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A molecularly imprinted polymer as the sorptive phase immobilized in a rotating disk extraction device for the determination of diclofenac and mefenamic acid in wastewater

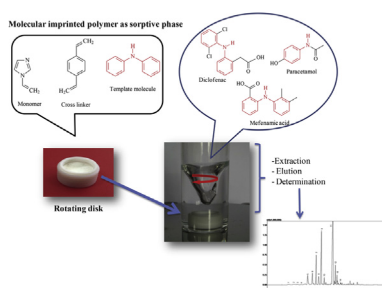
Valentina Manzo [a], Karla Ulisse [a], Inés Rodríguez [b], Eduardo Pereira [b, **], Pablo Richter [a, *]

[a] Department of Inorganic and Analytical Chemistry, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, P.O. Box 653, Santiago, Chile
[b] Department of Analytical and Inorganic Chemistry, Faculty of Chemical Sciences, University of Concepción, Chile

HIGHLIGHTS

- A MIP immobilized in a rotating disk sucessfully extracts NSAIDs from wastewater.
- MIP had remarkably superior binding properties compared to NIP for diclofenac and mefenamic acid.
- Significantly higher absolute recoveries for the MIP used in this work with respect to its commercial counterpart.
- A simple, green and inexpensive determination is accomplished.

GRAPHICAL ABSTRACT



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ABSTRACT

The microextraction of diclofenac and mefenamic acid from water samples was performed by using rotating disk sorptive extraction (RDSE) with molecularly imprinted polymer (MIP) as the sorptive phase. The MIP was synthesized from the monomer 1-vinylimidazol (VI) together with the cross-linker divinylbenzene (DVB) using diphenylamine as the template molecule. Scanning electron microscopy (SEM) analyses of the MIP revealed clusters of spherical particles having a narrow size distribution, with diameters of approximately 1 μm.

The optimized extraction conditions involved a disk rotation velocity of 3000 rpm, an extraction time of 120 min, a sample volume of 50 mL, and a sample pH of 2 as well as 25 mg of MIP immobilized in the disk. Desorption of the extracted analytes was performed with 5 mL of methanol for 10 min. Analysis by gas chromatography-mass spectrometry (GC–MS) was carried out after derivatization of the analytes with N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA).

Nonmolecularly imprinted polymer (NIP) was also synthesized for comparison. It was observed that under the same conditions, MIP extracted significantly more NSAIDs containing diphenylamine (or part of this molecule) in their structure than NIP. Higher significant differences between MIP and NIP were observed for diclofenac, mefenamic acid and paracetamol, clearly indicating the effect of the template on the extraction.

Recoveries of the method were between 100 and 112%, with relative standard deviations of 5–6%. The limits of detection were between 60 and 223 ng L^{-1}. Water samples from a wastewater treatment plant (WWTP) of Santiago de Chile, were found to contain concentrations of these acidic drugs between 1.6 and 4.3 μg L^{-1} and between 1.4 and 3.3 μg L^{-1} in the influent and effluent, respectively.

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* Corresponding author.
** Corresponding author.
E-mail addresses: epereira@udec.cl (E. Pereira), prichter@ciq.uchile.cl (P. Richter).

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1. Introduction

Despite the increasing selectivity and sensitivity achieved by current analytical techniques, sample preparation remains a critical issue in a chemical measurement process, particularly in complex samples in which the analytes are very dilute and a number of unknown interferences are present. In this context, the extraction and cleanup steps are of paramount importance in the sample preparation.

Extraction techniques have been the focus of intensive research over the last two decades, with advances in automation, miniaturization, and simplification driving this evolution [1,2]. The need to develop analytical processes that can replace toxic reagents and polluting solvents as well as minimizing the consumption of energy, reagents, and samples to reduce waste generation is an equally important driver of novel extraction techniques [3].

Solid-phase extraction (SPE) is currently the most widely used replacement for liquid–liquid extraction (LLE) for both the enrichment of organic pollutants in water samples [4,5] and cleanup purposes. According to recent reviews [6–8], an important vein of research in analytical chemistry has focused on the development of SPE systems that are combined with chromatography in both the thermal and solvent desorption modes. SPE, together with minimizing the use of solvents, has a number of additional advantages with respect to LLE, such as the more complete extraction of the analyte, more efficient separation of interferences from the analytes, no emulsion formation, easier collection of the analytes, more convenient manual procedures, removal of particulates, and ability to be more easily automated [5].

The selection of the SPE sorbent has a direct relationship with the analytical selectivity of the method. Selectivity will be achieved when significant differences occur between the analyte-solid phase interactions with respect to the interference-solid phase interactions. In this regard, when an organic molecule is highly hydrophobic, its interaction with an apolar support, for example C18, will be the basis for the removal of any polar interferences. However, if the analyte is capable of forming hydrogen bonds, besides containing apolar groups, it would be advisable to use a polymeric support containing N-vinylpyrrolidone and divinylbenzene (commercially available as Oasis HLB).

Although an Oasis HLB phase provides a hydrophilic-lipophilic balance for a good matching interaction with amphiphilic molecules, these interactions are not fully specific for the analyte alone and interactions with interfering molecules can also occur, reducing the active sites available to the analytes. A powerful and reproducible manner to achieve greater selectivity in SPE is the use of molecularly imprinted polymers (MIPs) as the solid phase (molecularly imprinted solid phase extraction, MISPE) [9].

MIPs are obtained by the copolymerization of mono- and polyfunctional monomers in the presence of a template. After polymerization, the template molecules are removed from the polymeric network, leaving selective sites for other molecules that are complementary in size, shape and functionality to the template. The resulting MIPs are stable in wide pH and temperature ranges and in different solvents [10,11].

MISPE is the most advanced technical application of MIPs; however, non-exhaustive sorptive microextraction techniques, such as solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE), have also implemented the use of MIPs as the sorptive material achieving the selective extraction of analytes from real samples [12,13]. The methods developed for the synthesis of MIP fibers for SPME or MIP stir bars for SBSE are rather simple and robust; therefore, their use will be extended to analytical laboratories in the coming years [13].

In 2009, our research group developed a new sample preparation technique called rotating disk sorptive extraction (RDSE), which is an alternative to the current microextraction/cleanup techniques and provides a number of advantages [14–27]. The extraction device used in RDSE exhibits an extraction phase with a high surface area-to-volume ratio, and it can be stirred at much higher velocities than the stir bar used in SBSE without damaging the phase because the extraction phase is only in contact with the liquid sample. Thus, higher rotating velocities facilitate analyte mass transfer to the sorptive surface.

Two configurations of the extraction device have been proposed for RDSE, providing a high versatility because any sorptive material used in either SPE or SBSE can be immobilized on the rotating disk. In addition, RDSE provides some advantages over SPE, especially that it allows the recirculation of the sample through the extraction phase and thus maximizes its sorptive capacity (in SPE the sorption occurs while the sample passes unidirectionally through the solid support). Furthermore, in RDSE, the interface is continuously renewed during the extraction process, which minimizes the involved cleanup steps for complex samples, which are required with SPE. Other important characteristics of RDSE are related to the shape design of the extraction device, which allows an easier automation of the extraction process [21], direct spectroscopic measurements in the extraction phase [17–19,22], and feasibility of its use in bioavailability studies [25].

A proof-of-concept application of the RDSE associated with a MIP sorptive material is presented for the determination of some NSAIDs in water samples. These drugs, which are widely used and can be acquired without a medical prescription, are emerging pollutants [23] currently found in waste and natural waters. A number of determinations of NSAIDs based on the use of MISPE have been reported, with most of them using the same analyte as the template molecule and 2-vinylpyridine or 4-vinylpyridine as the monomer in the synthesis of the MIP [28–33]. The MIP that we prepared in the present case is synthesized using diphenylamine (DPA) as the template and the monomer VI together with the cross-linker DVB (Fig. 1). As seen in Fig. 2, diphenylamine is part of the molecules of diclofenac, mefenamic acid and paracetamol, and consequently, a better match between the MIP and these molecules is expected compared with other NSAIDs.

2. Experimental

2.1. Reagents

Water from a Millipore Milli-Q Plus water system (Billerica, MA) was used throughout the experiment. All nonsteroidal anti-inflammatory drugs (ketoprofen, ibuprofen, naproxen, diclofenac, acetylsalicylic acid, and mefenamic acid) and the internal standard (meclofenamic acid) were purchased from Sigma–Aldrich

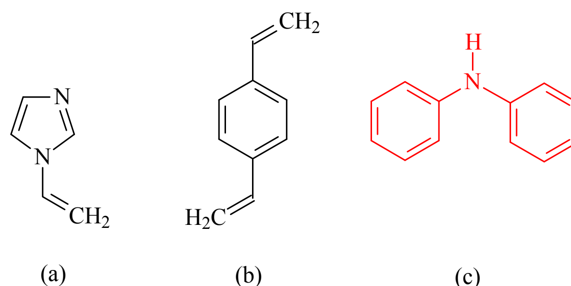

Fig. 1. Molecules used in the synthesis of the MIP: (a) VI; (b) DVB; and (c) template DPA.

(Milwaukee, WI, USA). Paracetamol and the syringe standard (hexachlorobenzene) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). The standard stock solutions of the analytes (50 mg L^{-1}) and the internal standard (2 mg L^{-1}) were prepared separately in methanol (GC–MS/pesticide analysis grade, Fisher Scientific, Fair Lawn, NJ, USA), and the syringe standard solution (20 mg L^{-1}) was prepared in ethyl acetate from Merck (Darmstadt, Germany). The pH was adjusted with 37% p.a. hydrochloric acid (0.1 mol L^{-1}) and p.a. sodium hydroxide (0.1 mol L^{-1}) from Merck.

Nitrogen with a purity of ≥99.995% and helium with a purity of ≥99.999% were purchased from Linde (Santiago, Chile), and they were used in the evaporation of the final extract and as the chromatographic carrier gas, respectively. Acetone, acetonitrile, toluene, ammonium formate, hexane, potassium dihydrogen phosphate (99.5% purity) and sodium chloride (99.5% purity) were all purchased from Merck. MTBSTFA was provided by Sigma Aldrich and was used as a derivatizing agent. The reagents 1,4-divinylbenzene (DVB, 80%), 1-vinyl-imidazole (VI, ≥99%), and 2,2-azobisisobutyronitrile (AIBN) were provided by Sigma Aldrich. Absolute ethanol (ETOH, p.a. grade), absolute ethanol (HPLC grade), and diphenylamine (DPA, 98%) were provided by Merck. A SupelMIP™ NSAIDs SPE Column from Sigma Aldrich was used for comparison with the proposed MIP associated with the RDSE technique.

2.2. Instruments

A Thermo Scientific Trace 1300 gas chromatograph (Milan, Italy) coupled to a Thermo Fisher Scientific ISQ mass-selective detector (Austin, TX, USA) was used to analyze the samples. The fused silica capillary column used was a Restek RTX-5MS (Bellefonte, PA, USA) (30 m × 0.25 mm id; 0.25 μm film thickness) coated with 5% phenyl/95% methylpolysiloxane. Two microliters of the derivatized sample extract was injected into the gas chromatograph using the splitless mode. The injector temperature was 250 °C. The initial oven temperature schedule was 100 °C, which was maintained for 1 min, followed by heating to 280 °C at a rate of 50 °C min^{-1}. Each chromatographic run required a total of 39 min with a solvent delay of 14 min, a transfer line temperature of 250 °C, an ionization source temperature of 200 °C, and a carrier gas flow rate of 1 mL min^{-1}. A dwell time of 0.1 s was employed for each m z^{-1}. The ions used in the selective ion monitoring (SIM) mode for the quantification and confirmation of the compounds are shown in Table 1.

The vial containing the sample and the rotating disk was placed on an MMS-3000 Boeco magnetic stirrer (Hamburg, Germany). The pH values were determined with a WTW Model pMX 3000 pH meter (USA). A KMC-1300V vortex mixer (Vision Scientific Co., Ltd., Korea) and an analog heatblock evaporator (VWR, USA) were employed during the extraction process.

2.3. Synthesis of the MIP and NIP

The required amount of monomer VI was weighed and placed into a 50-mL polymerization flask. Then, the solvent (ETOH) and the proper amount of the imprinting molecule (DPA) were added. The mixture was stirred for 5 min to obtain a homogeneous solution, and after that, it was allowed to stand for 30 min. Then, the required volume of cross-linker reagent (DVB) was added and stirred for an additional 5 min. Next, the required amount of initiator (AIBN) was dissolved in the minimum amount of solvent and added to the reaction mixture. This mixture was bubbled with $N_{2(g)}$ using a Pasteur pipette for 5 min, and then, the tube was filled with a balloon inflated with $N_{2(g)}$ for generating an inert atmosphere. The tubes were brought into a thermostatic bath at 70 °C for 24 h. Once the synthesis was complete, the MIP was removed from the polymerization flask and placed in a crucible coupled to a vacuum filtration system. The polymer was washed with 300 mL of ethanol and 200 mL of milliQ water. After washing the resin, it was put into a Petri dish in an oven at 40 °C until a constant weight was reached. The non-molecularly imprinted polymer (NIP) was synthesized under the same conditions as the MIP but in the absence of the imprinting molecule DPA.

2.4. Removal of the imprinting molecule

The removal of the imprinting molecule (DPA) was performed

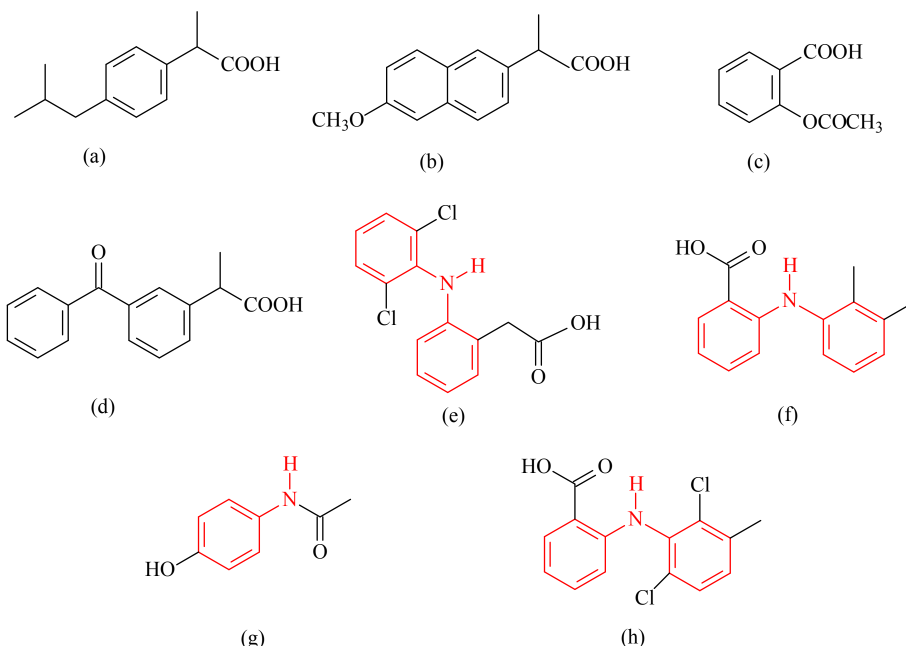

Fig. 2. Chemical structures of NSAIDs: (a) ibuprofen; (b) naproxen; (c) acetylsalicylic acid; (d) ketoprofen; (e) diclofenac; (f) mefenamic acid; (g) paracetamol; and (h) meclofenamic acid.

Table 1
Retention times, quantification ions (m z^{-1}) and confirmation ions (m z^{-1}) selected for each analyte, internal standard and syringe standard.

Analyte	Retention time (t_R)	m z^{-1}	
		Quantification ion	Qualifier ion
Hexachlorobenzene	19.14	284	286
Mefenamic acid	32.57	224	298
Diclofenac	34.21	352	354
Meclofenamic acid	35.57	243	352

using the dry MIP. The dry MIP was placed into a 250-mL beaker; 150 mL of methanol was added, and the mixture was stirred for a few min and was then allowed to stand for 1 h. Finally, the supernatant was removed through decantation. The procedure was repeated 3 or more times until the supernatant was free of DPA, which was confirmed by HPLC analysis. Afterwards, the resin was placed into a crucible coupled to a vacuum filtration system, filtered and placed in a Petri dish to subsequently carry the resin to the oven where it was dried at 40 °C until a constant weight was reached.

2.5. Grinding and sieving of the MIP and NIP

Once dried, the MIP and NIP were sieved using sieves with particle sizes of 250 μm, 180 μm and 100 μm. For all of the subsequent studies, the particle size fraction used was 100–180 μm. The resin remaining in the fraction with a size above 180 μm was taken to a mill to obtain the desired granulometry of the resin.

2.6. Characterization of the MIP and NIP through FT-IR and SEM

The infrared spectra of the MIP and NIP samples in KBr pellets were obtained on a Nicolet Magna 550 in the range of 4000–400 cm^{-1}. Moreover, the samples were analyzed by SEM in a JEOL JSM-6380 LV using an acceleration voltage of 20 kV and different magnification ranges.

2.7. Preparation of the rotating disks

The extraction device used included a Teflon disk (1.5 cm diameter) containing an embedded miniature magnetic stirring bar (Teflon-coated Micro Stir bar from VWR International). The disk has a 0.44 cm^3 cavity on one of its surfaces, in which 25 mg of the MIP or NIP sorbent was loaded. The cavity was covered with a fiberglass filter (1.4 cm diameter, 3 μm mean pore size) and sealed with a Teflon ring (Fig. 3). Before extraction, the MIP and NIP phase was conditioned with ethyl acetate, methanol and Milli-Q water for 5 min each.

2.8. Analytical procedure

A 50-mL aliquot of a standard or water sample was poured into a vial and adjusted to a pH of 2.0 with 0.1 M HCl. A 500-μL aliquot of a 2 mg L^{-1} internal standard (meclofenamic acid) was added to the real samples.

Fig. 3. Rotating disk used in this study.

The rotating disk containing the MIP phase was placed inside the vial, and the disk was rotated at 3000 rpm for 60 min at room temperature. After extraction, the disk was placed into a 10-mL vial containing 10 mL of methanol as a desorbing solvent and was stirred for 5 min at 2000 rpm. The methanol extract containing the concentrated analyte was then evaporated under a N_2 stream to dry. The extract was redissolved in 500 μL of ethyl acetate. The ethyl acetate extract was derivatized for 60 min at 60 °C with the addition of 20 μL of MTBSTFA. Prior to injection into the GC–MS, 20 μL of 20 mg L^{-1} HCB was added as a syringe standard. The same procedure was followed with the rotating disk containing the NIP phase for comparative purposes.

The internal standard was not considered for the normalization of the analyte signals when variables of the method were studied and optimized.

2.9. Real sample analysis

To evaluate the applicability of the method in a real sample, samples from WWTPs from Santiago, Chile were analyzed using the proposed method. These samples were collected from the influent and effluent of the plant, stored in polypropylene bottles and frozen until analysis. An aliquot of 50 mL of sample was collected and adjusted to a pH of 2 (in quadruplicate).

To compare the performance of the proposed MIP, the results were compared with those obtained by extraction involving the commercial version of MIP (SupelMIP™ NSAIDs SPE Column, Sigma Aldrich) [34] using GC–MS for quantification.

3. Results and discussion

3.1. MIP and NIP characterization

The yield obtained for MIP was 43.8%; meanwhile, the yield for the NIP was higher, reaching a value of 51.5%, indicating that the template molecule DPA slightly inhibited the polymerization of the radical. This yield is referred only to fraction of particle size, 100–180 μm. This fraction is obtained after the whole processes, washing, and removal of the template, grinding and sieving the polymers. The FT-IR spectra for the MIP and NIP are very similar to each to other, consistent with the fact that all of the products were synthesized based on same monomer, cross-linker and initiator. The spectra show the typical absorption bands associated with the functional groups of the MIP and NIP. The micrographs of MIP and NIP (Fig. 4) reveal clusters of spherical particles having a narrow size distribution with diameters of approximately 1 μm. Often, in a solution radical polymerization, the particles are irregular in shape and size due to the milling and sieving process, and some interaction sites can even be destroyed. Despite the difficulty of obtaining spherical particles, the use of an adequately porogenic solvent contributes to the formation of such particles because the polymer chains during formation are not able to occupy the total solvent volume to produce a dispersion of spherical particles

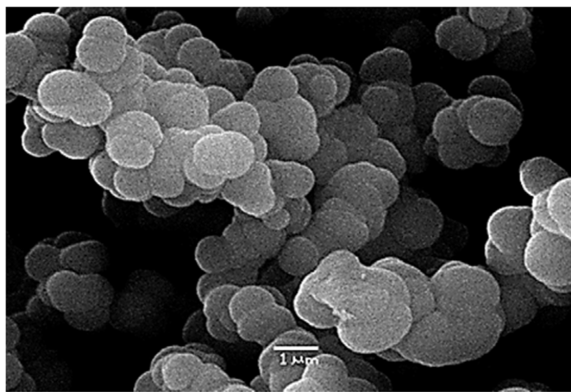

Fig. 4. Scanning electron microscope image of the MIP showing clusters of spherical particles.

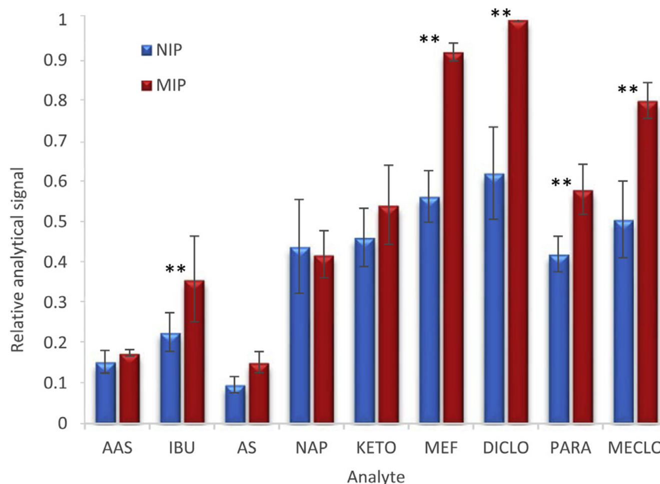

Fig. 5. Comparison in responses between the MIP and NIP for the studied NSAIDs (AAS: acetylsalicylic acid; IBU: ibuprofen; AS: salicylic acid; NAP: naproxen; KETO: ketoprofen; MEF: mefenamic acid; DICLO: diclofenac; PARA: paracetamol; and MECLO: meclofenamic acid). ** Significant differences between the MIP and NIP.

separate from the solution. This phenomenon occurs in this case, resulting in clusters of spherical particles that have better performance as far as their retention capabilities.

Previously, to the application of MIPs as sorptive phase, the synthesis of the MIPs was optimized in terms of the type and amount of monomers, acidic, basic, or neutral, the amount and type of solvent, the amount of crosslinker and finally the amount of initiator. All the variables of the synthesis were studied through experimental design and evaluated using multivariate analysis considering, the yield and the retention capacity, as the main factors to be considered in the performance of the MIPs. In this context, the best results were obtained for MIPs based on 1-vinylimidazole. Based on theoretical calculations, the most probable interactions between monomer and DPA are hydrogen bond and π- π stacking. Moreover as a process of characterization of MIPs, the selectivity between MIP and NIP was investigated for DPA using the value of Maximum Retention Capacity (Qmax). This value was obtained from isothermal studies at different temperatures and fitted to a Langmuir model. This Qmax values show that the retention capacity is two or three times higher for MIPs compared with NIPs. In addition, the reproducibility for retention of the template molecule within different batch of MIP ($n = 3$) was assessed, yielding a relative standard deviation of 3.4%.

3.2. Analytes selected according to the MIP effect

For the selection of the analytes to be determined in the water samples, extractions were performed with MIP and NIP separately on independent disks using the variables previously optimized for the retention of NSAIDs in Oasis HLB phase [26], which has similar chemical functionalities to the polymer used in this study. As recommended, NIP was used as a sorbent phase for comparison, verifying that the analytes are truly being extracted due to their affinity to binding sites present only in the MIP and not by nonspecific analyte/polymer interactions [35]. The chromatographic response of each drug after extraction with MIP and its comparison with the NIP results can be seen in Fig. 5.

The results indicated that the MIP had remarkably superior binding properties compared to NIP for the compounds having similar structures to the template molecule. Diclofenac and mefenamic acid (and the internal standard meclofenamic acid) showed signals 1.5–1.6 times higher when the extraction was performed with MIP with respect to extraction with NIP. Similarly, despite paracetamol showing a lower similarity with the template molecule, the difference between the response of MIP and NIP was also significant (approximately 30%). All of the other NSAIDs did not show significant differences ($p > 0.05$), except for ibuprofen to some extent.

These results suggest that cavities were created in the polymer network based on the interaction, size, shape and functionality of the diphenylamine molecule, and consequently, the NIP lacks adequate recognition site cavities retaining the analytes only through nonspecific adsorption [28]. According to these results, the analytes selected for further optimization were diclofenac and mefenamic acid. Due to the similar behavior of meclofenamic acid, this compound was used as internal standard when real samples were analyzed. As shown in Fig. 5, other NSAIDs can also be extracted at lower rates than diclofenac and mefenamic acid, however the selectivity of the determination can be strengthened by GC–MS.

3.3. Study of the variables

Different chemical and preconcentration factors were evaluated to obtain the highest extraction efficiencies of the selected drugs. In addition, the variables were studied in deionized water spiked with a known concentration of the selected NSAIDs, and consequently, the selectivity of the technique was able to assess the behavior of each variable.

The effect of the pH on the analytical signals of both analytes was studied between pH values of 1–12. Independent of the active cavity in the MIP, the retention of the analytes in the polymer always occurs through both π-π staking between aromatic groups and hydrogen bonds between the acidic hydrogen of the analytes and the nitrogen of the imidazole ring. As seen in Fig. 6, both analytes are more efficiently extracted in the pH range of 1–4, which is a consequence of their pKa values (4.15 and 4.2 for diclofenac and mefenamic acid, respectively) [36], indicating that at pH values over 6, the compounds are quantitatively dissociated, increasing their water solubility and decreasing their interaction by hydrogen bonds. The similar behavior of meclofenamic acid with both analytes observed in Fig. 6 shows that this compound is a good internal standard for the determination of the analytes in real samples.

Matrix modifiers are usually tested in microextraction techniques because they can enhance the extraction efficiency of a given analyte depending on the polarity. In the current case, similar to what was observed with RDSE of NSAIDs using Oasis HLB as the

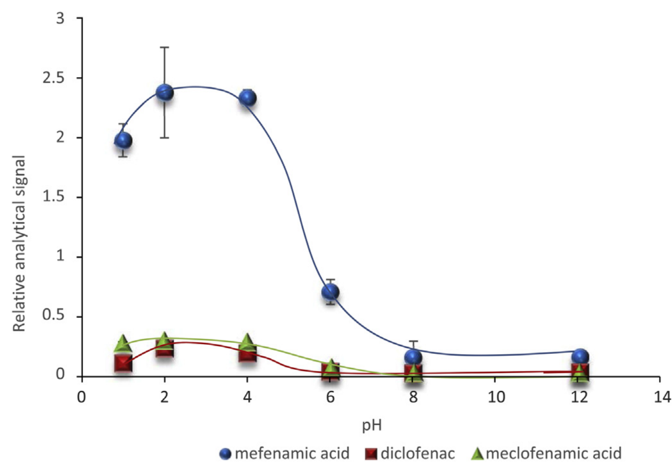

Fig. 6. Effect of the pH on the extraction of mefenamic acid, diclofenac and meclofenamic acid (internal standard).

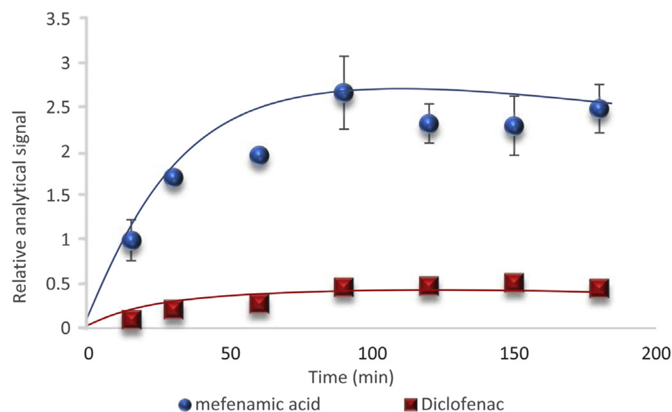

Fig. 7. Extraction profiles for mefenamic acid and diclofenac. The sample volumes were 50 mL.

sorptive material [26], neither methanol nor NaCl (both tested within the interval 0–15%) needed to be added as a matrix modifier because these analytes are semi-polar.

Another important hydrodynamic factor of RDSE is the rotation velocity of the disk. Efficient stirring of the sample in contact with the extraction phase is necessary to achieve the partition equilibrium as quickly as possible because the mass transfer of the analyte through the boundary layer that contacts the surface of the phase in microextraction techniques is the rate-determining step for its extraction. Consequently, in this study, this factor was studied between 200 and 3000 rpm. The dependence of the extraction efficiency on this factor was linear, and 3000 rpm was selected as the optimum value.

The extraction time for each analyte was determined under the selected experimental conditions for a 50-mL sample. The samples were extracted by RDSE for various times, ranging from 15 to 180 min, and the extraction profiles were then obtained. Fig. 7 shows the extraction profiles for diclofenac and mefenamic acid. The extraction time affects the amount of analyte concentrated in the MIP phase, as shown in Fig. 7, and the extraction yield increases with the extraction time until equilibrium is reached after approximately 60 min.

It has been previously observed [26] that the extraction time is directly related to the sample volume. In this case, for sample volumes of 100–250 mL with constant analyte concentrations in the solution, it was observed that equilibrium was not attained within the time range studied, although the preconcentration factor increased for the same extraction time. In this context, if more sensitivity is desired, the sample volume can be increased.

Regarding the amount of MIP loaded into the cavity of the disk, portions of 65, 50 and 25 mg of MIP were tested. It was found that increasing the amount of extraction phase used decreases the efficiency of the extraction device because when a higher amount is loaded into the cavity, it tends to be compressed, and thus, its interaction with the analytes decreases. When 25 mg of MIP was used, the extraction efficiency increased by approximately threefold for both analytes with respect to the use of 65 mg of MIP because the free movement of the MIP in the disk cavity allowed recirculation of the sample and better interaction with the analytes.

Once the compounds were extracted into the MIP, it was necessary to find a solvent that allows for the rapid desorption of the analytes with a small volume. Taking into account our previous experiences with the elution of acidic drugs [21,26] from other sorptive materials, an elution of 5 min with 10 mL of methanol gave rise to a quantitative desorption of the analytes from the MIP.

An important factor in the performance of microextraction techniques is the reusability of the sorptive material because this significantly reduces the cost of use. In the present case, considering a tap water sample enriched with the analytes, the portion of MIP loaded in a disk can be used in 6 sorption/desorption cycles, with absolute recoveries of 46 and 50%, for diclofenac and mefenamic acid, respectively, and relative standard deviations (% RSD) lower than 1% for both drugs, as the seventh cycle recoveries decrease by 33%.

3.4. Analytical figures of merit

The analytical features of the method together with the analysis of real samples were performed using GC–MS in the SIM mode to increase the sensitivity and selectivity of the determination. The chromatographic and derivatization conditions were selected with consideration of previous studies [37–40]. The analytical curve for each analyte was constructed using concentrations of standards ranging from 0.005 to 0.5 mg L^{-1}, which are 100 times lower if the preconcentration factor implicit in the method is considered.

Table 2 shows the correlation between the analyte concentration and the signal obtained from the GC–MS together with the detection limit, precision and recovery of the method.

The detection and quantification limits of the method were considered as the minimum concentrations of analyte, with signal-to-noise ratios of 3 and 10, respectively. They were determined for 10 drinking water sample aliquots spiked at a concentration of 5 μg L^{-1}.

The relative recoveries were between 99 and 100% by analyzing ($n = 6$) drinking water samples spiked at a concentration of 20 μg L^{-1}. The precision at the repeatability level ($n = 6$) sequentially using the same disk per sample was between 4 and 6%, and the intermediate precision, calculated using one disk per sample ($n = 6$), ranged from 5 to 6%.

A comparison between the results provided by RDSE using MIP and NIP as sorptive phase, and those obtained by following the same RDSE method but using a commercial MIP (SupelMIP™ NSAIDs SPE Column) was performed analyzing the same spiked drinking water sample (Table 3). As can be seen a clear difference between the absolute recoveries for mefenamic acid and diclofenac for both MIP phases (commercial and synthesized in this study) was obtained. In addition, the response of the commercial MIP was similar to that obtained for the NIP synthesized in this study. This behavior indicates that the commercial MIP does not have specific cavities for certain compounds, and it extracts all drugs with low

Table 2
Analytical features of the method.

Analyte	Linearity (r)	Slope $(\mu g\ L^{-1})^{-1}$	Intercept	LOD $(\mu g\ L^{-1})$	LOQ $(\mu g\ L^{-1})$	Relative recovery (% n = 6)	Precision interdisk (% RSD n = 6)	Precision intradisk (% RSD n = 6)
Mefenamic acid	0.9964	1.235	−0.0137	0.060	0.200	100	6	6
Diclofenac	0.9987	0.0965	−0.0003	0.067	0.223	99	5	4

and similar absolute recoveries. Similarly, when conventional Oasis HLB is applied to the determination of NSAIDs in analogous wastewater samples, recoveries for all drugs were similar, showing no specific preference for some analyte [26].

Table 3 shows absolute recovery values for the analytes between 46 and 50%, which can be considered a reliable value for equilibrium based extraction techniques.

3.5. Real sample analysis

The optimized and validated method was applied to real water samples obtained from the influent and effluent WWTP of Santiago, Chile. It was possible to quantify the studied drugs with internal standards, as stated above (Table 4).

The results show the presence of NSAIDs in the effluent of the treatment plants, which do not possess the ability to completely remove such contaminants. From the same sample, the method was also able to quantify paracetamol from the effluent with a concentration of $1.5 \pm 0.3\ \mu g\ L^{-1}$.

In addition to the recovery studies, accuracy of the method was also assessed by the comparative analysis of real samples. The results obtained from the proposed method were compared with their counterparts obtained by solid-phase extraction (SPE) with commercial MIP following the protocol described by Zorita et al. (2008) [34], which is the only extraction method reported for these analytes using this sorbent.

When comparing the results obtained by RDSE and SPE (Table 4), no significant differences ($p < 0.05$) were observed in most of the cases (two sample t-test, equal variances). A comparison between RDSE and its SPE counterpart indicates that RDSE is a simpler technique that does not require the use of vacuum pumps nor successive cleanup steps (with methanol and n-hexane). In addition, in the disk configuration, the sorbent can be used at least 6 times. However, the main disadvantage of RDSE is that the time involved in the extraction is longer than that of SPE (approximately 60 min vs 45 min).

4. Conclusion

The determination of diclofenac and mefenamic acid in water samples using RDSE containing MIP as the sorbent phase was feasible because the method presented extraction efficiencies between 99 and 100% with RSDs of less than 6%. Furthermore, the method based on the MIP was able to extract paracetamol with a significantly better efficiency with respect to the NIP because of its molecular similarity to the template used to synthesize the MIP.

Similar concentrations were found when the proposed method and that based on SPE using the commercial version of MIP were applied to real samples, indicating that RDSE is a reliable alternative as a sample preparation method. However, absolute recoveries were significantly higher for the MIP used in this work with respect to its commercial counterpart, clearly indicating the effect of the template molecule used in the MIP synthesis.

RDSE is a simple technique, and in the disk configuration, the sorbent can be used six times, assuring a good level of recovery and precision. The primary disadvantage of the present method is the relatively long extraction processing time compared to SPE methodologies.

The NSAIDs were determined in both the influent and effluent of WWTPs in Santiago, Chile, suggesting that these pollutants are reaching natural waters in similar concentrations to those observed in other countries.

Table 3
Absolute recoveries of NSAIDs from water using RDSE with commercial MIP, and with the MIP and NIP synthesized in this study. Bold numbers highlight the differences in absolute recovery for both analytes.

Analyte	MIP commercial (%)	NIP synthetic (%)	MIP synthetic (%)
Acetylsalicylic acid	8.0 ± 0.6	8 ± 1	9.0 ± 0.4
Ibuprofen	22 ± 5	11 ± 2	18 ± 5
Salicylic acid	6 ± 1	5 ± 1	8 ± 1
Naproxen	20 ± 3	22 ± 6	21 ± 3
Ketoprofen	36 ± 2	23 ± 4	27 ± 5
Mefenamic acid	**29 ± 1**	**28 ± 3**	**46 ± 1**
Diclofenac	**26 ± 5**	**31 ± 5**	**50.0 ± 0.2**

Table 4
Concentrations of mefenamic acid and diclofenac in the influent and effluent of a wastewater treatment plant in Santiago, Chile.

Concentrations found $(\mu g\ L^{-1})$				
Analyte	RDSE-MIP (this work)		SPE-MIP [34]	
	Influent	Effluent	Influent	Effluent
Mefenamic acid	4.3 ± 0.3	2.8 ± 0.1	4.3 ± 0.2	2.5 ± 0.2
Diclofenac	1.8 ± 0.1	1.3 ± 0.1	1.9 ± 0.2	1.5 ± 0.1

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