Magnetic molecular imprinted polymers as a tool for isolation and purification of biological samples

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Abstract: Technology of molecularly imprinted polymers (MIP) has become very popular in recent decades. MIPs are primarily used in medical diagnostics, chromatographic separation and solid phase extraction (SPE); also as sensors and catalysts. In recent years there have been reported benefits of combining molecular imprinted polymers with additional features, e.g. magnetic properties, through the build-up of this type of material on magnetite particles (Magnetic Molecularly Imprinted Polymer – MMIP). This method produces a multifunctional material which has high selectivity and the ability to isolate the analyte from biological and environmental samples, allowing effective purification from such interferents as proteins and fats. This developing branch of new materials for the preparation and purification of complex sample matrices is an interesting alternative to materials routinely used to date, particularly with regard to the immunosorbents.

This paper summarizes recent reports regarding MMIP preparation and their application for purification and isolation of compounds from biological matrices.

Keywords: Magnetic molecularly imprinted polymer, synthesis of MMIPs, solid phase extraction, biological samples, review.

1 Introduction

Polymeric sorbents are increasingly used in biomedical and environmental analyses. Despite the packing of chromatographic columns they are used for sample preparations, especially biological that contain e.g. proteins, fats and other interfering compounds. This step is the most time consuming part of the analysis and is the source of the majority of errors (up to 60%). As a result, it significantly contributes to the uncertainty of the results obtained by a given analytical procedure. The selection of the extraction technique depends mainly on the physico-chemical properties, physical state of the analyte and the chemical composition of the matrix. The method applied for sample isolation and purification is usually closely linked with the analytical technique used for identification and quantitation [1]. The compounds determined are very often present in the matrix at very low concentrations, which forces the analyst to use a very sensitive instrument, e.g. liquid chromatograph or gas chromatograph usually combined with mass spectrometer as a detector. These techniques require extensive purification of crude extracts from interfering compounds, which can significantly affect the interpretation of the analytical results [2,3]. Depending on the physical state of the samples, most commonly used methods are solvent extraction and solid phase extraction (SPE). The latter technique is the most popular method of purification and enrichment of crude extracts [4]. There is a wide range of SPE sorbents available on the market but the most commonly used materials are based on silica gel modified with various functional groups (-C8, -C18, -NH2, -CN, etc.). In the case of these adsorbents, the necessary step is to condition the packing bed. It should be noted that loss of performance is observed when the sorbent becomes dry during SPE procedure. Therefore, polymeric sorbents have recently become the main focus of interest. Their advantage is high resistance to extreme conditions during the preparation of samples, i.e. high and low pH, temperature and variation of pressure [5]. An interesting group of polymer sorbents are undoubtedly molecularly imprinted polymers (MIPs). MIPs are used in chemical analysis in various forms: crushed monolith [6], spherical particles or a polymer layer coated on another medium (e.g. magnetite or silica). Their advantage over the commercially available SPE columns is that they can be used many times without loss of selectivity. In recent years, MIPs have been combined with additional properties of the support, e.g.
magnetic properties. The magnetic molecularly imprinted polymers (MMIP) were presented for the first time in 1998 by Ansell and Mosbach [7]. Since then there have been numerous reports regarding application of MMIPs for the analysis of both environmental and biological samples (Fig. 1). MMIPs are attractive because, in addition to the advantages of classical MIP, such as high selectivity of dedicated analyte, they offer an increased contact with a sample as well as a simple method for sorbent separation from a sample by the application of magnetic field.

This work presents preparation methods for molecularly imprinted polymers with particular regard to magnetic polymers. An attempt was made to summarize the achievements in this area in the relation to the methods of sample preparation, especially biological samples.

2 Methodology of preparation of porous molecularly imprinted polymers

The process for MIP preparation is based on formation of a pre-polymerization complex between the template and the functional monomers which are able to produce specific non-covalent interactions with the template molecule in an appropriate solvent (a porogen). The next step in the synthesis is the addition of an appropriate amount of cross-linking agent and initiator which under the influence of physical factors, such as temperature or electromagnetic radiation, is able to initiate the polymerization reaction. Then the template is subjected to leaching by being removed from the “steric cavity”, which both in chemical and spatial terms corresponds to the substance isolated [9]. The scheme of MIP preparation is given in Fig. 2.

Molecularly imprinted polymers are present in various forms. The first form obtained was porous monoliths, which required a challenging process of crushing, fractionation and packing into the SPE columns [6]. Unfortunately, the resulting sorbent consisted of irregular grains, which caused destruction of steric imprinted cavities and consequently, a decrease in the efficiency of the extraction process. Therefore researchers began to work on obtaining spherical-shaped polymers. Spherical particles can be created using precipitation polymerization [10,11]. Another method of preparing spherical particles is the build-up of a polymer on the solid core, so called “core-shell” polymerization [12]. Apart from spherical particles, one can also find sorbents with a thin layer of polymer surface coated on a solid support, e.g. glass [13]. Fig. 3 illustrates different polymerization techniques used for MIP preparation.
Figure 3: Different polymerization techniques used for MIP preparation.

As it can be seen in Fig. 3, particles closest in form to a sphere are obtained through emulsion polymerization, suspension polymerization and by coating a polymer layer on a spherical support. In the case of precipitation polymerization the particles are of less regular shape and there is wider particle size distribution [14].

3 The synthesis of magnetic molecularly imprinted polymers

There are several preparation methods for spherical magnetic molecularly imprinted polymers. The process consists of four main stages as described below.

The first stage comprises obtaining iron(II, III) oxide (Fe$_3$O$_4$), called magnetite. Typically this compound is obtained by co-precipitation of hydrated iron(II) chloride (FeCl$_2$ $\times$ H$_2$O) and iron(III) chloride (FeCl$_3$ $\times$ 6H$_2$O). Iron in the second oxidation state can also be obtained from iron(II) sulphate (FeSO$_4$ $\times$ 7H$_2$O). Both reactions are carried out in ammonia [15] or in sodium hydroxide solution [16] at the temperature 80–100°C. Gao et al. [17] proposed another way to obtain magnetite. They mixed in basic solution of sodium hydroxide a trisodium salt of citric acid and sodium nitrate. The mixture was heated to a temperature of 100°C. Then iron ions in the form of tetrahydrated iron(II) sulfate were added. This method yielded nanoscale particles with narrow size distribution.

The next step is the modification of magnetite surface. The first method involves silanization of the surface. Magnetite nanoparticles are coated with a layer of SiO$_2$ by the reaction of tetraethyl orthosilicate (TEOS). Then the resulting silanol groups are functionalized by γ-MAPS [18] or other silanes that contain the multiple bonds necessary for the further polymerization process. The second method involves the use of surfactants, such as ethylene glycol or oleic acid. For example, oleic acid makes the surface of the polymer molecule amphoteric, which increases the possibility of its use in polar solutions (e.g. in water) [19]. In the literature, one can also find mention of the use of chitosan [20] and polichloromethylstyrene [21] to modify the surface of the magnetite prior to the polymerization step. Some researchers [19,22] omit the step of surface modification, going straight for the penultimate stage - the formation of molecularly imprinted polymer. This reaction may be carried out using different functional monomers and cross-linkers depending on the template used and the porogen solution.

The last stage involves washing out the template after polymerization as it occurs in the case of MIP synthesis. A commonly used mixture consists of methanol and acetic acid in the ratio 9:1 (v/v). The literature describes various ways of eliminating the imprinted molecule from the MMIP. Usually Soxhlet extraction is applied [23]. Much less frequently, repeated washing and sonication of the MMIP is used [24]. A diagram showing an example of MMIP preparation is shown in Fig. 4. Due to the properties of MMIPs, i.e. specific structure, highly expanded surface area and chemical stability, these particles are used as additives for the synthesis of nanotubes [25,26], wollastonite [27] or attapulgite [28].

After a four-step synthesis of the MMIP, the material is subjected to characterization. Compared to classic MIPs there are few methods of characterization presented in the literature (Table 1.) for MMIPs. The morphology of the sorbent is determined by imaging using scanning (SEM) and transmission electron microscopy (TEM). X-ray (XRD) and infrared spectroscopy (FT-IR) provide information about the chemical composition of the material obtained [19]. The magnetic properties of MMIPs are determined using the vibrating sample magnetometer [30] and thermal properties by thermogravimetric methods (TGA) [24].
4 Application of MMIPs for analyte isolation from biological samples

Biological samples contain interferents which can significantly affect the interpretation of results. Body fluids (blood, urine, plasma) and tissues contain a large amount of compounds, which results in the complexity of the obtained extracts subjected to the isolation process. In this type of sample compounds are present at very low concentrations, which necessitates the development of more effective isolation and purification methods [31]. Magnetic molecularly imprinted polymers have been mentioned in the literature for several years as an effective alternative to other techniques for isolation and purification. Application of MMIPs in solid phase extraction is not complicated. It offers the possibility to add an adsorbent directly to the liquid sample or a crude extract, which increases the contact of the sample with the analyte. The use of an external magnetic field can separate the sorbent from the solution. The scheme of the extraction using MMIP is shown in Fig. 5.

Primarily these materials are used for sample preparation prior to analysis utilizing different separation techniques, such as LC [32-34], GC [15, 35], and CE [36] or spectrophotometric [19] and spectrofluorimetric methods [37].

As presented in Table 2, magnetic molecularly imprinted polymers are used as a sorbent for a wide range of biological sample preparation. Data listed in the table are almost incomparable, however, some relationships can be drawn, for example lower recovery is obtained from solid samples compared to liquid samples. This is because...
Figure 5: A scheme of the extraction process using magnetic molecularly imprinted polymers: a – addition of MMIP to the sample; b – separation of MMIP from the sample with a magnet; c – analyte desorption; d - separation of MMIP from the analyte solution; e - analysis.

Table 2: Examples of MMIP application in the analysis of biological samples.

| Compound                  | Matrices       | Recovery         | LOD              | Method        | Literature |
|---------------------------|----------------|------------------|------------------|---------------|------------|
| Sulfonamides              | Chicken meat   | 95–99%           | 0.5–150 μg kg⁻¹ | HPLC-UV       | [38]       |
| Paracetamol               | Plasma sample  | 83–91%           | 0.17 μg L⁻¹      | HPLC-UV       | [39]       |
| Lamotigine                | Urine plasma   | 96–98%           | 0.5–0.7 μg L⁻¹   | HPLC-UV       | [40]       |
| β-agonists                | Pork and pig liver | 80.4–90%   | 0.52–1.04 ng mL⁻¹ | HPLC-FLD     | [24]       |
| Catecholamines            | Urine          | 92–102.8%        | 0.04–0.06 μM     | Capillary electrophoresis DAD | [18] |
| Gatifloxacin              | Urine          | 92%              | ~ 0.08 μg mL⁻¹   | Spectrophotometric | [19] |
| Tetracycline              | Tissue         | 72.8% to 96.5%   | less than 0.2 ng g⁻¹ | LC-MS        | [22]       |
| Sterols                   | Serum          | 72.4–88.2%       | 1.1–3.6 μg L⁻¹   | GC-MS         | [41]       |
| Naproxen drug             | Human urine    | 96.5–102.2%      | 2 ng mL⁻¹        | Spectrofluorimetric | [37] |
| Estrogens                 | Plasma         | 87.8–93.1%       | 0.3–0.4 ng mL⁻¹  | HPLC-UV       | [42]       |
| Carvedilol                | Serum          | 85–93%           | 0.13 ng mL⁻¹     | HPLC-UV       | [43]       |
| Estrogens                 | Fish, pork     | 73.8–96.7        | 0.4–1.7 ng g⁻¹   | HPLC-DAD      | [44]       |
| Polychlorinated biphenyls | Fish           | 73.41–114.21%    | 0.0028–0.0068 ng mL⁻¹ | GC-MS       | [45]       |
| Polychlorinated biphenyls | Fish           | 79.90–94.23%     | 0.0035–0.0070 ng mL⁻¹ | GC-MS       | [46]       |
| Auxin                     | Plant          | 70.1–93.5%       | 3.9–7.4 ng mL⁻¹  | HPLC-UV       | [47]       |
| Tramadol                  | Urine          | 98.2–100.1%      | 1.5 ng mL⁻¹      | Spectrophotometric | [48] |
| Anthraclyclines           | Urine          | 93.9–100%        | 0.6–2.4 ng mL⁻¹  | HPLC          | [49]       |
| Dopamine                  | Urine          | 94–110%          | 1.5 ng mL⁻¹      | Chemiluminescence | [50] |

[18] Capillary electrophoresis DAD
[19] Spectrophotometric
[22] LC-MS
[24] HPLC-FLD
[37] Spectrofluorimetric
[41] GC-MS
[42] HPLC-UV
[43] HPLC-UV
[44] HPLC-DAD
[45] GC-MS
[46] GC-MS
[47] HPLC-UV
[48] Spectrophotometric
[49] HPLC
[50] Chemiluminescence
an additional procedure for analyte extraction, from solid matrix to liquid environment, is required.

Besides their application in sample preparation for analysis of low molecular weight compounds, MMIPs may also be used as biosensors or sorbents for the detection and purification of specific groups of proteins. Application of such large molecules as a model for the molecular fingerprint in a polymer matrix is a big challenge. This is caused by poor mass flow, poor fit into the polymer structure and the formation of a heterogeneous fingerprint, which is the result of the complex chemical and steric structure of a protein [51]. The most commonly impressed molecule in this type of material is bovine serum albumin (BSA) [17]. These are either synthesized by polymerization carried out in the same manner as in the case of small molecules, however, using other monomers, for example by copolymerization of γ-aminopropytrimethoxysilane with TEOS on the FeO₂⁻ surface [52]. Another polymerization method is the use of atom transfer radical polymerisation (ATRP) [53] under mild conditions, increasing the sorption capacity and selectivity of the material to BSA. Multi-walled carbon nanotubes (MWNTs) are also very useful in the synthesis of magnetic molecularly imprinted polymers. They have been used for the synthesis of MWNTs@FeO₂⁻ MIPBSA which was applied to isolate BSA from bovine blood [25,26]. Satisfactory results were obtained with recovery in the range of 92.0–97.3%.

5 Summary

Due to their high selectivity, magnetic molecularly imprinted polymers have been applied in bioanalytics with increasing frequency. The combination of MIP extraction with advanced separation techniques such as LC, GC and CE allows separation and identification at very low concentrations of both small organic molecules as well as proteins by non-covalent binding to the polymer. Their unquestionable advantage is a low manufacturing cost, lower consumption of solvents, and extraction in a single piece of glassware, which reduces contamination of the sample with other compounds. Expanded surface area of the sample component that has contact with the sorbent materials creates new opportunities in analytics of trace amounts of the compounds present in a multicomponent matrix.

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