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Molecularly Imprinted Polymers for Pharmaceutical Compounds: Synthetic Procedures and Analytical Applications

Lawrence Madikizela, Nikita Tavengwa and
Vusumzi Pakade

Abstract

In this chapter, the synthetic procedures for molecularly imprinted polymers (MIPs) for pharmaceutical compounds are discussed. Regardless of its limitations, such as production of irregular particles and loss of sample during processing (crushing and sieving), bulk polymerization has been widely used compared to say precipitation and suspension polymerization partly due to its simplicity in synthesis and robustness. A comparison of indomethacin removal from aqueous solution by MIP particles prepared using bulk polymerization to those obtained from suspension polymerization showed that the particles from the former exhibited higher adsorption capacity. Furthermore, the chapter explores the strengths and limitations relating the use of pharmaceutical compounds as uni-templates, multi-templates and dummy templates. Also, the analytical applications of MIPs are discussed in more details with particular focus on molecularly imprinted solid-phase extraction (MISPE) of pharmaceuticals from environmental samples. This application (MISPE) is currently the most exploited in literature as more pharmaceutical drugs find their way into environmental water bodies.

Keywords: molecularly imprinted polymer, polymerization process, pharmaceuticals, polymer composites, analytical applications

1. Introduction

The development of molecularly imprinted materials is an area of intense research ever since its introduction by Wulff and Sarhan in 1972 [1]. Generations of scientists have been intrigued

by the binding phenomena involved in interactions that occur between natural molecular species such as antibodies and biological receptors. As such, over the years, numerous approaches have been used to mimic these interactions [2]. Molecular imprinting technology is today a viable synthetic approach to design robust molecular recognition materials able to mimic these natural phenomena. The main advantages of molecularly imprinted polymers (MIPs) are their high selectivity and affinity for the target molecule used in the imprinting procedure. Applications of molecularly imprinted materials have grown to include areas such as separation sciences and purification as extraction and chromatographic sorbents [3, 4], chemical sensors [5], catalysis [6], drug delivery [7] and biological antibodies and receptors system [8]. Among the polymeric materials developed, MIPs are also one of the most attractive materials for bioanalytical and biomedical applications [9]. Due to the high selectivity of MIPs towards pharmaceutical compounds, there are a number of companies that have gone to an extent of commercializing the MIP sorbent. These companies include Supelco in Belefonte (PA, USA), Biotage in Barcelona (Spain, Europe) and MIP Technologies in Lund (Sweden, Europe).

On the other hand, the occurrence of pharmaceutical compounds in the environment, particularly in surface water and wastewater, has also intrigued the scientific community. Pharmaceutical compounds are drugs that are used for the purpose of preventing, curing, treating disease and improving the health of their consumers [10]. To date, different classes of pharmaceuticals are known such as non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, antiretroviral drugs and steroid hormones. Pharmaceutical compounds enter the aquatic environment through various sources that include households, wastewater treatment plants (WWTPs), hospitals and industrial units [11]. Also, some pharmaceuticals are known to be transported into the environment through human excretion as metabolites and as unaltered parent compounds [12]. For example, the NSAIDs such as ibuprofen, naproxen, ketoprofen and diclofenac are eliminated from the human body with 10, 70, 80 and 10% of unaltered compounds, respectively [12]. The eliminated compounds are often swept into WWTPs. High number of research studies have demonstrated the inability of WWTPs to completely remove pharmaceuticals during the sewage treatment processes [13–15].

The formulation and the preparation of new pharmaceutical compounds conducted by companies can represent a danger for the environment because of their toxic potential. Despite concerns about potential risks associated with the presence of pharmaceuticals and personal care products in the environment, few toxicological data address the health and environmental effects of these compounds [16]. A recent review by Madikizela et al. [17] pointed out that there are indeed some traces of pharmaceutical compounds in water bodies even in many African counties that are least developed. Clofibric acid is regarded as one of the most persistent drug residues with an estimated persistence in the environment of 21 years, being frequently detected in environment monitoring of pharmaceuticals all around the world [18].

Conventional methods such as biodegradation, photo catalysis and advanced oxidation have been applied for the treatment of pharmaceutical contaminants [19]. Analytical tests are required for environmental monitoring of pharmaceutical drugs in order to evaluate the success of the treatment method. The complexity of the environmental samples has commanded the availability of selective analytical methods for the quantification of these

pharmaceutical compounds. Therefore, MIPs are developed and used in sample preparation for the purpose of increasing selectivity of analytical method, and sensitivity when applied as a sorbent in the sample pre-concentration step. Other sorbents that have been used for the extraction of pharmaceuticals compounds include biochars, chitosan, silica, zeolites, graphene, clays and carbon [20].

Selectivity is an important parameter in analytical chemistry. However, many materials that are used for the extraction of pharmaceuticals such as hydrophilic lipophilic balance and C_{18} sorbents lack this feature. On the other hand, MIPs have long since known to be attractive in this regard. **Figure 1** [21] shows the chromatograms (red line) which illustrates the efficiency of the molecularly imprinted polymer—solid-phase extraction (MIP-SPE) procedure (extraction rate of about 90%) and the advantages of both the concentration and sample clean-up with very low background and no interferences close to the retention time of 17β-estradiol. It is also noteworthy to point out that not only selectivity is enhanced but MIPs have also the capacity to pre-concentrate pharmaceutical compounds. This is especially important as pharmaceuticals are detected and quantified in very low concentrations. This further implies that other ordinary cheap instruments can be used for environmental analysis beside the mass spectrometry (MS) detection that is known to have low detection limits and can detect pharmaceuticals at trace levels.
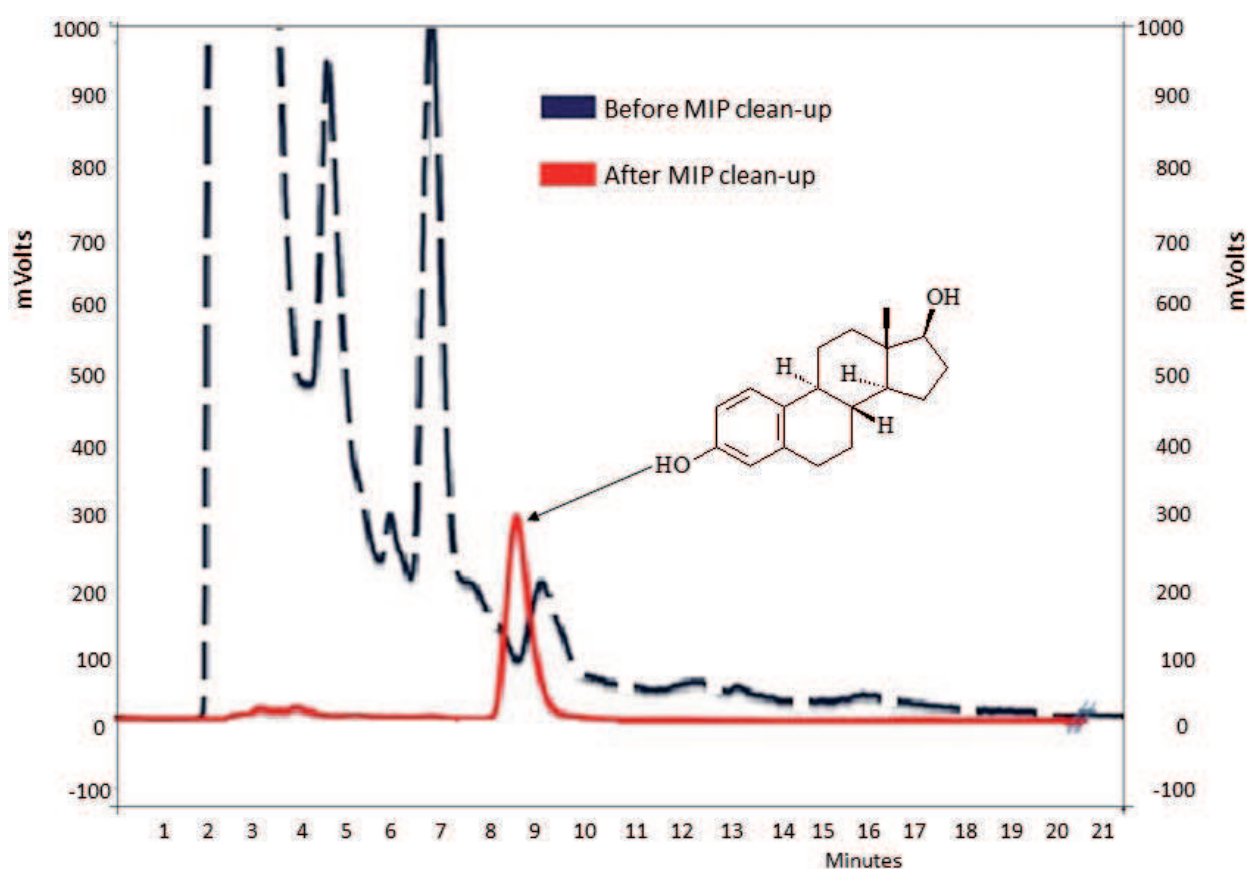

Figure 1. HPLC-FLD chromatograms obtained after extracts clean-up with MIP-SPE (AFFINIMIP® SPE Estrogens; Polyintell) of 100 mL of seine water spiked at 0.5 ng.mL⁻¹ with 17β-estradiol (—) and before MIP clean-up (—) [21].

2. Synthetic approaches

The molecularly imprinted polymerization techniques and methods have been well described in literature [22]. Basically, the imprinting process involves self-assembled of selected functional monomer around a template molecule followed by polymerization in the presence of a cross-linker [23]. The template is then removed from the polymer matrix, thus, leaving behind a cavity complementary in functional group, size and shape, which is available to strongly bind compounds that are closely related to the template molecule. This is demonstrated in **Figure 2** using the synthesis of MIP for fluconazole, an antifungal agent, as an example [24]. In their synthetic reaction [24], they used methacrylic acid, ethylene glycol dimethacrylate and fluconazole as functional monomer, cross-linker and template, respectively. Despite its
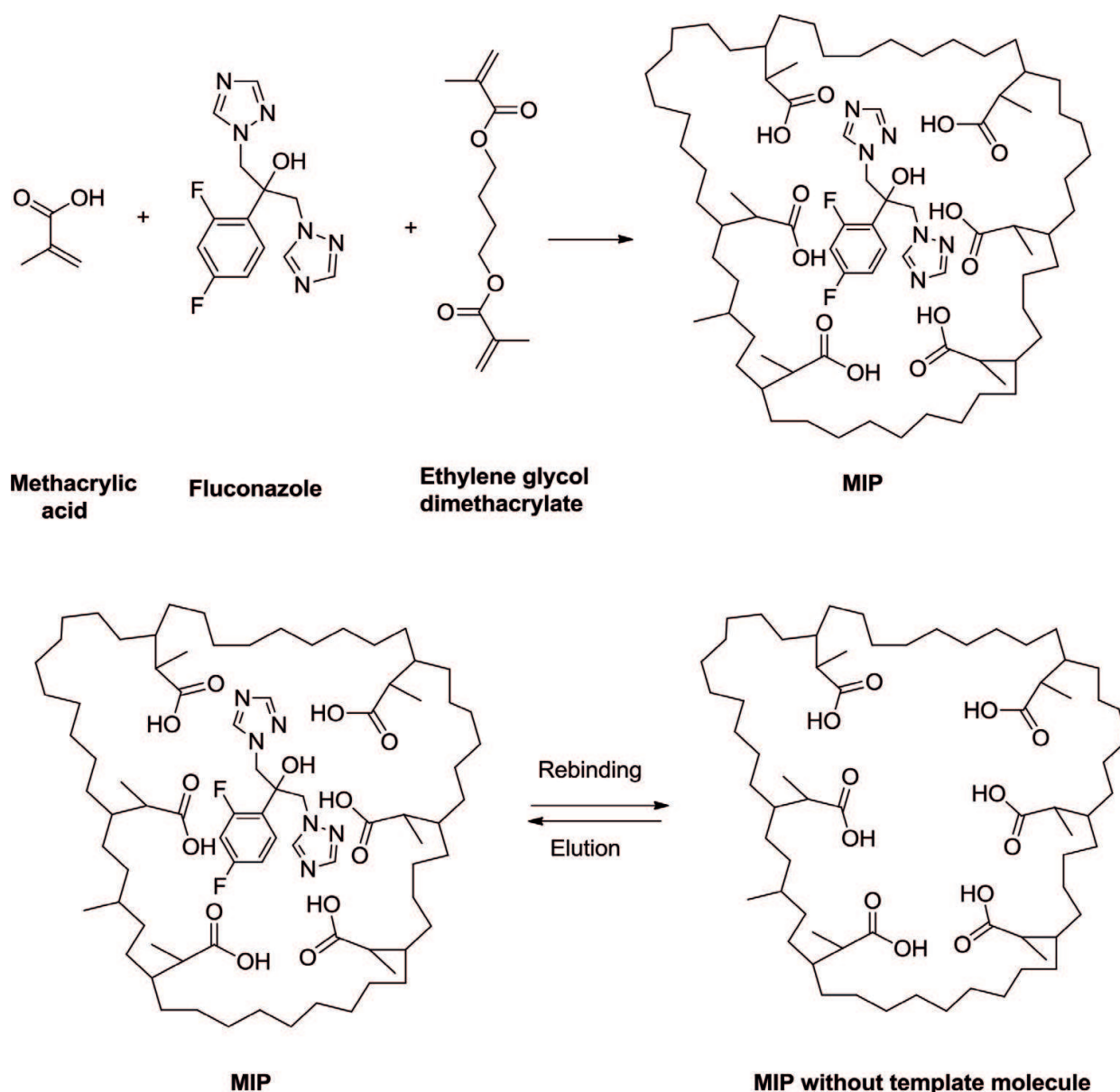

Figure 2. Synthetic scheme of a MIP for fluconazole adapted from Manzoor et al. [24].

disadvantages like wastage of material during processing (sieving and crushing), bulk polymerization remains the most common imprinting method for pharmaceutical compounds compared to other polymerization techniques such as precipitation, suspension and emulsion polymerizations [25]. Bulk polymerization has been practically proven to give higher binding capacity for indomethacin when compared to suspension polymerization [25]. In this instance, indomethacin binding capacities for MIP prepared by bulk and suspension polymerization were approximately 0.35 and 0.15 mg.g^{-1}, respectively.

3. Pharmaceutical compounds as templates

Templates are the most important reagent in the spatial arrangement of functional monomers during polymerization. Many pharmaceutical compounds of different therapeutic classes have been imprinted using the single-template and multi-template synthetic approaches [26–28]. Some of the imprinted compounds are given in **Table 1**. It has been observed that the non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics with the exception of fenoprofen which are frequently detected in environmental waters have been imprinted [29]. NSAIDs are known as the most consumed pharmaceuticals and in most cases their MIPs have been applied as selective SPE sorbents [29]. Most pharmaceutical drugs have hydroxyl and carbonyl functional groups which makes the imprinting process easy by utilizing their ability to form hydrogen bonding with several functional monomers. Also, due to the presence of aromatic rings in some pharmaceutical compounds and functional monomers, it is possible to have electrostatic interactions between the rings of different molecules. In this aspect, Farrington and Regan [30] demonstrated computationally the electrostatic interaction between the aromatic rings of ibuprofen (template) and 4-vinyl pyridine (functional monomer). Currently, MIPs are developed using one pharmaceutical compound as the template (uni-templating and dummy-templating). Also, there are procedures that have been shown in literature where many pharmaceuticals are used as multi-templates [26, 28]. In multi-templating approach, equal amounts of templates are added simultaneously into the polymerization mixture. Once removed at the end polymerization reaction, the resulting MIP can selectively re-bind the removed compounds from the environmental samples.

3.1. Physico-chemical properties of pharmaceutical compounds

Few pharmaceutical compounds that have been used in molecular imprinting as template molecules are given in **Table 1**. It can be seen from their molecular structures that pharmaceutical compounds compose of a variety of functional groups. The presence of functional groups in the template molecule makes it easy to undergo the molecular interactions such as hydrogen bonding. For example, the presence of carboxylic group in ibuprofen allows for hydrogen bonding in acidic conditions with 2-vinyl pyridine functional monomer [31]. Such hydrogen bonds are easy to break by washing the MIP with acetic acid which is a small molecule that can penetrate the pores of the MIP, thereby, disrupting the molecular interactions. This leads to easy regeneration of the MIP.
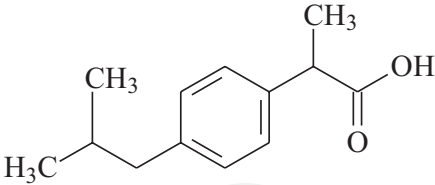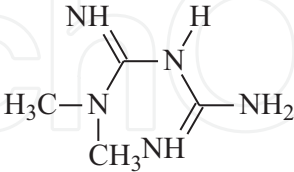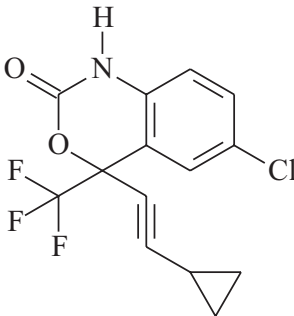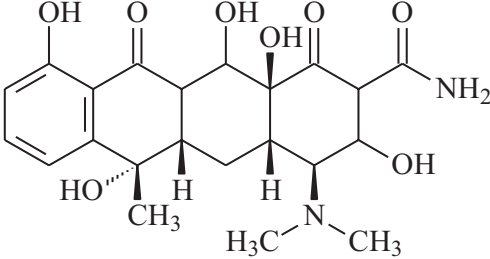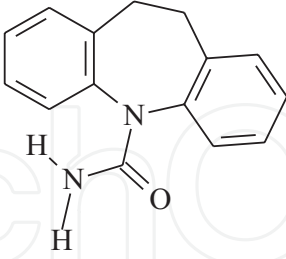
Therapeutic class	Compound	Chemical structure
NSAID	Ibuprofen	
Antidiabetic	Metformin	
ARV	Efavirenz	
Antibiotic	Tetracycline	
Anti-epileptic	Carbamazepine	

Table 1. Physico-chemical properties of selected imprinted pharmaceuticals.

The pK_a of a drug influences lipophilicity, solubility, protein binding and permeability which in turn directly affects pharmacokinetic (PK) characteristics such as absorption, distribution, metabolism and excretion [32, 33]. It is very important to understand the physico-chemical properties of templates and functional monomers prior to any MIP application. This is highlighted in the work reported by Dai et al. [34]. In their work, the adsorption efficiency of clofibric acid decreased significantly with the increase of pH when the pH was between 6 and 12.
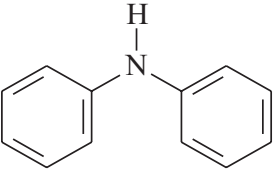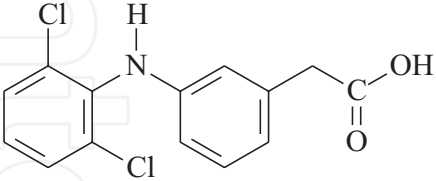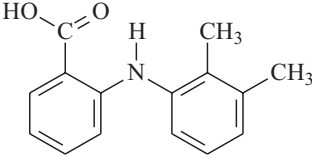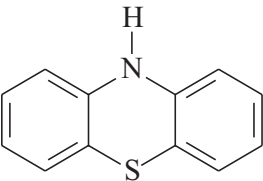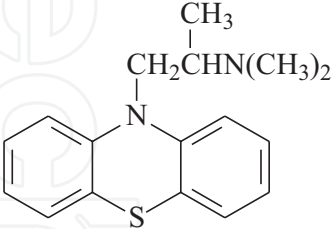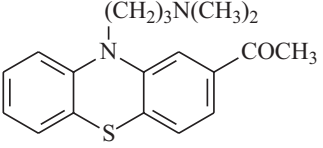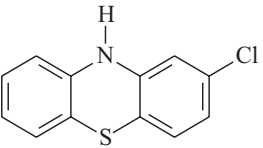
This phenomenon could be explained by the ionization of clofibric acid (pK$_a$ of 3.18) which could occur under strong basic condition. Therefore, clofibric acid was negatively charged. On the other hand, the functional monomer of 2-vinyl pyridine (pK$_a$ of 4.98) used in the synthesis of MIP could also be negatively charged. It is known that the ─COOH groups in the selective binding cavity of MIP play a key role in the rebinding of target compounds. The adsorption at basic pH could be due to the hydrophobic interactions [34].

3.2. Uni-templating

In most cases, uni-templating is done in order to extract one target analyte based on one attractive attribute such as bioactivity or ease excretion from human metabolism or the negative effect of that analyte such as toxicity or widespread in the environment. In the synthesis of MIPs, the target compounds are usually used as the template molecules. A diversity of pharmaceutical compounds have been detected in environmental waters [17, 35], therefore, most pharmaceuticals have been imprinted for the purpose of developing selective analytical methods. Conventionally, a single-template is imprinted which subsequently lead to the isolation of one pharmaceutical compound from water matrix [36]. 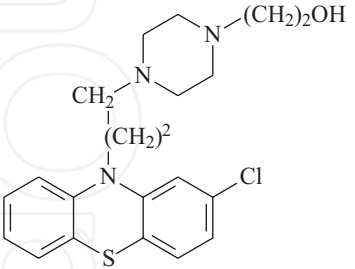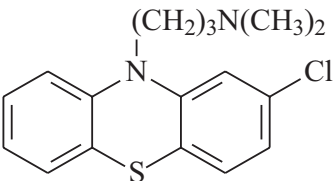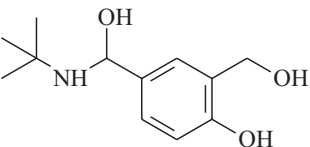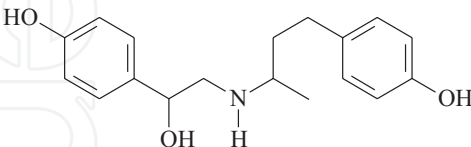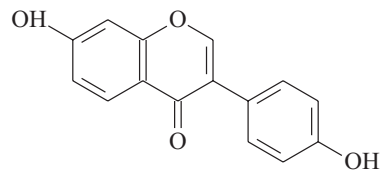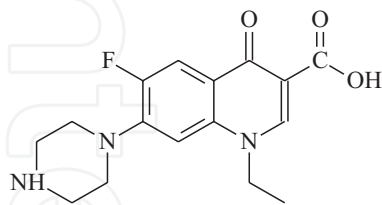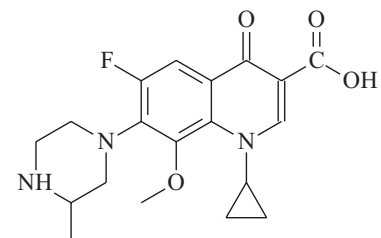Many pharmaceuticals that include ketoprofen [3], indomethacin [25], 17β-estradiol [27] and diclofenac [37] have been imprinted using the single templates. MIPs that have been synthesized using this approach usually possess high selectivity towards the compound that was used as template molecule. High selectivity maybe due to molecular recognition that could be strongly influenced by functional groups, shape and size of the target compound. In this case, selectivity is usually evaluated by extracting a mixture of organic compounds that consist of the target compound and other structurally related compounds. Zunngu et al. [3] tested selectivity of ketoprofen MIP for its ability to extract similar compounds (triclosan, gemfibrozil and fenoprofen) along the target compound from spiked deionized water. Their results showed accepted recovery (104%) for ketoprofen, and for competitors the recoveries did not exceed 20%. In a different study, Ming et al. [27] demonstrated the competitive adsorption ability of 17β-estradiol MIP towards the target compound in the presence of estriol, estrone, bisphenol A and hexestrol in aqueous solutions. Single-template MIPs usually lead to superior sample clean-up that subsequently results in cleaner chromatograms, however, this approach do not allow for a simultaneous multi-compound analysis. Multi-compound analysis can only be performed using uni-templating procedure after physical mixing a number of MIP for individual compounds. However, this approach is not financial feasible as it is expensive to synthesize a number of MIPs whose mixtures will be used to target a number of pharmaceuticals.

3.3. Multi-templating

One of the advantages of MIPs is the selective extraction of analytes in complex matrices. However, at times it is desired that a class/many compounds are removed or extracted from the environmental or real samples simultaneously. To achieve this, researchers have explored the possibility of imprinting multiple templates all at once for the

Dummy template	Target compounds		Selectivity	Recovery (%)	Reference
Diphenylamine	Diclofenac	Mefenamic acid	Selective in the presence of ibuprofen, naproxen, ketoprofen and salicyclic acid	100–112	[44]
Phenothiazine 2-chlorophenothiazine	 	 	Selective in the presence of tetracycline, naphthalene and anthracine	93–98	[42]
Salbutamol	Ractopamine		Selectivity was done chromatographically using clenbuterol, terbutaline and adrenaline as competitors.	—	[41]

Dummy template	Target compounds		Selectivity	Recovery (%)	Reference
Daidzein	Fluoroquinolones[*](1) Norfloxacin	(2) Gatifloxacin		64–103	[43]

[*]Fluoroquinolones—only two chemical structures are given, however, there were eight target compounds (fleroxacin, ofloxacin, norfloxacin, pefloxacin, ciprofloxacin, lomefloxacin, enrofloxacin and gatifloxacin).

Table 2. Chemical structures of dummy template molecules, their relative extracted compounds and analytical performance.

pre-concentration and extraction of a certain group of compounds where a cocktail of pharmaceutical compounds is used in the polymerization set-up. Practical examples for this include the work of Duan et al. [26] where five acidic pharmaceuticals which are ibuprofen, naproxen, ketoprofen, diclofenac and clofibric acid, were used as multi-templates in a synthesized MIP that showed selective recognition and ability to extract these target compounds from lake water and WWTP effluent using molecularly imprinted solid-phase extraction (MISPE) technique. In their work, Duan et al. [26] obtained the recoveries that were greater than 95% for all five acidic pharmaceuticals in lake water and wastewater spiked with 1 μg.L^{-1} of each compound. Dai et al. [38] have also reported the selective removal of the same group of pharmaceutical compounds using a multi-template MIP from contaminated water. These researchers demonstrated the selectivity of the multi-template MIP in the presence of fenoprofen and carbamazepine (both pharmaceutical compounds). In their study, the removal efficiency for the five target pharmaceuticals in water was greater than 80%, whereas, less than 40% was reported for fenoprofen and carbamazepine used as competitors. In the same aspect, a dual template (naproxen and ketoprofen) MIP has been reported [28], where the ability to recognize the template molecules was tested chromatographically in the presence of structural analogues (ibuprofen, fenbufen, fenoprofen and flurbiprofen). Also, Dai et al. [34] prepared a novel double-template MIP by precipitation polymerization using carbamazepine and clofibric acid as the double templates.

For a multi-template (naproxen, ibuprofen and diclofenac) MIP, it was observed that the selectivity collapses easily during the extraction of target compounds from aqueous phase [31]. This could be strongly influenced by bigger cavities that are created due to the usage of multi-templates. This could allow the easy access of many presumably smaller molecules into the binding sites. It has been demonstrated that the untargeted compounds can be selectively washed off from the multi-template MIP surface due to their weaker non-specific interactions with the polymer [31]. Contrarily, some researchers have indicated that the use of a dummy template during polymerization increased the selectivity of the final MIPs that target more that target one compound [39, 40].

3.4. Dummy-templating

The usage of target compounds as template molecules could have negative impact on the analysis of real samples due to their bleeding upon application into the sample matrix. This could be severe in the cases of incomplete template removal. To avoid this problem, the use of dummy templates for the synthesis of MIPs has been proposed in many studies [41–43]. In certain instances, the selected dummy templates exhibit the properties of more than one compound, and its chemical structure is closely related or similar to more than one pharmaceutical drug [42, 44]. In such cases, the prepared MIP is able to selectively extract more than one compound. This has been demonstrated by the synthesis of a MIP using diphenylamine as the template whose chemical structure closely resembles that of both diclofenac and mefenamic acid (**Table 2**) [44]. As shown in **Table 2**, the dummy molecule (diphenylamine) for both diclofenac and mefenamic acid can be characterized by two

phenyl groups which are both attached to the amine group, such groups also appear on the structures of the target compounds. Similarly, the approach has also been reported in the synthesis of MIP required for SPE of phenothiazines from meat samples, where phenothiazine and 2-chlorophenothiazine were used as dummy templates, thereby taking the advantage of their core chemical structures that compose of phenothiazine [42]. Both MIPs synthesized with phenothiazine and 2-chlorophenothiazine dummy templates were able to capture four different phenothiazines (acepromazine, promethazine, perphenazine and chlorpromazine), simultaneously. In a different work, a compound, daidzein, was used as a non-poisonous dummy template in the synthesis of MIP for fluoroquinolone antibiotics [43]. Their synthesized MIP was successfully applied for matrix solid-phase dispersion extraction of eight fluoroquinolones from fish samples. Such work gave high recoveries and selectivity for target compounds (**Table 2**). Similarly, the analysis of single pharmaceutical drug, ractopamine, commonly used for the treatment of asthma has been performed using dummy template MIP which has been synthesized using salbutamol as a dummy template [41]. The drawback that could be associated with the use of dummy templates in molecular imprinting could rise during the applications in real samples. Selectivity can be reduced greatly due to the differences in the physico-chemical properties of the dummy template and the targeted compound(s). For instance, in **Table 2**, it can be seen that diphenylamine (dummy template) is relatively smaller, in terms of size, as compared to the diclofenac (target compound). The same can be observed in the case of phenothiazine presented in **Table 2**. This could lead to easy binding of smaller molecules with similar functional groups into MIPs.

4. Synthesis of polymer composites

Traditional imprinting produce particles with adsorption sites embedded deep into the polymer matrix resulting in difficulties with mass transfer of analyte molecules [45]. Dummy templating has been used to address the problem of elution of template molecules during MIP application in real world samples. This problem occurs due to incomplete template removal caused by molecules occupying deeper pores in the polymer matrix. However, as discussed earlier, the recognition sites created by the dummy might not perfectly fit the target analyte leading to inferior extraction efficiencies as when the template was used. Surface imprinting (polymer composite) has been proposed as an alternative to dummy templating for addressing the MIP template bleeding problem. Various materials including graphene oxide [46, 47], magnetic cross-linked chitosan [48, 49], silica [49, 50], carbon nanotubes [51], quantum dots [52] and nanoparticles [53, 54] have been used as scaffolds in the preparation of imprinted polymer composites for pharmaceutical compounds. The template is imprinted at or near the proximity of the surface, therefore, there are no deeper lying cavities that may cause slow release of template molecules and subsequently bleeding. The materials used as anchors usually have large surface area to volume ratio resulting in lower mass transfer resistance and faster rebinding due to accessibility of the binding site [47]. Cl-TiO$_2$ imprinted photo catalyst exhibited higher photo degradation rate (72%) of tetracycline under visible

irradiation than non-imprinted photo catalyst [53]. Molecular imprinting magnetic γ-Fe_2O_3/cross-linked chitosan composites prepared by a microemulsion process were applied for the adsorption and degradation of norfloxacin (NOR) [48]. The MIP showed superior adsorption of NOR than its non-imprinted counterpart [48] and excellent selectivity of NOR adsorption in comparison to sulfadiazine, ofloxacin and phenol [49]. Elsewhere, an electrochemical sensor constructed by grafting MIP to multi-walled carbon nanotube (MWCNTs) surface immobilized on glass carbon electrode was evaluated for the determination of ceftazidime from human serum [51]. The functionalized MWCNTs played two roles, increasing the conductivity of a sensor and the amount of binding sites. The sensor demonstrated good precision, stability, sensitivity and selectivity for the target analyte. Recent work showed the synthesis of polymer composites for pharmaceutical drugs, where such materials are prepared for the purpose of sensory applications [52]. Prasad et al. [52] synthesized a novel monomeric graphene quantum dots—MIP-based nanocomposite directly at the surface of screen printed carbon electrode and applied for electrochemical detection of an anticancerous drug ifosfamide in biological and pharmaceutical samples. Their sensor gave the detection limit of 0.11 ng.mL^{-1} (S/N = 3), without any matrix effect, cross-reactivity and false-positives.

5. Reusability

MIPs sorbents are easily regenerated by washing with organic solvents to remove the adsorbed compounds. Therefore, they can be applied for various extractions repeatedly. In this aspect, acetylsalicylic acid MIP successfully adsorbed (removal efficiency 75–78%) the target compound from acidic aqueous solutions six times [55]. After desorption, MIP was regenerated with a mixture of methanol and acetic acid (7:3, v/v) followed by methanol. There are numerous examples of reusability in literature that include those cited in **Table 3** for the MIPs prepared for selective extraction of acetylsalicylic acid [55], diclofenac [36] and multi-templates (ibuprofen, naproxen, ketoprofen, diclofenac and clofibric acid) [26]. Such work, clearly demonstrate that MIP can be reused many times without losing its adsorption capacity. This is an excellent advantage for MIP as it is a common knowledge that many adsorbents are discarded after a single use.

Template/target compound	Regeneration solvent	Successful applications	Reference
Acetylsalicylic acid	Methanol/acetic acid (7:3, v/v) and methnol	6 repeatable adsorption/desorption experiments with 75–78% removal efficiency	[55]
Diclofenac	Methanol/acetic acid (9:1, v:v)	30 binding/regeneration cycles with ≥95% recovery	[36]
Multi-templates*	Methanol/acetic acid (9:1, v:v)	20 adsorption and desorption cycles gave constant recovery (≥95%)	[26]

*Multi-templates were ibuprofen, naproxen, ketoprofen, diclofenac and clofibric acid.

Table 3. Examples for reusability of MIPs.

6. Analytical applications

In relation with pharmaceutical compounds, MIPs are used in analytical applications such as sample preparation as SPE sorbents [37], chromatographic stationary phase [56] and biological sensing [51]. The analytical applications of MIPs are very useful for various reasons, such as they provide higher selectivity than the conventional sorbents, they also reduce the matrix influence on the resulting chromatograms and they lead to high sample enrichment factors [57]. Besides the use of MIPs in analytical applications such as sample preparation, they are widely evaluated as selective adsorbents in contaminated water. MIPs are introduced as clean-up adsorbents in environmental waters for removal of pharmaceuticals (batch adsorption) [31, 55].

6.1. Sample preparation

Sample preparation for the determination of pharmaceuticals in aqueous samples is, in most cases, preceded by a filtration method prior to pre-concentration [58]. However, this step may lead to loss of some compounds bound to particulate matter. Typical example to this, is the detection of trace levels of mefenamic acid (a hydrophobic compound) at $\mu g.kg^{-1}$ on the suspended solids following filtration [59]. Therefore, removal efficiencies and mass loadings may be affected by the filtration step [58]. Solid-phase extraction (SPE) techniques, on the other hand, have shown fairly good pre-concentration and extraction efficiencies for hydrophobic compounds. However, commercial reversed phase-based adsorbents used in SPE have not shown satisfactory efficiencies for the pre-concentration of polar organic compounds. It has been suggested that the molecular recognition brought about by MIPs could address this downfall of SPE [58]. Instead of conventional sorbents, the SPE cartridges are in this case packed with MIP particles. That is, the synthesized MIP particles are slurry or dry packed in between two frits inside the solid-phase extraction cartridge [60, 61], referred to as MISPE. After packing, the cartridge is then conditioned prior to the loading of sample solutions. Thereafter, the MISPE cartridge is washed for removal of sample interferences and the target compounds are eluted with a suitable organic solvent. MISPE has been widely used for selective extraction of pharmaceuticals from various matrices that include plasma, urine and water samples [25, 57, 62]. In most cases, MISPE is applied where target compounds are extracted from solution into the solid material.

In addition, other mode of solid-phase extraction for ARV drug (abacavir) such as solid-phase microextraction (SPME) has been reported [63]. The molecularly imprinted SPME technology for drug analysis has been described in great details by Ansari and Karimi [22]. These authors focussed on the progress, challenges and trends in trace determination of different drugs.

6.1.1. Molecularly imprinted solid-phase extraction

Pharmaceuticals from different classes have been extracted and pre-concentrated using MISPE (**Table 4**). Most common non-steroidal anti-inflammatory drugs (NSAIDs) with the exception of fenoprofen have been imprinted and their MIPs were applied in the form of MISPE from environmental samples [29]. This is expected as NSAIDs are classified as the most consumed

pharmaceuticals by humans with antipyretic activities in some countries such as South Africa [64]. The emerging environmental pollutants such as antiretroviral drugs (ARVs) are imprinted [63, 65], however, there is still limited/no information on their environmental extraction using MISPE. Most MISPE applications for ARVs are based on their extraction from biological samples [66, 67]. The application of MISPE allows for pre-concentration of various analytes from environmental samples which in turn lead to very low detection limits in µg.L^{-1} to ng.L^{-1} levels (**Table 4**). Based on higher extraction efficiency or percent recoveries for pharmaceuticals, MIPs show strong ability to extract such drugs from complex sample matrices such as wastewater. As can be seen in **Table 4**, various amounts of MIPs are used in SPE. Small quantities as demonstrated by Zunngu et al. [3] are the significant of potential application in miniaturization techniques.

6.2. Chromatographic analysis

One of the most important applications of MIPs is their usage as the chromatographic stationary phases. This is done by slurry packing the prepared MIP into the stainless still chromatographic column. During the application, the imprinted molecule binds strongly to the packing material, which results in its strong retention and longer retention time [56]. This application was demonstrated in literature where a chiral stationary phase for the enantioselective separation of naproxen was reported [56]. In their work [56], a MIP was synthesized using (*S*)-naproxen as the template and evaluated for chromatographic separation. Racemic naproxen was efficiently resolved on the MIP with (*S*)-naproxen eluted last. Similarly, Haginaka and Sanbe, [70], synthesized a uniformly sized MIP for (*S*)-naproxen that gave good enantioselectivity and resolution for naproxen. In addition, uniform-sized MIP material for (*S*)-propranolol when applied as chromatographic stationary phase has shown the ability to separate (*S*)-propranolol from a mixture that contains some structurally related β-adrenergic antagonists [71]. Due to the strong binding of target compound onto MIP, peak tailing on the chromatogram is usually evident. Therefore, there are opportunities relating to the improvement of the quality of the resulting chromatograms.

Analyte	Sorbent amount (mg)	Environmental sample loading	Elution	Analytical method and detection limit	Reference
Diclofenac	35	1000 mL wastewater and river water	2 mL of methanol/ acetic acid (9:1, v:v)	LC–MS/MS LOD – Not reported	[36]
Carbamazepine	200	100 mL wastewater effluent at pH 11	5 mL of methanol	LC-UV LOD – 25 µg.L^{-1}	[68]
Metformin	50	50 mL aqueous samples including wastewater, pH 10	1 mL of acetic acid and methanol (1:9)	LC-DAD-ESI/MS LOD – 1.5–3.4 ng.L^{-1}	[69]
Ketoprofen	14	50 mL wastewater at pH 5	1 mL of methanol	LC-UV LOD – 0.23 µg.L^{-1}	[3]
Indomethacin	200	100 mL river water at pH 5	2 mL methanol	LC-UV LOD – 0.03 µg.mL^{-1}	[25]

Table 4. MISPE of pharmaceuticals from environmental waters.

7. Results and suggestions

In the earlier sections of this chapter, the evidence of the improved selectivity due to the application of MIPs in analytical methods has been demonstrated (**Figure 1**). In numerous occasions, the quantification of some pharmaceutical drugs in the environment has been performed after sample clean-up and analyte(s) pre-concentration using MISPE. After pre-concentrated with MISPE, pharmaceutical compounds have been detected at concentration levels that range from low ng.L^{-1} to µg.L^{-1} [3, 26, 37, 44, 61]. In this chapter, it was further elaborated that there are MIP sorbents that are available commercially, and therefore, it is suggested that more sorbents should be available in the near future as more pharmaceuticals are being detected in the environment. Moreover, there are no reports in literature for MIPs synthesized for a number of pharmaceutical compounds more especially those that are new in the market. For example, in their recent review paper, Madikizela et al. [29] observed that the MIP for fenoprofen is yet to be developed. In different perspective, the potential for MIPs to be applied as chromatographic stationary phases for separation of complex mixtures such as enantiomers have been investigated extensively in the early 2000 [56, 70, 71]. Due to the promising results reported in previous years, it is suggested that this area should be exploited more carefully in order to improve the quality of chromatographic peaks, that could lead to better quality of analysis and results that are more reliable can be achieved. This of cause could lead to the introduction of new stationary phases by the manufacturers of chromatographic equipment and consumables.

Author details

Lawrence Madikizela[1]*, Nikita Tavengwa[2] and Vusumzi Pakade[3]

*Address all correspondence to: lawremcem2@dut.ac.za

1 Department of Chemistry, Durban University of Technology, Durban, South Africa

2 Department of Chemistry, University of Venda, Thohoyandou, South Africa

3 Department of Chemistry, Vaal University of Technology, Vanderbjlpark, South Africa

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