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1 **Application of molecularly imprinted polymers in analytical chiral separations and**
2 **analysis**

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18
19 **Abstract**

20 Over the last two decades the process of development and application of a new types of
21 molecular imprinted polymer (MIP) sorbents in the field of analytical chemistry have been
22 widely described in the literature. One of the new trends in analytical chemistry practice is the
23 use of new types of MIP sorbents as specific sorption materials constituting the stationary
24 phase in advanced separation techniques. The following review paper contains comprehensive
25 information about the application of a specific and well defined MIP sorbents (with the data
26 base in the paper about the reagents used in MIP preparation process) as stationary phases in
27 separation techniques including high performance liquid chromatography and capillary
28 electrochromatography. Coverage includes newly created types of stationary phases (MIP
29 sorbents) used for chiral recognition, with the focus on applications in enantioselective
30 separation.

31
32 **Keywords:** chiral separation, high performance liquid chromatography, capillary
33 electrochromatography, molecularly imprinted polymers, enantiomers.

34

35 **1. Introduction**

36

37 It is widely known that almost every biochemical process occurring in the cells of living
38 organisms is based on the specific, stereoselective interactions between reacting molecules
39 and catalysts. Therefore, the stereochemistry of molecules involved or affecting those
40 processes should be considered. A rapid growth of the branches of scientific activity dealing
41 with the stereochemistry occurred with these considerations. As a consequence, almost all
42 newly designed, biologically active substances such as drugs or pesticides are compounds
43 with strictly defined stereochemistry. There is great interest in obtaining enantiomerically pure
44 biologically active compounds with this being achieved by different approaches for example
45 stereoselective synthesis and/or crystallization, biotransformation or chiral separation of
46 isomeric mixtures.

47 The separation of isomeric mixtures is a complex process requiring enantiomer to
48 diastereoisomer formation to create differences in physicochemical properties. The key
49 problem for the modern analytical chemistry is to develop procedures using appropriate
50 techniques to conduct separation processes in an effective way. In most cases enantiomers
51 might be separated applying the wide spectrum of methods such as: crystallization, extraction,
52 chromatographic techniques, membrane techniques and electromigration techniques. For the
53 assessment of optical purity of particular enantiomers the following analytical techniques
54 have been successfully applied: NMR, polarimetry, immunoanalytical methods, chiral
55 sensors, isotopes dilution, chromatographic techniques and capillary electrophoresis (CE)[1,
56 2].

57 The separation of enantiomers for chiral molecules is crucial, particularly in the
58 pharmaceutical industry, since enantiomers can present different, and even opposite
59 pharmacological and toxicological properties. The development of effective methods and
60 techniques to prepare drugs with highly enantiomeric purity taken places at the beginning of
61 1980s. Since this time, high performance liquid chromatography (HPLC) has become the
62 most extensively applied approach for chiral separation, while capillary
63 electrochromatography (CEC) is attracting increasing interest recently [3]. Generally, the
64 enantiomers resolution may be carried out by chromatography of the racemic mixture on a
65 chiral stationary phase (CSP). These commonly includes cyclodextrins (CDs), crown ether,
66 several types of derivatives of cellulose and amylose, pirkle type phases, macrocyclic
67 antibiotics and cyclofructans. Recently a common course of action is the use of molecularly
68 imprinted polymers (MIPs) as the stationary phase for the separation of racemates. This type

69 of stationary phase can be classified as a specific group of CSP [4]. Molecular imprinting is a
70 promising technique for the preparation of polymers which possess highly selective
71 recognition properties and serve as separation media, especially for chiral molecules. In
72 comparison to traditional stereoselective selector systems, chiral imprinting of polymers has
73 several advantages, for example low material costs, ease of preparation, scalability, and
74 flexibility to design various self-supporting formats. Moreover, these polymers have
75 demonstrated improved stability toward mechanical and thermal stress and to tolerate a broad
76 range of solvents, bases, acids, and salts, making them particularly well-suited to operation in
77 challenging environments [5].

78 Taking into account the chemical nature of the interactions between the templating molecule
79 and the interactive functional groups, molecular imprinting technology may be divided into
80 covalent and non-covalent approaches [5,6]. Bulk polymerization is the most commonly used
81 technique to prepare new types of LC column filling medium (stationary phase) because of
82 the highly efficient process and low laboratory equipment costs. The LC columns filled with
83 MIP stationary phases prepared by bulky polymerization techniques are mostly used in
84 preparative separation of racemic mixtures (for example in organic chemistry to clean and
85 separate the reaction products) and not in analytical chemistry – in the field of assess the
86 optical purity of defined chemical compound. The main drawback that should be pointed out
87 is that the synthesized polymer particles of a developed stationary phase often are
88 characterized by irregular shapes, forms and dimensions [7-12]. To obtain more regular
89 shapes and optimal dimensions of MIP particles used as fillings of HPLC columns it is
90 recommended to prepare new MIP material using for example silica-gel surface modification
91 polymerization technique. The surface characteristic of MIP particles using the above
92 mentioned polymerization technique is much more appropriate for the packing material of
93 HPLC columns, but is characterized by lower efficiency than bulky polymerization [13, 14].

94 The first report on the application of the MIPs for enantioseparation has taken place in 1978
95 [15]. In this published work, the template 4-nitrophenyl- α -D-mannopyranoside was covalently
96 linked to a monomer to form 4-nitrophenyl- α -D-mannoside-2,3,4,6-di-o-(4-
97 vinylphenylboronate), which was then co-polymerized with styrene and divinylbenzene. In
98 other works, non-covalently molecular imprinting has been reported to be a more direct and
99 flexible approach because of its use of a larger range of compounds including chiral
100 molecules that can be imprinted [16,17]. The most extensively applied as the templates have
101 been several compounds and its derivatives including L-Phenylalanine anilide, L-
102 phenylalanine, (S)-naproxen, (2)-nicotine and other chiral drugs [17, 18]. Methacrylic acid

103 (MAA) has been usually used as the functional monomer. Normal formats of MIPs for
104 enantioseparation mainly include monoliths, particles, and membranes. Other important
105 milestones reported in the field of enantioseparation process using wide spectrum of MIP
106 materials are outlined in Figure 1 [19-26].

107 Molecularly imprinted chiral stationary phases (MICSP) have predetermined selectivity, since
108 they are prepared by using one of the pure enantiomers as template, which is rarely obtainable
109 with conventional CSP. For example, in the case when the enantioseparation is carried out in
110 an HPLC column packed with a given (R)-enantiomer-imprinted stationary phase, the
111 corresponding (S)-enantiomer will elute before the (R)-enantiomer, since the latter will be
112 more retained [5, 6]. In general, these systems allow the enantioresolution with selectivity
113 factors (α) ranging from 1.5 to 5 or even greater. However, in many cases enantiomers are not
114 completely separated due to the large peak broadening and tailing, especially of the more
115 retained enantiomer. Obviously, this drawback becomes more problematic when a separation
116 of more than two compounds is necessary. The observed peak broadening and tailing is
117 probably connected with the heterogeneity of binding sites, in terms of both affinity and
118 accessibility, and different association and dissociation kinetics [6].

119 Although many other competing technologies exist, MIP-mediated chiral recognition
120 phenomena continue to attract interest from the scientific community. An ever-increasing
121 body of data accumulated in numerous studies, however, has provided a basis for clearer
122 understanding of true potential and inherent limitations of MIP materials in chiral recognition
123 applications [5]. The interest of such methodologies applying MIP materials in the field of
124 chiral separation techniques over the years 1991 to 2017 (obtained from Scopus Web Site data
125 base) were illustrated on the Figure 1. Moreover, MIPs sorbents or filling mediums prepared
126 for separation techniques are characterized by many advantages including easy preparation
127 methodology, high physical and chemical stability (resistance to high temperatures, organic
128 and inorganic solvents and pH conditions). In addition, this type of sorption/filling medium
129 might be used repeatedly by applying an appropriate regenerative procedure. Taking into
130 account the advantages previously mentioned, the MIP materials become suitable solutions as
131 a stationary phases/mediums for the following separation techniques: chromatography
132 (especially HPLC); CECs, electrochemical and biomimetic sensors; quartz crystal
133 microbalance; solid phase extraction (SPE) and in the field of membrane separation [27].

134 The aim of this review is to provide a critical overview of the current role of MIP-type
135 affinity materials in the multidisciplinary field of chiral recognition, with the focus on
136 applications in enantioselective separation by chromatographic techniques. The coverage of

137 this review is selective rather than exhaustive, and concentrates on innovative concepts rather
138 than incremental improvements. We conclude that MIPs are very promising materials to be
139 used as selective stationary phases in chromatography although further developments are
140 necessary in order to fully exploit their potential.

141

142 **2. MIPs: a selective sorption medium for enantioresolution by liquid chromatography**

143

144 Enantiomers of bioactive compounds may exhibit different physiological effects on
145 pharmacological activity, metabolism processes, and toxicity when being ingested by living
146 organisms [28]. Thus, effective separation of chiral compounds (both analytical and
147 preparative enantioseparations origin) is important and often required in fields including food
148 and agrochemical industries, medical chemistry, drug development, enzyme engineering,
149 catalyst technology and the material sciences. Chiral HPLC, possessing advantages of
150 accuracy and generality, is still the most important technique for the analysis of enantiomeric
151 optical purity and rapid achievement of enantiomerically pure materials, although it is
152 relatively time-consuming and labour intensive [28]. HPLC has been the most extensively
153 applied technique for chiral separation in last few decades.

154 In HPLC, indirect and direct resolution methods can be used for enantioseparation. In indirect
155 mode, the two enantiomers interact and form stable diastereoisomers with strong bonds are
156 formed before the chromatographic separation can take place in non-chiral stationary phase
157 and require the use of high purity derivatizing reagents. Moreover, this mode is time
158 consuming and purification steps may be required [29]. Thus, the direct method is mainly
159 employed enantioresolution mode. Here the CSP is present into the column interacting
160 continuously with the enantiomers to be separated. Diastereomeric complexes with the
161 involvement of weak bonding are formed during the LC and then achieving their separation
162 [30].

163 Many efficient CSP's exist although most of the chiral recognition elements incorporated into
164 these CSPs are non-target-specific in nature, and the reliable prediction of the separability and
165 order of elution of a given pair of enantiomers is still elusive [5]. In 1985, the application of
166 MIP materials combined with the LC technique for the separation of amino acid derivatives
167 was described [24]. Since then, MIPs have become increasingly popular as CSPs in HPLC.
168 The MIPs are introduced to overcome some limitations of conventional CSPs, and offer the
169 unique opportunity to tailor CSPs with predefined chiral recognition properties by using the
170 enantiomers of interest as binding-site-forming templates. Moreover, due to the simplicity of

171 operation chirality transfer from a templating enantiomer to the polymer network also
172 eliminates the need for lengthy synthetic routes, sophisticated receptor designs, and elaborate
173 immobilization procedures. In addition, MICSPs are characterized by excellent chiral
174 recognition properties for the templating chiral species, which are manifested in high
175 enantioselectivity, pronounced substrate-specificity, and predictable order of elution, with the
176 enantiomers employed as templates being the more strongly retained species [5]. In
177 consequence, technology of molecular imprinting has been extensively applied to
178 manufacture of target-specific CSPs for a wide range of chiral compounds including a variety
179 of drugs of abuse [23] and pharmaceuticals [3, 31], naturally occurring compounds [32],
180 amino acid derivatives [33] and many other specific chemical compounds determining by
181 HPLC technique.

182 However, some difficulties and drawbacks of MICSPs in HPLC exist. The most important are
183 difficulties associated with the engineering of suitable chromatographic formats as well as the
184 inherently poor mass-transfer characteristics of imprinted polymers [34]. Since
185 chromatographic columns for chiral recognition are mainly packed with particles derived
186 from bulk polymers by the traditional grinding and sieving procedure, irregular particles with
187 relatively broad size distributions exist resulting in packing's of irreproducible quality which
188 manifests in poor column efficiency and high column back pressure. Moreover, although this
189 method can be easily carried out in any laboratory, it is not appropriate for large-scale
190 production. In addition, limited commercial application of MIP-CSPs was are apparent
191 because of previously mentioned reasons. More specifically peak broadening and tailing have
192 both thermodynamic and kinetic characteristics [35], which depends on the association
193 constant and sample load with an increasing trend for high values. To improve
194 chromatographic efficiency, the replacement of non-covalent imprinting with covalent and the
195 use of several strategies for obtaining uniformly sized spherical microspheres is practiced.

196 In the last few years, several polymerization strategies have been proposed in the literature
197 including precipitation, suspension, and multi-step swelling and polymerization. Application
198 of these polymerization techniques gives the possibility to prepare the spherical imprinted
199 particles, with a narrow size distribution, and polymer monoliths which may be used as
200 chromatographic stationary phases (Table 1). Much research effort has been invested in
201 establishing dedicated MIP formats for chromatographic applications, for example porous
202 monoliths, spherical beads, and silica-supported films. Normal formats of MIPs for
203 enantioseparation in HPLC include monoliths, particles, and membranes. The application of
204 these formats in HPLC are described generally and listed in Table 1.

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2.1. MIPs particles used in liquid chromatography

As it was previously mentioned, MIPs particles characterized by appropriate morphological and physicochemical properties, might be prepared by application such methods as precipitation, suspension, and multi-step swelling polymerization, which were briefly described in Table 1. However, due to the fact that the preparation of MIPs particles by bulk polymerization is inefficient, and they present poor separation behavior when are applied as CSPs, the surface imprinting technique (SIT) was introduced [47]. To manufacture surface molecular imprinted polymers (SMIPs), the MIP should be typically grafted on the supporting materials surface, such as silica gel, however, the recognition ability of this kind of SMIP was sensitive to the grafting conditions. Schematic representation of SMIP-CSP preparation is presented in Figure 2. Silica gel particles surface-coated with chiral selectors as CSPs for chiral separation by HPLC was first time reported in 1986 [48]. This coating method has now been extensively applied for enantioseparations mainly due to its high separation efficiency and simple preparation process. Compared to the surface grafting method, the coating method process is simpler and the final surface is more homogeneous. In SMIPs, the recognition sites are more easily accessible with favorable binding kinetics. In SIT, less template molecules are applied in comparison to what is used in conventional imprinting techniques since the template is only used in the surface coating step [47]. This technique has been applied in the imprinted coating on numerous different types of nanomaterials including silica particles [49], nanowires [50], nanotubes [51] and magnetic nanoparticles [52]. The SIT is important for the formation of MIPs on the support particles surface. MIPs created by this technique present highly uniform shape and size, and therefore, more efficient particles can be prepared. As a results faster mass transfer, higher binding capacity, and easier adsorption and removal of templates than traditional MIP particles are obtained [3].

An example of the application of this method to enantio-separation was presented by Dong et al. [53]. In the work, SMIP-coated CSPs (SMIP-CSPs; poly-methacrylic acid as the matrix) were successfully prepared by coating an (R)-DABN (1,1 -Binaphthalene-2,2 -diamine) imprinted polymer on silica gel particles which showed an excellent resolution ability for the racemic DABN by HPLC. The prepared SMIP-CSP showed the highest separation factor (3.39) for the resolution of the DABN racemate, more than their previous work (2.14) using MIPs produced by bulky polymerization technique [54].

238 In SIT, the selection of functional monomers also has an extreme difference what is illustrated
239 in a paper published in 2012 [55]. A L-Phe imprinted polymer based on monodisperse hybrid
240 silica microspheres (MH-SiO₂) with –CH=CH₂ groups was synthesized by SIT, while the
241 MH-SiO₂ was synthesized by a sol–gel process in aqueous media using tetraethylorthosilicate
242 (TEOS) and 3-methylacryloxypropyl trimethoxysilane (MATES) as the precursors. Compared
243 with the imprinted polymer prepared with β-cyclodextrin (β-CD) or MAA as functional
244 monomer, the imprinted polymer prepared with both β-CD and MAA as binary functional
245 monomers holds the highest adsorption capacity. Under the optimum chromatographic
246 conditions, a complete baseline separation of phenylalanine racemates was observed using the
247 column packed with the imprinted polymer prepared with both β-CD and MAA as binary
248 functional monomers. The separation factor and resolution of the imprinted polymer towards
249 phenylalanine racemates were calculated as 1.41 and 1.46, respectively, which is higher than
250 that of other imprinted polymers [55].

251 One of the most important parameter of MIP-based CSP on the silica-gel bead is the thickness
252 of the film, due to the fact that it has an important impact on the separation results. The
253 thickness and morphology of the film of MIP-based CSP are very difficult to control.
254 However, an application of the “grafting from” approach in which the grafting reaction can
255 proceed by polymerization from the surface can effectively control the thickness of grafted
256 polymer. This was approached has been used and demonstrated, e.g. for the separation of the
257 enantiomers of the citalopram [56]. For this purpose, the iniferter-mediated grafting approach
258 to develop a surface-imprinted CSP was employed. Firstly, MIP chiral selectors were grafted
259 to the surface of porous silica particles, after which a homogeneous material was formed. This
260 material had a stronger interaction with the S-enantiomer of the drug. In this way, an optimal
261 thickness was obtained which provided the best resolution of the analysed racemate.

262 Another method which allow the tuning of the morphology of the film of MIP-based CSP as
263 well as complex framework, and functionality of a well-defined MIP, is reversible-
264 deactivation radical polymerization (RDRP), especially addition fragmentation chain transfer
265 polymerization (RAFT) [3]. The latest technique makes use of a chain transfer agent (CTA) in
266 the form of a thiocarbonyl compound (or similar) to afford control over the molecular weight
267 and polydispersity during a free-radical polymerization. RAFT has been proven to be able to
268 reduce heterogeneity of resultant polymers. The RAFT would contribute much to the
269 controllable development of MIP-based CSPs on the supporting materials to obtain excellent
270 MIPs with high homogeneity and capacity [3]. Information on application of organic
271 polymer-based particles MIPs in HPLC are provided in Table 2.

272

273 *2.2. MIP monolithic materials*

274

275 Due to the advantages such as low cost, ease of preparation, good stability, high
276 reproducibility, rapid mass transfer and versatile surface chemistry, monolithic materials have
277 been widely used for various applications in LC. Among these materials MIPs are of high
278 importance and have recently been applied extensively in HPLC for chiral separations. The
279 preparation process of monolithic MIPs is more straightforward and convenient in
280 comparison to particles. The combination of monolithic column and MIPs combines the high
281 efficiency of chromatography as well as the high selectivity provided by MIPs. The in situ
282 polymerization method employed in 1993 [58], was used to prepare molecularly imprinted
283 monolithic polymer rods. In the procedure of MIP preparation, a template compound, a cross-
284 linker, and a functional monomer were mixed in a stainless steel column and heated. Thus, the
285 polymerization occurred in the column, greatly shortening the pre-preparation time. After
286 polymerization, the template and porogenic solvents are removed by exhaustive washing with
287 an acetic acid-methanol mixture. It need to be noted that a suitable porogenic solvent should
288 meet three criteria [64]: (i) template molecules, initiator, monomer, and cross-linker must be
289 soluble in the porogenic solvents; (ii) the porogen should be able to create large pores, which
290 can modify the flow-through property of the resulting polymer; and (iii) the porogenic
291 solvents should have low polarity. Low polarity can have weak interferences to the interaction
292 between the imprint molecule and the monomer during polymerization, being important to
293 obtain MIPs with high selectivity.

294 In situ technology integrates the advantages of monolithic column and molecularly imprinted
295 technology, which is prepared by a very simple, one-step, free-radical polymerization process
296 directly within a chromatographic column without the tedious procedures of grinding, sieving
297 and column packing [25].

298 Two MIP monolithic matrices exist: organic polymer-based monoliths (the major MIP
299 monolith matrices) and silica-based molecularly imprinted monolith (mainly used for chiral
300 recognition by electrochromatographic techniques). The first type of MIP monolithic matrices
301 has been extensively investigated since a lot of variety of monomers are available and stable
302 in different pH environments. The most common monomers used to prepare this type of MIP
303 includes MAA, 4-vinylpyridine (4-VP) and acrylamide (AA).

304 To minimize the template consumption as well as to improve the kinetic properties, another
305 method was developed to prepare the MIP on the glass microspheres in the column [65]. The
306 column was pre-packed with glass microspheres. Next, the pre-polymerization mixture was
307 injected into the interstitial volume of the column. The polymerization took place *in situ* and
308 the column could be directly used for HPLC after the template had been removed. The MIPs
309 obtained exhibited higher efficiency, better kinetic properties, and low back pressure of the
310 column.

311 To improve the separation efficiency, more effective imprinted sites are needed. It is often the
312 case that the number of effective imprinted sites mostly depends on the ratio of monomers. In
313 fact, the permeability is bad for traditional volatile organic solvents with high monomer
314 content [3] and here room-temperature ionic liquids (RTIL) are used to overcome these
315 drawbacks. Due to properties including low vapour pressure, excellent solvation qualities, and
316 good chemical and thermal stability, RTILs are of high importance nowadays in separation
317 science. In addition, RTIL may be classed as green solvents, which have temperate effects on
318 the environment. Taking into account advantages of RTILs, these compounds present
319 immense potential as replacements for traditional solvents in the MIP preparation process. For
320 example, a MIP monolith with good permeability was successfully achieved using a strategy
321 involving a high content of monomers in a dimethyl sulfoxide-dimethylformamide
322 ([BMIM][BF₄])-based green solvent [59]. The imprinted monolith was prepared with ketoprofen
323 or naproxen as a template, 4-VP as the functional monomer, and ethylene glycol dimethacrylate
324 (EGDMA) as a crosslinking monomer. Column efficiency and permeability of the MIP monolith
325 can be tuned by a mixture of [BMIM][BF₄]/DMSO. The approach allowed the creation of an
326 imprinting system in a short polymerization time (<1.5 h) and higher imprinting factor (IF - 8.64)
327 than the MIP prepared in a traditional volatile solvent. In another study, a new CSP based on MIP
328 was prepared in RTIL by use of the metal pivot concept. Imprinted monoliths were
329 synthesized by use of a mixture of R-mandelic acid (template molecule), 4-VP, EGDMA, and
330 several metal ions as pivot between the template and functional monomer. A ternary mixture
331 [BMIM][BF₄] containing metal ions was used as the porogenic system. Separation of the
332 enantiomers of racemic mandelic acid was successfully achieved on the MIP thus obtained,
333 with resolution of 1.87, whereas no enantiomer separation was observed on the imprinted
334 monolithic column in the absence of metal ions. The results reveal that use of metal ions as a
335 pivot, in combination with ionic liquid, is an effective method for preparation of a highly
336 efficient MIP stationary phase for chiral separation. Information on the application of organic
337 polymer-based monolith MIPs in HPLC are listed in Table 2.

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339 *2.3. Molecularly imprinted polymer-based membranes*

340

341 For preparative applications, membranes can be used as the separation matrices, with the
342 benefit that a continuous process can be designed, as compared to the batch wise operation of
343 chromatography. MIP-based membranes were presented as feasible in HPLC for chiral
344 separation in 1990s [62]. In the beginning, membranes were prepared either as free-standing
345 thin films [66] or thin polymer films on the surface of solid supports [67] following standard
346 imprinting recipes. Others have employed a phase-inversion precipitation technique starting
347 from linear polymer precursors [62]. Imprinted polymer membranes can be also prepared
348 by casting an imprinted polymer in the pores of a porous solid support, such as a
349 polypropylene membrane. This solution was applied for enantioseparation of CBZ-
350 tyrosine [62]. However, new developments of MIP-based membranes have appeared
351 during recent years [3].

352 It is reported that both permselectivity and flux are important properties for membrane
353 separation and it can be challenging to improve the flux of a MIP membrane without
354 deterioration of permselectivity. Therefore, efforts have been made to maintain the quality of
355 these two parameters. For example, in 2012, a molecularly imprinted nanofibre membranes
356 (MINFMs) were synthesized and compared with traditional molecularly imprinted membranes
357 (MIPMs) [61]. It was shown that the fluxes through the MINFMs gave one to two orders of
358 magnitude higher than those of standard normal MIPMs without depression of
359 permselectivity.

360 Additionally, other approaches to modify the properties of MIPMs exist. For example, an
361 appropriate selection of substrate of MIPMs was presented to be a very important parameter
362 [63]. For example, the synthesis of ractopamine MIPs nanotube membranes on anodic
363 alumina oxide (AAO) nanopore surface by atom transfer radical polymerization (ATRP) was
364 described, in which MAA was selected as functional monomer. AAO has a highly-ordered
365 hexagonal nanopore array as well as adjustable pore diameter, thickness and shape, so the
366 resultant polymers usually have uniform shape and size. Compared with the traditional
367 methods, the method combined of imprinted layer-coated nanostructures with surface
368 enrichment of the targets can significantly improve the binding capacity and kinetics of
369 imprinted materials by increasing the number of binding sites at the material's surface [63].
370 Moreover, AAO-MIPs have a small dimension with a high specific surface area. The
371 emergence of AAO as a nanoreactor for molecular imprinting can eliminate the limitations of

372 traditional imprinting, such as incomplete removal of the template, small binding capacity,
373 slow mass transfer, and irregularity in the shape of materials. Information on application of
374 organic polymer-based membranes MIPs in HPLC are provided in Table 2.

375

376 *2.4. MIPs template in capillary electrophoresis*

377

378 Capillary electrophoresis (CE) is one of the techniques used for separating enantiomers. This
379 technique is characterized by high efficiency, low consumption of solvents and selectors,
380 simple instrumentation, as well as short analysis time when applied in practical problem
381 solving in various industries including; chemical, pharmaceutical, biomedical, food and
382 environmental. In general, the separation of enantiomers is obtained by adding the chiral
383 selectors to the running buffer [68]. Various types of chiral selectors, including CDs and their
384 derivatives, different classes of antibiotics, polysaccharides, proteins, crown ethers, chiral
385 metal complexes, surfactants, chiral ion binding reagents have been successfully used to
386 separate enantiomers [69]. At present, CDs and their derivatives (anionic and cationic CDs),
387 remain the most commonly used chiral selectors in CE. Beyond chromatographic and
388 electrokinetic techniques, hybrid technique such as CEC can be used to obtain pure
389 enantiomers [70]. Application of MIP for analytical scale separation in CE and CEC are
390 becoming more popular than in LC nowadays due to the intrinsic character of high separation
391 efficiency and minimized requirement of the amount of MIP template in CE and CEC [71].

392 CE is an effective method of analysis for a wide range of applications because it is fast and
393 requires a small amount of solvents and reagents [72]. CE has proved to be a powerful
394 technique for separation of chiral compounds. Since it has the advantages of high resolution
395 of such compounds one of the most successful areas of application of CE is chiral amino acid
396 analysis. To obtain a high resolution of the target enantiomers, the choice of separation mode
397 is one of the most important issues in the CE analysis of both amino acid and other compound
398 enantiomers. Separation modes in CE involve the addition of appropriate CSs into a
399 background solution (BGS). The most commonly used modes are: (i) cyclodextrin-modified
400 capillary zone electrophoresis (CD-CZE); (ii) CD electrokinetic chromatography (CDEKC);
401 (iii) micellar EKC (MEKC); (iv) CD modified MEKC (CD-MEKC); (v) chiral ligand-
402 exchange CE (CLE-CE); (vi) affinity CE (ACE) and (vii) non-aqueous CE (NACE) [73].

403 One of the key separation modes for CE enantiomers analysis is CD-CZE in which "neutral"
404 CDs are added to the BGS as CSs. The migration of ionic compounds in the CDs zone results

405 in a chiral separation. In CE-CZE natural α -, β - and γ -CDs and derivatized CDs which include
406 hydroxypropyl- α -CD (HP- α -CD), HP- β -CD, methyl- β -CD (Me- β -CD) and dimethyl- β -CD
407 (DM- β -CD) can be used [74]. Besides labelled amino acids [75] CD-CZE enantioseparation
408 of alkyl and aryl monoesters of *N*-blocked aminophosphonic acids [76], structurally complex
409 basic drugs [77], deprenyl and its major metabolites [78], cyclic antidepressants [79] have
410 also been applied.

411 In the CDEKC separation mode “charged” CDs are added to BGSs as the CS in contrast to
412 CD-CZE mode. The charged CDs functions act as a pseudo-stationary phase for the enantio-
413 separation. When the ionic CDs interacts with a racemic mixture, analytes transfer from the
414 surrounding water phase due to the electrophoretic migration of the charged CDs [87]. For
415 CDEKC anionic [80] and cationic CDs [81] are produced, however, the dominant CDs group,
416 due to its resolution powers and their commercial availability, are highly sulfated CDs (HS-
417 CDs) [82]. Various types of CDs are highly effective for separation of racemic amino acids
418 and their derivatives using the CDEKC [83, 84]. Recently progressed analytical approaches
419 employing CDEKC in the area of enantioselective analysis of drugs metabolites, and
420 biomarkers in biological samples have been described [85].

421 MEKC which can separate both neutral and charged analytes by the capillary electrophoretic
422 technique, was developed in 1982 and the first paper was published in 1984 [86]. MEKC is
423 also another important mode of CE which is also widely used for the enantio-separation. In
424 this solution the chromatographic principle is based on the distribution equilibrium between
425 immiscible phases and distinct from the ACE binding stoichiometry between analytes and
426 chiral micelles which is not required and also more complex relationship with the
427 concentration of the chiral selector compared to the complexing components [87]. A fully
428 automatized MEKC-MS method was developed for the chiral analysis of d- and l-amino acids
429 [88]. As one of many possibilities of using the MEKC model, the enantio-separation of four
430 stereoisomers of palonosetron hydrochloride (PALO) [89] might be carried out.

431 Coupling MEKC using achiral and/or chiral surfactants with chiral recognition ability of CDs
432 (CD-MEKC mode) uses a micellar solution containing CDs as a BGS. Since the MEKC mode
433 gives good resolution of compounds closely related to each other based on small differences
434 in partition coefficients with respect to the micelle, it is suitable for separation of many types
435 of enantiomers in samples which are often characterised by complex matrix composition [73].
436 In fact, many applications of CD-MEKC to real sample analyses have been reported. The
437 content of catechins and methylxanthines in green tea has been determined by a CD-MEKC
438 method with the addition of hydroxypropyl- β -cyclodextrin [90]. A CD-MEKC method with

439 HP- γ -CD as chiral selector for the enantiomeric separation of econazole have also been
440 reported [91]. There are reports where CD-MEKC method has been developed for the
441 simultaneous separation of a group of parent phthalates [92] or separating conjugated linoleic
442 acid (CLA) isomers [93]. In all cases the CD-MEKC method was simpler, safer and more
443 economical, than HPLC and GC methods.

444 CLE-CE technique was used for the first time in 1985 for the enantioseparation of D,L-amino
445 acid and because of low cost, high convenience and controllable enantiomer migration order it
446 is of growing interest. The widely-used chiral ligands in CLE-CE mode are L-AAs, D-AAs,
447 L-AAs derivatives and some chiral organic acids [94].

448 NACE was first introduced in 1984 however, the first publications on the separation of
449 enantiomers with the NACE mode were available in 1996 [95]. NACE has been proved to be
450 a powerful tool to achieve the enantioseparation of, nine structurally similar chiral
451 anticholinergic drugs [96] and for the chiral separation of some β -blockers and β -agonists
452 using di-n-amyl L-tartrate-boric acid complex as the chiral selector [97]. Isomers of amino
453 acids are separated by both NACE and ACE mechanisms [73].

454 A number of publications on biological, food and pharmaceutical applications of the CE
455 chiral separation have appeared. It has been shown that CE is an appropriate technique for the
456 quantitative determination of many enantiomers even in complex sample matrices [73].

457

458 **3. MIPs: a sorption medium for enantioresolution by capillary electrochromatography** 459 **technique**

460

461 CEC is a hybrid separation technique that combines elements and advantages of LC and
462 electrophoresis [98]. The stationary phase used in CEC have been greatly developed due to
463 the various requirements of microscopic separation. Due to the low cost and favourable
464 molecular recognition capability and stability of MIPs, they have also found use in
465 chromatography [99]. The combination of the MIP technique with capillary CEC takes
466 advantage of the selectivity of MIPs and utilises the high CEC efficiency [98]. The approach
467 was first applied in 1994 [26], since then, this has been a commonly used technique in
468 separation sciences [98] and highly selective stationary phases within chiral separations [100].
469 The development of MIP-charged capillary columns suitable for CEC applications, was more
470 difficult than for HPLC, essentially, it is required to develop dedicated MIP formats. In the
471 literature several protocols allowing the synthesis of MIPs using the appropriate
472 characteristics in CEC have been proposed [5]. First attempts were performed by packing

473 MIP particles inside the capillary [6]. In Table 3 currently used examples of different
474 approaches to MIP-type capillary formats were generally described.

475 Examples of applications of MIPs as a sorption medium in CEC chiral separation techniques
476 are currently one of the most attractive topics in chromatography. For example, a number of
477 short OT- MIP columns for chiral separation of various pharmaceuticals (especially NSAIDs)
478 and other compounds was presented where excellent efficiencies in chiral separation of
479 template enantiomers as well as non-chiral separation of nonpolar and polar test solutes was
480 obtained [110]. In other studies, monolithic MIP for chiral separation of nateglinide and its L-
481 enantiomer, using an in situ method was designed and prepared. Experiments have shown that
482 chiral detection was dependent on stereochemical structures and arrangement of functional
483 groups in the MIP cavities. The thermodynamics of the enantioseparation indicated an
484 enthalpy-controlled process [111]. Selectivity of (S)-naproxen MIP monoliths, which was
485 prepared by an in situ thermal-initiated polymerization, was also examined. The study results
486 showed that good chiral recognition was not only dependent on the MIP monoliths, but also
487 on the CEC parameters such as amount of organic solvent, pH range of buffer solution, salt
488 concentration, column temperature and addition of for example surfactants [112]. Use of
489 MIPs for CEC enantiomer separation of propranolol using a partial filling technique was also
490 reported. This method allows altering of the amount of MIP used for a certain separation
491 which, in turn, is beneficial for fast optimization [112]. MIP stationary phases synthesised by
492 an in situ photo-initiated polymerisation reaction for rapid separation of propranolol were
493 studied as well [113].

494 MIPs as a sorption medium in CEC represent a novel method and a promising tool for the
495 demanding or special analytical separation tasks, such as chiral separation. The MIP-CEC
496 system might be employed in miniaturised analysis systems in general. Promising potential of
497 MIP-CEC has been increasingly evident since more and more CEC-based MIPs stationary
498 phases have been successfully prepared and increasingly used in fields such as drug or food
499 analysis [113].

500

501 **4. MIPs: a selective solid materials in capillary liquid chromatography**

502

503 Capillary LC and CEC are well known as powerful analytical techniques based on the
504 differential distribution of analytes between the mobile and stationary phases leading to their
505 general migration patterns. The use of in situ modified capillaries in both HPLC or high
506 performance capillary electrophoresis (HPCE) instruments offers many mechanical and

507 optical advantages [114]. In tubular, especially porous layer open tubular (PLOT), formats
508 faster regeneration and higher linear velocity can be achieved in comparison to packed bed
509 capillaries. There are many types of stationary phases that can be used in the nano-analytical
510 separations in capillaries. MIPs have a number of advantages (high resolution and
511 reproducible retention, fast analysis time and very conservative use of reagents) when applied
512 as CSP materials. The development of enantioselective MIPs have great interest in the context
513 of being used for capillary LC as well as CEC mode in monolith or PLOT [115]. One
514 example is when MIP-coated capillaries have been evaluated in separations of the ketoprofen
515 racemate [115] or racemic amlodipine and naproxen [116].

516

517 **5. Summary and future challenges**

518

519 From the analytical chemistry point of view (assessing the purity of obtained chemical
520 compounds) it is necessary to successfully improve and developed new types of sorption
521 materials which might be considered and applied as a stationary phases in advanced analytical
522 separation techniques. Suitably developed and well characterized material employed as a
523 stationary phase allows for effective separation and optimal identification of chemical
524 compounds in enantio-separation processes, especially in a case of biological, medical,
525 environmental and pharmaceutical samples. The possibility of “creative design” of stationary
526 phase for a specific chemical compound using molecularly imprinted techniques gives an
527 opportunity to increase the selectivity and sensitivity of applied analytical methodology.

528 Nevertheless, it should be highlighted that such polymeric materials employed as stationary
529 phases in different types of separation techniques, must be characterized with appropriate
530 particle size (mesh), particles diameter and geometry, high porosity and specific surface area.
531 Due to this fact, the whole process of preparation and characterization of new types of
532 polymer material as a stationary phase in separation techniques requires appropriate
533 knowledge, skills, time, and adequate laboratory facilities. The improperly prepared polymer
534 material might lead not only to poor analysis results, but also cause damage (permanent or
535 temporary) to expensive analytical equipment (e.g. applied detectors, such as mass
536 spectrometers).

537 One of the main challenge in the field of application process of MIP stationary phases in
538 advanced separation analytical techniques to assess the optical purity of chiral compounds,
539 described in detailed in scientific literature, is to develop a MIP stationary phase that will be
540 characterized by sufficient homogeneity and high density of the binding sites, having

541 excellent chiral recognition properties and high mass transfer kinetics. Moreover, when
542 preparing the new type of MIP stationary phase it is vital to select reaction conditions /
543 method in such way to ensure high uniform of size and shape of synthesized MIP particles. In
544 the case of developing a new preparation process, the most popular practice is the bulky
545 polymerization technique. However, the solution for this problem, mentioned in many
546 research papers, is to applied other polymerization techniques which helps to ensure the
547 optimal shape and size of obtained MIP material particles, such as *in situ* multi-step swelling,
548 suspension polymerization, or the surface molecular imprinting technique (using so-called
549 microspheres) on a specific materials like silicagel, nanofibers or magnetic particles.
550 Application of microspheres is one of the most optimal solution in a case of stationary phases
551 in the field of enantio-separation process, due to the optimal size and shape of particles, which
552 increase the efficiency of the separation and identification of chiral chemical compounds.
553 However, using mentioned microsphere based polymerization techniques in many cases
554 requires large amounts of reagents and solvents to prepare the optimal MIP stationary phase
555 [1,5,7,117].

556 Furthermore, it is important for the preparation process of almost every MIP materials to
557 select an optimal template molecule. In some cases it is difficult and expensive to source the
558 chemical compounds. Moreover, the template monomer which will be the most suitable to
559 develop new stationary phase is not soluble in any porogen solution and it is hard to perform
560 the polymerization reaction. The solution for this problem might be the application of
561 structural analogues of template molecules (dummy template imprinted polymers). However,
562 the main drawback is the possibility to achieve an inadequate degree of selectivity of a
563 developed sorption material to the specific chiral compound. The important challenge is to
564 develop stationary phases in advanced separation techniques which might be applied
565 successfully in large-scale enantio-separation processes and in every day chemical analysis,
566 i.e. pharmaceutical or biotechnological origins within on-line systems. One of the main future
567 trends in analytical chemistry origin concerning the rapid separation of chiral chemical
568 compounds might be the newly developed enantioselective electrochemical sensors (in-situ
569 rapid analysis). To designed and developed a desired electrochemical sensor it is important to
570 cover the electrode's surface with a functionalized thin film layer which will be capable to
571 "recognize" only one enantiomer. Such small-scale analytical devices might be impregnated
572 with a thin film of a specific MIP material as a stationary phase, which greatly simplifies the
573 qualitative and quantitative analysis factors of a selected enantiomer in biomedical,
574 biochemical, pharmaceutical and environmental samples [118].

575

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579

580 **7. Conflict of interest**

581 The authors declare that they have no conflict of interest

582

583 **8. References**

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918 **9. Figure Captions**

919 **Figure 1.** The milestones of MIPs development and the general degree of MIPs applications
920 in the field of stationary phases used in analytical separation techniques (Scopus Web Site
921 data base).

922 **Figure 2.** Schematic representation of SMIP-CSP preparation: 1) pretreatment; 2) coating on
923 silica gel; 3) surface polymerization; 4) removal of template.