SOLID-PHASE EXTRACTION OF SELECTED ACIDIC PHARMACEUTICALS FROM WASTEWATER USING A MOLECULARLY IMPRINTED POLYMER

by

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DECLARATION

I hereby declare that this dissertation, which I herewith submit for the research qualification

MASTERS OF APPLIED SCIENCES IN CHEMISTRY

to the Durban University of Technology, Department of Chemistry, is apart from the recognized assistance of my supervisors, this is my own work and has not been previously submitted by me to another institution to obtain a research diploma or degree.

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To God be the glory for making this happen!!!.

DEDICATION

I dedicate this work to my daughter Sithandiwe, my mother Nonhlanhla Dlamini, grandparents Maduyaza and Dlokovu and to the whole Phangela family.

ABSTRACT

In this study, molecular modeling was used to investigate the intermolecular interactions between the functional monomer and ketoprofen which is an acidic pharmaceutical that possesses anti-inflammatory and analgesic activities. Ketoprofen is widely employed in medical care for treating musculoskeletal injury. This led to rational design of a molecularly imprinted polymer (MIP) that is selective to ketoprofen. Density functional theory (DFT) at B3LYP/6-31 level was used to investigate the intermolecular interaction between functional monomers and ketoprofen. Binding energy, ΔE, was used as an indication of the strength of the interaction that occurs between functional monomers and ketoprofen. 2-vinylpyridine (2-VP) as one of the functional monomers gave the lowest binding energy when compared to all the functional monomers investigated. Monomer-template interactions were further experimentally investigated using spectroscopic techniques such as Ultraviolet-visible and Fourier transform infrared (FTIR).

A selective MIP for ketoprofen was synthesized using 2-vinylpyridine, ethylene glycol dimethacrylate, 1,1'-azobis(cyclohexanecarbonitrile), toluene/acetonitrile (9:1, v/v), and ketoprofen as a functional monomer, cross-linker, initiator, porogenic mixture, and template, respectively. The polymerization was performed at 60 °C for 16 h, and thereafter the temperature was increased to 80 °C for 24 h to achieve a solid monolith polymer. The non-imprinted polymer (NIP) was synthesized in a similar manner with the omission of ketoprofen.

Characterization with thermogravimetric analysis (TGA) and powder X-ray diffraction (XRD) showed that the synthesized polymers were thermally stable and amorphous. Morphology of the particles were clearly visible, with MIP showing rough and irregular surface compared to NIP on the scanning electron microscopy (SEM). The characterization of the prominent functional groups on both MIP and NIP were performed using FTIR and nuclear magnetic resonance (NMR). The existence of hydroxyl was observed in the MIP; this was due to the presence of ketoprofen in the cavity. Prominent carbonyl group was an indication of the cross-linker present in both polymers.

The synthesized MIP was applied as a selective sorbent in the solid-phase extraction of ketoprofen from the water. The extracted ketoprofen was monitored by high performance liquid chromatography (HPLC) coupled with UV/Vis detector. Several parameters were investigated for maximum recovery of ketoprofen from the spiked deionized water. The optimum method involved the conditioning of 14 mg MIP sorbent with 5 mL of methanol followed by equilibrating with 5 mL of deionized water adjusted to pH 2.5. Thereafter, 50 mL sample (pH 5) was loaded into the cartridge containing MIP sorbent followed by washing and eluting with 1% TEA/H_2O and 100% methanol, respectively. Eluted compounds were quantified with HPLC.

MIP was more selective to ketoprofen in the presence of other structural related competitors. The analytical method gave detection limits of 0.23, 0.17, and 0.09 mg L^{-1} in wastewater influent, effluent, and deionized water, respectively. The recovery for the wastewater influent and effluent spiked with 5 µg L^{-1} of ketoprofen was 68%, whereas 114% was obtained for deionized water. The concentrations of ketoprofen in the influent and effluent samples were in the ranges of 22.5 - 34.0 and 1.14 - 5.33 $mg.L^{-1}$, respectively. The relative standard deviation (RSD) given as ± values indicates that the developed analytical method for the analysis of ketoprofen in wastewater was rapid, affordable, accurate, precise, sensitive, and selective.

TABLE OF CONTENTS

List of figures

List of tables

List of abbreviations

DFT	: density functional theory
FTIR	: Fourier transform infrared spectroscopy
GC	: gas chromatography
HLB	: hydrophilic-lipophilic balance
HPLC	: high-perfomance liquid chromatography
LC	: liquid chromatography
LOD	: limit of detection
LOQ	: limit of quantification
MIMSPE	: molecularly imprinted micro-solid phase extraction
MIP	: molecularly imprinted polymer
NIP	: non-imprinted polymer
NMR	: nuclear magnetic resonance
NSAIDs	: non-steroidal anti-inflammatory drugs
NVT	: number, volume, and temperature
SEM	: scanning electron microscopy
SPE	: solid phase extraction
SPME	: solid phase micro-extraction
TEA	: triethylamine
TGA	: thermogravimetric analysis
WWTPs	: wastewater treatment plants
XRD	: X-ray diffraction

ANNEXURES

Annexure A: MAP of WWTPs in Durban, KwaZulu-Natal, South Africa

Annexure B: Published paper on this work

Chapter 1:

Introduction

1.1 Introduction

The presence of the pharmaceuticals was first identified in surface and wastewater in the United States and Europe in the 1960s [1]. Pharmaceuticals are designed to be biologically active. Their occurrence and fate in the environment have enticed the devotion of the scientific community to assess the effectiveness of the environmental policy [2-5]. Pharmaceuticals are recognized class of environmental pollutants; hence they are becoming increasingly problematic contaminants of either surface water or ground water around industrial and residential communities. The sources of pharmaceuticals in the wastewater treatment plants are household, effluent from pharmaceutical industries, hospitals, and health service centers, which enter the water system and combined with diffused sources [1, 6-8]. Pharmaceuticals are classified as recalcitrant bio-accumulative compounds and they are regarded as hazardous chemicals in the aquatic environment. Pharmaceuticals are easily transported from wastewater to other water matrices. This is because water is a good carrying medium for polar and semi-polar compounds [9]. The fate of pharmaceuticals in the environment has already been the subject of advanced investigations and yet still has severally methodological challenges to be overcomed [10]. The limitation of the pharmaceuticals in the environment is caused by the low concentrations which are found in μg L^{-1} or ng L^{-1} level in the wastewater [11, 12]. In order to determine these compound a highly sensitive, specialized analytical instrument and analyte preconcentration method is required.

1.1.1 Target compound

Ketoprofen also known as [(RS)-2-(3-benzoylphenyl)propanoic acid] with CAS number 22071-15-4 Figure 1 is an acidic pharmaceutical that was selected in this study. The selection of this pharmaceutical drug in this project was guided by the limited information on the occurrence of ketoprofen in South African environment. The limitation of this pharmaceutical drug was due to its being not commonly used in many medications. Other non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, naproxen, etc, have been monitored extensively in the South African environment [13-16]. The occurrence of ketoprofen in South African aqueous

environment has only been reported in 3 studies [11, 17, 18]. Due to the large quantity of ketoprofen that is consumed by humans, the compound is world widely detected with other NSAIDs in wastewater and surface water [19-21]. When NSAIDs are overdose they can increase life-threatening heart problems such as heart attack or strokes, may also have stomach or intestinal effect such as bleeding. Ketoprofen possesses anti-inflammatory and analgesic activities due to its ability to inhibit cyclooxygenase enzymes that promote inflammation [22, 23]. Ketoprofen is widely employed in medical care as it is able to treat inflammatory diseases and musculoskeletal injury [24]. Ketoprofen is used to treat pain and/or inflammation caused by arthritis. Therefore, after intake by humans it is excreted through urine and then it is transported from household. Ketoprofen presence is caused by direct disposal to aquatic systems, water run-off from landfill sites and incomplete removal during wastewater treatment [17].

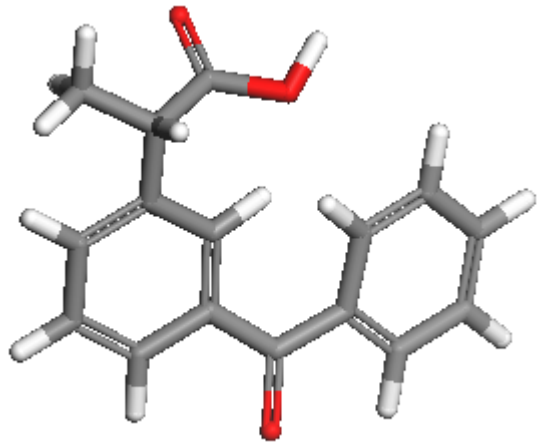


Figure 1: Structure of ketoprofen; with white as hydrogen, red for oxygen and grey for carbon.

1.1.2 Analysis

MIPs are increasingly being used as selectivity supports in liquid chromatography, capillary electrophoresis and solid-phase extraction and as catalysts, bionic sensors and artificial antibodies [25-29]. For quantification of NSAIDs, liquid chromatographic technique are usually carried out after extraction of ketoprofen from aqueous matrices. Solid-phase extraction, solid phase micro-extraction (SPME) and molecularly imprinted micro-solid phase extraction (MIMSPE) are the widely used

techniques for sample development and clean-up of wastewater samples [30, 31]. These techniques are based on adsorption of the ketoprofen onto the adsorbing material.

SPE is the preferred sample preparation technique to extract pharmaceuticals from environmental aqueous samples because of its higher recoveries, elimination of emulsions and less organic solvent usage. SPE improves selectivity when MIP is used as the adsorbent [32, 33]. SPE is the widely used procedure to extract traces of organic compounds from environment samples. Extractions have been performed with the C18 cartridge, weak cation exchange sorbents, hydrophilic-lipophilic balance polymer resin (e.g. Oasis HLB) [85, 86]. However, these methods have shown limited recovery for polar compounds, and they are time-consuming and lack selectivity [4, 5, 87]. The SPE stationary phases which are currently used have limited sorbents with higher selectivity such as immune sorbents. Thus MIPs are developed and applied to specifically target certain compounds, because they are customized synthetic polymers with highly specific recognition ability for target molecules [33, 87].

Acidic pharmaceuticals that include ketoprofen have been widely quantified using chromatographic techniques such as gas chromatography (GC), liquid chromatography (LC) with various detection systems such as flame ionization detection, diode array UV–Vis detector, mass spectrometry [34-37]. However, with the LC-MS the disadvantage in quantitative analysis is that it is sensitive to a matrix effect [38, 39]. This is often the case with complex matrices found in the wastewater samples. To eliminate this problem, the analytical method requires the use of an effective sample clean-up. Therefore, SPE method using MIP is used to eliminate the matrix effect.

HPLC is used after appropriate sample pre-treatment. Different studies of the analysis of ketoprofen using solid phase extraction [31] have been reported in foreign countries [40]. One of the commonly employed sample prep techniques is SPE. In SPE, a sorbent is used for the extraction of target compounds from the water samples. Sorbents available in literature for this application are Oasis HLB, MIPs, Oasis MAX and C18 cartridge [41-45]. SPE offers better detection limits and reduces matrix effects. There are currently few published reports for the

determination of these pollutants in South African wastewater systems [4, 11, 17]. These reports are based on liquid chromatographic analysis after solid-phase extraction of target compounds using Oasis hydrophilic-lipophilic balance (HLB) cartridges. Therefore LC is a preferred technique for non-volatile compounds. Among these sorbents, MIPs are relatively new hence in this project they were evaluated for their suitability in the extraction of ketoprofen from wastewater.

1.1.3 Molecularly imprinted polymers

Molecular imprinting technique was firstly introduced in 1972 and was modified to advance technology in 1980 [25]. Molecularly imprinted polymers (MIPs) are known as smart materials with a predetermined selectivity. Nowadays, molecular imprinting is a world known effective method for obtaining a synthetic media with a high selectivity for the target molecule [46, 47]. Molecular imprinting technology is a rapidly developing technique for the preparation of polymers and is used for the preparation of highly specific binding sites for small molecules [48]. Molecularly imprinted polymer (MIP) is a promising material for sensing, separation of chemical and biological molecules for the synthesis of a highly selective polymer. Molecular imprinting technology involves the generation of cavities containing functional sites within a highly cross-linked polymer matrix [49, 50].

Most commonly, the target compounds interact with MIP through non-covalent bonding, ionic interaction and hydrophobic interaction methods [30]. For a MIP to possess such interactions, the polymerization is usually carried out following the bulk polymerization, in situ polymerization, suspension polymerization, and multi-step-swelling polymerization approaches [51-53]. MIPs are prepared using a functional monomer (s), which permits the interactions with the functional group of a target molecule and they are synthesized in the presence of a cross-linking monomer. The imprinted molecule is removed from the polymer in order to create the molecularly imprinted cavity with the corresponding binding sites for the target molecule.

Once synthesized, the nature of adsorption of target compounds onto MIPs is investigated using the adsorption isotherms which are classified into two categories:

(i) Models of discrete distribution in which homogeneous surface is supposed,

(ii) Continuous distribution models which take into account the surface heterogeneity.

The first category of discrete distribution model focuses on the Langmuir isotherm, which is based on three following assumptions [54]:

(a) Adsorption cannot proceed beyond monolayer coverage,
(b) All surface binding sites are equivalent and can take up the adsorbed target molecule,
(c) The ability of a target molecule to bind at a given site does not depend on the occupation of neighboring sites.

On the other hand, the continuous distribution model is studied using Freundlich isotherm. This method concentrates on the surface heterogeneity and the continuous energy distribution for the sorption process which is detected when using different solid surfaces. The Freundlich model is an oversimplification of the Langmuir model applied to a heterogeneous surface with an energy distribution equivalent to an exponential decrease [32, 54-56]. There are other isotherms known in literature such as, Langmuir-Freundlich, Jovanovich, Jovanovich-Freundlich and Allosteric [54, 57].

1.1.4 Computational simulation

The formation of a complex between the target molecule and functional monomers is the first step in the preparation of MIP. The binding energy is used in order to quantitatively evaluate the interaction between the target molecule and the functional monomer. For any given target molecule, a monomer-template complex of higher ΔE is more suitable for the synthesis of the polymer, compared to those of the lower ΔEs [46]. However, the purpose for this study was basically not to rationally design a MIP based on the binding energy which were obtained from different target molecules and the functional monomer, but it was to screen different functional monomers. Due to limited time and cost of preparing MIPs which include a costly washing to desorb the template living out the cavity of interest only one template was studied for this project.

The focus on this study was the development of a fast and reliable screening method to aid the design and synthesis of MIP with high loading capacity and selectivity for ketoprofen.

1.2 Problem statement

One of the most relevant investigations in environmental analytical chemistry is water quality. However, the increased use of pharmaceutical compounds by human and in animals is becoming a new environmental problem because of their increased presence in the environment and their possible adverse effects on human and ecological systems [7, 58, 59]. In recent years, NSAIDs that include ketoprofen have been detected in South African aquatic resources [3, 4, 11, 60-62]. Therefore, in order to perform full evaluation of these emerging pollutants in water, selective and sensitive methods are required for sample preparation and quantification. The application of chromatographic techniques for quantification of ketoprofen is well understood. However, in South Africa only Oasis Hydrophilic Lipophilic Balance (HLB) SPE cartridges have been applied in the extraction of ketoprofen from wastewater [11, 18, 63]. These SPE sorbents often suffer from poor selectivity, hence MIPs are proposed in this study for selective extraction of ketoprofen from wastewater.

Furthermore, the ability for South African WWTPs to remove ketoprofen during the treatment process is not known. Therefore, the initial assessment in terms of the feasibility for the South African WWTPs to remove ketoprofen during the treatment process was investigated.

1.3 Justification

In the last few decades, reports on the accumulation of pharmaceuticals in the environment have increased [53, 64, 65]. As analytical techniques become more sensitive and deployed, more drugs are discovered in the wastewater samples. MIPs are useful adsorbents for pharmaceuticals in water. The removal of pharmaceuticals from the wastewater using multi-template MIP has been reported [66]. However, the

performance of such polymers in the single template in South Africa has not been fully evaluated in the complicated sample matrix such as wastewater influent and effluent. And yet it has the largest number of WWTPs that are mainly used for domestic wastewater treatment and their potential for removal of pharmaceuticals such as ketoprofen is not known. To understand the risk of aquatic life and water consumers from suffering the health effects caused by pollutant levels, it is necessary to fully evaluate the occurrence of ketoprofen in water resources. This performance is important because although MIPs are selective to particular functional groups they are not specific for certain molecules. The backbone polymer of molecularly imprinted sorbent can absorb some compounds based on functional groups present especially for aqueous samples.

To address this problem, the development of highly sensitive and selective methods for trace determination of compounds such as ketoprofen in complex wastewater matrix is required. One of the most suitable methods of ketoprofen analysis is the use of chromatographic methods that are equipped with a very sensitive mass spectrometry detector [35, 67]. However, the operation, maintenance, and cost, of mass spectrometry detector are expensive. Therefore, some laboratories had opted for the use of a cheaper and readily available UV-visible detector. The sensitivity of the UV-visible detector is usually improved by the use of solid-phase extraction [31] for clean-up, preconcentration of target compounds and removal of interferences also improves [68].

1.4 Aim

The aim of the study was to quantify the levels of ketoprofen in wastewater using molecularly imprinted polymer as selective sorbent in solid-phase extraction.

1.5 Objectives of the study

- Synthesis of MIP using computational approach for modelling.
- The synthesized MIP to be suitable for the selective extraction of ketoprofen from wastewater.

- To develop the SPE method that can be used for the selective isolation and pre-concentration of ketoprofen from wastewater.
- To quantitatively determine ketoprofen in wastewater using HPLC-UV.
- To evaluate the removal efficiency of ketoprofen during the wastewater treatment process.

1.6 Dissertation outline

This dissertation is presented in seven chapters as described below:

Chapter 1

This chapter covers the introduction, problem statement, justification, aims and objectives of this study.

Chapter 2

This chapter outlines a literature review of the relevant reports that informs this study. Detailed literature of ketoprofen as a drug in wastewater, different polymerization methods, sample methods and sample preparation techniques are discussed in this section. This chapter also include the background information of the characterization techniques used in this study.

Chapter 3

The detailed experimental procedures for computationally design of MIP, synthesis, solid phase extraction optimization, characterisation techniques of the polymers and on the evaluation of the acidic pharmaceutical in wastewater are covered in this chapter. Also, the chapter outlines the use of density functional theory (DFT) methods to simulate the interactions of ketoprofen and the functional monomer.

Chapter 4

This chapter outlines the results and discussion obtained from the studies of polymers including the characterization and application for the determination of ketoprofen in wastewater. The characterisation of the synthesised MIPs, optimization of the SPE that uses MIP as absorbent, selectivity, validation of the method and quantitatively determination of ketoprofen in wastewater are covered in this chapter.

This was achieved by packing the SPE cartridges with the MIP's, which was optimized for the analysis of target compound. The analysis was done on the HPLC-UV, peak area was used to calculate the recoveries and concentration of the ketoprofen.

Chapter 5

This chapter highlights the important achievements, conclusions, and recommendation of this study.

Chapter 2:

Literature review

2.1 Environmental trends

In the view of preserving the precious resource such as water, there is a need to understand the extent of all widely used pharmaceuticals in the environment. It has been previously reported that wastewater treatment plants (WWTPs) are the primary sources of pharmaceuticals in river water. In other countries such as United States, Sweden and Spain, pharmaceuticals that include ketoprofen have been detected in wastewater (Table 1) [69-71] There are currently few studies that have been reported on the presence of ketoprofen in South African aquatic conditions [11, 14, 17, 18]. In other countries the concentration of ketoprofen in the WWTPs was less than 2 μg L^{-1}, while in South Africa it was detected in higher concentrations [14, 18]. The concentrations of the pharmaceuticals are found in μg L^{-1} or ng L^{-1} level in the wastewater. In Table 2, the removal efficiency of ketoprofen from wastewater is not 100% removed in most treatment plants as this is caused by the low concentration levels.

Table 1: Detected concentrations of ketoprofen (μg.L^{-1}) in wastewater.

WWTP – City - Country	Influent	Effluent	Reference
Darvill – Pietermaritzburg – South Africa	3.15	0.38	[14]
Amanzimtoti – Durban – South Africa	8.6	1.55	[18]
Northern – Durban – South Africa	<10	1.0	[17]
Baltimore Back River – MD – United States	1.20	0.28	[69]
Kallby – Lund - Sweden	1.35	0.48	[70]
North - Seville city - Spain	1.36	0.41	[72]
Riverside- CA – United States	1.5	<LOQ	[73]

Table 2: Removal efficiency of ketoprofen from wastewater.

Country	%Removal	Reference
Korea	94	[62]
Czech Republic	72	[74]
Spain	40 - 100	[75]
Tokyo	45	[76]
Sweden	69	[77]
Brazil	48	[78]

2.2 Sampling and extraction of ketoprofen from water

The collection of wastewater samples is usually performed using the grab sampling approach [22, 79-82], however, there are few studies reported where passive sampling has been developed [31, 80, 82]. Grab sampling is the most convenient approach as it allows for collection of samples any time of the day. During the analytical method development, samples are usually collected to validate the new methods. Whereas, in passive sampling, the analysis can only be performed after approximately two weeks of the sampling process.

Nowadays, the use of MIPs in solid-phase extraction [31], so-called molecularly imprinted SPE (MISPE) belongs to the most advanced technical application of the polymers [83, 84]. According to the literature review, MIPs have been applied to extract different NSAIDs, mainly naproxen, diclofenac and ibuprofen from river water and wastewater samples [33, 49]. The application of MIP-based solid phase extraction to biological and environmental samples has been studied [84]. This technique is referred as MIP-SPE or MISPE and its benefits are the selectivity to the pre-determined choice of target molecule employed in the preparation. Also, this improves sensitivity when larger sample volume is extracted because the MISPE is

selective to a target molecule. The problem with non-specific adsorption often occurs with the hydrophobic nature of the polymer and this can be reduced when a small amount of the MIP is used and appropriate washing schemes prior to elution [85, 86]. The challenge for MIPSPE is the presence of the target molecule which remains in the MIP, which reduces the capacity of the binding sites for the analyte.

MISPE are packed with methanol solution of MIP as a slurry into an SPE cartridge. Polytetrafluoroethylene (PTFE) frits will be placed above and below the polymer particles sorbent bed. A similar method of a blank SPE cartridge is also prepared using non-imprinted polymer (NIP) as a sorbent. The optimization of MISPE cartridge is done as follows, conditioning, loading, washing, and elution, and lastly the isolation of the target compounds from real samples. In the conditioning step, the MIP cartridge is washed with the eluting solvent and then with loading solvent for equilibration. The function of this step is to activate the binding sites of the MIPs for maximizing their interactions with the target analyte in the samples [33, 83].

2.3 Molecular simulation

In order to prepare MIPs, a functional monomer, and a cross-linker are polymerized in the present of a target molecule and a porogenic solvent. The target molecule is then extracted from MIP, leaving sites which are complementary to those of the template. Since an optimal target molecule and functional monomer interaction has a determining impact on successful imprinting, the choice of appropriate monomers plays a critical role in the successful imprinting process [87]. The best functional monomers are selected based on a trial and error method which is expensive and time-consuming [88-90]. Nowadays, with the appreciation of the advent of computational chemistry, the computer-aided study of MIPs has been investigated as a rational and fast technique to search for optimal imprinting conditions [88]. Also, the use of molecular dynamics simulations and computational screening to identify functional monomers capable of strong interaction with the target molecule have been investigated [88, 91].

Although, the molecular imprinting technique has been widely used for polymer synthesis, few studies have shown the drive of the imprinting process with the aid of

a computational modeling and mono-target molecule in the MIP recognition. Molecular dynamics simulations are a powerful tool to investigate complex systems made of thousands of atoms at reasonable computational costs [92-94]. The properties of molecules are affected by the surrounding environment and accurate molecular dynamic simulations must include a proper description of the existing interactions. Simulations are based on classical mechanical force fields that describe non-covalent interactions, H-bonding, van-der-Waals forces, π–π interactions and electrostatic interactions [91]. Molecular dynamics simulation in canonical ensemble at constant atom number, volume and temperature (NVT) has been utilized to fully understand the interactions of ketoprofen with 2-vinyl pyridine [48, 94]. These is based on charges of the compounds, whereby ketoprofen acts as a proton donor and 2-vinyl pyridine as a proton acceptor.

2.4 Polymerization methods

An important step in the polymer synthesis is when the complex is formed between the target molecule and the functional monomer in the pre-polymerization step. The product of this step and polymerization reaction determines the quality and performance of the polymer synthesized. However, the quantity and quality of the MIP recognition sites is a direct function of the mechanisms and extent between the target molecule and functional monomer interaction present in the pre-polymerization reaction [95].

MIP is a molecular signature of the target molecule (analyte) that is imprinted in the polymeric network; the cavities are produced after target molecule is extracted from the polymer and are readily accessible for rebinding of the analyte from the environmental samples. The target molecule is subsequently removed from the polymer leaving behind the cavities which are complementary in chemical functionality, size and spatial arrangement [96].

The challenge of designing and synthesizing a MIP can be a discouraging overlook into the unqualified practitioner. This includes the absolute number of experimental variables involved, such as the nature and level of the target molecule, functional monomer(s), cross-linker(s), solvent(s) and initiator, the method of initiation and the duration of the polymerization. However, MIP has the advantages of physical

robustness, resistance to elevated temperature and pressure, inertness to acids, bases, and aggressive organic solvents, low cost and eases to prepare [97, 98].

The target molecule and functional monomer can interact via reversible covalent, non-covalent and non-covalent interactions. Non-covalent interaction involves hydrogen bonds, ionic bonds, hydrophobic interactions and van der Waal forces are shown in Figure 2. Where (i) formation of reversible interactions between the template and the functional monomer may involve one or more of the following reciprocal actions: (A) reversible covalent binding; (B) covalent attachment of polymerizable binding groups that are activated for non-covalent interactions by template cleavage; (C) electrostatic interactions; and, (D) hydrophobic or van der Waals.[98] {ii} A subsequent polymerization in the presence of a cross-linking monomer. (iii) Template removal from the polymer. (iv) The analyte selectively binding in the cavities vacated by the template. Non-covalent imprinting is used more because of its great selection of different functional monomers with the target molecule.

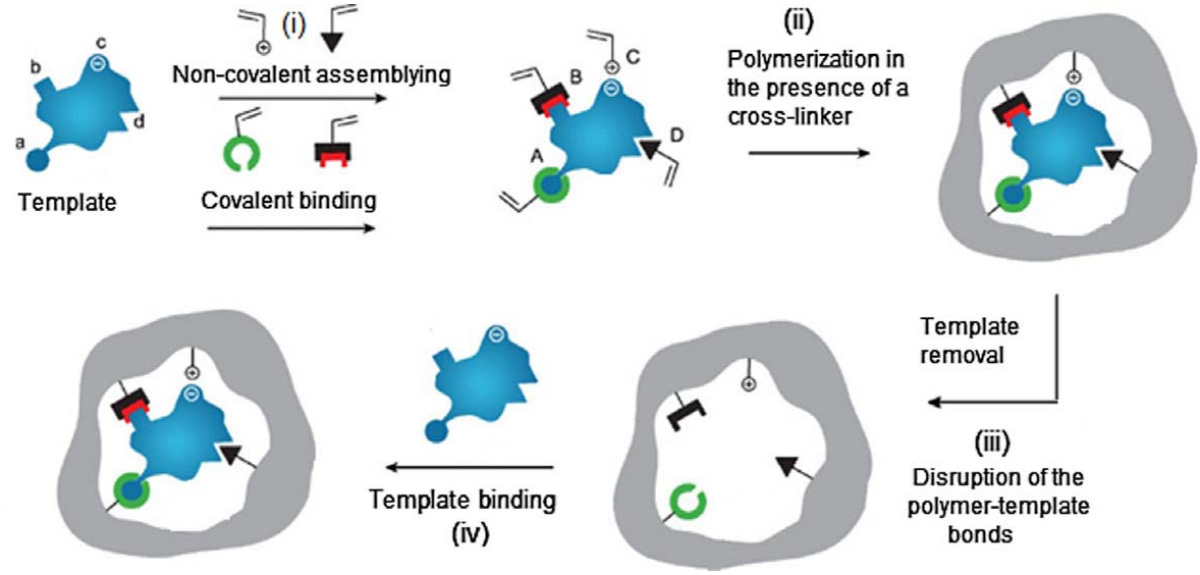


Figure 2: Molecular imprinting illustration [97].

The target molecule is important because it directs the organization of the functional groups pendent to the functional monomer. Functional monomer is responsible for the binding interactions in the imprinted binding sites. In the non-covalent molecular imprinting, functional monomer is added in excess relative to the number of moles of

the target molecule to favor the formation of the target molecule, functional monomer assemblies. There are three functions of a cross-linker in the polymerization process:

(1) For controlling the morphology of the polymer matrix,

(2) To stabilize the imprinted binding site and

(3) It imparts mechanical stability to the polymer matrix.

The solvent is there to bring all the components in the polymerization into one phase. And it is also responsible for creating the pores in macroporous polymers. The solvent in the synthesis is then referred as the porogen. The porogen also has other functions as a solvent in a non-covalent imprinting polymerization which must be cautiously chosen to maximize the likelihood of target molecule, functional monomer complex formation [99].

Different polymerization methods can be used in the preparation of MIP, these methods are summarized in Table 3 with their advantages and disadvantages [100, 101]**.** Many preparations of MIP use the bulk method because of its simplicity and easily controlled. The reaction mixture of the target molecule, functional monomer, cross-linker, initiator, and porogen are combined in a vessel, the mixture is purged with nitrogen to remove oxygen and sealed under vacuum. The bulk polymer monolith is then crushed, ground and sieved to obtain particles which are between 25-50 μm sizes [102, 103].

The method of suspension consists of a heterogeneous polymerization method which produces the spherical beads in a broad size range between μm to mm sizes. This technique involves suspension in either water or perfluorocarbon. The polymerization mixture containing higher molar concentrations of the target molecule and the functional monomer is suspended as droplets into an excess of a continuous dispersion phase by agitation in the present of a stabilizer.

Polymerization by precipitation requires a larger amount of porogen than that used in bulk method, which can be ten times more. During the process of polymerization, the growing of polymers chains become insoluble in the liquid phase and then form a precipitate. When using the precipitation method, the binding sites are inside their network which then causes a slow mass transfer of target molecules.

Table 3: Summary of different polymerization methods for the preparation of MIP.

MIP format (polymerization)	Advantages	Disadvantages
Bulk	Polymerization minimalism and universality, Not require particular skills	Tedious procedures of grinding, sieving and column packing, Uneven particles size and shape, low performance
Suspension	Globular particles, Highly reproducible results, Large scale possible	Phase partitioning of complicates system, Water is incompatible with most imprinted procedures, Specialist surfactant polymers required
Precipitation	Imprinted microspheres, Uniform size and high yields	Large amount of target molecule, High dilution factor

2.5 Characterization and evaluation of MIPs

2.5.1 Fourier transform infrared spectroscopy (FTIR)

FTIR is a technique capable of determining the functional groups in the structure of a molecule. It particularly uses a sample which is in a solution or in the solid state. FTIR spectroscopy is a powerful method for studying hydrogen bonding (H-bonding) due to the technique being able to demonstrate the structure of the functional groups; this is applied in the analysis of MIP in the pre-polymerization complexes. FTIR provides higher information content of the spectra that in some cases allows not only detecting but also to quantify the analyte and study the mechanism of interaction of the target molecule with the MIP [95]. The function of the method

depends ultimately on a careful choice of the target molecule and MIP to avoid overlapping FTIR bands.

The imprinting process begins with a complexation between the target molecule and functional monomer generally via hydrogen bonding. FTIR is used to identify the formation of the bond since the stretching frequency of hydroxyl or amino groups (hydrogen bond donors) and carbonyl group (hydrogen bond acceptors) are displaced and observable shift can be identified [95].

2.5.2 Scanning electron microscopy (SEM)

Microscopy techniques are used in different ways to probe imprinted polymers on a variety of scales length and also used to study the macropores, shape, volume, and average particle size. SEM provides composition information obtained from the contrast between MIP and target molecule.

2.5.3 Thermal analysis

Thermal analysis has been widely use in the last decades for rapidly assessing the thermal stability of various substances [104, 105]. Therefore, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) will be used to study the thermal stability of the prepared polymers. In TGA, when a polymer is thermally stable, there should be no observed mass change and it also gives the upper use temperature of a material (polymer). Beyond this temperature the material will begin to degrade.

2.5.4 Zeta Potential

Zeta potential is a scientific term for electrokinetic potential in colloidal dispersions. In the colloidal chemistry, it is usually symbolized using the Greek letter zeta (ζ), hence *ζ-potential*. Zeta potential is frequently used for the understanding the surface reactivity of the polymers. This is achieved by measuring the magnitude of the

electrostatic or charge repulsion or attraction between particles. According to Smoluchowski theory which applies to particles of all shapes and concentration, and not to surface conductivity [106] States that if zeta potential reads zero, it indicates an uncharged surface and when it is greater than ±25 mV it is highly charged [107].

2.5.5 Powder X-ray diffraction (XRD)

XRD is used for phase identification of a crystalline material and can provide information on unit cell dimensions. This is achieved when an analyzed material is grounded, homogenized and average bulk composition is determined. X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference when conditions satisfy Bragg's Law shown in equation (1).

$$n\lambda = 2d\ sin\ \theta \qquad (1)$$

Where *n* is a positive integer, λ is the wavelength of incident wave, *d* is the spacing between layers of a atoms and *θ* is the angle between the incident rays and the surface of the crystal [108].

2.5.6 Elemental micro-analysis

Elemental micro-analysis is used to measure the percentage by mass of carbon, hydrogen, nitrogen, oxygen, etc. within samples. This technique is also applied in the analysis of copolymers by calculating the comonomer composition of the polymer [99]. Hence, this method is not sufficiently sensitive to detect the trace quantities of target molecule remaining in the polymer.

2.5.7 Solid-state NMR

Solid-state NMR techniques avoid the use of a solution and therefore enable the NMR spectra of insoluble materials to be analyzed. In the polymer network, its gives an insight different of the chemical environments present in the sample and obtain the information on the degree of chemical [99]. NMR spectroscopy was used to gain insight into the interaction of the polymers.

2.6 Chromatographic Techniques

2.6.1 Gas chromatography (GC)

In a GC, the components of a vaporized sample are separated as a result of being partitioned between a mobile gaseous phase and a liquid or a solid stationary phase held in a column. When performing a gas chromatographic separation, the sample is injected and vaporized into the head of a chromatographic column. Elution is brought about by the flow of an inert gaseous mobile phase. In other chromatography methods, the mobile phase does not interact with the molecules of the analyte but its only function is to transport the analyte through the column. GC is suitable for the analysis of volatile and thermally stable compounds, and if the compound is non-volatile derivatization is required. Derivatization is the process of chemically modifying a compound to produce a new compound which has properties that are suitable for analysis using a GC. And the derivatization process of the compounds may lead to sample loss, which afterward might leading to errors in quantitative analysis.

2.6.2 High-perfomence liquid chromatography (HPLC)

In liquid chromatography, the mobile phase is a liquid solvent containing the sample as a mixture of solutes. This technique required instruments capable of much higher pumping pressures than the simple devices that preceded them. When the sample is introduced into the column, some of the analytes are adsorbed onto the stationary phase and some are passing along with the mobile phase depending on the polarity of the analytes.

Both of the chromatography techniques are efficient, highly selective, and widely applicable, required small sample and readily adapted to quantitative analysis. HPLC can accommodate non-volatile, thermally unstable compounds and is also applicable to inorganic ions as well.

Chapter 3:

Methodology

3.1 Chemicals

Ketoprofen (≥98%), triclosan (≥97%), 2-vinylpyridine (97%), HPLC-grade methanol (≥99.9%), 1,1'-azobis-(cyclohexanecarbonitrile) (98%), ethylene glycol dimethacrylate (98%) and toluene (99.7%) were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC-grade acetonitrile (≥99.9%) and glacial acetic acid (100%) were purchased from Merck (Darmstadt, Germany). Formic acid (approx. 98%), fenoprofen (≥97%) and HPLC grade triethylamine (≥99%) were purchased from Fluka (Steinheim, Germany).

3.2 Computational approach

The computational approach was done to investigate the interaction of target molecule and the functional monomer. Molecular dynamics simulations in the canonical ensemble at constant atom number, volume, and temperature (NVT) were utilized to fully understand the interactions of ketoprofen and the functional monomer(s). All simulations were executed using the Discover Module of Materials Studio (version 7.0) [48]. The molecular modeling was performed using the Materials Studio software. All calculations were carried out using COMPASS force field for the intermolecular interaction of ketoprofen. All systems were subjected to energy minimization for geometry optimization using minimizer incorporated in the discover module of Materials Studio 7.0 before molecular dynamics simulations were conducted. For minima calculation, a maximum iteration of 100000 was used with an ultra-fine convergence level. The molecular dynamic simulation using NVT lasted for 100 ps with a time step of 1 fs. And for calculation of the molecular volumes, the Accelrys DS Viewer program was used [49].

The calculation of ΔE was performed using Discover Module of Materials Studio with the Materials Studio 7.0 software [109]. First, the conformations of the template and functional monomers were optimized and the energy of the molecules with the optimized conformation was calculated. Then the confirmation optimization and energy calculation was applied to the complex formed between target molecule and functional monomers. Finally, the binding energy of target molecule with the monomers was obtained from equation (2):

$$\Delta E = \left| E_{(complex)} - E_{(template)} - E_{(monomer)} \right| \quad (2)$$

Where ΔE is the binding energy of KJ mol^{-1}, $E_{(complex)}$ is the total energy of the complex, $E_{(template)}$ is the total energy of the target molecule and $E_{(monomer)}$ is the total energy of the functional monomer.

3.3 Synthesis of molecularly imprinted polymer

Published work was adopted with slight modifications for the synthesis of MIP for ketoprofen [50, 110]. The synthesis was carried out by mixing 25 mg of ketoprofen with 54 µL of 2-vinylpyridine in a ratio of 1:5, respectively. The mixture was stirred at room temperature in a 250 mL round-bottomed flask containing 10 mL of acetonitrile/toluene (1:9, v/v) porogenic mixture for 30 minutes. Thereafter, the reaction vessel was placed on ice to prevent unwanted polymerization. Ethylene glycol dimethacrylate (4.77 mL) and 100 mg of 1,1'-azobis-(cyclohexanecarbonitrile) were added. The mixture was purged with nitrogen gas for 10 minutes, sealed and stirred in an oil bath at 60 °C for 16 hours to initiate polymerization. After 16 hours, the temperature was increased to 80 °C and maintained for 24 hours to achieve a solid monolith polymer. The polymer was dried to constant mass at 80 °C followed by grinding and sieving. NIP was synthesized and treated likewise with the omission of ketoprofen in the reaction mixture.

Thereafter, both MIP and NIP particles that were ranging from 25 to 90 µm were collected and washed repeatedly with a mixture of acetic acid: acetonitrile (1:9; v/v) until ketoprofen could not be detected in the HPLC system.

3.4 Template removal

The resulting polymer particles were thereafter washed off repeatedly with 40 mL of methanol/acetic acid (9:1, v/v) making a total of 1000 mL of the solvent used to remove the template in the polymer. This was agitated for 30 minutes at 150 rpm using Labcon 3100U shaker and centrifuged for 10 minutes at 3000 rpm using

Rotofix 32A from Hettich. Then 20 µL of supernatant was injected into HPLC in order to confirm the total removal of templates from the MIPs. The rest of the supernatant was discarded and the fresh solvent was used for template removal. The procedure was repeated several times until templates could not be detected in the washing solution. MIP was further washed 3 times with 100 mL methanol to remove residual acetic acid. MIP was then filtered and dried overnight in an oven set at 50 $^{\circ}$C.

3.5 Characterization of polymers

Surface charge of the polymer matrix dispersed in deionised water was studied using a zeta-potential analyzer. The Zeta potential and hydrodynamic diameter results were obtained from a Zeta-sizer Nano ZS90 (Malvern, UK) instrument in water. This was equipped with a blue 405 nm laser. The temperature of the cell was kept constant at 25 °C.

A scanning electron microscope (SEM), JOEL model JSM 6700F (Tokyo, Japan) was used to study the morphology of the polymers. Polymers were mounted on 10 mm diameter metal mounts using carbon tape. Those were coated with gold under vacuum in an argon atmosphere. The samples were then studied at an accelerating voltage of 20 keV and desired magnification.

In order to confirm the molecular dynamics simulation predictions, Fourier transform infra-red (FTIR) equipped with attenuated total reflection (ATR) from Perkin Elmer FTIR spectrometer (Llantrisant, United Kingdom) was used [3]. A small amount of the polymers were mounted in the ATR. Spectra of the polymers were recorded in the range of 400-4000 cm^{-1} in a solid state.

Thermal analysis was performed using TGA/DSC 1 Star^{e} system that was obtained from Mettler Toledo (Columbus, USA). Thermograms were recorded using a heating rate of 10 $^{\circ}$C.min^{-1} from 25 $^{\circ}$C to 700 $^{\circ}$C, under a nitrogen atmosphere at a rate of 100 mL min^{-1}.

Agilent VNMRS Wide Bore 500 MHz nuclear magnetic resonance (NMR) spectrometer with a ^{1}H frequency of 500 MHz and a ^{13}C frequency of 125 MHz was used for characterization. The spectra were acquired utilizing a dual-channel 4 mm

Chemagnetics TM T3 HX MAS probe using 4 mm zirconia rotors. The cross-polarization (CP) spectra were recorded at 25 °C with proton decoupling using a recycle delay of 10 s. The CP pulse power parameters were optimized for the Hartmann-Hahn match using a Glycine standard sample. The radio frequency fields for the match were $\gamma_C B_{1C} = \gamma_H B_{1H} \approx 55$ kHz. The contact time for cross-polarization was optimized to 2.0 ms. Magic-angle-spinning (MAS) was performed at 10 000 revolutions per second (10 kHz).

Elemental analysis was performed using Series II CHNS/O analyzer 2400 obtained from Perkin Elmer (Llantrisant, United Kingdom). Instrument used helium, air and oxygen gases. About 2,5 mg of the sample was weighed into the Tin capsule which was 8 mm.

Diffraction patterns (10 – 90°) of the synthesized polymers were determined using an X-ray diffraction (XRD) equipped with XRD commander for data collection and Eva software for processing. XRD system was obtained from Bruker AXS (Karlsruhe, Germany). Cu K_α radiation source at a seam rate, of 2° min^{-1} was employed.

3.6 Binding analysis - Optimization of adsorption experiments

Batch adsorption experiments were carried out at room temperature using deionized water that was spiked with ketoprofen. Various parameters such as adsorption solvent, sample pH (3-9), polymer amount (8-25 mg), contact time (5-60 min) and initial concentration of target molecule (0.001-70 $mg.L^{-1}$) were optimized. Only one parameter was changed at a time during the optimization. For example, while verifying the contact time, the adsorption solvent, the sample pH, the amount of the polymer and concentration of the target molecule in spiked deionized water were kept constant. All experiments were carried out in triplicate. Extraction efficiency and adsorption capacity were determined using Eqs. (3) and (4), respectively.

$$\textbf{Extraction efficiency}\ (\%) = \frac{(\boldsymbol{C_0 - C_e})}{\boldsymbol{C_o}} \times \mathbf{100} \qquad (3)$$

$$\textbf{Adsorption capacity}\ (\mathbf{mg\ g^{-1}}) = \frac{(\boldsymbol{C_o - C_e})\mathbf{V}}{\boldsymbol{W}} \qquad (4)$$

Where C_o represent the initial concentration ($mg.L^{-1}$) before the adsorption and C_e the final concentration ($mg.L^{-1}$) of target molecule remaining in solution after adsorption. V is the volume of the solution in liters and W represents the mass of the polymer in grams [32, 111].

3.7 Preparation of molecularly imprinted solid-phase extraction (MISPE) cartridge

Empty SPE cartridges (1 mL) that were purchased from Supelco (Bellefoute, USA) were mounted into the SPE manifold and washed with methanol prior to their use. The MIP slurry was prepared with 1 mL of methanol and transferred into the empty 1-mL SPE cartridge. Two frits were placed at the bottom and top of the MIP sorbent to safeguard against sorbent losses. The same procedure was followed in the preparation of the NIP cartridge.

Solid-phase extraction manifold was purchased from Phenominex (Califonia, USA), and it was connected to a vacuum pump that was obtained from Merck Millipore (Massachusetts, USA).

3.8 Optimization of the MISPE

The optimization of sample pH, amount of sorbent, sample volume, elution solvent and ionic strength of the sample were carried out using spiked deionized water (1000 $\mu g.L^{-1}$ of ketoprofen compound) by varying one parameter while keeping others constant. Each optimization procedure was repeated three times (n = 3).

Sample pH was optimized by using various values of the pH of deionized water spiked with 1000 $\mu g.L^{-1}$ of ketoprofen. The pH was adjusted to 3, 5, or 7 with HCl (1 $mol.L^{-1}$) and NaOH (0.6 $mol.L^{-1}$). Ketoprofen was extracted using MISPE prior to HPLC-UV analysis. The following parameters were retained constant while varying the pH of the spiked sample, each cartridge was conditioned with 1 mL of methanol followed by equilibration with 1 mL of deionised water at pH 2.5 with a flow rate of 1 $mL.min^{-1}$. A 50 mL volume of the sample was loaded onto the MISPE cartridge at a

flow rate of 5 mL.min^{-1} and sent to waste. The cartridge was vacuum-dried for 10 min, followed by washing with 1 mL of 5% (v/v) triethylamine (TEA) in water. The retained ketoprofen was eluted with 1 mL of methanol at a flow rate of 1 mL.min^{-1}.

The quantity of the polymer added in the cartridge was investigated by packing the cartridge with different polymer amount ranging in between 8 to 14 mg. Each cartridge was conditioned with 1 mL of methanol and equilibrated with deionised water at pH 2.5, consecutively. A 50 mL volume of the sample at pH 5 was loaded onto the cartridge and sent to waste. The cartridge was vacuum-dried for 10 min, followed by washing with 1 mL of 5% (v/v) triethylamine (TEA) in water. The retained ketoprofen was eluted with 1 mL of methanol at a flow rate of 1 mL. min^{-1}.

The effect of sample volume was investigated by passing different volumes of deionized water (pH 5) spiked with 1000 µg.L^{-1} of each compound through the MISPE cartridge. The volumes of spiked deionized water were in between 10 to 100 mL. The retained ketoprofen in the MISPE cartridge were eluted with methanol. The other experimental parameters were kept constant while varying the sample volume.

Three solvent mixtures were investigated in order to get the best elution solvent for the MISPE cartridge and they were (a) methanol, (b) methanol and acetic acid, 9:1 (v/v), and (c) 0.2% formic acid and acetonitrile, 4:6 (v/v). The other experimental parameters were retained constant while varying the elution solvent.

The effect of ionic strength of the sample was tested by spiking deionized water with varies amounts of sodium chloride in between the range of 0.5–2.0 % (w/v).

3.9 Sampling and sample pre-treatment

Samples analyzed consisted of raw influent collected after wastewater screening for solid removal and final effluent sampled after disinfection of treated water with chlorine. The physicochemical quality of water samples are shown in Table 4. Wastewater samples were collected once per week in the month of May in 2016 from Amanzimtoti (GPS: S30.00749° E30.91720°), Kingsburgh (GPS: S30.07445° E30.85687°) and Umbilo (GPS: S29.84556° E30.89103°) WWTPs located around the city of Durban in KwaZulu-Natal Province of South Africa (MAP in annexure A).

Samples were filtered through a 0.22 µm syringe filter, thereafter pH was adjusted to 5 using 0.1 M sodium hydroxide/2% formic acid. Samples were refrigerated at 4^{o}C until analysis.

3.10 Extraction of ketoprofen from wastewater

Water samples were percolated through the packed cartridge using the pre-optimized SPE conditions. This was achieved by conditioning the cartridge with 1 mL of methanol followed by equilibration with 1 mL of deionised water. 50 mL of wastewater sample (pH 5) was loaded at 1 mL.min^{-1}. The cartridge was vacuum dried for 10 min, followed by washing with 1 mL of 5% (v/v) triethylamine (TEA) in water. The retained ketoprofen was eluted with 1 mL of methanol and injected into HPLC.

After each use, the cartridges were regenerated by washing with 3 mL of deionised water and 3 mL of methanol.

Table 4: Physicochemical properties of water in the studied sources.

WWTP		Parameter					
		pH	DO[a] ($mg\ L^{-1}$)	Conductivity ($\mu s\ cm^{-1}$)	TDS[b] ($mg\ L^{-1}$)	Salinity (psu)	ORP[c]
Amanzimtoti	Influent	6.61	0.91	1172	613	0.61	-18.7
	Effluent	7.27	3.91	1009	490	0.49	-50.7
Kingsburg	Influent	7.37	0.24	921	465	0.46	-36.3
	Effluent	7.11	4.72	572	288	0.27	-27.8
Umbilo	Influent	7.13	0.38	822	413	0.40	-23.8
	Effluent	6.67	5.36	711	359	0.35	-31.2

(a) Dissolved oxygen, (b) total dissolved solids and (c) oxidation-reduction potential.

3.11 HPLC Analysis

High performance liquid chromatography (HPLC) system that consisted of an online mobile phase degasser unit (Model: DGU-20A5), 20 µL sample loop, pump (Model: LC-20AT), and UV-visible detector (Model: SPD-20A) obtained from Shimadzu Corporation (Kyoto, Japan) was used. The mobile phase conditions consisted of a mixture of acetonitrile and 0.2% formic acid in water (60:40, v/v) at a flow rate of 1 mL/min. Separation was performed on a Gemini C_{18} HPLC column of 150 x 4.6 mm x 5 µm dimensions from Phenominex (California, USA). Shimadzu LC solutions software was used for data collection and processing. Detector wavelength was set at 255 nm.

3.12 Quality assurance

A stock solution of 100 $mg.L^{-1}$ ketoprofen was prepared in acetonitrile. The stock solution was further diluted in order to prepare the working standards. The working standards were analyzed using HPLC. Calibration curve, limit of detection and limit of quantification (LOQ) were computed. The accuracy and precision of analytical method were validated using deionised water that was spiked with ketoprofen at concentration levels ranging from 5 to 1000 $\mu g.L^{-1}$.

Chapter 4:

Results and discussion

4.1 Computational

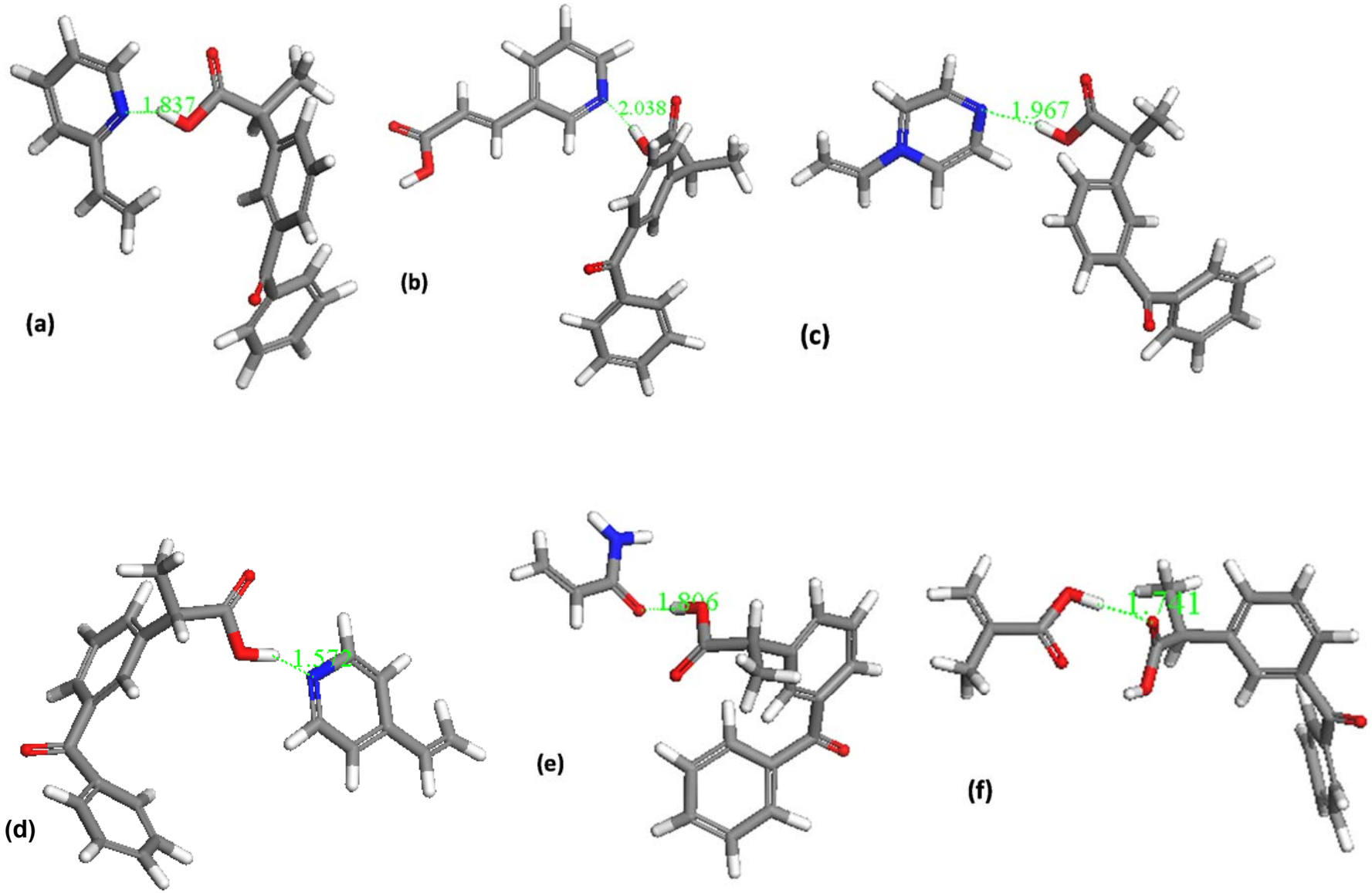
4.1.1 Study of template-monomer interactions

In order to develop a MIP for selected pharmaceutical compound, one had to understand the properties of these sorbents at the molecular level. Firstly, the interactions were studied using the bonding distance between the functional monomers and the target molecule shown in

Figure 3. In this work, three functional monomers, *i.e.* 2-vinylpyridine (2-VP), 1-vinylimidazole (1-VI) and 4-vinylpyridine (4-VP) were theoretically selected as possible functional monomers because of the hydrogen bonding length between the functional monomers and the template molecules which gave the highest binding energy in Table 5. Since they was no significant differences in between the three functional monomers binding energy, they were further studied [50].

1-vinylimidazole had the lowest binding energy (Table 6), however, it had the longest bond length. This phenomenon is very significant in MIP processing especially when one had to remove the template which in turn creating a target cavity which is specific for selected molecule. In case where the binding energy is very strong, this would have drastically increased the cost as more solvent would be required to desorb template from its pocket. It is therefore without any doubt that 2-vinylpyridine was deemed to be the most suitable functional monomer for complexation with ketoprofen due to its relatively low binding energy value. Given the presence of a carboxylic acid functionality on the ketoprofen

Figure 3a, it was expected that the reaction will go under electrostatic interaction and potentially hydrogen bonding with the nitrogen of 2-vinylpyridine.


1.837
(a)
2.038
(b)
1.967
(c)
1.572
(d)
1.806
(e)
1.741
(f)

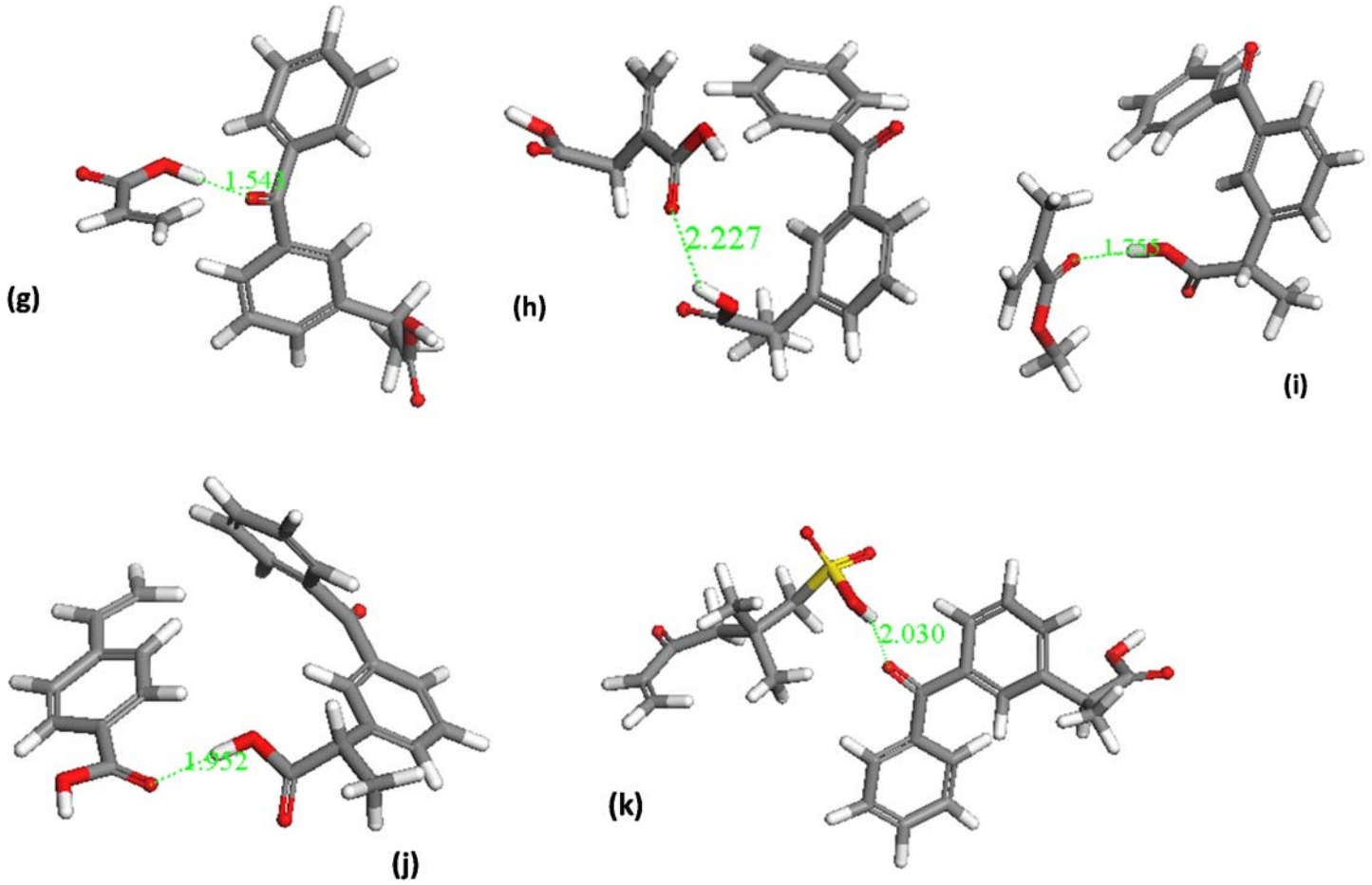


Figure 3: Target molecule-monomer interaction (target molecule = ket), functional monomers (a) 2-vinylpyridine, (b) Trans-3-(3-pyridyl)-acrylic Acid, (c) 1-vinylimidazole, (d) 4-vinylpyridine, (e) Acrylamide, (f) Methacrylic Acid, (g) Acrylic Acid, (h) Itaconic Acid, (i) Methyl methacrylic Acid, (j) p-vinylbenzoic Acid p-vinylbenzoic Acid and (k) Acrylamido-2-methyl-1-propane-sulphonic Acid.

Table 5: Summary of calculations of ΔE and bond length target molecule-monomer.

Functional monomer		Bond length (Å)	ΔE (KJ mol^{-1})	Bonding type
2-vinylpyridine	2VP	1.837	-11.97	≡N---H-O-
1-vinylimidazole	1VIA	1.967	-15.94	≡N---H-O-
4-vinylpyridine	4VP	1.572	-9.39	≡N---H-O-
Acrylamide	AAM	1.806	-22.60	=O---H-O-
Acrylic Acid	AA	1.543	-27.22	=O---H-O-
Itaconic Acid	IA	2.227	-25.20	=O---H-O-
Methacrylic Acid	MA acid	1.741	-22.23	=O---H-O-
Methyl methacrylic Acid	MAA	1.755	-23.57	=O---H-O-
p-vinylbenzoic Acid	PVA	1.952	-24.23	=O---H-O-
Trans-3-(3-pyridyl)-acrylic Acid	TPAA	2.038	-27.52	≡N---H-O-
Acrylamido-2-methyl-1-propane-sulphonic Acid	AMPSA	2.030	-59.54	=O---H-O-

Table 6: Summary of calculations for ΔE and bond length of target molecule-monomer with cross linker and initiator present.

Complexes	Bond length (Å)	ΔE (KJ mol^{-1})
2-vinyl pyridine	1.724	-15.56
1-vinylimidazole	1.924	-8.08
4-vinyl pyridine	2.557	-17.90

4.1.2 Molecular dynamics simulation

The mulliken charges of atoms present in ketoprofen and 2-vinyl pyridine were tabulated according to their structures represented in Figure 4. These calculations were done to predict the atoms that are most likely form hydrogen bonding [48, 112]. According to these charges, it was found that the possible proton donor for ketoprofen was H_{33} which is the hydrogen atom of the hydroxyl group and proton acceptor for 2-vinyl pyridine was N_6. The hydrogen bonding interaction that occurs between target molecule and the functional monomer was as predicted from the binding energy calculations. Even though 4-vinyl pyridine had the highest binding energy, its bond length was far apart from each other. These results then is in

agreement in selecting 2-vinyl pyridine as the functional monomer, having the shortest bond distance of 1.724 Angstrom (Å).

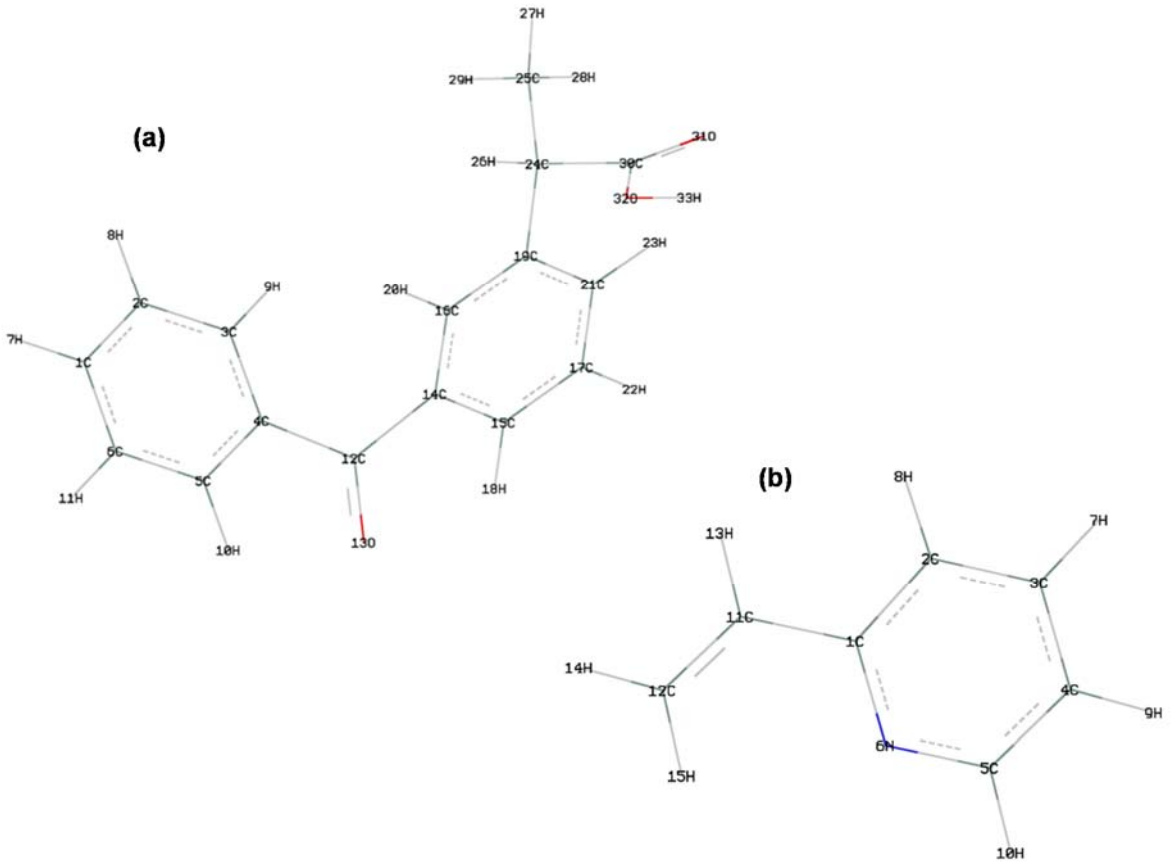


Figure 4: Computational derived structures of ketoprofen (a) and 2-vinyl pyridine (b).

Table 7: Mulliken charges of ketoprofen and 2-vinyl pyridine.

Compound	Number	Atom	Atomic charges
(a) *Mulliken charges of ketoprofen*			
	1	C	-0.090
	2	C	-0.068
	3	C	-0.097
	4	C	0.026
	5	C	-0.062
	6	C	-0.103
	7	H	0.112
	8	H	0.132
	9	H	0.129
	10	H	0.156
	11	H	0.114

12	C	0.263
13	O	-0.473
14	C	0.015
15	C	-0.065
16	C	-0.102
17	C	-0.113
18	H	0.158
19	C	0.031
20	H	0.144
21	C	-0.091
22	H	0.115
23	H	0.102
24	C	-0.099
25	C	-0.386
26	H	0.127
27	H	0.165
28	H	0.184
29	H	0.111
30	C	0.515
31	O	-0.459
32	O	-0.714
33	**H**	**0.323**

(a) *Mulliken charges of 2-vinyl pyridine*

1	C	0.192
2	C	-0.103
3	C	-0.103
4	C	-0.104
5	C	0.051
6	**N**	**-0.314**
7	H	0.100
8	H	0.082
9	H	0.089
10	H	0.095
11	C	-0.105
12	C	-0.254
13	H	0.097
14	H	0.118
15	H	0.161

4.1.3 Molecular structures vibrational spectra

The chemical structure of functional monomer and target molecules was further verified by the use of infrared analysis. The spectrum obtained using computational modeling was compared to the experimentally obtained spectra. Spectra obtained experimentally and theoretically for the target molecule (ketoprofen) and functional monomer (2-vinylpyridine) are presented in Figure 5 and Figure 6, respectively. Vibrational assignments for template, functional monomer and template-monomer interactions have been calculated using B3LYP/6-31+G**//B3LYP/3-21G methods and are given in Table 8, Table 9 and Table 10. Ketoprofen has 33 atoms showing 93 vibrational degrees of freedom. The observed bands are based on the theoretical spectra. The bands observed at 1380, 1747, 3000 and 3516 cm-1 in the spectrum of ketoprofen correspond to the vibrations of C-O, C=O, C-H and O-H groups, respectively. In Table 8, it was observed that at 3677 cm-1 represent an OH band, the evidence of this was shown from the experimental data of this band at 3268 cm-1 which revealed a redshift of ~409 cm-1 which is an evidence of the intermolecular hydrogen bonding as shown in Table 10.

The OH stretch in the theoretical calculation was found to be sharp with increased intensity at 3677 cm-1, which was found to be a very weak band at 3268 cm-1 for experimental. This was due to the intensity which depend on the mass of the sample in experimental which does not affect the theoretical and as well as the matrix effect which is not considered in the theoretical calculations. Several other bands were identified for CH in the form of CH3 at 1437, 3064, 3132 and 3152 cm-1 which were red shifted by ~161 cm-1 to those of the experimental. C=O showed vibrational assignments at v36 rocking, v78 and v79 symmetric stretching with the red shift as well. The spectroscopic signature of 2-vinylpyridine is allocated in Table 9 which has 39 functional vibrations. It is an aromatic structure showing CN and CH stretching vibrations, with a slide red shift in experimental spectra. CN showed vibrational assignment at v24 and v29 with the red shift by ~66 cm-1. CH at 812, 1185 and 3165 cm-1 with redshift to experimental by ~87 cm-1. Vibration noise at v34 of CH which was symmetric stretching was found to be sharp with intensity at 3165 cm-1, which had a weak band at 3078 cm-1 [113]. Due to the fact that theoretical vibrations are more intense and distinctive than the experimental vibrations, CN bands were

invisible in experimental IR spectra. The nature of the calculation allows theoretical NIP to be analyzed as an isolated system which excludes any noises or interferences, which cannot be controlled experimentally [114].

Ketoprofen binds into the polymeric cavity created by 2-vinylpyridine molecules. The complex of ketoprofen and 2-vinylpyridine has the largest system of 48 atoms with 138 vibrational modes. The hydrogen of NH group in 2-vinylpyridine molecule exerts interaction, under the influence of electrostatic environment with COOH group of ketoprofen. The experimental and theoretical frequencies of the complex corresponded with those of the individual bands for ketoprofen and 2-vinylpyridine. The vibrational assignment of the complex is summarized in Table10. Formation of the hydrogen bond between the target molecule and monomer molecules was proven by the high-intensity shift band at 3004 cm-1 on the RB3LYP/6-31G(d), these apparently matching with the observed 3000 cm-1 of experimental. This result suggests electrostatically driven hydrogen bonding interaction during complexation between target molecule and monomer.

Table 8: Vibrational wavenumber, obtained for Ketoprofen using RB3LYP/6-31G (d) method.

Vibration Nos.	Experimental wavenumber (cm^{-1})	Theoretical wavenumber (cm^{-1})		Approximate description of vibrations
	FT-IR	Freq	Infrared	
v1	-	23.16	0.0541	
v2	-	35.42	2.4988	
v3	-	39.70	1.5471	
v4	-	50.81	0.6493	
v5	-	68.42	0.5327	
v6	-	109.57	0.4180	
v7	-	133.69	2.1760	
v8	-	158.25	0.8055	
v9	-	188.80	0.3202	
v10	-	219.43	0.1085	
v11	-	224.08	0.8872	
v12	-	253.54	5.8228	
v13	-	293.22	0.0828	
v14	-	293.95	0.1435	
v15	-	332.09	1.8814	
v16	-	388.02	6.4714	
v17	-	419.85	0.1974	

v18	-	432.72	7.4507	
v19	-	442.83	1.0687	
v20	-	456.73	1.8058	
v21	-	512.40	2.6996	
v22	566	566.25	15.1070	βWhole mol.
v23	-	600.72	21.6380	
v24	-	632.86	0.4585	
v25	-	636.41	57.4418	
v26	657	654.62	43.4043	βWhole mol.
v27	684	674.16	41.1912	
v28	-	704.24	10.1510	
v29	715	715.64	23.4261	βWhole mol.
v30	716	718.40	4.7203	νWhole mol.
v31	-	736.39	67.2294	
v32	-	786.46	23.4811	
v33	-	803.26	3.1630	
v34	818	823.87	11.1895	βC=C
v35	841	844.60	10.9373	ρCH
v36	853	868.49	3.7564	ρC=O
v37	-	873.31	30.3377	
v38	-	931.00	14.7297	
v39	-	951.30	1.8909	
v40	968	956.84	0.1764	ρMid. Ring C-H_2
v41	972	973.27	30.0915	βCH=CH_2
v42	975	982.78	7.2812	τCH=CH_2
v43	-	997.78	3.0042	
v44	-	1005.79	1.2056	
v45	-	1015.36	0.7901	
v46	-	1018.57	0.9178	
v47	-	1019.96	1.2843	
v48	1080	1057.93	1.1483	$^{\delta}$CH=CH_2
v49	-	1100.08	47.3521	
v50	-	1105.07	10.1074	
v51	-	1114.02	3.0218	
v52	-	1131.68	11.1579	
v53	1158	1167.17	9.8512	ωCH=CH_2
v54	-	1181.24	220.2120	
v55	-	1194.60	0.1963	
v56	-	1207.14	9.5451	
v57	-	1210.25	31.3235	
v58	-	1226.96	27.2430	
v59	1285	1295.81	4.3964	τ adjucent CH
v60	-	1302.73	303.8124	
v61	1314	1324.79	4.2193	τ adjucent CH

v62	-	1349.68	31.9880		
v63	-	1361.50	13.7776		
v64	-	1366.45	2.0628		
v65	1398	1369.76	5.3694	v_{as} Mid. ring C=C	
v66	-	1415.86	56.0398		
v67	1448	1437.23	9.8527	δCH_3	
v68	-	1477.11	13.9723		
v69	-	1493.06	13.7501		
v70	-	1520.83	3.7789		
v71	-	1526.80	8.8649		
v72	-	1531.87	4.3773		
v73	-	1539.16	1.9982		
v74	-	1635.11	22.3181		
v75	1636	1637.23	10.2358	$v_{as}C=C$	
v76	-	1655.64	1.0087		
v77	-	1657.75	19.9593		
v78	1652	1739.99	159.7524	$v_sC=O$	
v79	1728	1836.07	235.8886	v_s Adjucent C=O	v_s
v80	2936	3063.64	22.6765	v_aCH_3	sy
v81	-	3085.64	7.3580		mm
v82	2988	3131.64	28.4780	$v_{as}CH_3$	etri
v83	2991	3151.92	11.8498	$v_{as}CH_3$	c
v84	-	3185.26	0.0727		stre
v85	3113	3193.24	9.3892	v_{as}Mid. Ring C-H_2	tchi
v86	-	3195.67	11.2500		ng,
v87	-	3202.65	12.2224		v_{as}
v88	-	3206.87	23.3937		asy
v89	-	3209.52	6.7246		mm
v90	-	3216.58	10.9273		etri
v91	-	3223.85	7.8623		c
v92	-	3226.28	5.4953		
v93	3268	3677.38	51.6671	v_sOH	

c stretching, β in-plane bending, γ out- of-plane bending, ρ rocking, $^{\delta}$ scissoring, ω wagging, τ twisting, mol. The molecule, mid. Middle.

Table 9: Vibrational wavenumber, obtained for 2-VP using RB3LYP/6-31G(d) method.

Vibration Nos.	Experimental wavenumber (cm^{-1})	Theoretical wavenumber (cm^{-1})		Approximate description of vibrations
	FT-IR	Freq	Infrared	
v1	-	99.87	1.8278	
v2	-	212.17	1.2868	
v3	-	225.61	1.3859	
v4	407	416.54	3.5467	Ring Breath
v5	-	450.44	0.6654	
v6	460	466.42	2.2996	Whole mole. Breath
v7	587	593.19	4.3457	
v8	631	638.25	2.0164	
v9	-	658.39	0.8107	
v10	796	760.99	14.5549	ωCH=CH_2
v11	802	812.38	1.0404	$^{\delta}$CH
v12	-	826.35	31.7766	
v13	-	906.76	3.1773	
v14	930	961.36	25.3924	ωAdjacent CH_2
v15	975	977.38	0.0050	ωCH=CH_2
v16	990	1001.20	0.5364	ωCH=CH_2
v17	-	1007.10	3.5880	
v18	-	1033.68	18.6388	
v19	1050	1053.96	3.9014	ρCH=CH_2
v20	-	1079.79	3.5467	
v21	-	1133.83	4.4021	
v22	1157	1185.38	5.3919	$_{\gamma}$CH
v23	1257	1241.58	5.0549	ωC-C
v24	1300	1309.91	3.5678	v_{as}C-N
v25	-	1342.30	3.6708	
v26	-	1352.16	2.0218	
v27	1467	1452.40	5.4422	$^{\delta}$CH=CH_2
v28	1474	1481.21	24.9943	$^{\delta}$CH=CH_2
v29	1589	1522.63	25.8858	v_{as}C-N
v30	-	1621.94	18.6024	
v31	1648	1644.95	50.7026	$_{\gamma}$C=C
v32	-	1713.94	1.0551	
v33	-	3161.26	13.0127	
v34	3078	3165.30	27.5814	v_{as}CH
v35	3097	3180.17	9.8927	v_sAdjucent CH=CH_2
v36	-	3188.65	5.9636	
v37	-	3202.84	25.0629	
v38	-	3216.21	17.6288	

v39	-	3266.17	7.5369

v_s symmetric stretching, v_{as} asymmetric stretching, β in-plane bending, γ out- of-plane bending, ρ rocking, δ scissoring, ω wagging, τ twisting, mol. The molecule, mid. Middle.

Table 10: Vibrational wavenumber, obtained for Ket-2VP using RB3LYP/6-31G(d) method.

Vibration Nos.	Experimental wavenumber (cm^{-1})	Theoretical wavenumber (cm^{-1})		Approximate description of vibrations	
	FT-IR	Freq	Infrared	KET	2-VP
v1	-	7.44	0.0941		
v2	-	10.89	0.0748		
v3	-	16.85	0.4648		
v4	-	36.90	2.3941		
v5	-	39.28	0.0287		
v6	-	48.11	0.1603		
v7	-	53.10	0.5543		
v8	-	61.36	0.6036		
v9	-	77.42	1.7576		
v10	-	88.27	2.8161		
v11	-	107.02	1.0961		
v12	-	124.23	1.3411		
v13	-	133.39	2.6787		
v14	-	146.26	1.1999		
v15	-	165.41	4.8597		
v16	-	196.58	1.7124		
v17	-	207.73	1.0932		
v18	-	222.13	0.5093		
v19	-	227.48	0.8001		
v20	-	249.64	7.9058		
v21	-	270.48	18.9634		
v22	-	291.65	0.1993		
v23	-	301.43	0.9877		
v24	-	342.96	6.6180		
v25	-	390.14	6.4391		
v26	-	420.32	0.2480		
v27	-	422.31	1.5007		
v28	-	442.14	0.6339		
v29	-	447.13	0.5140		
v30	-	457.52	4.2362		
v31	-	463.92	4.2632		
v32	-	467.07	3.8953		

v33	547	518.92	4.2632	βWhole mol.	
v34	-	574.79	1.9370		
v35	-	597.82	3.1066		
v36	-	616.88	12.8823		
v37	-	632.23	0.2135		
v38	-	649.14	15.5065		
v39	-	653.56	1.2867		
v40	-	662.30	28.5152		
v41	-	678.25	17.1236		
v42	-	697.86	2.8205		
v43	712	713.03	27.1522	vWhole mol.	
v44	-	718.51	12.8533		
v45	-	738.17	47.0434		
v46	-	767.02	15.6554		
v47	-	783.71	20.9070		
v48	-	810.66	0.8830		
v49	-	812.09	1.6654		
v50	-	825.29	32.1182		
v51	-	828.86	4.1095		
v52	-	843.68	6.2820		
v53	-	869.36	4.1095		
v54	-	884.21	49.7512		
v55	-	912.07	0.7656		
v56	-	932.70	6.6842		
v57	-	951.54	4.0235		
v58	968	956.19	1.1645	ρMid. Ring C-H_2	ρAdjacent CH_2
v59	-	957.49	3.1287		
v60	-	975.18	32.4941		
v61	-	981.10	99.0150		
v62	-	983.75	7.5907		
v63	-	997.45	1.1645		
v64	1000	1003.06	2.0373		ρCH=CH_2
v65	-	1005.25	1.0099		
v66	-	1015.62	1.9046		
v67	-	1018.53	0.8939		
v68	-	1019.79	1.8027		
v69	-	1023.48	25.0393		
v70	-	1029.20	9.2213		
v71	-	1036.15	24.3914		
v72	-	1057.80	1.1961		
v73	-	1060.97	4.1472		
v74	1092	1087.35	16.5864		δCH=CH_2
v75	-	1105.69	13.6836		
v76	-	1107.46	7.6932		
v77	-	1114.42	5.1783		

v78	-	1132.78	4.7365		
v79	-	1139.40	1.2083		
v80	1142	1169.02	9.4536	$_{\gamma}$CH	
v81	-	1190.89	7.2724		
v82	-	1194.14	0.2434		
v83	-	1206.00	12.9554		
v84	-	1211.01	29.9801		
v85	1219	1222.55	51.5020	$_{\gamma}$CH	
v86	-	1244.45	11.3540		
v87	-	1252.36	235.7574		
v88	-	1302.37	204.1692		
v89	-	1310.39	140.2213		
v90	-	1311.58	23.4721		
v91	-	1347.78	12.4721		
v92	-	1350.20	28.9700		
v93	-	1363.71	12.3648		
v94	-	1367.07	2.3939		
v95	-	1369.34	12.8250		
v96	-	1370.76	10.2735		
v97	1379	1377.40	146.6196	ν_{as}C-C	$^{\delta}$CH=CH$_2$
v98	-	1434.88	7.4178		
v99	1466	1458.03	24.0200	$^{\delta}$OH	$^{\delta}$CH=CH$_2$
v100	-	1475.76	16.4624		
v101	-	1491.81	11.5464		
v102	-	1492.93	15.4685		
v103	-	1507.26	6.7940		
v104	-	1520.96	4.7719		
v105	-	1528.00	8.4596		
v106	-	1531.84	4.7891		
v107	1571	1533.23	38.4763	$_{\gamma}$CH	$_{\gamma}$CH
v108	-	1539.00	2.2306		
v109	-	1624.73	31.4541		
v110	-	1634.54	29.5758		
v111	-	1636.87	7.8039		
v112	1746	1651.12	30.1542		$_{\gamma}$C=C
v113	-	1655.42	0.4813		
v114	-	1657.63	19.0404		
v115	-	1717.89	1.1234		
v116	2148	1737.95	167.1700	ν_sC=O	
v117	2305	1791.33	181.0355	ν_s Adjucent C=O	
v118	3000	3004.43	2386.6238	ν_sOH	
v119	-	3061.32	24.5993		
v120	-	3085.34	8.4530		
v121	-	3128.11	27.8501		
v122	-	3151.01	12.8337		

v123	-	3163.55	6.5320
v124	-	3184.62	0.1031
v125	-	3187.26	14.9769
v126	-	3192.02	12.2091
v127	-	3194.26	13.8468
v128	-	3195.62	13.3568
v129	-	3201.86	14.9341
v130	-	3203.85	15.1379
v131	-	3206.71	21.2783
v132	-	3210.85	8.5276
v133	-	3211.92	4.8520
v134	-	3218.43	9.6345
v135	-	3223.73	15.3234
v136	-	3223.91	7.3880
v137	-	3225.69	5.9002
v138	-	3274.93	5.8577

ν_s symmetric stretching, ν_{as} asymmetric stretching, β in-plane bending, γ out- of-plane bending, ρ rocking, δ scissoring, ω wagging, τ twisting, mol. The molecule, mid. Middle.

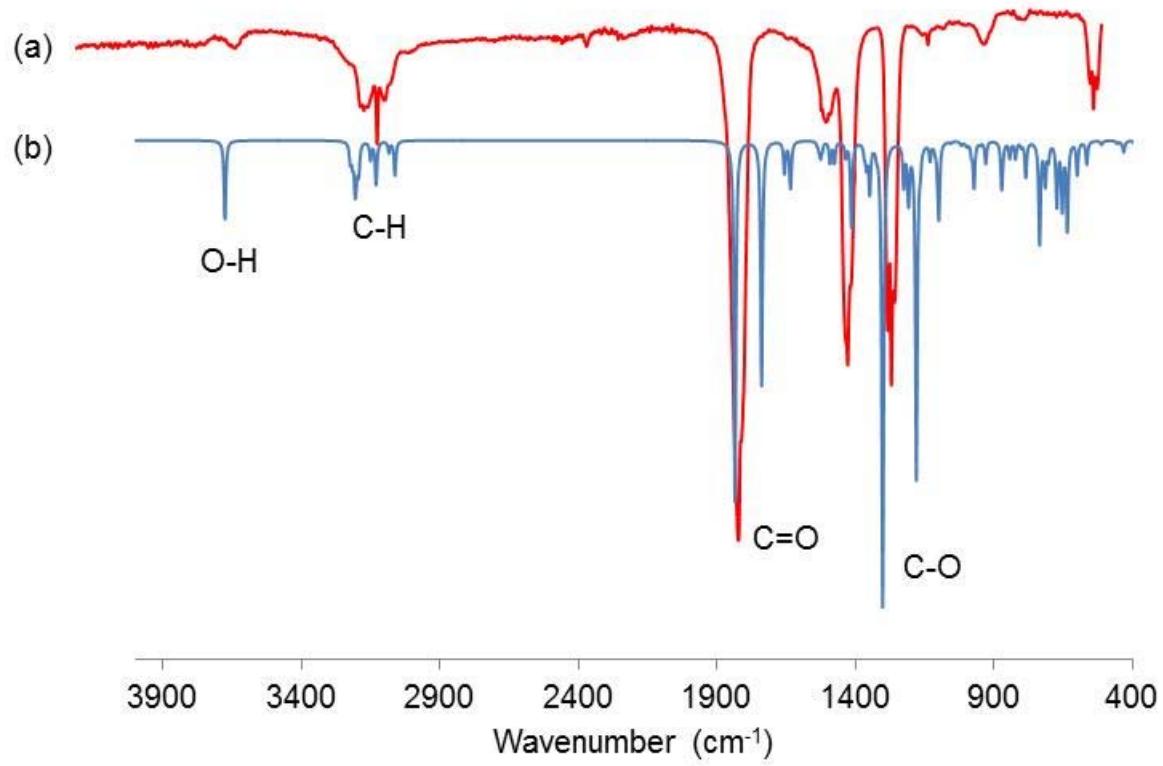


Figure 5: FTIR spectra of the target molecule (Ketoprofen), (a) Experimental and (b) theoretical experimental.

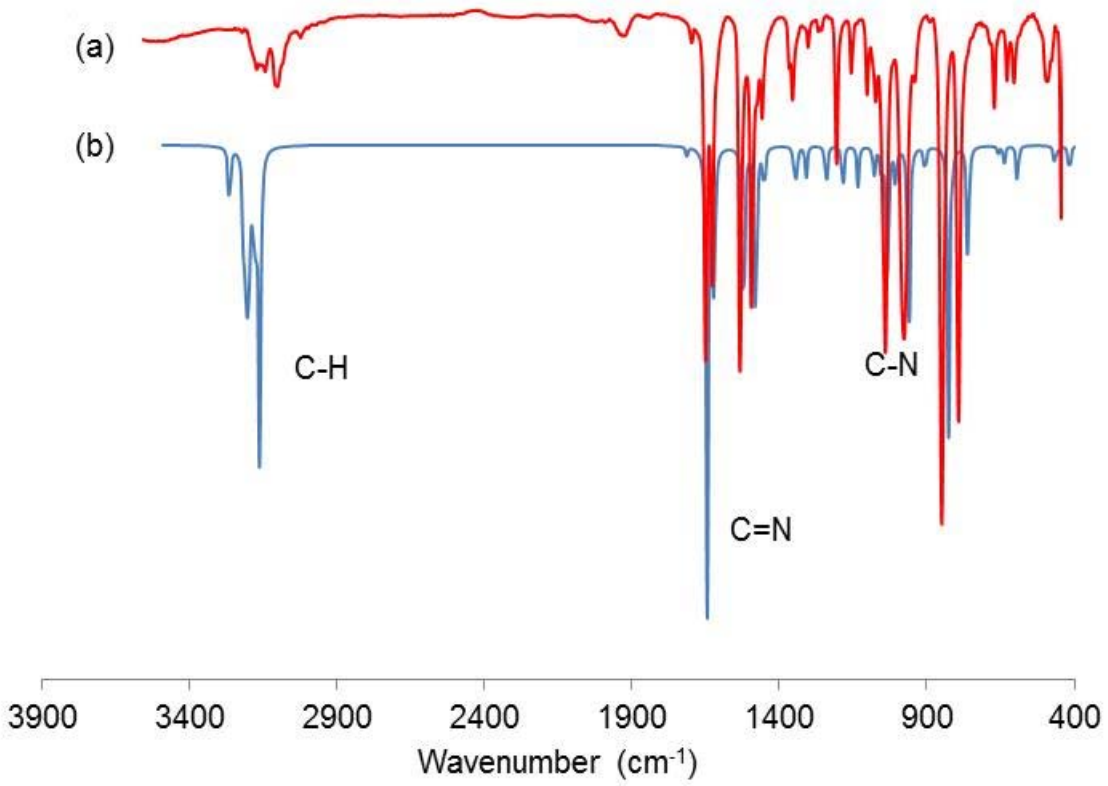


Figure 6: FTIR spectra of the functional monomer (2-vinylpyridine), (a) Experimental and (b) theoretical experimental.

4.2 Characterization

4.2.1 Zeta-potential

Zeta potential measurements of washed polymers were performed in deionised water after sonication in order to disperse the polymer particles in solution. The hydrodynamic diameter in Table 11 for MIP was found to be larger than that of NIP, which was due to the target compound being present in the MIP. The zeta-potential average for MIP and NIP was found to be 0.4 and 2.40 mV, respectively. The NIP was found to be more positively charged than the MIP due to the presence of the ketoprofen in MIP which reduces the charge by blocking functional groups which contribute to this charge. The data obtained in Figure 7 indicates the difference in surface charge of the polymers [115]. The values of both polymers do not exceed the ± 25 mV necessary for the polymer to collapsed and distributed in solution. The

higher surface charge on the NIP is responsible for the stabilization of the polymer dispersion.

Table 11: Zeta-potential and hydrodynamic diameter for the polymers.

Measurements		Polymers	
		MIP	NIP
Hydrodynamic diameter	Nm	79.9 ± 4.9	151.4 ± 32.0
Zeta-potential	mV	0.4 ± 0.5	2.0 ± 0.1

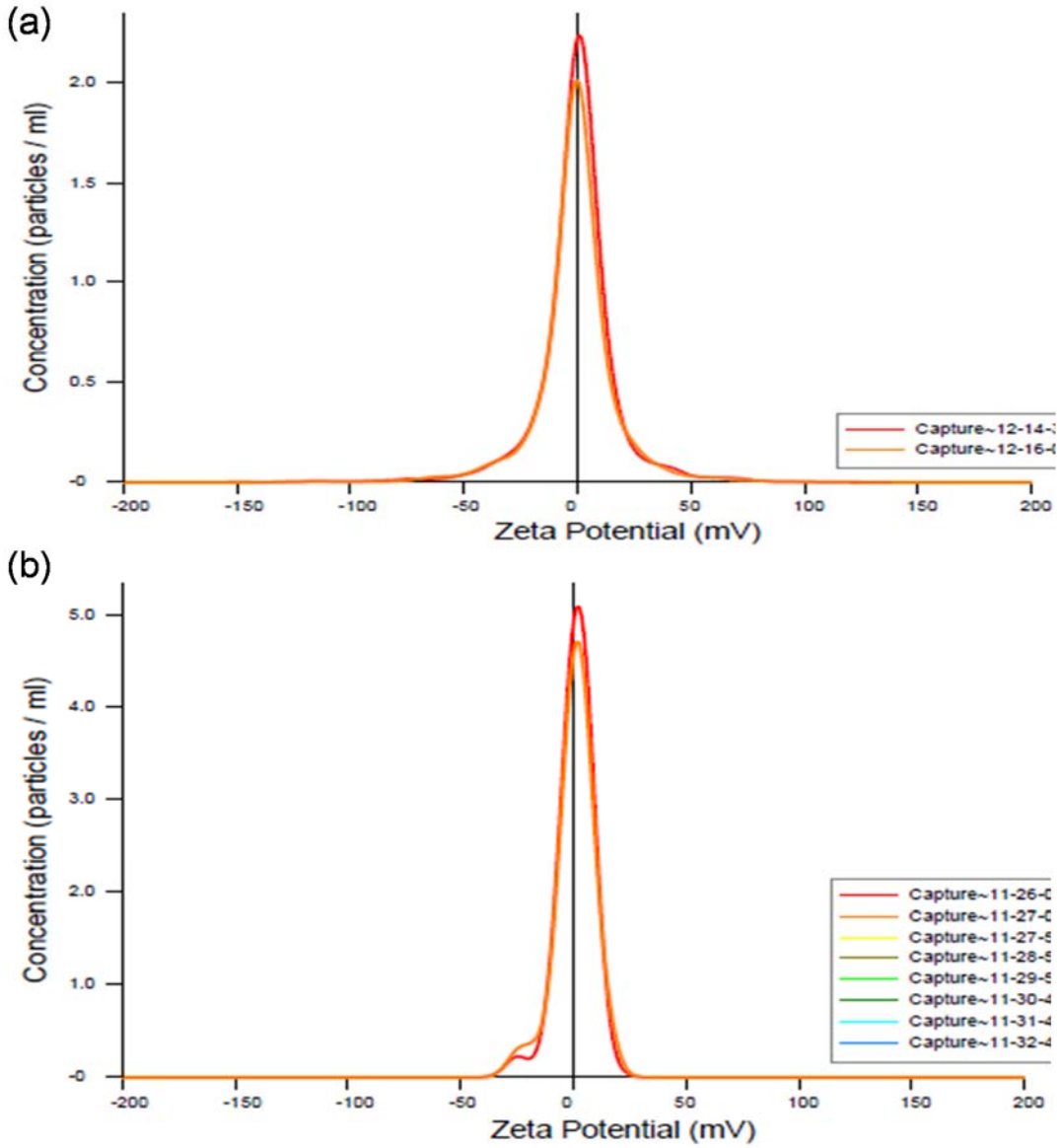


Figure 7: Zeta-potential of the (a) molecularly imprinted polymer and (b) non-imprinted polymer.

4.2.2 SEM analysis

The samples were then studied at the desired magnification for the morphology of the particles as shown in Figure 8 . It was also observed that unwashed particles for both polymers (Figure 8 a and b) showed a mixture containing smaller particle sizes, which were not clear visible. Morphology of the washed polymers (Figure 8c and d) particles was clearly visible, with MIP showing rough and irregular surface compared to NIP. Clearly the smaller particles were removed during the washing process compared to unwashed samples. In Figure 9 MIP shows rough and large pore sizes of the surface than the NIP which is nearly smooth.

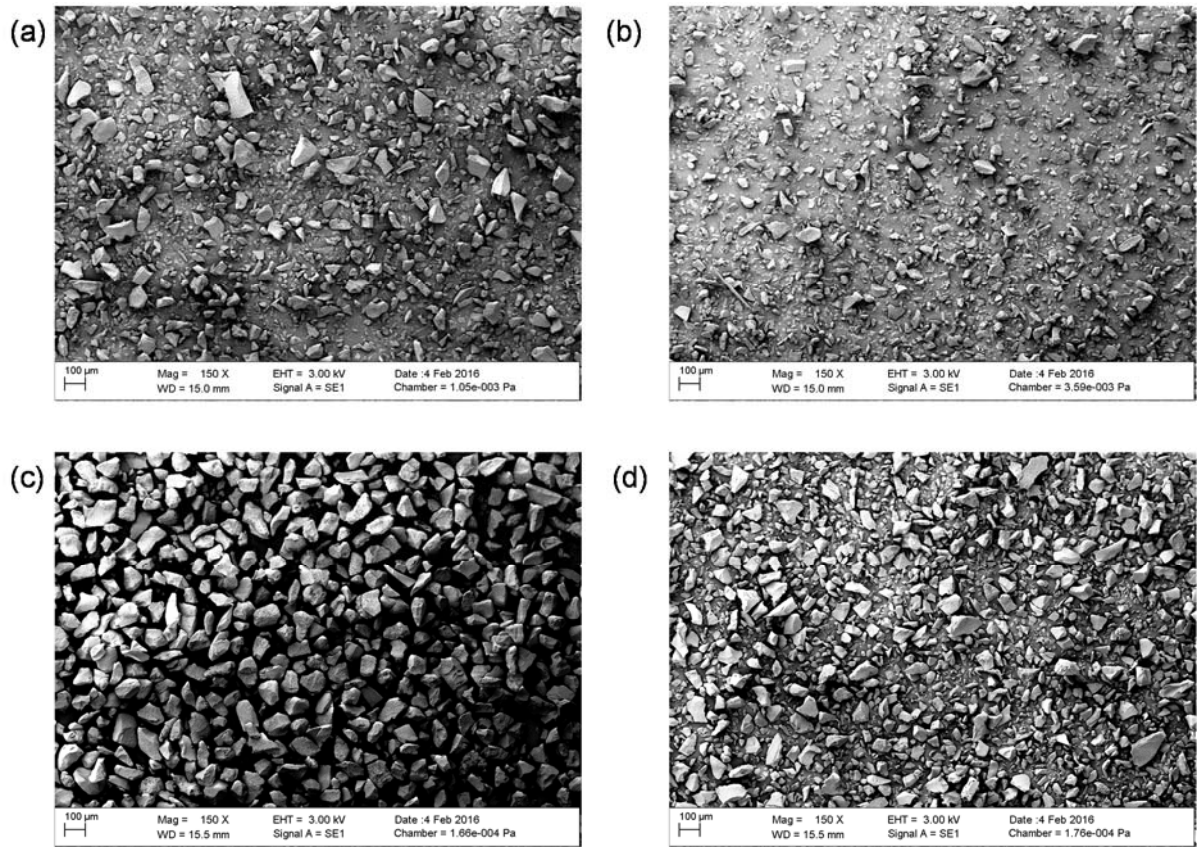


Figure 8**:** SEM micrographs of (a) unwashed MIP, (b) unwashed NIP, (c) washed MIP and (d) washed NIP which was done at a low voltage of 3.0 kV.

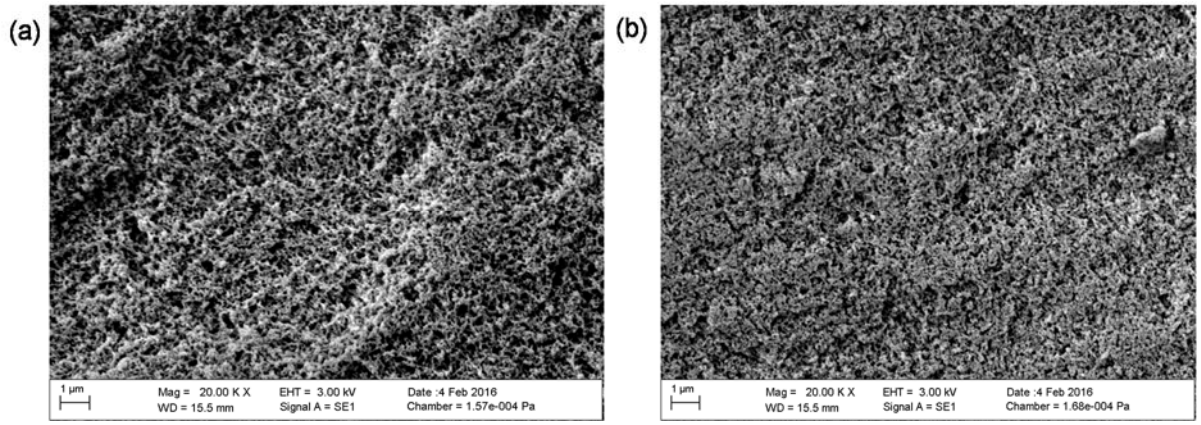


Figure 9: SEM micrographs of (a) MIP and (b) NIP, at 20 000 K X magnified.

4.2.3 Spectroscopic analysis

The FTIR spectra of washed MIP and NIP (Figure 10) showed similar backbone structure. These indicate the high degree of cross-linking (EGDMA) agent in both polymers, as strong vibration bands at ≈1765 and ≈1382 cm^{-1} are caused by the C=O and C-O functional group of EGDMA, respectively [113, 116]. The appearance of C≡N band at ≈1323 cm^{-1} in all polymers spectrum was caused by the presence of cyanide groups in polymers that originate from the functional monomer and initiator. Broadband obtained at ≈ 3590 cm^{-1} for the synthesized polymers was caused by the existence of hydroxyl groups that originates from the template molecules [112]. FTIR analysis confirmed the existence of the hydrogen bonding interactions and validity of the mechanism that takes place during the synthesis of the polymers. FTIR of the polymers was identical in all peaks, which was an indication that they were similarly synthesized with the omission of ketoprofen for NIP.

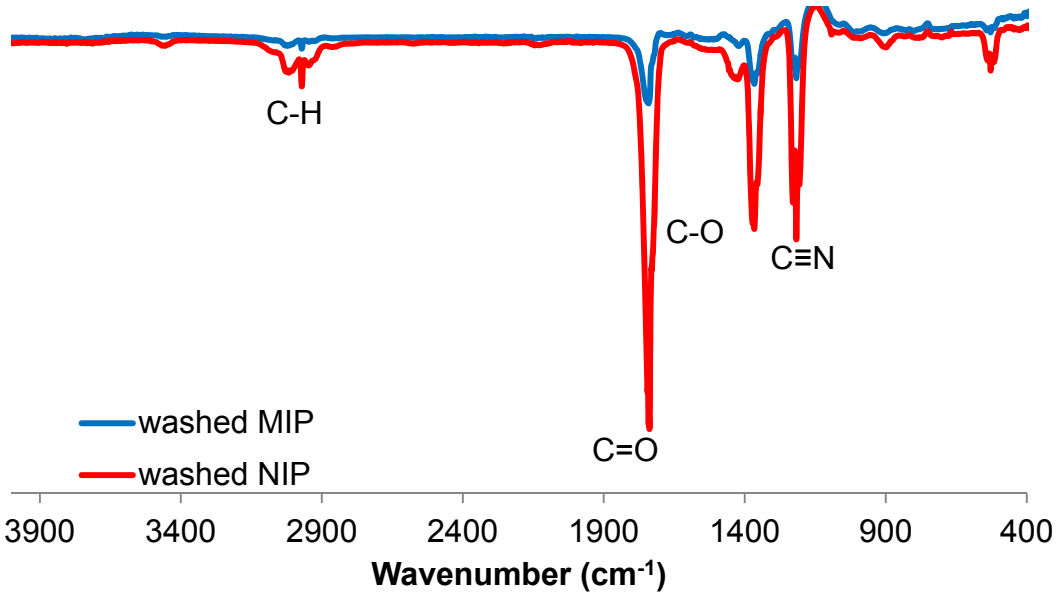


Figure 10: FTIR spectra of the synthesized MIP and NIP.

4.2.4 Thermal analysis

Thermogravimetric analysis was performed for the washed MIP and NIP (Figure 11). At 40 ˚C, the washed polymers had a mass loss of approximately 4%. This was probably due to the presence methanol used in the template removal step [117]. Further thermal decomposition of polymers was observed at 290 °C which was marked as the temperature where the polymer backbone collapses. In a separate study, the polymer backbone collapsed at 250 °C for a MIP that was synthesized for 1,3-diisopropylurea [117]. It was further observed that at 425 °C, there was 100% thermal decomposition for NIP, whereas the corresponding mass loss was 90% for MIP. The difference might have been caused by structural variations that could have happened during template removal process.

Differential scanning calorimetry thermograms (Figure 12) of MIP and NIP were similar with an endothermic peak at 355 °C which is associated with the temperature where there is complete thermal decomposition of the polymers. Similarities in the thermograms could be due to similar structural arrangements of the MIP and the NIP.

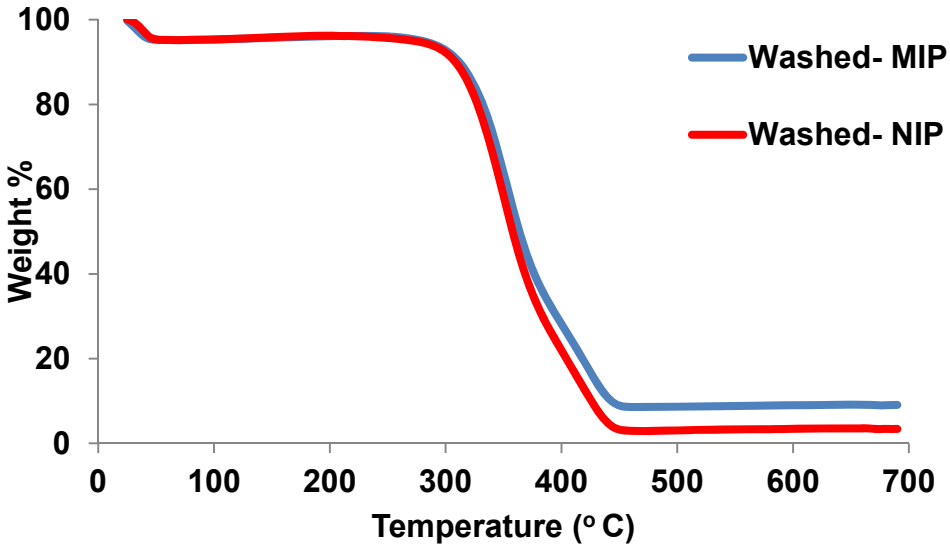


Figure 11: Thermogravimetric analysis of the synthesized polymers.

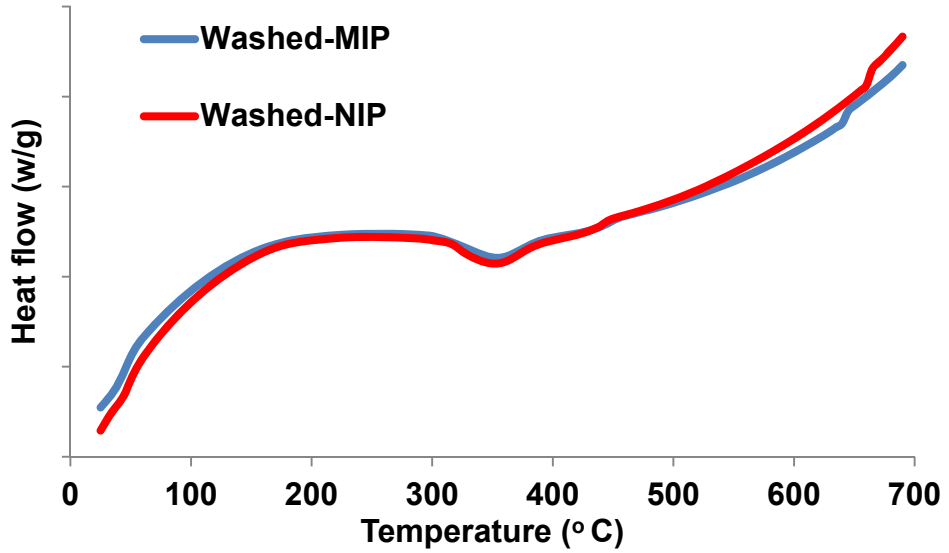


Figure 12: DSC curves for the polymers.

4.2.5 Solid-state NMR

The solid-state ^{13}C CP/MAS NMR spectra for the washed polymers Figure 13 were similar to those obtained from previous studies [118]. The results showed no differences in the resonance pattern, which proved that the MIP and NIP were synthesised from the same components [119-121]. The resonance observed was the evidence of various functional groups, methyl group in CH_3 broad peak at proximity 17 ppm, R-CH_2-R group at 45 ppm and a methylene group in R-CH_2-O at 61 ppm.

The resonances at 175 ppm represent carbonyl group in R-COO-R. The observed signals were in agreement with the synthesized polymers.

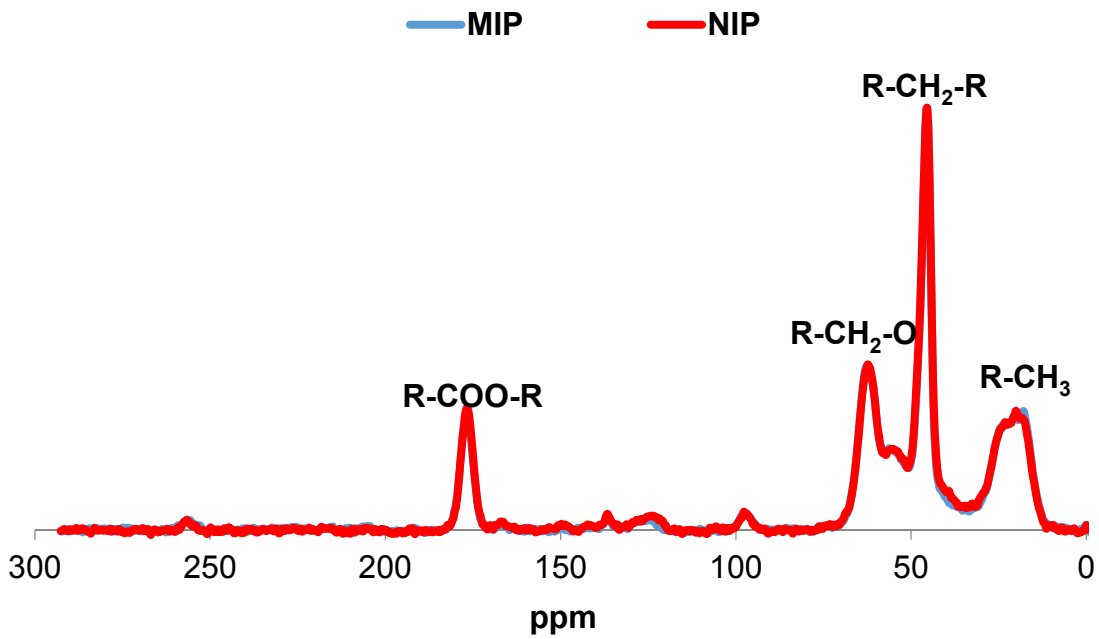


Figure 13: Solid-state ^{13}C CP/MAS NMR Spectra of the MIP and NIP.

4.2.6 Elemental analysis

The synthesized polymers were analyzed in terms of their carbon, nitrogen and hydrogen contents using the elemental analyzer. As indicated in

Table **12** the carbon and hydrogen contents were identical due to similar conditions in the synthesis. Also, this was an indication of a successful template removal as the presence of ketoprofen in the MIP could have resulted in higher carbon and hydrogen contents. The sources of nitrogen in the polymers are the initiator and functional monomer used in synthesis. The differences in the nitrogen contents of polymers can be explained by the possible disruptions in the chemical structure of the MIP during excess washing while removing ketoprofen. This was evident in the chromatograms obtained during template removal where there were unknown peaks recorded for the MIP solutions. Oxygen is the only other element (not quantified) known to be present in both polymers. The sources of oxygen in polymers are cross-linker and template used in polymerization [117].

Table 12**:** Measured percentage by mass of carbon, hydrogen, and nitrogen of the polymers using CHN analyzer.

Polymer	Carbon (%)	Hydrogen (%)	Nitrogen (%)
Washed MIP	58.28	7.39	0.33
Washed NIP	59.68	7.33	1.0

4.2.7 X-ray diffraction

The structural properties of synthesized polymers were analyzed by XRD Figure 14. The patterns of the synthesized polymers display a reflection peak in the 2θ^{o} region of 63^{o}, which is a broad peak. The behaviour of amorphous polymers for both MIP and NIP was similar as their structural properties were the same. And their diffractograms had a poor intensity for both polymers, which did not have significant differences. This was due to the template being successfully removed from the MIP.

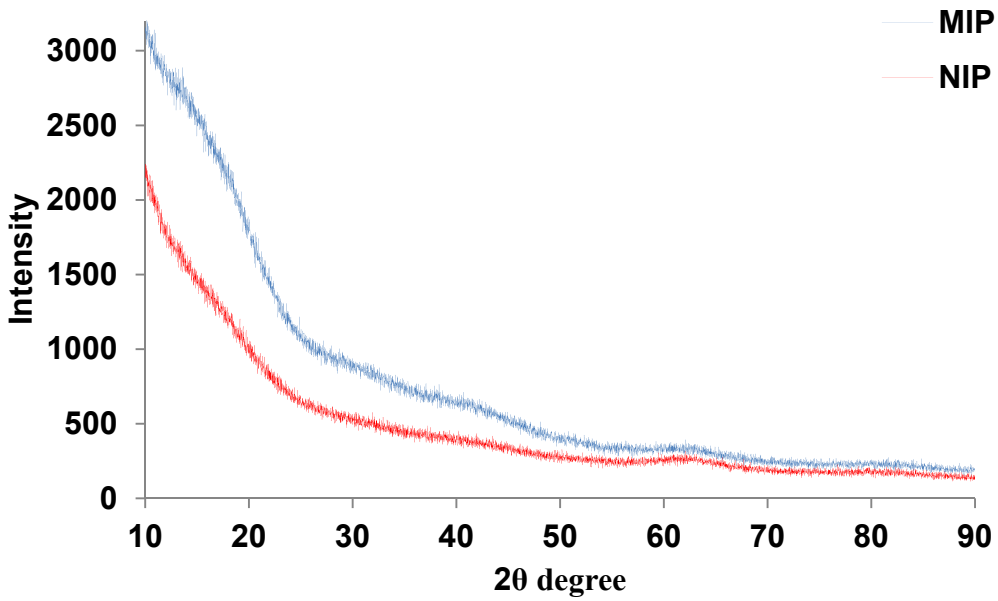


Figure 14: X-ray diffractograms for washed MIP and NIP.

4.3 Template removal

After polymerization, templates were removed from the polymers in order to form cavities of complementary size and shape of the target compound. A solvent mixture

of methanol/acetic acid (9:1, v/v) was used to extract the template from the polymer matrix. Then after, the polymer matrix was continually washed with methanol to remove residual acetic acid which was initially used to break non-covalent interactions between the polymer and target molecule. Template removed was quantitatively analyzed using HPLC. The solvent used to remove template was changed in each time of the 25 cycles (Figure 15), in each cycle, the amount of ketoprofen removed from the polymer was decreasing. After 15th cycle, ketoprofen compound was not detected by a UV detector, but the polymers were washed further to ensure the complete removal of templates.

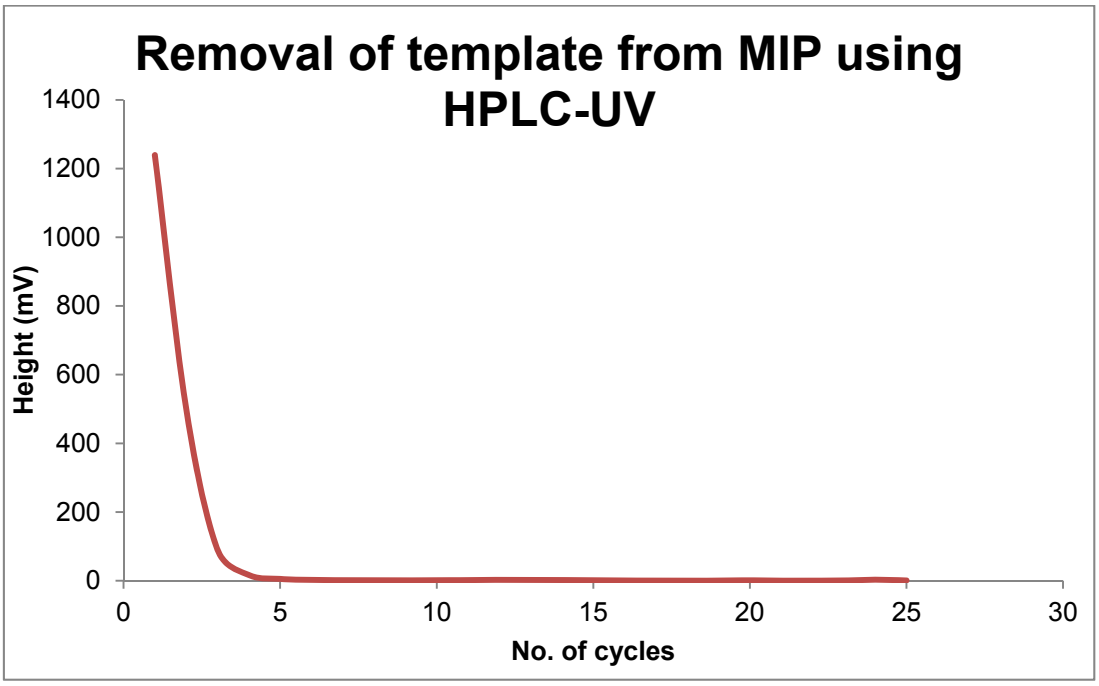


Figure 15: Extraction profile for ketoprofen removed from the polymer (MIP).

4.4 Binding analysis

4.4.1 Adsorption experiments

4.4.1.1 Effect of solvent

The binding capacity of molecularly imprinted polymers is influenced by the type of solvent used in the particular application of the MIP [121, 122]. The effect of absorption solvent for ketoprofen was investigated in order to determine whether the

NIP and MIP were capable of binding with the template of interest when the two polymers were suspended in different solvents. This was achieved using the following solvents; toluene, deionised water, acetonitrile, and chloroform at constant conditions. The constant conditions were polymer amount (8 mg), contact time (45 min), sample volume in aqueous solution (10 mL) and concentration of target molecule (1 $mg.L^{-1}$). In Figure 16 it was observed that when toluene was used alone as the adsorption solvent it was absorbing more onto the NIP than the MIP while it was a porogen solvent in a mixture used for the synthesis. Acetonitrile had the lowest extraction efficiency due to the solvent being used in the porogen mixture of the synthesized polymers and deionized water with the highest extraction efficiency due to the partitioning effect of the lyophilic ketoprofen into the hydrophobic polymers. Acetonitrile resulted in being smaller than toluene it penetrated the pores as such washing off the analytes from the cavities while toluene could not penetrate easily as such once the analytes are adsorbed into the cavity they could not be easily washed off. Chloroform gave equal extraction efficiency on the polymers, this was because chloroform is a very harsh solvent that dissolves most organic polymers. The polymers are not very water soluble, hence they should extract more into the solid phase, and they cannot extract more when the organic solvents are used due to their solubility in the organic solvents. It was found that deionised water was the better solvent with extraction efficiency >96% of MIP compare to organic solvents which resulted in conditions which are unfavorable for binding.

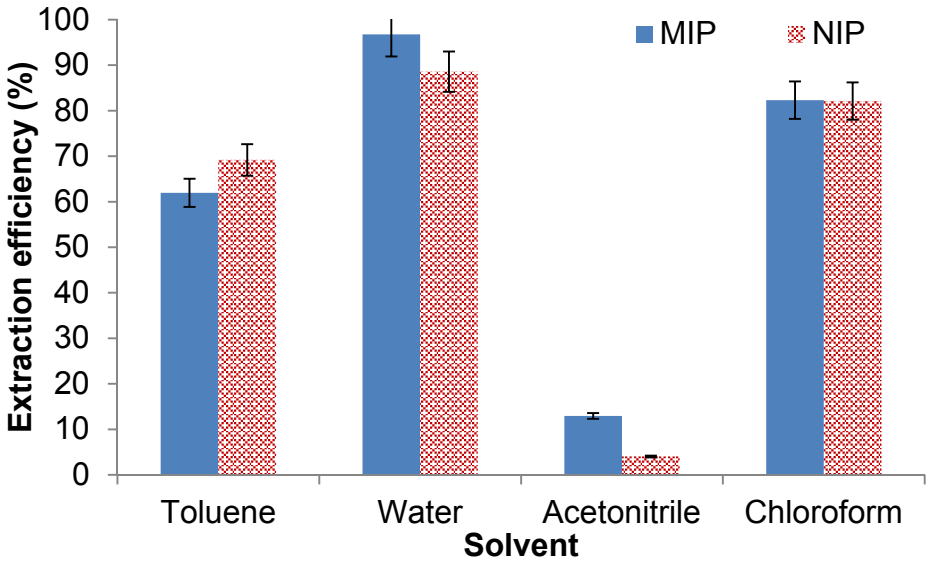


Figure 16: Effect of suspension solvent on the extraction efficiency of ketoprofen using MIP adsorbent.

4.4.1.2 Effect of sample pH

The effect of sample pH is important in order to achieve a maximum extraction of the target molecule. The sample pH varied over the pH range of 3 – 9 and while other experimental conditions were kept constant. These constant conditions were adsorption solvent (deionised water), polymer amount (8 mg), contact time (45 min), sample volume (10 mL) and concentration of target molecule (1 $mg.L^{-1}$). The suitable retention of a target compound on the on the adsorbent in the aqueous media depends on the pH of the medium [110]. It was observed that the extraction efficiency for the target molecule was highly affected by the sample pH based on the results in Figure 17. It was also observed that when the target molecule was more acidic it was protonated which resulted in the extraction efficiency to be higher (> 90%). However, when the sample pH was increased to basic, ketoprofen was deprotonated causing the poor extraction efficiency. Therefore, pH 5 was selected for adsorption experiments based on the different extraction efficiency between the MIP and NIP, while pH 3 was higher and had no significant difference for the retention of ketoprofen in between the two polymers.

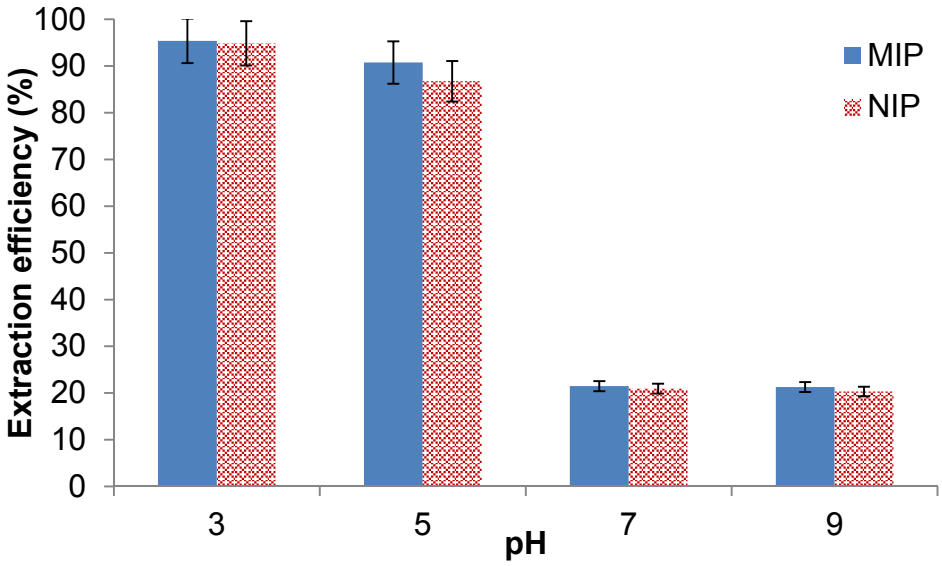


Figure 17: Effect of sample pH on the extraction efficiency of ketoprofen using MIP/NIP adsorbent.

4.4.1.3 Effect of contact time

The relationship between the amount of ketoprofen adsorbed and contact time with the polymer is shown in Figure 18, where the following experimental conditions were used: varying contact time, polymer amount (8 mg), sample pH (5), sample volume (10 mL) and concentration of target molecule (1 $mg.L^{-1}$). It was observed that there was an increase in the amount of ketoprofen adsorbed by the polymer from 5 – 30 min, after which equilibrium was reached. After 30 min it was observed that the maximum extraction efficiency was achieved and 45 min was used in a subsequent experiment to ensure the maximum uptake of ketoprofen. This is important in order to allow the sufficient adsorption of target compound from complex samples such as wastewater. In this way, even the non-targeted species that can be adsorbed un-intentionally could be released back into the solution due to poor adsorption. The adsorption performance of the MIP was higher than of the corresponding NIP.

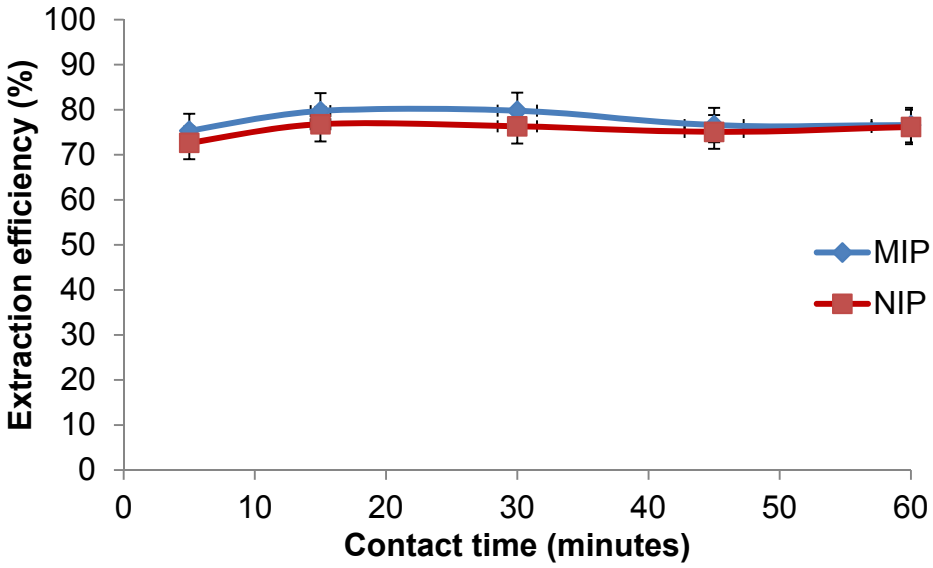


Figure 18: Effect of contact time on the extraction efficiency of ketoprofen using MIP adsorbent.

4.4.1.4 Effect of polymer amount

The dependency of the amount of polymers adsorbed on the quantity of the adsorbent used is shown in Figure 19, where the following experimental conditions were used: varying polymer amount, sample pH (5), sample volume (10 mL), contact time (45 min) and concentration of target molecule (1 mg L^{-1}). It was found that increasing dosage of the polymer adsorbent, the amount of ketoprofen had no significant effect on the extraction efficiency which was >97%. Therefore, subsequent adsorption experiments were further carried out using 15 mg of the polymer.

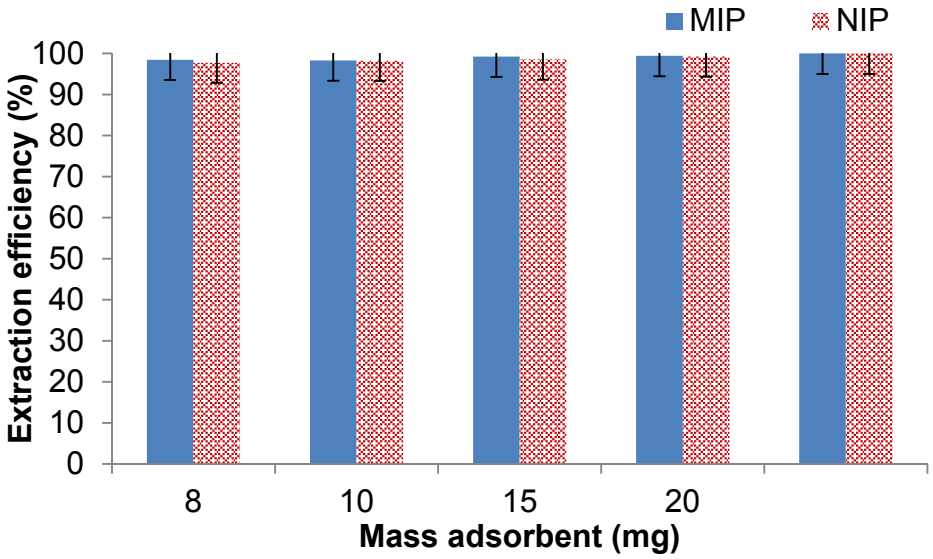


Figure 19: Effect of polymer amount on the extraction efficiency of ketoprofen using MIP adsorbent.

4.4.1.5 Effect of initial concentration

The results obtained for the adsorption capacity as a function of initial concentration is depicted in Figure 20. During the optimization of initial concentration, the polymer amount (14 mg), sample pH (5), sample volume (10 mL) and contact time (45 min) were kept constant. The adsorption capacity increased linearly as a function of initial concentration, this was observed until 70 $mg.L^{-1}$, therefore it was noted that at high concentration the adsorption capacity was also increasing. In this experiment, the concentration was not increased beyond 70 $mg.L^{-1}$ as the target compound was expected to be in $\mu g.L^{-1}$ level of wastewater samples.

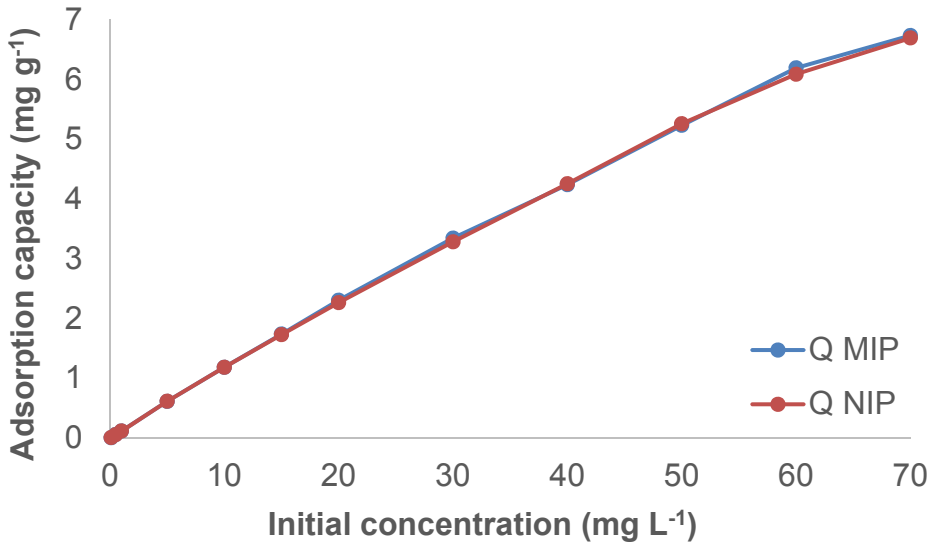


Figure 20: Effect of initial concentration on the adsorption capacity of ketoprofen using MIP adsorbent.

4.4.2 Binding sites characterization

4.4.2.1 Kinetic modeling

The adsorption process was described by employing Eqs.(5)$\log(Q_e - Q_t) = \log Q_e - \frac{k_1 t}{2.303}$ (5) and (6) for pseudo-first-order and pseudo-second-order kinetic models, respectively.

$$\log(Q_e - Q_t) = \log Q_e - \frac{k_1 t}{2.303} \quad (5)$$

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^{\,2}} + \frac{1}{Q_e} t \quad (6)$$

Where Q_t is the adsorption capacity at any time (mg.g^{-1}), Q_e is the adsorption capacity at equilibrium (mg.g^{-1}), t is the constant time (min), k_1 and k_2 are pseudo-first-order (min^{-1}) and pseudo-second-order sorption rate constants (g.mg^{-1} min^{-1}), respectively [32, 55]. The kinetic data result shown in Table 13, based on the correlation coefficients (R^2) for polymers, pseudo-second-order model produced

better quality of linearization compared to the pseudo-first-order model, which indicates that the adsorption is of chemical nature [56]. The adsorption capacity values obtained in Table 10 were closer to those reported before in this work for MIP and NIP, respectively. The adsorption capacities obtained for polymers when employing pseudo-second-order model were 0.2457 and 1.2473 $mg.g^{-1}$ min^{-1} for MIP and NIP, respectively. Therefore, the results prove that the target molecule binds to two or more processes at the adsorbent surface with different binding energies since the data falls in the pseudo-second-order kinetic model. The implication of this is that the adsorption of ketoprofen onto the polymers occurred via a chemisorption process.

Table 13: Calculated parameters for the kinetic models.

polymer	Pseudo-first-order	Pseudo-second-order		
	R^2	R^2	k_2 ($g.mg^{-1}$ min^{-1})	Q_e ($mg.g^{-1}$)
MIP	0.0140	0.9956	0.2457	3.2216
NIP	0.2014	0.9981	1.2473	3.1857

4.4.2.2 Adsorption isotherms

The isothermal analysis of the polymers was analyzed using Eqs.(7) and (8) for the linearized forms of Freundlich and Langmuir isotherms, respectively.

$$\log Q = m \log C_e + \log a \quad (7)$$

$$\frac{C_e}{Q} = \frac{C_e}{Q_{max}} + \frac{1}{Q_{max} K_L} \quad (8)$$

Where Q is the amount of the adsorbed molecule at equilibrium ($mg.g^{-1}$), *m* is the adsorption intensity or surface heterogeneity, C_e is the equilibrium concentration of the target molecule ($mg.L^{-1}$), *a* is the adsorption capacity of target molecule ($mg.g^{-1}$),

Q_{max} is the maximum adsorption capacity ($mg.g^{-1}$) and K_L is Langmuir adsorption equilibrium constant [32, 56]. From the results obtained it showed that the data fitted well with Langmuir isotherm which indicated the homogeneous nature of binding sites [55]. The results of K_L and Q_{max}, constants were determined by using the intercepts and slope of the linear plots of C_e/Q versus C_e and the results are given in Table 14. The maximum adsorption capacities obtained for the polymers were higher than that obtained in kinetic modelling of MIP and NIP (3.2216 and 3.1857), respectively. Therefore, the results which were obtained previously on the maximum adsorption capacities in section 4.5.1.5 of MIP and NIP (6.7353 and 6.6940, respectively) was still consistent with the result reported in this section.

Table 14: Data extracted from the adsorption isotherms.

Polymer	Langmuir			Freundlich
	R^2	K_L ($L.mg^{-1}$)	Q_{max} ($mg.g^{-1}$)	R^2
MIP	0.9929	0.2618	9.3633	0.8389
NIP	0.9912	0.1549	8.2440	0.8243

4.5 MISPE optimization

Optimization was carried out by employing deionised water spiked with 1 $mg.L^{-1}$ of ketoprofen as the sample. SPE parameters investigated include sample pH, sorbent mass, sample volume, and washing and elution solvents. The fact that the target compound is polar, methanol was used for its elution from SPE cartridge. During optimization, only one parameter was changed at a time while keeping other parameters constant. Each experiment was conducted in triplicate, where average results are discussed.

4.5.1 Influence of sample pH

For the adsorption of acidic pharmaceuticals, it has been reported that the extraction is based on hydrogen binding of the target compound and functional monomer (2-vinylpyridine) [48]. In this context, pH of the 20 mL of water at a flow rate of 1 $mL.min^{-1}$ solutions was adjusted in order to promote the monomer-template interactions. pH was investigated in the range of 3-7, where the highest recovery was obtained at pH 5 (Figure 21). Therefore, pH 5 was selected as optimum and used in all subsequent experiments. At selected pH, the recovery for ketoprofen using NIP was 69.5% which could be because of the adsorption based on non-specific interactions, whereas for the corresponding MIP, 88% was obtained because of molecular recognition. pH values greater than 7 were not investigated due to the deprotonation of ketoprofen which could lead to unavailability of adsorption sites. This behavior is somewhat similar to the behavior of other acidic pharmaceuticals on MIP [55].

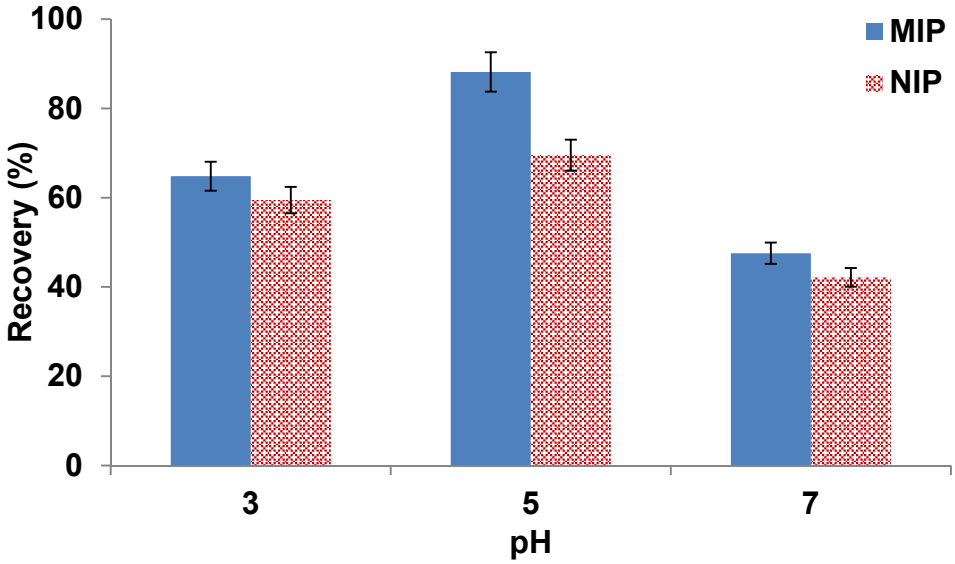


Figure 21**:** Effect of pH on the recovery of ketoprofen from the polymers. Extraction conditions were: sample volume (20 mL), washing solvent (1 mL of deionised water) and elution solvent (1 $mL.min^{-1}$ methanol).

4.5.2 Influence of the amount of polymer materials

The quantity of the polymer for the optimum extraction of ketoprofen was investigated. It was discovered that low mass of the polymers resulted in poor recoveries which could be a result of the maximum occupation of the binding sites. However, the accepted recovery was achieved by employing 14 mg of the polymer (Figure 22). It should be noted that this work was investigated using 1 $mg.L^{-1}$ of ketoprofen in a 50 mL solution which is much higher than the concentrations expected in wastewater samples. Therefore, the amounts of polymers beyond 14 mg was not investigated.

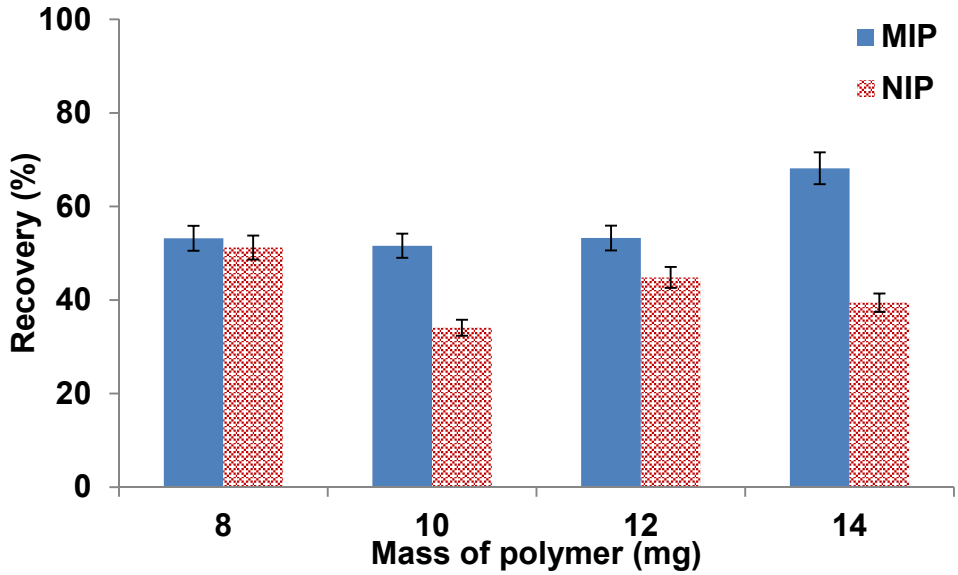


Figure 22: Effect of polymer amount on the recovery of ketoprofen. Extraction conditions were: sample volume (50 mL) with 1 $mL.min^{-1}$ flow rate, washing solvent (1 $mL.min^{-1}$ of deionised water), elution solvent (1 $mL.min^{-1}$ methanol) and pH 5.

4.5.3 Influence of sample volume

High sample volumes are often required in the environmental analysis as they tend to lead to high preconcentration factors which in turn produces better sensitivity. The results in Figure 23 show that the recovery increased from the sample volume of 10-20 mL for both polymers because of limited analyte interaction with the polymer as not enough volume was available to saturate the whole sample through the sorbent. Accepted recoveries (>70%) were achieved in the sample volumes of 20 and 50 mL

for MIP. However, 50 mL was selected as optimum and could not keep using more solution as it will generate unwanted waste. Recoveries decreased beyond the sample volume of 50 mL for MIP which could be because of the capacity of the polymers being exceeded. Because of limited binding sites in NIP, maximum recovery was obtained when the sample volume was 20 mL.

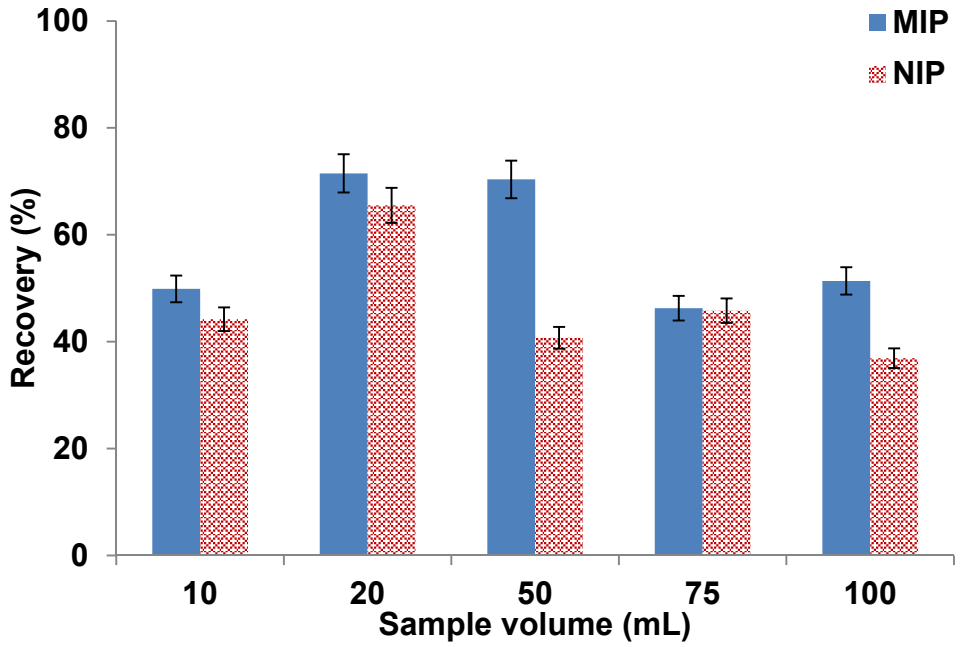


Figure 23: Effect of sample volume on the recovery of ketoprofen. Extraction conditions were: washing solvent (1 mL of deionised water), elution solvent (1 mL methanol), sorbent mass (14 mg) and pH 5.

4.5.4 Influence of elution solvent

Polar organic solvents were investigated for the elution of retained compound. Such solvents were expected to disrupt the hydrogen bond between ketoprofen and the MIP. Results in Figure 24, show that methanol was the most suitable solvent than 2% formic acid in deionized water/acetonitrile (40:60, v/v) and methanol/acetic acid (9:1), for removal of ketoprofen from the SPE cartridge. MIP recoveries were poorer with other mixtures compare to methanol; this was because of the binding strength which was weak. Due to the high cost of organic solvents and problems that are associated with their waste disposal, the elution volume of 1 mL was used. However, it is expected that high elution volumes may lead to dilution of the sample in turn.

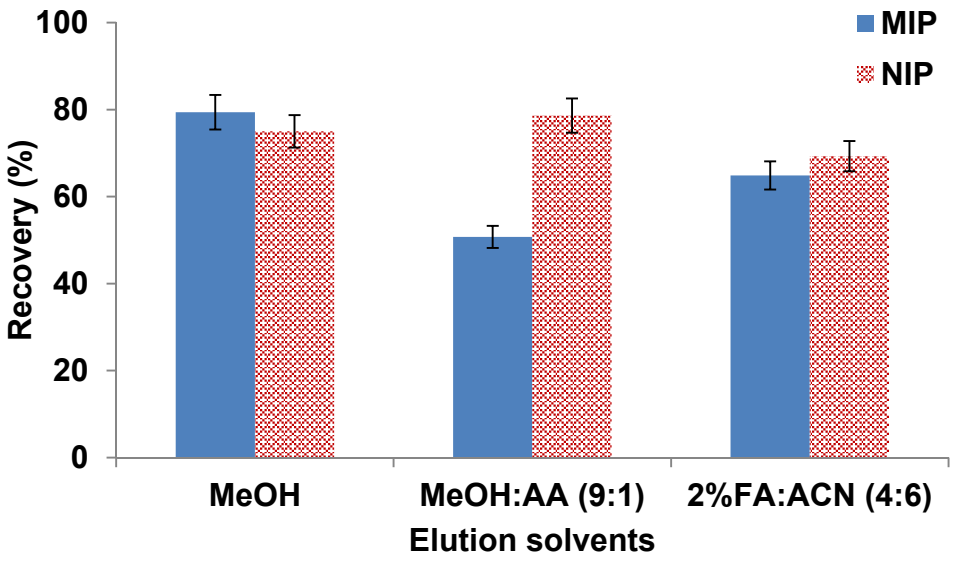


Figure 24**:** Effect of elution solvent on the recovery of ketoprofen. Extraction conditions were: washing solvent (1 mL of deionised water), sorbent mass (14 mg), sample volume (50 mL) and pH 5. (*MeOH: methanol; AA: acetic acid; FA: formic acid; ACN: acetonitrile.)*

4.5.5 Influence of salt concentration

The effect of ionic strength was investigated by spiking the deionized water to different concentrations of sodium chloride in the range of 0.5 – 2 % (w/v). It was noted that percentage of sodium chloride has no significant effect on the recovery of ketoprofen in the MIP, but slightly different with NIP which was decreasing the salt concentration in water solution was increased (Figure 25). From the results obtained it was noted that the synthesized MIP was not capable of analyzing aqueous samples containing salt for ketoprofen, because of levels of salt content in the sample as the results of recoveries were > 60%.

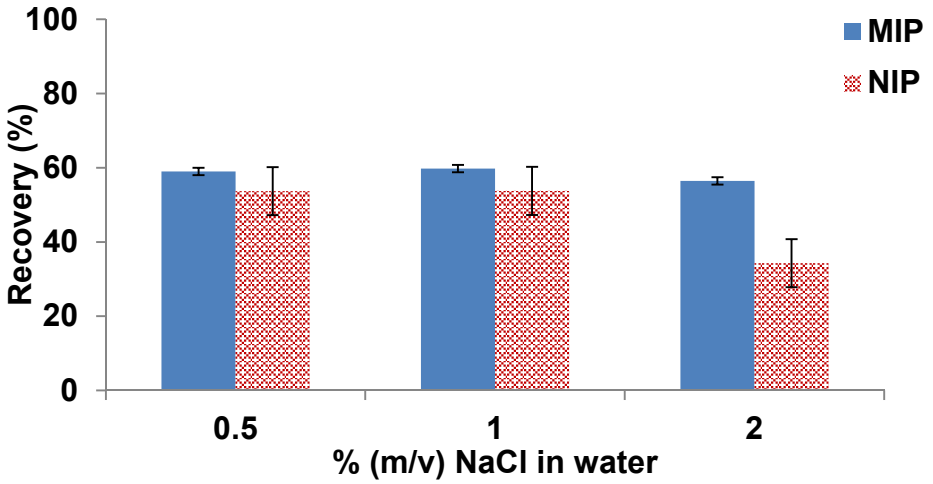


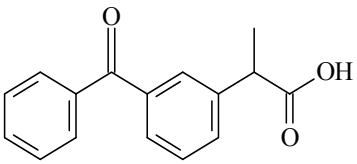
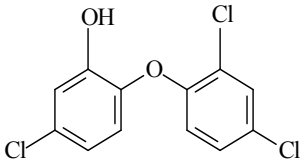
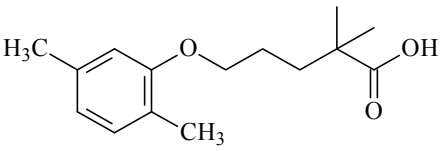
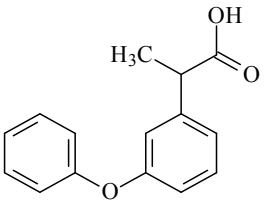
Figure 25: Effect of salt concentration on the recovery of ketoprofen. Extraction conditions were: washing solvent (1 mL of deionised water), sorbent mass (14 mg), sample volume (50 mL) at pH 5 and elution solvent methanol.

4.6 Selectivity

4.6.1 Molecularly imprinted solid-phase extraction

The selectivity of the polymers was investigated using deionised water that was spiked with ketoprofen, fenoprofen, gemfibrozil and triclosan. Fenoprofen and gemfibrozil are acidic pharmaceuticals that contain similar functional groups as ketoprofen (Table 15). These two pharmaceuticals are usually present in wastewater solutions that contain ketoprofen [62, 123]. Other pharmaceutical drug such as gemfibrozil is used to lower the risk in humans caused by taking too much of the NSAIDs [124]. Triclosan is an anti-bacterial compound that is usually detected in wastewater with ketoprofen [17, 125]. These competitors were expected to be adsorbed by MIP through the formation of hydrogen bonds due to the presence of hydroxyl groups in their molecules.

Table 15: Molecular structures of ketoprofen and competitors.

Compound	Molecular Structure
Ketoprofen	
Triclosan	
Gemfibrozil	
Fenoprofen	

The results show low recoveries (less than 20%) for all competitors which may be because of differences in their molecular shapes and sizes compared to ketoprofen. Therefore, the prepared polymers were highly selective towards ketoprofen (Table 16 and Table 17). Selective washing was investigated in this case. Ten percent was recovered for triclosan when the MIP cartridge was washed with water, 17% was obtained for the corresponding NIP. Further washing solvents were investigated for selective removal of competitors which could result in cleaner chromatograms when the method is applied in wastewater analysis. The addition of organic solvents in the washing solutions resulted in elution of ketoprofen together with competitors for the NIP cartridges. This is a result of non-specific binding. Therefore, the cartridges packed with NIP were discarded because of poor selectivity. However, in the case of the MIP, only competitors were removed during washing as a result of molecular

imprinting. The introduction of acetonitrile in the washing solvent resulted in the reduction of ketoprofen recovery in the case of the MIP as well.

Table 16: Effect of washing solvent in the solid-phase extraction of ketoprofen using MIP.

	% Recovery				
Compound	H_2O	MeOH/H_2O (5:95, v/v)	TEA/ACN (1:99, v/v)	TEA/H_2O (1:99, v/v)	TEA/H_2O (5:95, v/v)
Ketoprofen	69.06	67.61	33.39	107.85	103.98
Fenoprofen	5.81	1.98	2.49	1.17	2.68
Gemfibrozil	0.96	1.03	0.59	1.85	0.15
Triclosan	9.93	9.56	2.31	13.76	12.64

MeOH: methanol; TEA: triethylamine; ACN: acetonitrile; H_2O: water

Table 17: Effect of washing solvent in the solid-phase extraction of ketoprofen using NIP.

	% Recovery				
Compound	H_2O	MeOH/H_2O (5:95, v/v)	TEA/ACN (1:99, v/v)	TEA/H_2O (1:99, v/v)	TEA/H_2O (5:95, v/v)
Ketoprofen	87.44	61.63	2.01	50.34	5.61
Fenoprofen	5.43	0.99	2.35	1.34	2.09
Gemfibrozil	0.90	0.90	0.57	1.77	0.08
Triclosan	17.59	10.12	3.31	14.39	12.93

MeOH: methanol; TEA: triethylamine; ACN: acetonitrile; H_2O: water

4.6.2 Selectivity in batch adsorption studies

The selectivity of MIP for ketoprofen batch rebinding experiment was achieved under these conditions initial concentration (C_i) (1mg.L^{-1}), the polymer amount (14 mg), sample pH (5), sample volume (10 mL) and contact time (45 min) were kept constant for all polymers. Once the system was equilibrated, the concentration of adsorbed ketoprofen (C_f) in solution was measured and the mass (*m*) of the target molecule

adsorbed to the MIP calculated. K_D, distribution factor ($mL.g^{-1}$) of ketoprofen between the MIP particles and the aqueous solution was determined by using the following equation:

$$K_D = \frac{(C_i - C_f)}{mC_f} V \quad (9)$$

Where V is the volume of initial solution. A selectivity coefficient for ketoprofen ($K^{sel}_{ketoprofen}$) relative to the competitor compounds was defined as the ratio of distribution factors (K_D) of ketoprofen and competitor compounds (j).

$$K^{sel}_{\frac{ketoprofen}{j}} = \frac{K_D^{ketoprofen}}{K_D^j} \quad (10)$$

The relative selectivity factor K' was determined by the following equation:

$$K' = \frac{K'(MIP)}{K'(NIP)} \quad (11)$$

Where K'(MIP) and K'(NIP) are selectivity coefficient for MIP and NIP, respectively [126, 127]. These parameters are given in Table 18, which indicated that MIP was highly selective for ketoprofen recognition than NIP in aqueous samples.

Table 18: Selectivity of ketoprofen.

Analyte	K_D (MIP) ($mL.g^{-1}$)	K_D (NIP) ($mL.g^{-1}$)	K^{sel} (MIP)	K^{sel} (NIP)	K'
Ketoprofen	1065.29	264.98	–	–	–
Fenoprofen	15.94	30.32	66.85	8.74	7.65
Gemfibrozil	174.84	88.83	6.09	2.98	2.04

4.7 Validation of the analytical method

The chromatographic analysis of ketoprofen was achieved on a reverse-phase stationary phase (Figure 26). The performance of the proposed analytical method that uses MIP as a selective sorbent for SPE was validated based on sensitivity,

accuracy, and precision. A linear plot with R^2 greater than 0.99 was achieved for a seven-point calibration curve in the concentration range of 0.001 to 10 mg.L^{-1}. On the basis of LOD and LOQ results shown in Table 19, the developed method is highly sensitive. LOQ and LOD are defined as concentrations that gave the signal-to-ratio of 10 and 3, respectively [97]. Greater preconcentration factor in the proposed method led to better method sensitivity as compared with the data in the literature [128]. The results in Table 19 show that the analytical method was highly accurate as indicated with recoveries in the range of 68-114%. The relative standard deviation (RSD) given as ± values indicate that the developed method is precise.

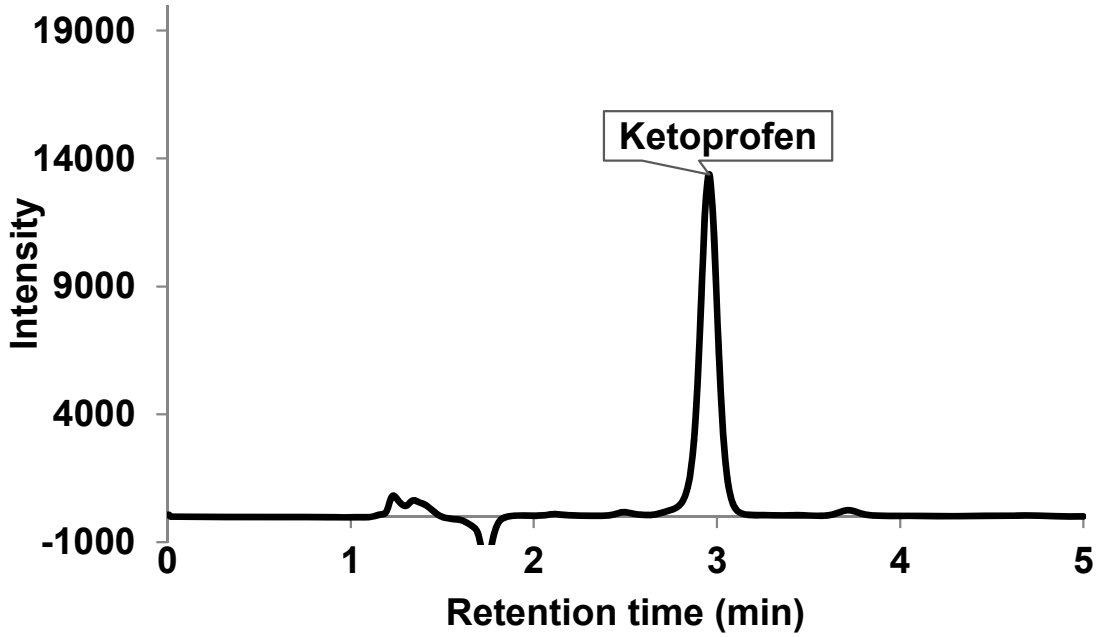


Figure 26: Chromatographic analysis of 1000 µg.L^{-1} ketoprofen standard solution.

Table 19: LOD, LOQ, recovery (%) and RSD (%) values (n = 3) achieved for the deionized water spiked with ketoprofen in the concentration range of 5 to 1000 µg.L^{-1}.

Spiked sample	LOD (µg.L^{-1})	LOQ (µg.L^{-1})	Recovery (%) ± RSD (%)		
			1000 µg.L^{-1}	25 µg.L^{-1}	5 µg.L^{-1}
Deionized water	0.09	0.30	68 ± 3	80 ± 17	114 ± 16
Influent	0.23	0.78			68 ± 10
Effluent	0.17	0.55			68 ± 15

4.8 Quantification of ketoprofen in wastewater

After successful optimization of SPE, the developed procedure was applied on extraction of ketoprofen from wastewater before high-performance liquid chromatographic quantification. The identification of ketoprofen in the extracted wastewater samples was based on similar retention times with a standard solution, and the quantification was performed used the external calibration method. Ketoprofen was detected in all wastewater samples Table 20 with concentrations greater than 2 $\mu g.L^{-1}$ in the effluent, whereas, the levels were much higher in the influent. Chromatograms were cleaner for the effluent when compared to influent samples because of effective wastewater treatment process in Figure 27. Detected levels of ketoprofen were compared with previous concentrations reported locally and in other countries. Previous work [17] reported the concentration range of 1-6 $\mu g.L^{-1}$ in Amanzimtoti WWTP influent, whereas the present study reports the average concentration of 28.4 $\mu g.L^{-1}$. The differences could be due to seasonal variations as sampling were collected from August to October in the previous work [17], whereas the samples of the present study were collected in May. Also, the variations in population dynamics might be another factor to be considered. Overall, the concentrations of ketoprofen detected in these plants were higher than the levels reported in South Africa and the rest of the world (Table 21) because of differences in WWTP designs and consumption of the drug in foreign countries [69, 129, 130]. Most recently, ketoprofen is one of the pharmaceuticals that have been detected in wastewater and surface water from several countries including Algeria and China [2, 131].

Table 20: Concentrations ($\mu g.L^{-1}$) ± standard deviations (n = 3) of ketoprofen in wastewater samples.

WWTP	Influent	Effluent
Amanzimtoti	28.4 ± 1.1	3.50 ± 2.4
Kingsburgh	28.2 ± 12	3.40 ± 3.1
Umbilo	27.3 ± 0.57	2.90 ± 2.5

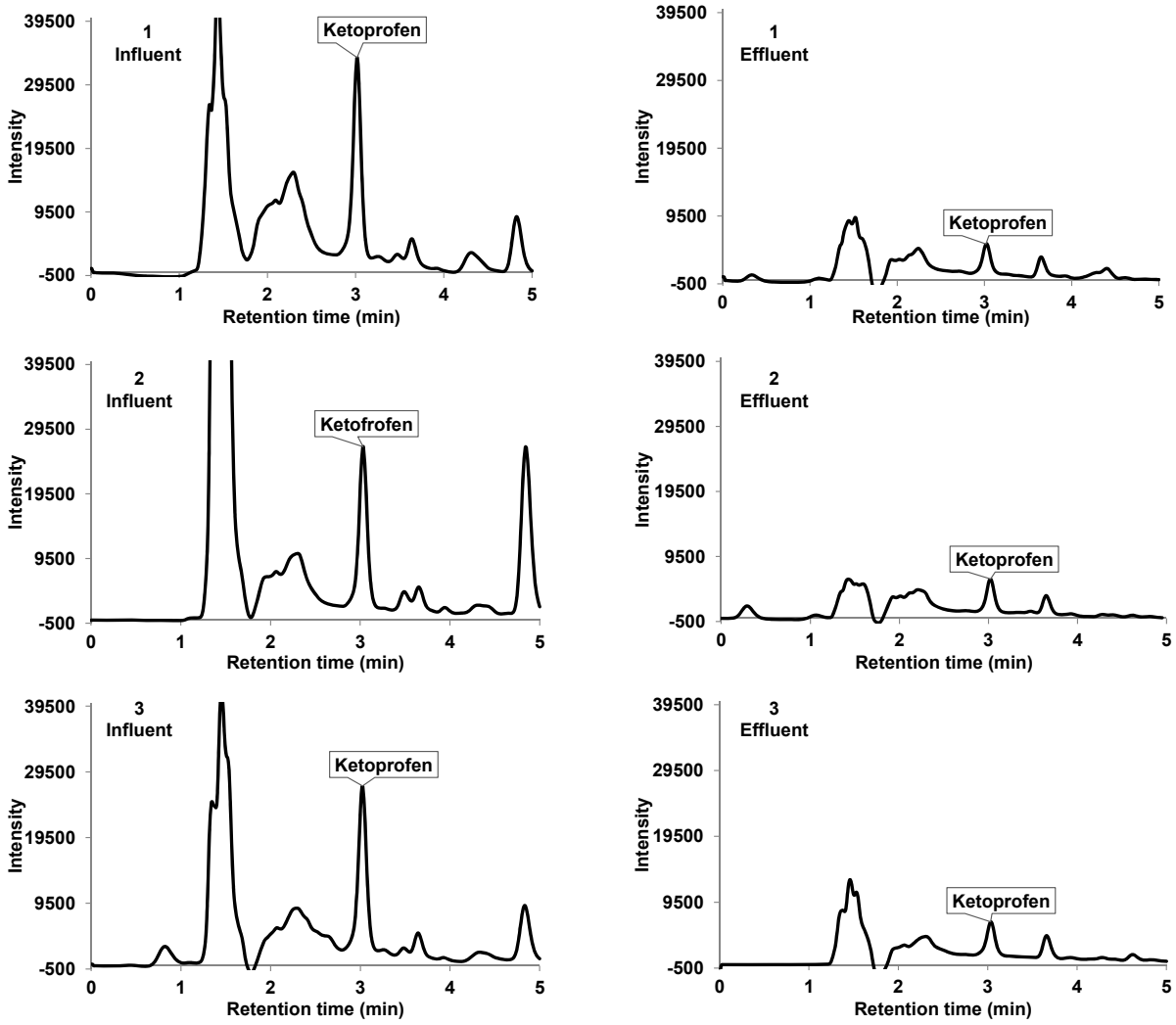


Figure 27: Typical Chromatograms obtained for wastewater collected at Amanzimtoti (1), Umbilo (2) and Kingsburgh (3) WWTPs.

Table 21: Concentrations (μg.L^{-1}) of ketoprofen reported in literature

WWTP – City – Country	Influent	Effluent	Reference
Northern – Durban – South Africa	<10	1.0	[17]
Baltimore Back River – MD – United States	1.20	0.28	[69]
Darvill – Pietermaritzburg – South Africa	3.15	0.38	[14]
Amanzimtoti – Durban – South Africa	8.6	1.55	[18]
Kallby – Lund - Sweden	1.35	0.48	[70]

4.9 Removal of ketoprofen from wastewater

To the best of our knowledge, this is the first detailed study based on the occurrence and removal rate of ketoprofen in Amanzimtoti, Kingsburgh and Umbilo WWTPs. Removal rate was determined based on the concentrations present in both influent and final effluent. Therefore, the performance of these WWTPs in terms of reducing ketoprofen during treatment process was compared to various plants in the world (Table 22). It was observed that the sampled WWTPs performs in a similar manner and sometimes better when compared with various plants for the reduction of ketoprofen during the wastewater treatment [74-76, 125].

Table 22: Literature data on the removal rates of ketoprofen from WWTPs.

Country	%Removal	Reference
South Africa	88-90	This work
Korea	94	[123]
Spain	40 - 100	[131]
Czech Republic	72	[132]
Tokyo	45	

Chapter 5:

Conclusion and recommendations

5.1 Conclusion

The aim of this study was to computational design a model and synthesize a molecularly imprinted polymer that is capable of extracting ketoprofen from the wastewater. A computational approach using the binding energy (ΔE) as a measurement of the molecular interaction between the functional monomer and the target molecule was presented as a tool for screening functional monomer that is required for the preparation of MIP. From the computational calculations of binding energy, the interaction with the lower ΔE was selected for synthesis of MIP. FTIR analysis of both unwashed and washed MIP confirmed the occurrence of H-bonding between the template and functional monomer. The results gave an insight into MIP in molecular level and showed that the computational approach based on DFT method can be used for screening suitable monomer that is required in the synthesis.

A MIP with binding sites was successfully synthesized by a bulk polymerization method. Morphology of the particles was clearly visible, with MIP showing rough and irregular surface compared to NIP. The polymers were thermally stable and amorphous. Thermal decomposition of polymers was observed at 290 °C and an endothermic peak at 355 °C on DSC, which was marked as the temperature where the polymer backbone collapses. The synthesized MIP and NIP showed similar backbone structure when studied in the solid-state NMR. The carbon and hydrogen contents were identical due to similar conditions in the synthesis while nitrogen differences were caused by disruptions in the chemical structure of the MIP during excess washing while removing ketoprofen.

The highest extraction efficiencies achieved for ketoprofen when using molecularly imprinted polymer as the adsorbent was used. The optimum conditions for ketoprofen adsorption onto MIP cartridge for sample pH, amount of sorbent, sample volume and elution solvent were 5, 14 mg, 50 mL, and methanol, respectively. The MIP synthesized demonstrated a high selectivity towards ketoprofen rather than the structural related competitors.

For the first time, a selective molecularly imprinted polymer for ketoprofen was synthesized and applied as the SPE sorbent. SPE technique optimized was used

with HPLC for the quantitative determination of ketoprofen in Umbilo, Amanzimtoti, and Kingsburgh WWTPs. For wastewater analysis, ketoprofen was detected in all samples. The average limit of detection for deionized water spiked with ketoprofen, influent and effluent were 0.09, 0.23 and 0.17 $\mu g.L^{-1}$, respectively. The average concentrations of ketoprofen found in the influent and effluent were greater than 27 and 2 $\mu g.L^{-1}$, respectively. These concentrations were higher when compared to what has been reported for WWTPs located in Europe. The removal rate during domestic wastewater treatment was in the range of 88 to 90%. These results call for a detailed screening of ketoprofen in other South African water bodies including river and dam water. The analytical methodology used for wastewater analysis was fast, sensitive, selective and gave results with good precision.

5.2 Recommendations

Further studies are required that apply computational chemistry in selecting the functional monomer that is suitable for binding with target molecule. In such studies, they should be more investigation on experimental work for support on selection of the functional monomer.

Further detailed characterization of the nature of binding sites in the synthesised polymer using other isotherms will be necessary for understanding the binding mechanism.

More information on quantity, quality, and toxicity of this pharmaceutical and metabolites are definitely needed especially when attempting to reuse wastewater. And other techniques for analysis such as LC-MS should be considered as they are more sensitive and discriminatory, as well as the extraction methods for clean-up and pre-concentration the wastewater. This will include in few extraction methods such as liquid- liquid extraction (LLE), stir-bar sorptive extraction (SBSE), and ultrasonic solvent extraction (USE).

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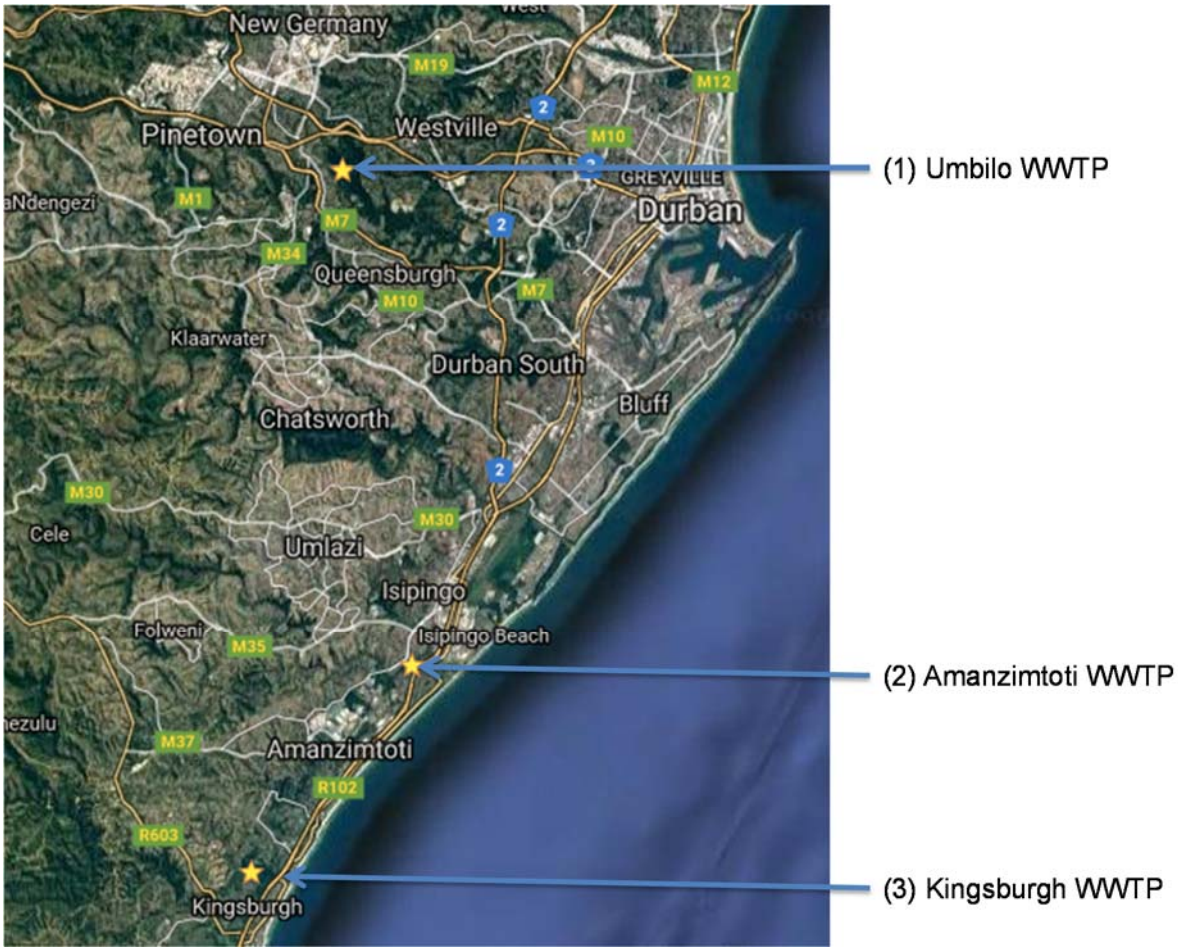
Annexures:

MAP showing the sampled WWTPs in Durban, KwaZulu-Natal, South Africa



(1) Umbilo WWTP,
(2) Amanzimtoti WWTP and
(3) Kingsburgh WWTP

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Synthesis and application of a molecularly imprinted polymer in the solid-phase extraction of ketoprofen from wastewater

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ABSTRACT

Ketoprofen is a nonsteroidal anti-inflammatory drug widely consumed by humans as it possesses analgesic activities. A selective molecularly imprinted polymer (MIP) for ketoprofen was synthesized and applied as a solid-phase extraction sorbent. MIP was synthesized using 2-vinylpyridine, ethylene glycol dimethacrylate, 1,1′-azobis(cyclohexanecarbonitrile), toluene/acetonitrile (9:1, v/v), and ketoprofen as a functional monomer, cross-linker, initiator, porogenic mixture, and template, respectively. The polymerization was performed at 60 °C for 16 h, and thereafter the temperature was increased to 80 °C for 24 h to achieve a solid monolith polymer. Nonimprinted polymer was synthesized in a similar manner with the omission of ketoprofen. Characterization with thermogravimetric analysis and X-ray diffraction showed that the synthesized polymers were thermally stable and amorphous. Solid-phase extraction cartridges packed with MIP were used with high-performance liquid chromatography for quantitative analysis of ketoprofen in wastewater. The analytical method gave detection limits of 0.23, 0.17, and 0.09 μg/L in wastewater influent, effluent, and deionized water, respectively. The recovery for the wastewater influent and effluent spiked with 5 μg/L of ketoprofen was 68%, whereas 114% was obtained for deionized water. The concentrations of ketoprofen in the influent and effluent samples were in the ranges of 22.5–34.0 and 1.14–5.33 μg/L, respectively. Overall, the analytical method for the analysis of ketoprofen in wastewater was rapid, affordable, accurate, precise, sensitive, and selective.

1. Introduction

Ketoprofen, also known as [(*RS*)-2-(3-benzoylphenyl)-propionic acid], is a commonly used pharmaceutical drug which possesses anti-inflammatory and analgesic activities because of its ability to inhibit cyclooxygenase enzymes that promote inflammation [1]. Ketoprofen is widely used in medical care because it is able to treat inflammatory diseases and musculoskeletal injury [2]. Because of the large quantity of ketoprofen consumed by humans, the compound is widely detected with other nonsteroidal anti-inflammatory drugs (NSAIDs) in wastewater and surface water [3–5]. Once consumed, 80% of ketoprofen is eliminated as unchanged drug and its degradation in wastewater treatment plants (WWTPs) depends on the biological treatment efficiency [6]. It has been previously reported that WWTPs are the primary source of pharmaceuticals in river water [5].

To date, many reports have emerged on the occurrence of NSAIDs such as ibuprofen, naproxen, and diclofenac in South African environment [7–13]. However, there are

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currently few studies that have reported on the presence of ketoprofen in South African aquatic conditions [11–13]. With the view of preserving the precious resource such as water, there is a need to understand the extent of all widely used pharmaceuticals in the environment. South Africa has a large number of WWTPs that are mainly used for domestic wastewater treatment and their potential for removal of pharmaceuticals such as ketoprofen is not known. There is currently a lack of data on the toxicity of ketoprofen in aquatic life. To understand the risk of aquatic life and water consumers from suffering the health effects caused by pollutant levels, it is necessary to fully evaluate the occurrence of ketoprofen in water resources.

To address this problem, the development of highly sensitive and selective methods for trace determination of compounds such as ketoprofen in complex wastewater matrix is required. One of the most suitable methods of ketoprofen analysis is the use of chromatographic methods that are equipped with a very sensitive mass spectrometry detector [14,15]. However, the operation, maintenance and cost, of mass spectrometry detector is expensive. Therefore, some laboratories had opted for the use of a cheap and readily available UV–visible detector. The sensitivity of UV–visible detector is usually improved by the use of solid-phase extraction (SPE) for cleanup and preconcentration of target compounds [16].

In SPE, the most widely used extraction media for ketoprofen are Strata X, C_{18}, and Oasis hydrophilic lipophilic balanced (HLB) sorbents [17,18]. Although the application of these sorbents leads to the improvement of sensitivity, they often lack selectivity and their single use results in massive generation of solid waste. Nowadays, molecularly imprinted polymers (MIPs) are developed for SPE applications because of their properties that include high selectivity, reusability, mechanical strength, and resistance against acids, bases, and organic solvents [19]. The development and application of MIPs for the selective analysis of single NSAID such as ibuprofen and diclofenac is well documented [20,21]. The use of multitemplate MIPs for ketoprofen and several NSAIDs in wastewater analysis has been explored [22]. MIPs synthesized using the multitemplate approach are usually selective toward a group of compounds. However, these may not be useful in the analysis of a single analyte as it is important to obtain cleaner chromatograms which subsequently lead to more accurate measurements. Currently, there is a lack of available information for the synthesis of MIP that can selectively extract ketoprofen from aqueous samples.

Ketoprofen has been identified as one of the pharmaceutical drugs that contaminate Umgeni River which is found in the northern part of Durban city in South Africa [11,13]. With the exception of the work published by Madikizela et al. [12], there are currently no reports on the occurrence of ketoprofen in the southern region of Durban. Apart from these studies [11–13], there is currently no other South African study that has focused on the analysis of ketoprofen in water resources. Therefore, the aims of this study were to evaluate the occurrence and removal efficiency of ketoprofen in WWTPs located in the southern part of Durban city, South Africa. To achieve these aims, an MIP was synthesized, characterized, and applied in selective SPE of ketoprofen from wastewater before high-performance liquid chromatographic quantification.

2. Experimental section

2.1. Materials

Ketoprofen (≥98%), triclosan (≥97%), 2-vinylpyridine (97%), high-performance liquid chromatography (HPLC) grade methanol (≥99.9%), 1,1′-azobis-(cyclohexanecarbonitrile) (98%), ethylene glycol dimethacrylate (98%), and toluene (99.7%) were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC grade acetonitrile (≥99.9%) and glacial acetic acid (100%) were purchased from Merck (Darmstadt, Germany). Formic acid (approximately 98%), fenoprofen (≥97%), and HPLC grade triethylamine (≥99%) were purchased from Fluka (Steinheim, Germany).

2.2. Synthesis of MIP

Published work was adopted with slight modifications for the synthesis of MIP for ketoprofen [23,24]. Synthesis was carried out by mixing 25 mg of ketoprofen with 54 μL of 2-vinylpyridine. The mixture was stirred at room temperature in a 250 mL round-bottomed flask containing 10 mL of a acetonitrile/toluene (1:9, v/v) porogenic mixture for 30 min. Thereafter, the reaction vessel was placed on ice to prevent unwanted polymerization. Ethylene glycol dimethacrylate (4.77 mL) and 100 mg of 1,1′-azobis-(cyclohexanecarbonitrile) were added. The mixture was purged with nitrogen gas for 10 min, sealed, and stirred in an oil bath at 60 °C for 16 h to initiate polymerization. After 16 h, the temperature was increased to 80 °C and maintained for 24 h to achieve a solid monolith polymer. The polymer was dried to constant mass at 80 °C followed by grinding and sieving. Particles ranging from 25 to 90 μm were collected and washed repeatedly with a mixture of acetic acid/acetonitrile (1:9; v/v) until ketoprofen could not be detected in high-performance liquid chromatographic system. Nonimprinted polymer (NIP) was synthesized and treated likewise with the omission of ketoprofen in the reaction mixture.

2.3. Apparatus

HPLC system consisting of an online mobile phase degasser unit (model DGU-20A5), 20 μL sample loop, pump (model LC-20AT), and UV–visible detector (model SPD-20A) obtained from Shimadzu Corporation (Kyoto, Japan) was used. The mobile phase conditions consisted of a mixture of acetonitrile and 0.2 formic acid in water (60:40, v/v) at a flow rate of 1 mL/min. Separation was performed on a Gemini C_{18} HPLC column of 150 × 4.6 mm × 5 μm obtained from Phenominex (California, USA). Shimadzu liquid chromatography (LC) solutions software was used for data collection and processing. Detector wavelength was set at 255 nm.

For characterization, thermal analysis was performed using thermogravimetric analysis/differential scanning

calorimetry (TGA/DSC) 1 Star^e system obtained from Mettler Toledo (Columbus, USA). Thermograms were recorded using a heating rate of 10 °C/min from 25 to 700 °C, under nitrogen atmosphere at a rate of 100 mL/min.

Diffraction patterns (10–90°) of the synthesized polymers were determined using an X-ray diffraction (XRD) equipped with XRD commander for data collection and Eva software for processing. XRD system was obtained from Bruker AXS (Karlsruhe, Germany). Cu Kα radiation source at a rate of 2°/min was used.

Elemental analysis was performed using Series II carbon, hydrogen, nitrogen, sulfur and oxygen (CHNS/O) analyzer 2400 from Perkin Elmer (Llantrisant, United Kingdom).

For SPE, manifold was purchased from Phenominex (California, USA), and it was connected into vacuum pump that was obtained from Merck Millipore (Massachusetts, USA).

2.4. Sampling

Samples analyzed consisted of raw influent collected after wastewater screening for solid removal and final effluent sampled after disinfection of treated water with chlorine. Wastewater samples were collected once per week in the month of May in 2016 from Amanzimtoti (Global positioning system (GPS), S30.00749° E30.91720°), Kingsburgh (GPS, S30.07445° E30.85687°), and Umbilo (GPS, S29.84556° E30.89103°) WWTPs located around the city of Durban in KwaZulu-Natal Province of South Africa. The samples were transported into the laboratory, where they were filtered through a 0.22 μm syringe filter; thereafter, pH was adjusted to 5. Samples were refrigerated at 4 °C until analysis.

2.5. Preparation and application of SPE

Empty SPE cartridges (1 mL) purchased from Supelco (Bellefonte, USA) were mounted into the manifold and washed with methanol before their use. Thereafter, 14 mg of MIP particles were packed in between two polypropylene frits.

Water samples were percolated through the packed cartridge using the preoptimized SPE conditions. This was achieved by conditioning the cartridge with 1 mL of methanol followed by equilibration with 1 mL of deionized water. Fifty milliliters of wastewater sample (pH 5) was loaded at 1 mL/min. The cartridge was vacuum dried for 10 min, followed by washing with 1 mL of 5% (v/v) triethylamine in water. The retained ketoprofen was eluted with 1 mL of methanol and injected into HPLC.

After each use, the cartridges were regenerated by washing with 3 mL of deionized water and 3 mL of methanol.

2.6. Quality assurance

A stock solution of 100 mg/L ketoprofen was prepared in acetonitrile. The stock solution was further diluted to prepare the working standards. The working standards were analyzed using HPLC. Calibration curve, limit of detection (LOD), and limit of quantification (LOQ) were computed. The accuracy and precision of analytical method were validated using deionized water and wastewater samples that were spiked with ketoprofen at concentration levels ranging from 5 to 1000 μg/L.

3. Results and discussion

3.1. Characterization of polymers

Thermogravimetric analysis was performed for the washed MIP and NIP (Fig. 1). At 40 °C, the washed polymers had a mass loss of approximately 4%. This was probably because of the adsorbed methanol used in the template removal step [25]. Further thermal decomposition of polymers was observed at 290 °C which was marked as the temperature where the polymer backbone collapses. In a separate study, the polymer backbone collapsed at 250 °C for an MIP that was synthesized for 1,3-diisopropylurea [25]. It was further observed that at 425 °C, there was 100% thermal decomposition for the NIP, whereas the corresponding mass loss was 90% for the MIP. The difference might have been caused by structural variations that could have happened during template removal process.

Differential scanning calorimetry thermograms (Fig. 2) of the MIP and NIP were similar with endothermic peak at 355 °C which is associated with the temperature where there is complete thermal decomposition of the polymers. Similarities in the thermograms could be because of similar structural arrangements of the MIP and NIP. Structural similarities were further confirmed with X-ray diffractograms (Fig. S1). The lack of peaks in diffractograms is an indication of amorphous polymers.

The synthesized polymers were analyzed in terms of their carbon, nitrogen, and hydrogen contents using an elemental analyzer. As indicated in Table 1, the carbon and hydrogen contents were identical because of similar conditions in the synthesis. Also, this is an indication of a successful template removal as the presence of ketoprofen in the MIP could result in higher carbon and hydrogen contents. The sources of nitrogen in the polymers are the initiator and functional monomer used in synthesis. The differences in the nitrogen contents of polymers can be explained by the possible disruptions in the chemical

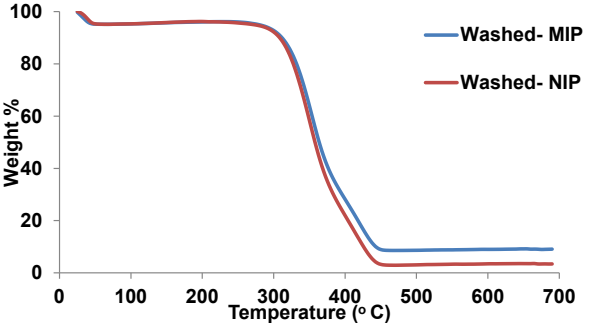


Fig. 1. Thermogravimetric analysis of the synthesized polymers.

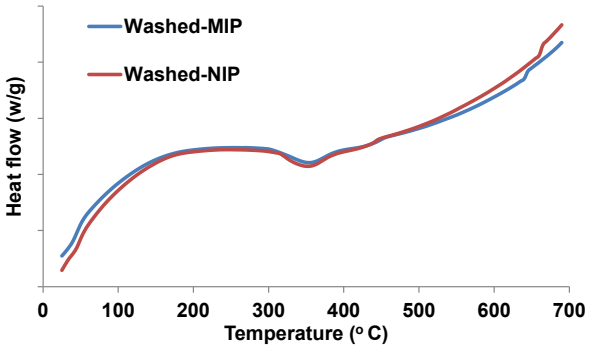


Fig. 2. DSC curves for the polymers.

Table 1
Measured percentage by mass of carbon, hydrogen, and nitrogen of the polymers using CHN analyzer.

Polymer	Carbon (%)	Hydrogen (%)	Nitrogen (%)
Washed MIP	58.28	7.39	0.33
Washed NIP	59.68	7.33	1.0

structure of the MIP during excess washing while removing ketoprofen. This was evident in the chromatograms obtained during template removal where there were unknown peaks recorded for the MIP solutions. Oxygen is the only other element (not quantified) known to be present in both polymers. The sources of oxygen in polymers are cross-linker and template used in polymerization.

3.2. SPE studies

3.2.1. Optimization

Optimization was carried out by using deionized water spiked with 1 mg/L of ketoprofen as the sample. SPE parameters investigated include sample pH, sorbent mass, sample volume, and washing solvents. Because the target compound is polar, methanol was used for its elution from the SPE cartridge. During optimization, only one parameter was changed at a time whereas keeping other parameters constant. Each experiment was conducted in triplicate, where average results are discussed.

For the adsorption of acidic pharmaceuticals, it has been reported that the extraction is based on hydrogen binding of the target compound and functional monomer (2-vinylpyridine) [26]. In this context, pH of the water solutions was adjusted to promote the monomer–template interactions. pH was investigated in the range of 3–7, where the highest recovery was obtained at pH 5 (Fig. 3). Therefore, pH 5 was selected as optimum and used in all subsequent experiments. At selected pH, the recovery for ketoprofen using NIP was 69.5% which could be because of the adsorption based on nonspecific interactions, whereas for the corresponding MIP, 88% was obtained because of molecular recognition. pH values greater than 7 were not investigated because of the deprotonation of ketoprofen which could lead to unavailability of adsorption sites. This behavior is somewhat similar to the behavior of other acidic pharmaceuticals on the MIP [27].

The quantity of the polymer for the optimum extraction of ketoprofen was investigated. It was discovered that low mass of the polymers resulted in poor recoveries which could be a result of maximum occupation of the binding sites. However, the accepted recovery was achieved by using 14 mg of the polymer (Fig. 4). It should be noted that this work was investigated using 1 mg/L of ketoprofen which is much higher than the concentrations expected in wastewater samples. Therefore, the amounts of polymers beyond 14 mg could not be investigated.

High sample volumes are often required in environmental analysis as they tend to lead to high preconcentration factors which in turn produce better sensitivity. The results in Fig. 5 show that the recovery increased from the sample volume of 10–20 mL for both polymers because of limited analyte interaction with the polymer as not enough volume was available to percolate the whole sample through the sorbent. Accepted recoveries (>70%) were achieved in the sample volumes of 20 and 50 mL for the MIP. However, 50 mL was selected as optimum as it leads to higher preconcentration factor. Recoveries decreased beyond the sample volume of 50 mL for the MIP which could be because of the capacity of the polymers being exceeded. Because of limited binding sites in the NIP, maximum recovery was obtained when the sample volume was 20 mL.

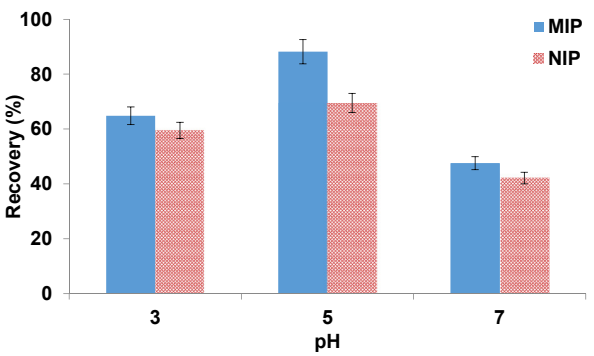


Fig. 3. Effect of pH on the recovery of ketoprofen from the polymers. Extraction conditions were as follows: sample volume, 20 mL; washing solvent, 1 mL of deionized water; and elution solvent, 1 mL methanol.

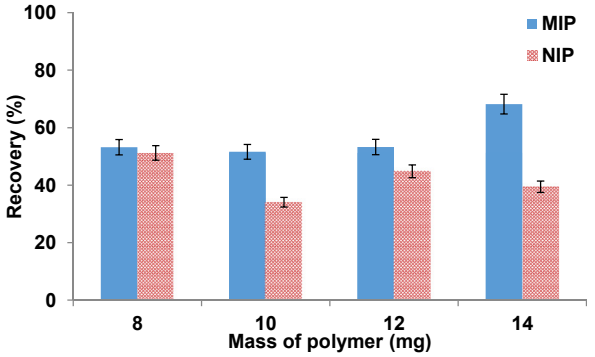


Fig. 4. Effect of polymer amount on the recovery of ketoprofen. Extraction conditions were as follows: sample volume, 50 mL; washing solvent, 1 mL of deionized water; elution solvent, 1 mL methanol; and pH, 5.

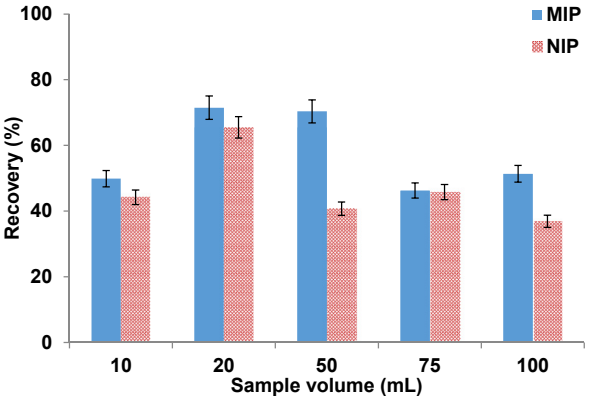
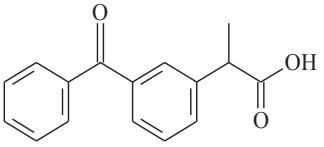
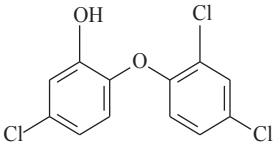
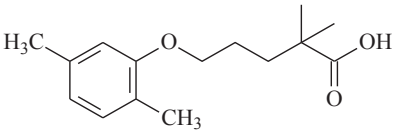
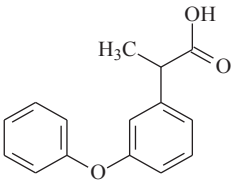


Fig. 5. Effect of sample volume on the recovery of ketoprofen. Extraction conditions were as follows: washing solvent, 1 mL of deionized water; elution solvent, 1 mL methanol; sorbent mass, 14 mg; and pH, 5.

Table 2
Molecular structures of ketoprofen and competitors.

Compound	Molecular structure
Ketoprofen	
Triclosan	
Gemfibrozil	
Fenoprofen	

3.2.2. Selectivity

The selectivity of the polymers was investigated using deionized water that was spiked with ketoprofen, fenoprofen, gemfibrozil, and triclosan. Fenoprofen and gemfibrozil are acidic pharmaceuticals that contain similar functional groups as ketoprofen (Table 2). These two pharmaceuticals are usually present in wastewater solutions that contain ketoprofen [28,29]. Triclosan is an antibacterial compound usually detected in wastewater with ketoprofen [12,30]. These competitors were expected to be adsorbed by the MIP through the formation of hydrogen bonds because of the presence of hydroxyl groups in their molecules.

The results show low recoveries (<20%) for all competitors which may be because of differences in their molecular shapes and sizes compared to ketoprofen. Therefore, the prepared polymers were highly selective toward ketoprofen (Tables S1 and 3). However, high ketoprofen recovery in the NIP could indicate a nonspecific adsorption which could be problematic considering the complex wastewater matrix. Therefore, selective washing was investigated in this case. Ten percent was recovered for triclosan when the MIP cartridge was washed with water, 17% was obtained for the corresponding NIP. Further washing solvents were investigated for selective removal of competitors which could result in cleaner chromatograms when the method is applied in wastewater analysis. Addition of organic solvents in the washing solutions resulted in elution of ketoprofen together with competitors for the NIP cartridges. This is a result of nonspecific binding. Therefore, the cartridges packed with NIP were discarded because of poor selectivity. However, in the case of the MIP, only competitors were removed during washing as a result of molecular imprinting. The introduction of acetonitrile in the washing solvent resulted in the reduction of ketoprofen recovery in the case of the MIP as well.

Table 4
LOD, LOQ, recovery (%), and RSD (%) values (n=3) achieved for the deionized water and wastewater influent and effluent spiked with ketoprofen.

Spiked sample	LOD (μg/L)	LOQ (μg/L)	Recovery (%) ± RSD (%)		
			1000 μg/L	25 μg/L	5 μg/L
Deionized water	0.09	0.30	68 ± 3	80 ± 17	114 ± 16
Influent	0.23	0.78	_	_	68 ± 10
Effluent	0.17	0.55	_	_	68 ± 15

3.3. Validation of the analytical method

The chromatographic analysis of ketoprofen was achieved on a reverse-phase stationary phase (Fig. S2). The performance of the proposed analytical method that uses MIP as a selective sorbent for SPE was validated based on

Table 3
Effect of washing solvent in SPE of ketoprofen using the MIP.

Compound	% Recovery				
	H_2O	MeOH/H_2O (5:95, v/v)	TEA/ACN (1:99, v/v)	TEA/H_2O (1:99, v/v)	TEA/H_2O (5:95, v/v)
Ketoprofen	69.06	67.61	33.39	107.85	103.98
Fenoprofen	5.81	1.98	2.49	1.17	2.68
Gemfibrozil	0.96	1.03	0.59	1.85	0.15
Triclosan	9.93	9.56	2.31	13.76	12.64

ACN, acetonitrile; H_2O, water; MeOH, methanol; TEA, triethylamine.

Table 5
Removal rates and concentrations (μg/L) ± standard deviations ($n = 3$) of ketoprofen in wastewater samples.

WWTP	Influent	Effluent	%Removal
Amanzimtoti	28.4 ± 1.1	3.50 ± 2.4	88
Kingsburgh	28.2 ± 12	3.40 ± 3.1	88
Umbilo	27.3 ± 0.57	2.90 ± 2.5	90

sensitivity, accuracy, and precision. A linear plot with R^2 greater than 0.99 was achieved for a seven-point calibration curve in the concentration range of 0.001–10 mg/L. On the basis of LOD and LOQ results shown in Table 4, the developed method is highly sensitive. LOQ and LOD are defined as concentrations that gave the signal-to-noise ratio of 10 and 3, respectively. Greater preconcentration factor in the proposed method led to better method sensitivity as compared with the data in the literature [31]. The results in Table 4 show that the analytical method was highly accurate as indicated with recoveries in the range of 68–114%. The relative standard deviation (RSD) given as ± values indicate that the developed method is precise.

3.4. Wastewater analysis

The developed procedure was applied in the SPE of ketoprofen from wastewater before high-performance liquid chromatographic quantification. Ketoprofen was detected in all wastewater samples (Table 5) with concentrations greater than 2 μg/L in the effluent, whereas the levels were much higher in the influent. Chromatograms were cleaner for the effluent when compared with influent samples because of effective wastewater treatment process (Fig. 6). Detected levels of ketoprofen were compared with previous concentrations reported locally and in other countries. Previous work [12] reported the concentration range of 1–6 μg/L in Amanzimtoti WWTP influent, whereas the present study reports the average concentration of 28.4 μg/L. The differences could be because of seasonal variations as the samples were collected from August to October in the previous work [12], whereas the samples of the present study were collected in May. Also, the variations in population dynamics might be another factor to be considered. Overall, the concentrations of ketoprofen

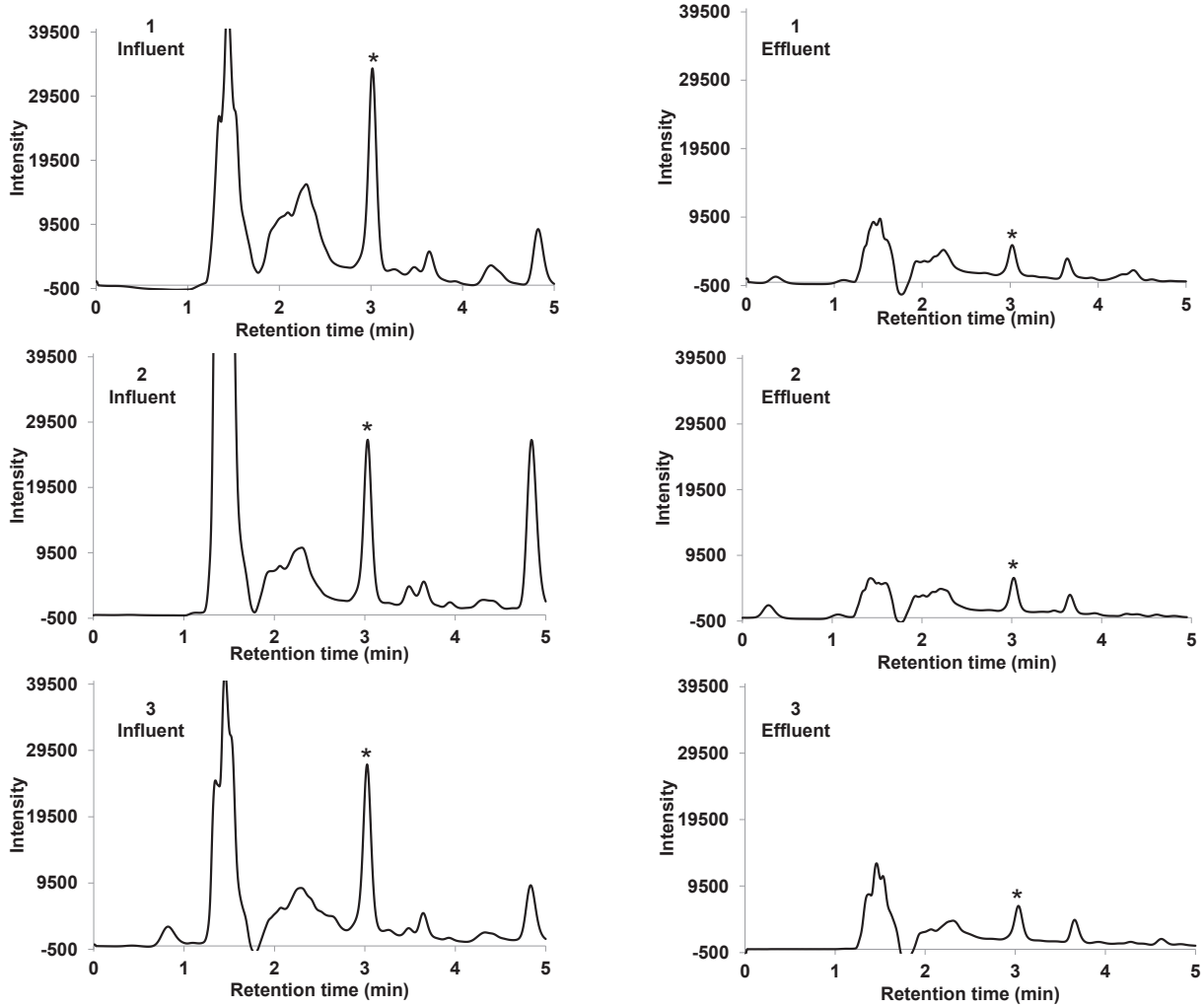


Fig. 6. Typical chromatograms obtained for wastewater influent and effluent collected from Kingsburgh (1), Umbilo (2), and Amanzimtoti (3) WWTPs. *Peak for the target compound (ketoprofen).

detected in these plants were higher than the levels reported in South Africa and the rest of the world (Table S2) because of differences in WWTP designs and consumption of the drug in foreign countries [32–36]. Most recently, ketoprofen is one of the pharmaceuticals that have been detected in wastewater and surface water from several countries including Algeria and China [6,37,38].

To the best of our knowledge, this is the first detailed study based on the occurrence and removal rate of ketoprofen in Amanzimtoti, Kingsburgh, and Umbilo WWTPs. Removal rates were determined based on the concentrations present in both influent and final effluent. Therefore, the performance of these WWTPs in terms of reducing ketoprofen during treatment process was compared with various plants in the world (Table S3). It was observed that the sampled WWTPs perform in a similar manner and sometimes better when compared with various plants for the reduction of ketoprofen during the wastewater treatment [30,39–41].

4. Conclusions

For the first time, a selective MIP for ketoprofen was synthesized and applied as the SPE sorbent. SPE technique was optimized and used with HPLC for the quantitative determination of ketoprofen in Umbilo, Amanzimtoti, and Kingsburgh WWTPs. For wastewater analysis, ketoprofen was detected in all samples. Concentrations of ketoprofen found in this study were higher when compared with what has been reported for WWTPs located in Europe. The removal rate of ketoprofen during domestic wastewater treatment was in the range of 88–90%. These results call for a detailed screening of ketoprofen in other South African water bodies including river and dam water. The analytical methodology used for wastewater analysis was fast, highly accurate, sensitive, and selective and gave results with good precision.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.crci.2016.09.006.

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