an electric field it was considered to be a good candidate to investigate our new approach.

After the imprinted and the non-imprinted capillaries had been prepared, preliminary studies were performed to test their behaviour under different run buffer compositions. The chosen buffer composition was 20 mM phosphate, while the pH values were changed from 3 to 5. We also verified that the acidic conditions of separation did not compromise the stability of penicillins.

The measurements performed by using DMSO as a neutral marker showed that no electroosmotic flow was measured at pH 3 and 4 despite the introduction of sulfonic groups on the capillary surface. This means that the charged sulfonic groups are probably shielded by the polymer grown from the capillary surface and that they do not bring to the formation of any charged double layer responsible of the electroosmotic flow. As a consequence, the electrophoretic runs at pH 3 and 4 were performed in reversed polarity mode as most of the tested molecules behave like anions because of the dissociation of the acidic function. The electrophoretic runs performed at pH 5 both on the imprinted and non-imprinted capillary showed a quite different behaviour of the capillary surfaces owing to the presence of a measurable electroosmotic flow of about

$1.2 \cdot 10^{-4}$ cm^2/Vs, probably related to the dissociation of the methacrylic acid present in the grafted layer.

In order to verify the presence of any molecular recognition behaviour due to the imprinting process, the comparison between the electrophoretic patterns of the penicillin V obtained in the imprinted and non-imprinted capillaries was performed in the experimental conditions reported in figure 2. The significant delay of the migration time observed in the imprinted capillary clearly shows the presence of molecular recognition properties of the polymer surface layer towards the chosen template molecule.

The next step was to investigate the presence of a selective recognition not only towards the template molecule but also towards strictly related molecular structures by the imprinted capillary. Such measurements were accomplished by taking into account different compounds whose chemical structures are reported in figure 3: penicillin V as the template molecule, penicillin G and dicloxacillin as molecules bearing different structures of the lateral chains, ampicillin and amoxicillin as zwitterionic structures due to the presence of an amino group in the side chain, and benzoic acid as a completely unrelated structure. Among the different experimental conditions tested before, a run buffer at pH 5 was chosen for the selectivity measurements since the presence of the electroosmotic flow allows the migration of the zwitterionic molecules as well.

Selectivity was determined for each considered compound by calculating its effective electrophoretic mobility in the imprinted and non-imprinted capillaries through the subtraction of the corresponding electroosmotic flow. Then, the difference of the effective electrophoretic mobility measured in the imprinted and non-imprinted capillary for each compound (taken as absolute value) was divided by that of the template molecule obtained in the same manner. This approach allowed us to define a measurable parameter of selectivity in order to compare the behaviour of different compounds with respect to the template penicillin V. The relative mobility values obtained for each compound can be compared with that of the template molecule (assumed as 1): the more the value is close to unity the more similar is the molecular recognition with respect to the template molecule. This is summarized in table 1, from which it is clear that compounds like penicillin G and dicloxacillin are well recognized by the imprinted polymer layer, while the same is not true for compounds as amoxicillin, ampicillin and benzoic acid which show a much reduced value of the selectivity

parameter. In particular, it is clear that the β-lactam structures where it is present an amino group in the side chain are not recognized at all by the imprinted polymer, probably for the presence of a positive charge which destabilizes the interactions in the polymer binding sites.

After the recognition properties of the imprinted capillary were settled, a further investigation concerned a study of the behaviour of the system in the presence of increasing amount of methanol in the run buffer. This was performed by using the same experimental conditions reported in figure 2 and by considering the template molecule only. The results obtained are reported in figure 4. It is possible to observe that the difference in the electrophoretic migration of penicillin V between the imprinted and the non-imprinted capillary is progressively lost, reaching a minimum for percentage concentration of methanol greater than 30 % (v/v). Thus, as the maximum differential mobility is observed in pure buffer, this denotes the achievement of the imprinting process in a very polar polymerization medium and the suitability of this approach to prepare molecularly imprinted layers showing molecular recognition abilities in pure aqueous environments.

CONCLUSIONS

This newly proposed approach produces a surface-grafted imprinted capillary suitable for the molecular recognition and separation of very polar analytes by capillary electrophoresis. The application of this approach to open tubular capillary electrochromatography represents a new way to prepare capillary affinity systems for the screening of the binding properties towards different kinds of polar templates. We retain also that the presence of molecular recognition effects in aqueous media can lead to the production of a new generation of imprinted materials suitable for the selective separation of very polar substances and for solid phase extraction and preconcentration of water soluble compounds from aqueous matrices. Moreover, by using this general approach, further synthesis strategies of imprinted materials could be implemented and quickly tested by capillary electrophoresis with net savings of time and materials.

BIBLIOGRAPHY

Barahona F, Turiel E, Cormack PAG, Martin-Esteban A. 2010. Chromatographic performance of molecularly imprinted polymers: core-shell microspheres by precipitation polymerization and grafted MIP films via iniferter-modified silica beads. *J.Polym.Sci.A* **48**: 1058-1066. DOI: 10.1002/pola.23860

Brueggemann O, Freitag R, Whitcombe MJ, Vulfson EN. 1997. Comparison of polymer coatings of capillaries for capillary electrophoresis with respect to their applicability to molecular imprinting and electrochromatography. *J.Chromatogr.A* **781**: 43–53.

Chang LM, Li Y, Chu J, Qi JY, Li X. 2010. Preparation of core-shell molecularly imprinted polymer via the combination of reversible addition-fragmentation chain transfer polymerization and click reaction. *Anal.Chim.Acta* **680**: 65-71. DOI: 10.1016/j.aca.2010.09.017

Chirica G, Remcho VT. 1999. Silicate entrapped columns - new columns designed for capillary electrochromatography. *Electrophoresis* 1999. **20**: 50–56.

Giovannoli C, Baggiani C, Anfossi L, Giraudi G. 2008. Aptamers and molecularly imprinted polymers as artificial biomimetic receptors in affinity capillary electrophoresis and electrochromatography. *Electrophoresis* **29**: 3349-65. DOI: 10.1002/elps.200800004.

Gao B, Wang J, An, F, Liu Q. 2008. Molecular imprinted material prepared by novel surface imprinting technique for selective adsorption of pirimicarb. *Polymer* **49**: 1230-1238. DOI:10.1016/j.polymer.2007.12.041.

Gao B, Lu, J, Chen Z, Guo J. 2009. Preparation and recognition performance of cholic acid-imprinted material prepared with novel surface-imprinting technique. *Polymer* **50**: 3275–3284. DOI:10.1016/j.polymer.2009.05.008.

Gao B, Liu S, Li Y. 2010a. Preparation and recognition performance of uric acid-imprinted material prepared with novel surface imprinting technique. *J.Chromatogr.A* **1217**: 2226–2236. DOI:10.1016/j.chroma.2010.02.027.

Gao B, Li Y, Zhang Z. 2010b. Preparation and recognition performance of creatinine-imprinted material prepared with novel surface-imprinting technique. *J.Chromatogr.B* **878**: 2077–2086. DOI:10.1016/j.jchromb.2010.06.007.

Gao B, Niu Q, Du R. 2010c. Preparation and recognition performance of cytisine alkaloid-

imprinted material prepared using novel surface molecular imprinting technique *J.Sep.Sci.* **33**: 1338–1348. DOI:10.1002/jssc.200900762.

Guo MJ, Zhao Z, Fan YG, Wang CH, Shi LQ, Xia JJ, Long Y, Mi HF. 2006. Protein-imprinted polymer with immobilized assistant recognition polymer chains. *Biomaterials* **27**: 4381–4387. DOI: 10.1016/j.biomaterials.2006.04.002.

Horvath J, Dolnik V. 2001. Polymer wall coatings for capillary electrophoresis. *Electrophoresis* **22**: 644-655.

Huang YC, Lin CC, Liu CY. 2004. Preparation and evaluation of molecularly imprinted polymers based on 9-ethyladenine for the recognition of nucleotide bases in capillary electrochromatography. *Electrophoresis* **25**: 554–561. DOI: 10.1002/elps.200305735.

Komiyama M. 2002. *Molecular Imprinting: From Fundamentals to Applications*. Wiley-VCH: Weinheim. DOI: 10.1002/352760202X

Li Z, Day M, Ding J, Faid K. 2005. Synthesis and characterization of functional methacrylate copolymers and their application in molecular imprinting. *Macromolecules **38***: 2620-2625. DOI: 10.1021/ma0478308.

Lin JM, Nakagama T, Uchiyama K, Hobo T. 1997a. Capillary electrochromatographic separation of amino acid enantiomers using on-column prepared molecularly imprinted polymer. *J.Pharm.Biomed.Anal*. **15**: 1351–1358.

Lin JM, Nakagama T, Uchiyama K, Hobo T. 1997b., Temperature effect on chiral recognition of some amino acids with molecularly imprinted polymer filled capillary electrochromatography. *Biomed.Chromatogr*. **11**: 298–302.

Lin JM, Nakagama T, Wu XZ, Uchiyama K, Hobo T. 1997c. Capillary electrochromatographic separation of amino acid enantiomers with molecularly imprinted polymers as chiral recognition agents. *Fresenius'J.Anal.Chem*. **357**: 130–132.

Lin JM, Uchiyama K, Hobo T. 1998. Enantiomeric resolution of dansyl amino acids by capillary electrochromatography based on molecular imprinting method. *Chromatographia* **47**: 625–629.

Matsui J, Tamaki K, Sugimoto N. 2002. Molecular imprinting in alcohols: utility of a pre-polymer based strategy for synthesizing stereoselective artificial receptor polymers in hydrophilic media. *Anal.Chim.Acta* **466**: 11–15.

Nawrocki J. 1997. The silanol group and its role in liquid chromatography.

J.Chromatogr.A **779**: 29-71.

Nilsson S, Schweitz L, Petersson M. 1997. Three approaches to enantiomer separation of beta-adrenergic antagonists by capillary electrochromatography. *Electrophoresis* **18**: 384–390.

Pan G, Zu B, Guo X, Zhang Y, Li C, Zhang H. 2009. Preparation of molecularly imprinted polymer microspheres via reversible addition-fragmentation chain transfer precipitation polymerization. *Polymer* **50**: 2819-2825. DOI: 10.1016/j.polymer.2009.04.053.

Perez-Moral N, Mayes AG. 2007. Molecularly imprinted multi-layer core-shell nanoparticles - A surface grafting approach. *Macromol.Rapid Commun.* **28**: 2170-2175. DOI: 10.1002/marc.200700532.

Quaglia M, De Lorenzi E, Sulitzky C, Massolini, G. Sellergren B. 2001. Surface initiated molecularly imprinted polymer films: a new approach in chiral capillary electrochromatography. *Analyst* **126**: 1495–1498. DOI: 10.1039/b105401p.

Quaglia M, De Lorenzi E, Sulitzky C, Caccialanza G, Sellergren B. 2003. Molecularly imprinted polymer films grafted from porous or nonporous silica: Novel affinity stationary phases in capillary electrochromatography. *Electrophoresis* **24**: 952–957.

Ruckert B, Hall AJ, Sellergren B. 2002. Molecularly imprinted composite materials via iniferter-modified supports. *J.Mater.Chem.* **12**: 2275-2280. DOI: 10.1039/b203115a.

Sasaki S, Ooya T, Takeuchi T. 2010. Highly selective bisphenol A-imprinted polymers prepared by atom transfer radical polymerization. *Polym.Chem.* **1**: 1684-1688. DOI: 10.1039/c0py00140f.

Schweitz L, Andersson LI, Nilsson S. 1997. Capillary electrochromatography with predetermined selectivity obtained through molecular imprinting. *Anal.Chem.* **69**: 1179–1183.

Schweitz L, Spegel P, Nilsson S. 2000. Molecularly imprinted microparticles for capillary electrochromatographic enantiomer separation of propranolol. *Analyst* **125**: 1899–1901. DOI: 10.1039/b007221o.

Schweitz L, Andersson LI, Nilsson S. 2001. Rapid electrochromatographic enantiomer separations on short molecularly imprinted polymer monoliths. *Anal.Chim.Acta* **435**: 43–47.

Schweitz L. 2002. Molecularly imprinted polymer coatings for open-tubular capillary

electrochromatography prepared by surface initiation. *Anal.Chem.* **74**: 1192–1196. DOI: 10.1021/ac0156520.

Schweitz L, Andersson LI, Nilsson S. 2002. Molecularly imprinted CEC sorbents: investigations into polymer preparation and electrolyte composition. *Analyst* **127**: 22–28. DOI: 10.1039/b105104k.

Spegel P, Schweitz L, Nilsson S. 2001. Molecularly imprinted microparticles for capillary electrochromatography: Studies on microparticle synthesis and electrolyte composition. *Electrophoresis* **22**: 3833–3841.

Titirici MM, Sellergren B. 2006. Thin molecularly imprinted polymer films via reversible addition-fragmentation chain transfer polymerization. *Chem.Mater.* **18**: 1773-1779. DOI: 10.1021/cm052153x.

Tsujii Y, Ohno K, Yamamoto S, Goto A, Fukuda T. 2006. Structure and properties of high-density polymer brushes prepared by surface-initiated living radical polymerization. *Adv.Polym.Sci.* **197**: 1–45. DOI: 10.1007/11569084.

Wei XL, Li X, Husson SM. 2005. Surface molecular imprinting by atom transfer radical polymerization. *Biomacromolecules* **6**: 1113-1121. DOI: 10.1021/bm049311i.

Williams A, Ibrahim IT. 1981. Carbodiimide chemistry: recent advances. *Chem.Rev.* **81**: 589-636.

Zhao B, Brittain WJ. 2000, Polymer brushes: surface-immobilized macromolecules. *Prog.Polym.Sci.* **25**: 677–710. DOI:10.1016/S0079-6700(00)00012-5

Zu BY, Pan GQ, Guo XZ, Zhang Y, Zhang HQ. 2009. Preparation of molecularly imprinted polymer microspheres via atom transfer radical precipitation polymerization. *J.Polym.Sci.A* **47**: 3257-3270. DOI: 10.1002/pola.23389.

TABLES

Table 1: selectivity of the imprinted capillary measured towards different analytes, assuming a relative mobility value of 1.0 for the template molecule penicillin V. Experimental conditions as in Figure 2 except for the buffer pH which is 5 instead of 4.

Analyte	Relative mobility
penicillin V	1.0
penicillin G	0.94
dicloxacillin	0.90
amoxicillin	0.18
ampicillin	0.11
benzoic acid	0.14

LEGEND OF FIGURES:

Figure 1: synthesis scheme of the imprinted capillary.

Figure 2: electropherograms of the migration of penicillin V at 50 mg/l: (A) imprinted capillary; (B) non-mprinted capillaries. Run buffer: sodium phosphate 20 mM, pH 4; voltage: 25 kV, reversed polarity ; detection at 205 nm; capillary 48.5 cm (40 cm), 25°C; hydrodynamic injection at 50 mbar for 20 s

Figure 3: chemical structures of compounds separated on imprinted and non-imprinted capillaries.

Figure 4: effect of increasing methanol amount on the difference between the electrophoretic migrations of MIP and NIP capillaries measured on the template molecule. Electrophoretic conditions as in figure 2.

figure 1

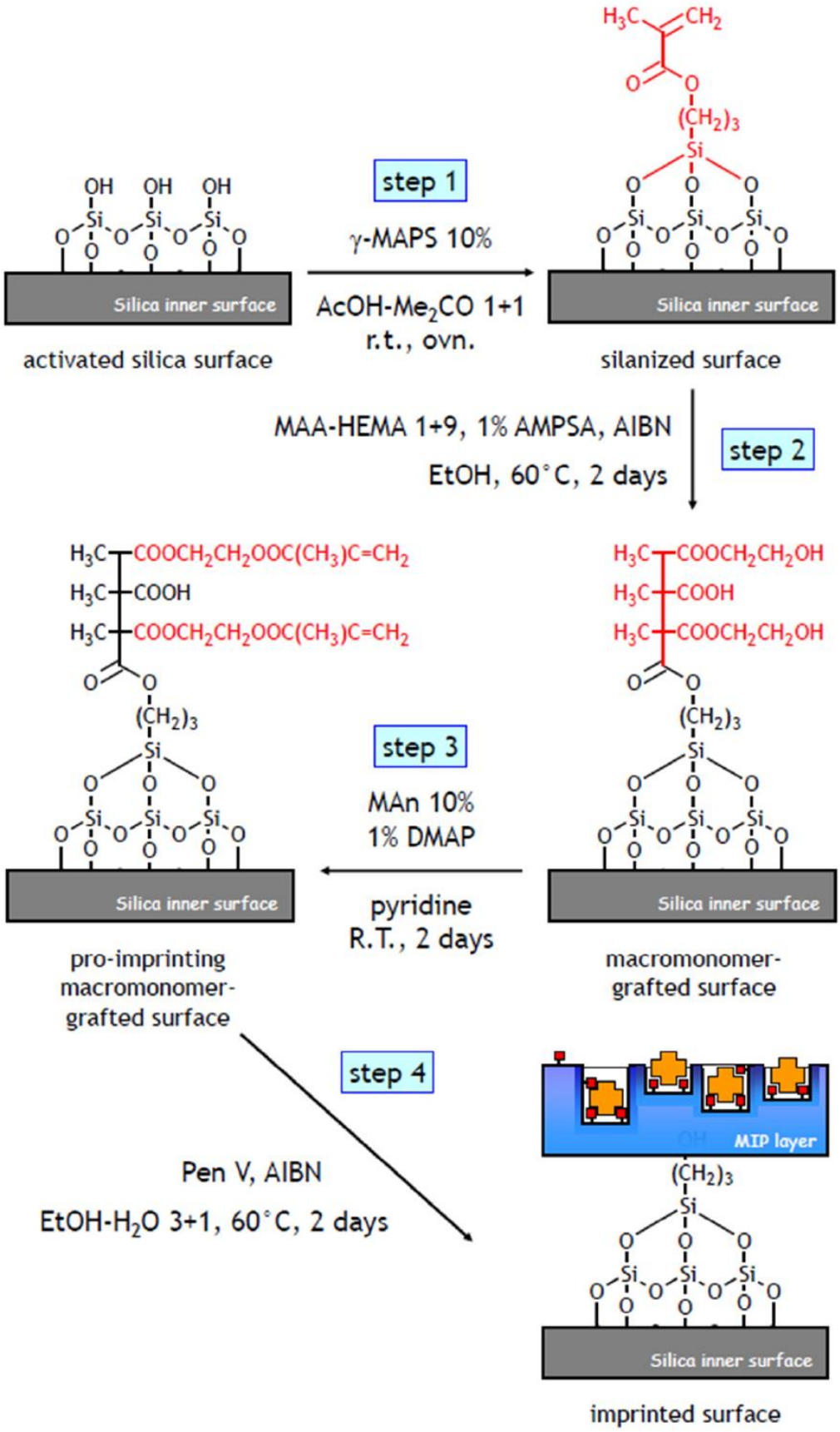

step 1
γ-MAPS 10%
AcOH-Me2CO 1+1
r.t., ovn.
Silica inner surface
activated silica surface
silanized surface
MAA-HEMA 1+9, 1% AMPSA, AIBN
EtOH, 60°C, 2 days
step 2
step 3
MAn 10%
1% DMAP
pyridine
R.T., 2 days
pro-imprinting macromonomer-grafted surface
macromonomer-grafted surface
step 4
Pen V, AIBN
EtOH-H2O 3+1, 60°C, 2 days
MIP layer
imprinted surface

figure 2

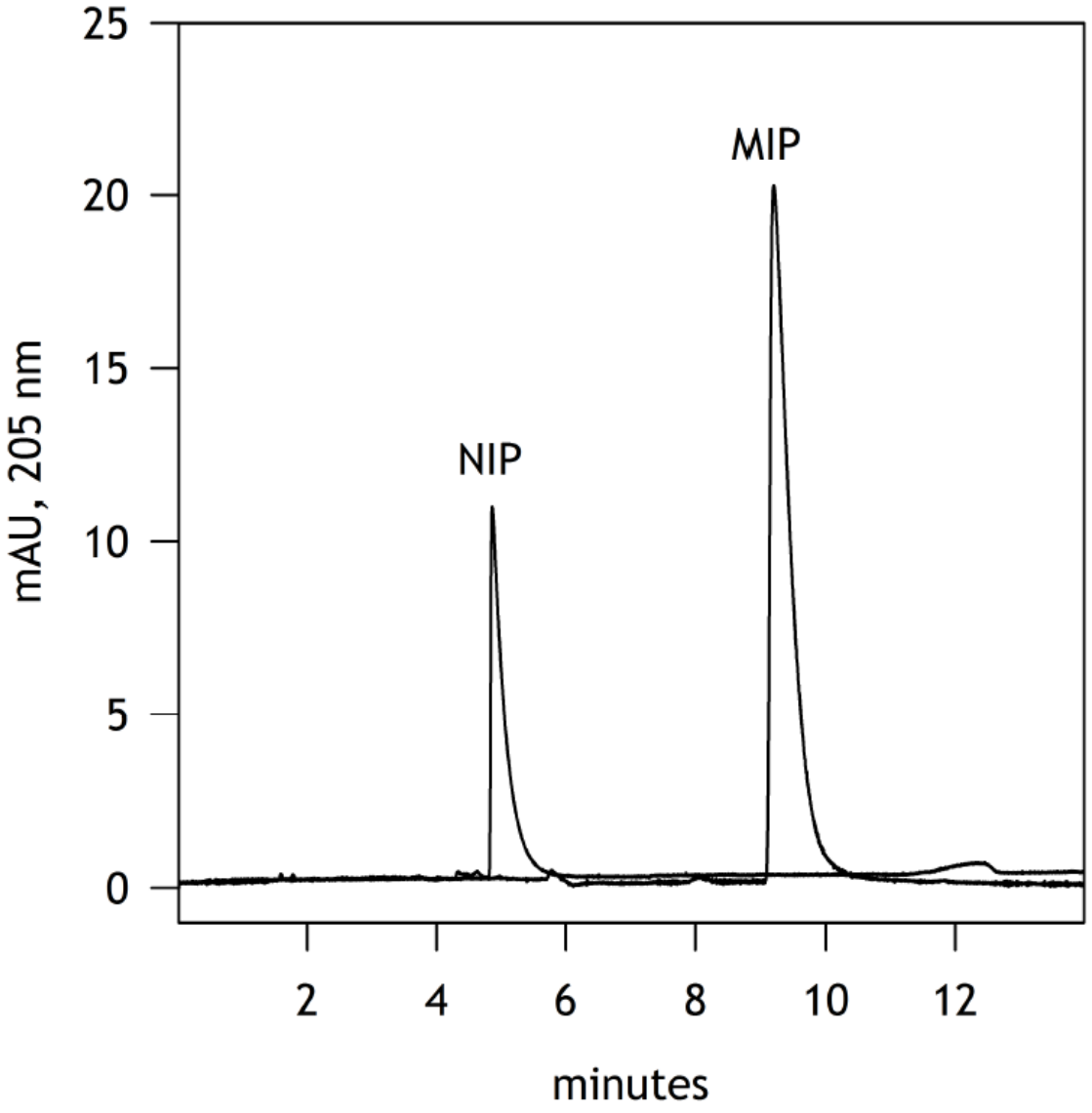
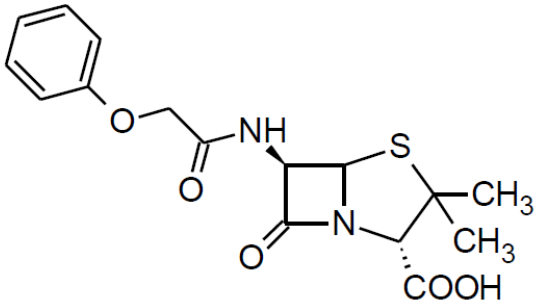
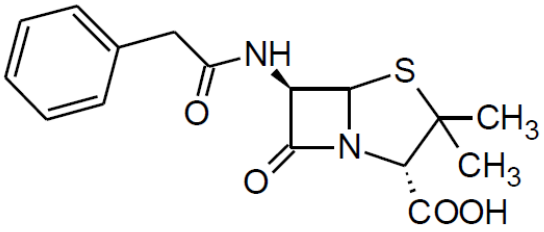


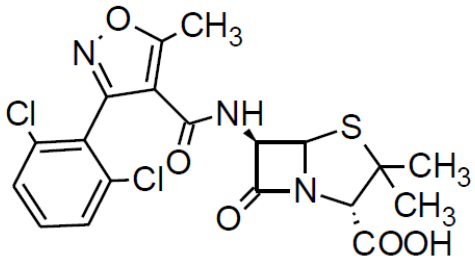
figure 3



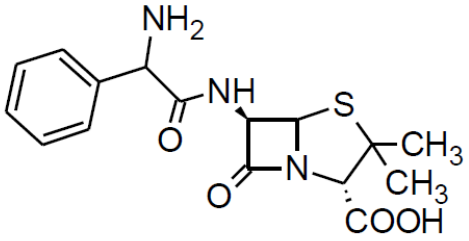
penicillin V, PV



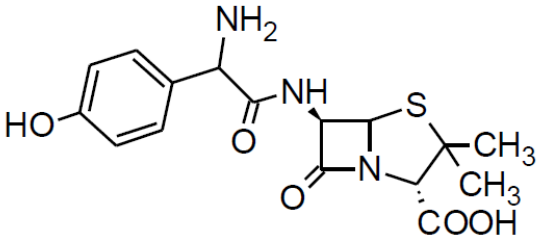
penicillin G, PG



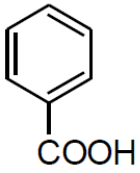
dicloxacillin, DIX



ampicillin, AMP



amoxicillin, AMX



benzoic acid, BA

figure 4