Preparation and Characterization of Methacrylic Acid-based Molecularly Imprinted Polymer as a New Adsorbent for Recognition of 1,4-dihydroxybenzene

Kehinde N. Awokoya[1*], Vincent O. Oninla[1], Iyanuoluwa T. Adeleke[1] and Jonathan O. Babalola[2]

[1]*Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.*
[2]*Department of Chemistry, University of Ibadan, Ibadan, Oyo State, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Author KNA designed the work and wrote the first draft of the manuscript. Author VOO co-designed the work, wrote the protocol and the final draft of the manuscript. Author ITA managed the analyses of the study. Author JOB is the research group leader and coordinated the present research that produced this manuscript. All authors read and approved the final manuscript.

Article Information

Original Research Article

ABSTRACT

This article presents the first example of sequentially reported template removal procedure in molecularly imprinted polymers (MIPs) using UV-spectrophotometer. Polymerization was achieved in a glass tube containing 1,4-dihydroxybenzene (DHB) template molecule, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), azobisisobutyronitrile (AIBN). The polymer matrix obtained was ground and the template molecule was removed from polymer particles by leaching with methanol/acetic acid, which leaves cavities in the polymer material. The polymer material both prior to and after leaching was characterized by Fourier transform infrared spectroscopy, Scanning electron microscopy, X-ray diffraction, and Brunauer-Emmett-Teller model. The rate of template

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Corresponding author: E-mail: knawokoya@gmail.com;

removal from the synthesized MIPs was studied at ten different times: 15, 30, 45, 60, 75, 90, 105, 120, 135, and 150 min. The percentage leached from the template increased sharply within 60 min, and over 90% of the template was removed within 75 min. The recovery of DHB achieved in aqueous solution, using the MIP sorbent, was found to range from 47.45 to 86.56%, while that of non-imprinted polymer (NIP) sorbent was found to range from 7.73 to 83.10%. The elastic retractive force between the polymer and solvent was found to be higher in chloroform than water and methanol. The study shows that MAA is a suitable matrix for the formation of binding sites for water soluble template molecules through molecular imprinting.

1. INTRODUCTION

Polymers are large molecules built up by the repetition of small, simple chemical units. Polymers can have different chemical structures, physical properties, mechanical behaviour, thermal characteristics, etc, and can be classified on the basis of origin as natural, derived, and synthetic polymers. Molecularly imprinted polymers (MIPs) are synthetic polymeric materials with specific recognition sites complementary in shape, size and functional groups to the template molecule, which is principally the imprinted molecule [1]. The synthesis of highly specific MIPs has been the goal of many research groups in recent times [2]. The template-monomer interaction can be through covalent interactions, non-covalent, and hybridization of covalent and non-covalent, which is also called semi covalent imprinting [3]. There is considerable attention in molecular imprinting due to its importance in a broad range of applications [4-5]. MIPs have been used as antibody and enzyme mimics in an increasing number of applications in which selective target recognition plays an important role. These include the use of MIPs in ligand binding assays, biomimetic sensors, and controlled organic synthesis. In all imprinting processes the template is fundamental in that it directs the organization of the functional groups pendent to the functional monomers. The template should have the highest number of polymerizable sites that will interact well with the monomer as much as possible [6].

However, before the imprinted material can be used in any application, the template molecules have to be extracted from the imprinted material [7]. More than two but not many extraction methods have been employed in the removal of templates from MIPs, these includes soxhlet extraction [8] washing/washing on-line [9] and solid phase extraction [10]. In recent times, accelerated solvent extraction (ASE) using various organic solvents containing acids or base additives has been reported [11] Ellwanger et al. [12] have also reported the removal of templates with supercritical fluid extraction, microwave-assisted extraction and ultrasonic assisted extraction. In addition, recent literatures have shown that all these removal techniques had impressive extraction efficiencies for template removal [7] but, no reports in the literature had shown the kinetics and rate of this removal. Studying the sequential removal would give a guidable explanation to the general comprehension on the chemical reaction rate at which the template is removed from the MIPs. To the best of our knowledge, this is the first scientific paper to sequentially report the removal of 1,4-dihydroxybenzene template and the adsorption properties of the methacrylic-based imprinted material. In this present work, a simple and efficient synthesis method, bulk polymerization, was employed to prepare the MIPs by using DHB as the template molecule. DHB is a suitable template for use in imprinting designs on account of its number of hydrogen bond interaction points (Fig. 1) suitable for forming non covalent interactions with the methacrylic acid, functional monomer.
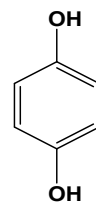
Molecular Weight: 110.11 g.mol^{-1}
Density: 1.3 gcm^{-3}
Appearance: White granular solid
Solubility in Water: Soluble
Chemical Formular: $C_6H_6O_2$

Fig. 1. Chemical structure and properties of 1,4-dihydroxybenzene

2. MATERIALS AND METHODS

2.1 Chemicals

Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), 1,4-dihydroxybenzene (DHB), azobisisobutyronitrile (AIBN), methanol (MeOH) and acetic acid were supplied by Sigma-Aldrich, Germany and used as obtained. Distilled water was supplied by Department of Chemistry, Obafemi Awolowo University, Nigeria. All chemicals used were of analytical grade.

2.2 Preparation of Molecularly Imprinted Polymer

The 1,4-dihydroxybenzene MIPs were prepared by bulk polymerization using DHB, MAA, EGDMA and AIBN as template molecule, functional monomer, crosslinker and initiator, respectively. DHB (400 mg, 3.63 mmol), MAA (1.6 mL, 18.96 mmol), EGDMA (17.5 ml, 92.79 mmol), and AIBN (5 mg, 0.02 mmol) were placed in distilled water (5 mL, 277.47 mmol) in a standard flask (scheme 1). The mixture was stirred, and transferred into a heating module. The temperature was increased from room temperature to 70 ^{O}C within 30 min, and then kept at 70 ^{O}C for 72 h. After polymerization process, the glass tube was broken and the resulting MIPs (brown solid polymers) was wiped free of the broken glass and the monolith was ground with a mortar and pestle. As control, a non imprinted polymer (NIP) was also prepared following the same procedure, except that the template molecule was omitted from the polymerization process.

2.3 Template Removal Method and Kinetics Consideration

Template was removed using the method described by Awokoya et al. [13] with slight modification. The polymer was thoroughly washed and stirred magnetically for 2.5 h with a mixture of MeOH and acetic acid (90:10, v/v). The reaction mixture was centrifuged and the supernatant liquids were tested until no DHB template was observed to leach from the polymer using a UV-vis spectrophotometer. Then, the polymer was washed several times with pure MeOH to remove the acetic acid and aid drying [14].

The rate removal of template molecule from the synthesized MIPs were determined at ten different times by washing the MIPs every 15 min (15, 30, 45, 60, 75, 90, 105, 120, 135, and 150) for 2.5 h and replacing the supernatant with fresh solution. The percentage leached of the template was calculated according to the Eq. 1.

$$\% \ Leached = \frac{Amount \ leached}{Amount \ loaded} \times 100 \qquad (1)$$
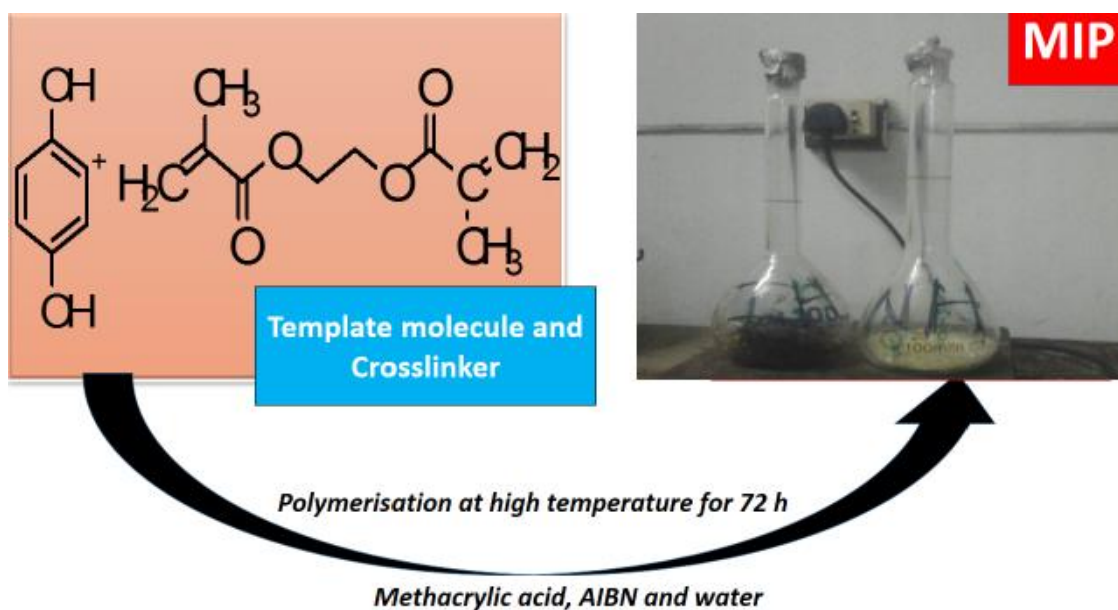
Scheme 1. Synthesis of MIP for DHB as template and methacrylic acid as functional monomer

2.4 Scanning Electron Microscopy (SEM)

Morphologies of the MIP and NIP particles were studied using a concise FEGSEM 6100 Zeiss ultra Plus Germany at an accelerated voltage of 20.0 kV with secondary electrons in low vacuum mode (LV). Prior to the SEM analysis, the samples were taken and dusted onto a carbon sticker, then coated with gold using a sputter coater for 30 min and then the images were recorded.

2.5 Brunauer-Emmett-Teller (BET) Surface Area and Pore Size Analysis

Gas adsorption analysis for both the MIP and NIP were carried out using Trister II 3020, Autosorb Automated gas sorption system analyzer (micrometrics, 2012 surface area and porosity, USA). The samples were first degassed under vacuum overnight to remove any adsorbed solvent. The adsorption isotherms of the degassed samples were then obtained using nitrogen as the adsorbate at a temperature of 77.35 K.

2.6 Fourier Transform Infrared (FT-IR) Spectroscopy

The Fourier transform infrared (FT-IR) spectra of the MIP and NIP particles were obtained using impact 330 series Nicolet Avatar FT-IR spectrometer model in the frequency range 4000 — 650 cm^{-1} using potassium bromide disks.

2.7 X-ray Powder Diffraction (XRD)

To get an insight into the structure of imprinted and non-imprinted polymers formed by using methacrylic acid functional monomer, powder XRD patterns were recorded using an X-ray diffractometer BRUKER D2 PHASER DOC-M88-EXX155 V4-07, 2010 Germany, using the Cu tube with 1.5418 (Å) radiation. Data were recorded during 2Θ range of 10°-90° under continuous scan mode using the scan rate of 4°/min.

2.8 Swelling Analysis

The solvent swell ratio was evaluated for water, chloroform and methanol based on a method described by Mashaelkar et al. [15] with slight modification. The MIP particle (300 mg) was packed in 1 ml syringe cartridges. The cartridges were each then filled with 1 ml of water,

chloroform and methanol respectively. After 6 h equilibration at room temperature, the weight of swollen polymer was measured. The swelling ratio (Sr) of the MIP was calculated using Eq. 2 [16] based on the volume of the dry polymer and volume of the wet polymer.

$$Swelling\ ratio\ (Sr) = \frac{volume\ of\ swollen\ polymer}{volume\ of\ dry\ polymer} \qquad (2)$$

2.9 Adsorption Studies

Adsorption test was carried out using the method described by Awokoya et al. [13] with slight modification. Adsorption of DHB was performed at room temperature by adding 30 mg of the leached MIP into10 mL of a DHB solution with initial concentrations of DHB in water ranging from 100 mg/L to 500 mg/L. After shaking for 5 h, MIPs were separated by centrifugation at 6000 rpm for 10 min, and the concentration of DHB in the mixed solution was determined by UV-vis spectrophotometer. Two replicate extractions and measurements were performed for each solution. The extraction percentage of hydroquinone was calculated from Eq. 3;

$$Extraction\ (\%) = \frac{c_i - c_f}{c_i} \times 100 \qquad (3)$$

where C_i is the initial DHB concentration (mg/L) and C_f is the DHB concentration at adsorption equilibrium (mg/L).

3. RESULTS AND DISCUSSION

### 3.1 Structural Characterization of MIP and NIP

3.1.1 SEM images

Scanning electron microscopy was employed to observe the morphological features of the MIP and NIP particles (Fig. 2). SEM is a technique which forms an image of a microscopic region of the polymer particles surface. From the SEM images, the results indicated that the nature of all the polymer particles (Fig. 2 a, b, and c) were all found to be irregular. As it could be seen, a flat and smooth surface was observed for the control polymer (NIP). On the other hand, if compared to the NIP, the unleached MIP exhibits a rough surface, which suggests a more accessible structure in the synthesized MIP. The comparatively rough surface and the appearance of perforations

in the leached MIP (Fig. 2a) can be attributed to successful leaching of the guest molecule during the washing process that might have generated the cavities in the MIP. Thus, more suited for extracting and adsorbing DHB template molecule.

3.1.2 XRD analysis

X-ray diffraction patterns of the leached MIP, unleached MIP, and NIP particles that were found to be similar are demonstrated in Fig. 3.

XRD method is a powerful tool for investigating the nature of polymer and extent of crystallinity present in the polymer. From the diffraction pattern results, it is obvious that the polymers contain both sharp as well as diffused peaks. Sharp peaks correspond to crystalline orderly arrangements of atoms through the interaction of electromagnetic radiation (structures are arranged in an orderly array or lattice), and diffused peaks correspond to amorphous regions. A series of characteristics peaks for all the polymers in the 2Θ range of $10 - 90°$
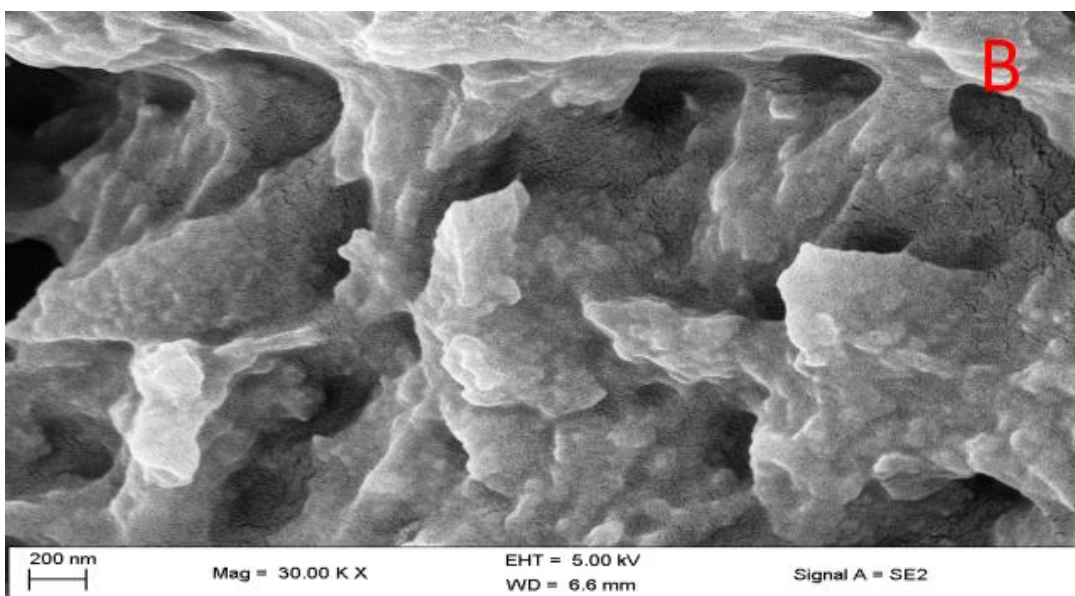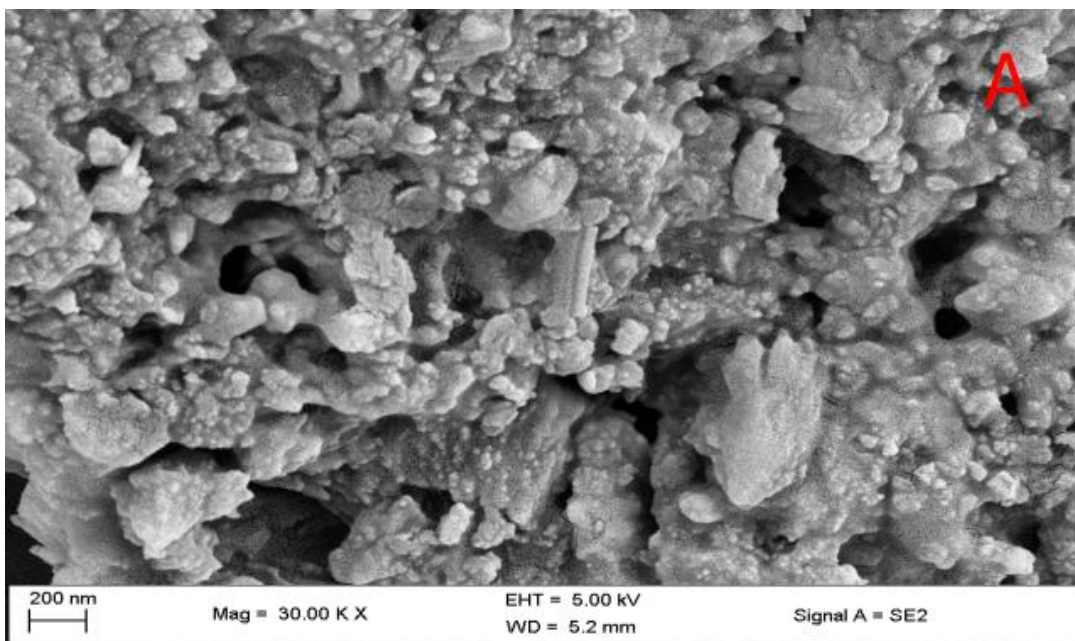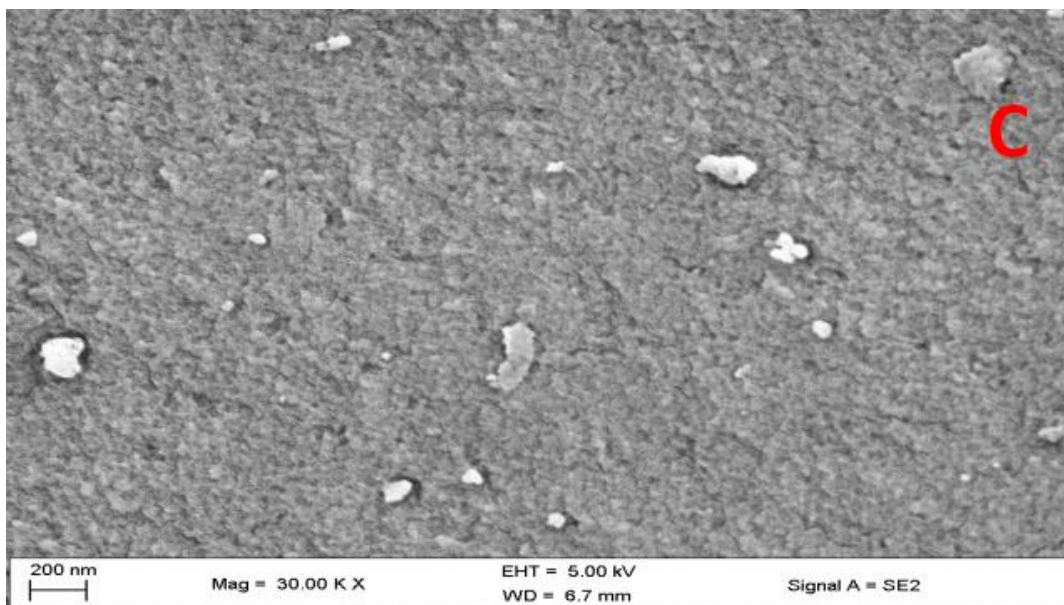
Fig. 2. SEM micrographs of the polymers: (A) leached MIP (B) Unleached MIP (C) NIP

were obtained at $2\Theta = 20°$, $30°$, and $43°$. The crystalline peaks at approximately $20°$ (2Θ) could be attributed to the anhydrous crystal size of the polymers. It was also observed that the intensity of diffraction at $20°$ (2Θ) was higher for NIP. In the corresponding unleached MIP, the relatively two weak diffraction intensity peaks occur at 2Θ values of about $20°$ and $43°$, while they have weakened further in the case of the leached MIP. This deflection is most possibly due to presence of numerous hydrogen bonds formed in the self-assembly process between the functional monomer and the template molecule for the MIPs. It could also be probably due to more homogeneity of NIP particle in comparison to MIPs. Similar observations have been reported by Saber et al. [17].

3.1.3 FTIR analysis

The FT-IR spectra of NIP, unleached MIP and leached MIP materials prepared using radical bulk polymerization are presented in Figure 4. FT-IR spectroscopy is useful for monitoring changes in the chemical environment resulting from the addition or removal of functional group in the imprinted polymer. Also can be applied to extract quantitative information on the composition of the polymers. Looking at the FT-IR spectra, all the polymers have comparable IR spectra indicating correlation in the backbone structure. Fig. 4a shows the FT-IR spectrum of synthesized unleached MIP. A broad peak was

observed at 3435.35 cm^{-1}, indicative of the existence of hydroxyl groups and thus, implies the successful imprinting of DHB template onto the MIP. After the removal of the template (Fig. 4b) i.e the leached MIP, the typical broad peak of OH existing in the spectrum of Fig. 4a shredded distinctly with significant decreased in intensity and the FT-IR spectrum became identical to that of the NIP (Fig. 4c). These results suggested that DHB has been sufficiently leached from the MIP. The characteristic peaks at 1155.40, 1159.26 and 1159.26 cm^{-1} (Fig. 4b, 4c and 4a) which correspond to C-N absorption confirmed the presence of azobisisobutyronitrile (initiator) in all the polymers. The absorptions due to carbonyl group stretch at 1724.42, 1726.35 and 1728.28 cm^{-1} (Fig. 4b, 4c and 4a) might have originated from methacrylic acid functional monomer and ethylene glycol dimethacrylate crosslinking monomer. All of these FT-IR bands proved that a sturdy and hardwearing MIPs had been successfully prepared.

3.1.4 BET surface area and pore characteristics

Determination of the specific surface areas and pore characteristics of imprinted polymer materials is crucial to express presence of reactive surface species. Table 1 displays the BET results for NIP and MIP. The surface area of NIP was 0.0276 m^{2}g^{-1} with low pore volume of 0.00075 cm^{2}g^{-1} and had average pore diameter

of 54.023 nm. Upon imprinting with DHB, there was an increase in the surface area (0.0740 m^2g^{-1}) and the pore volume (0.00125 cm^2g^{-1}) of the imprinted material with decrease in pore diameter of the MIP. The higher pore volume and surface area of the MIP compared to that of the NIP could results from the pore sockets and binding sites after DHB has been extracted. In line with IUPAC classification [18] both the MIP and NIP pore diameters belong to the macropore region, which is suitable for adsorption of DHB from aqueous media.

3.2 Swelling Studies of the MIP

The solvent effect on the swelling property was investigated in three different porogenic solvents: water, chloroform and methanol. A cross-linked polymer like molecularly imprinted polymers, when put in solvent, rather than dissolving, will sponge up portion of the solvent and in the end swell. As seen in Fig. 5, the elastic retractive force between the polymer and solvent was found to be more pronounced in chloroform compared to water and methanol. This result suggests that the degree of polymers swelling is dependent on the nature of the polymer-solvent interaction. Similar observations have been demonstrated by Crowther et al. [19].

Table 1. Pore characteristics of the imprinted and non-imprinted polymers

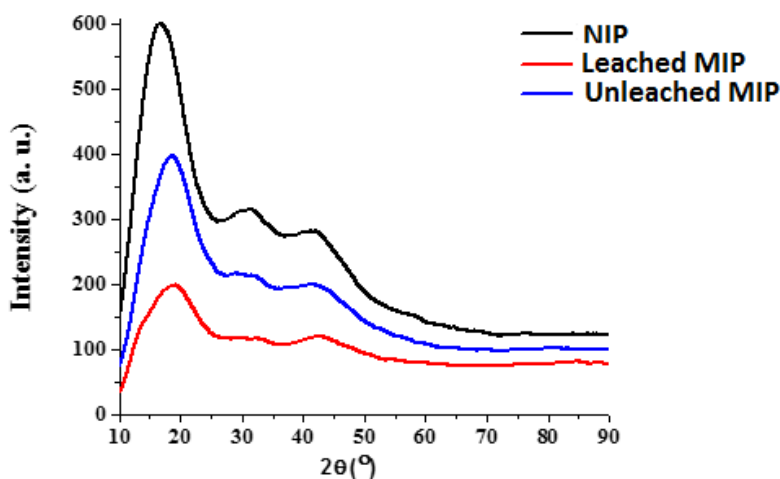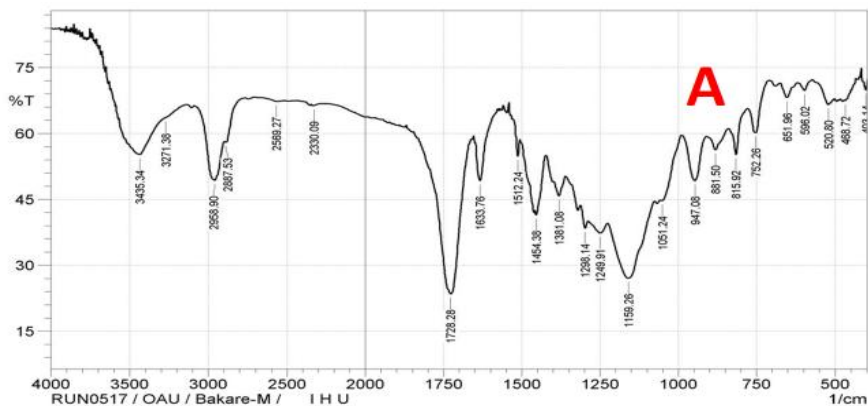| Sorbent	Surface area (m^2g^{-1})	Pore volume (cm^2g^{-1})	Average pore diameter (nm)
MIP	0.0740	0.00125	52.638
NIP	0.0276	0.00075	54.023

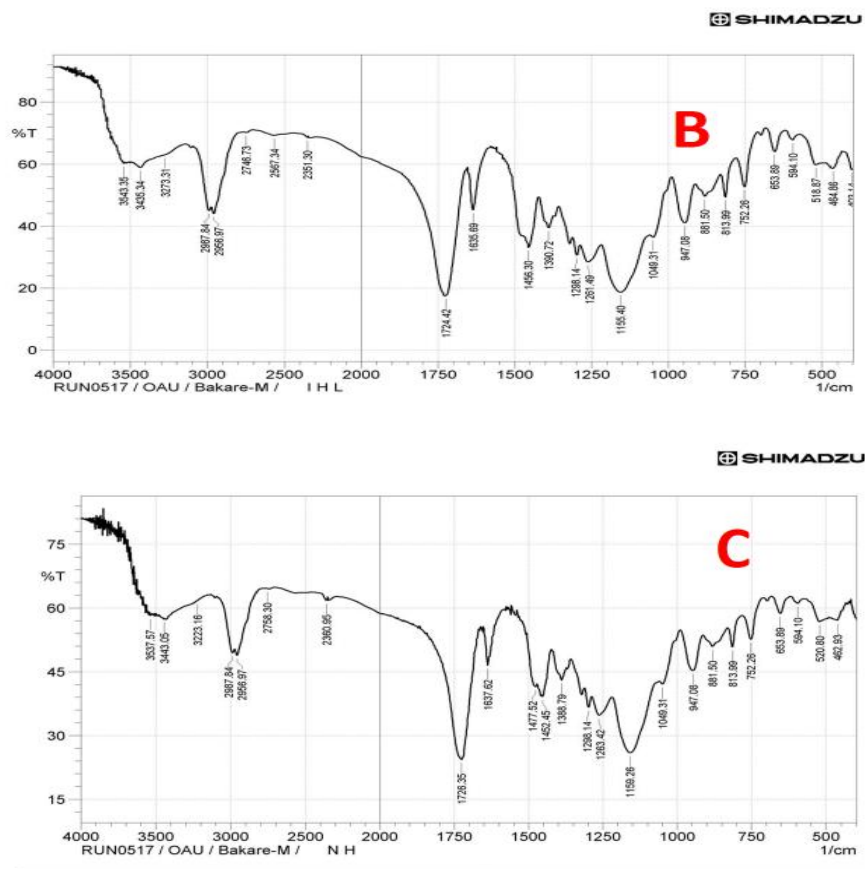**Fig. 3. XRD patterns of NIP, unleached MIP and leached MIP**

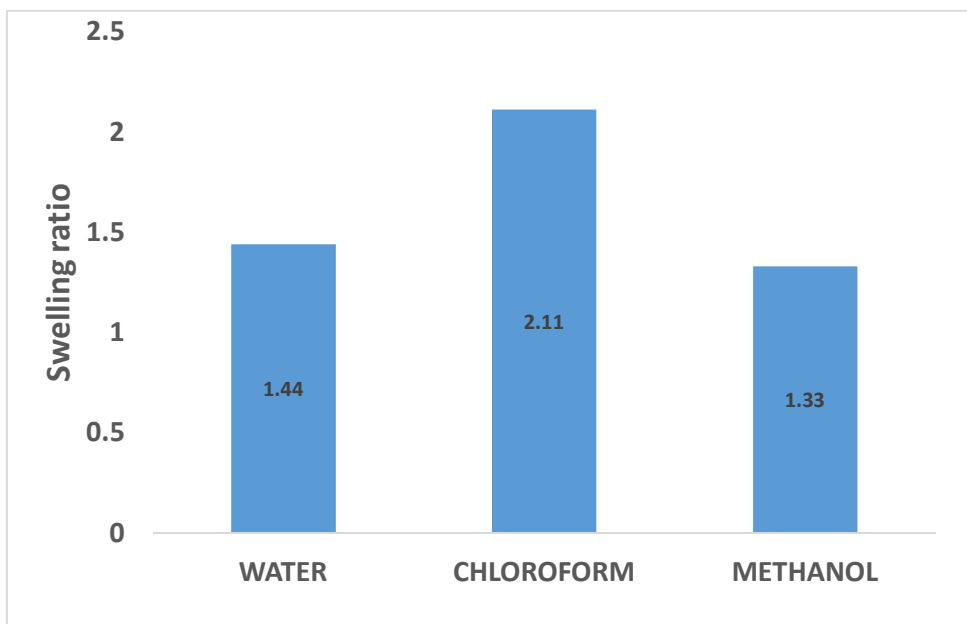**Fig. 4. FT-IR spectra for (A) unleached MIP (B) leached MIP (C) NIP**

Fig. 5. Swelling ratio of the MIP in different porogenic solvents

3.3 Kinetics Consideration of Template Removal

Template removal is a crucial step in the preparation of most molecularly imprinted polymers. When 400 mg of the template was loaded in the pre-polymerization mixture of the MIP, the percentage leached of the template from MIP using ethanol/acetic acid as leach liquors are shown in Fig. 6. The percentage leached from template increases sharply within the first 60 minutes, over 90% of the template was removed within 75 minutes. After 75 minutes the percentage leached of the template nearly remain constant. The result suggest that the complete process of leaching-off the template had been achieved in 75 minutes.
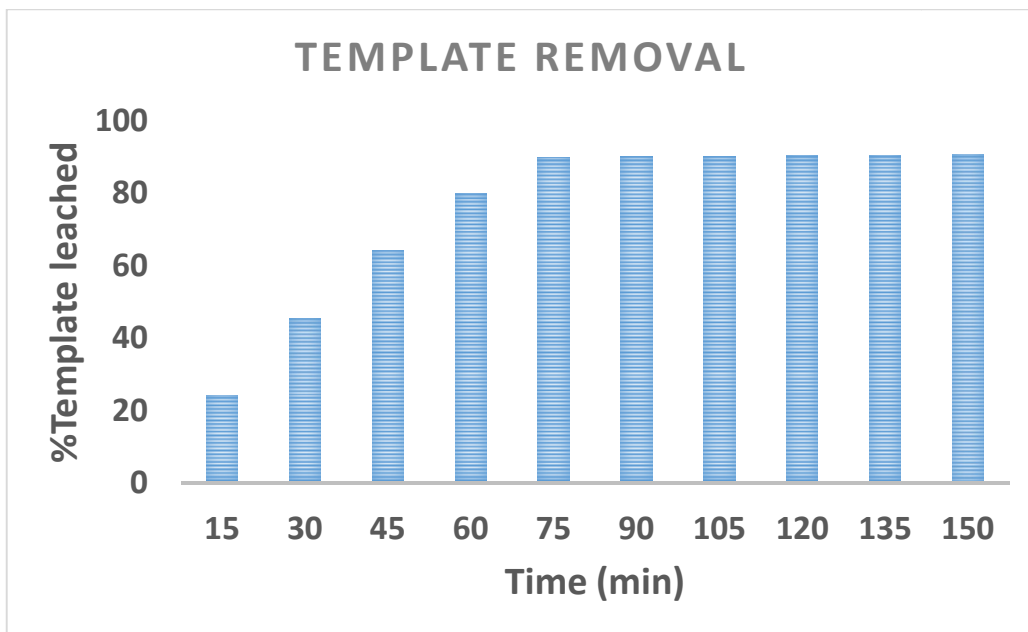

Fig. 6. Percentage template leached in each washing at 15 min intervals
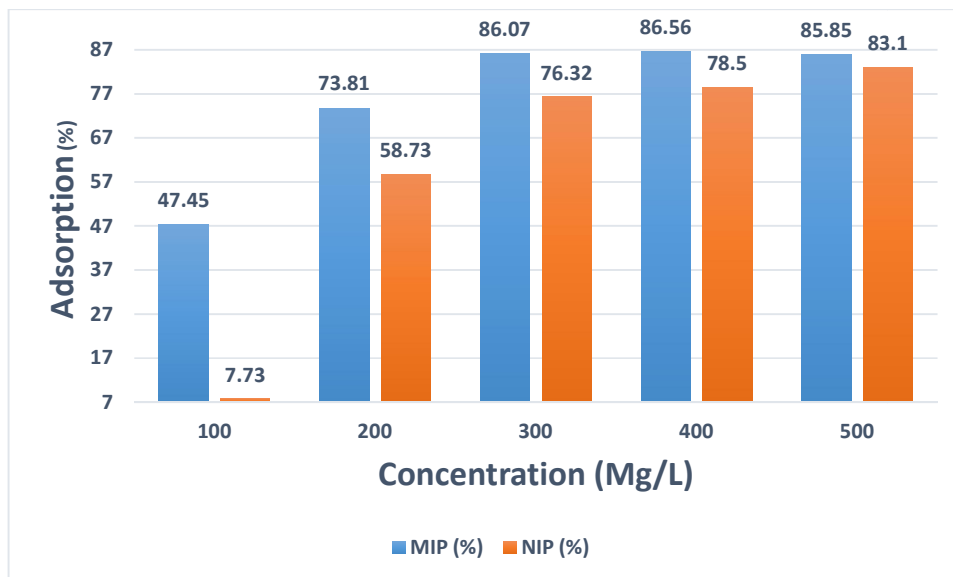

Fig. 7. Analysis of the rebinding of DHB unto MIP and NIP at room temperature for 5 h

3.4 Recognition Properties of DHB Imprinted Particles

To evaluate efficiency of MIP for selective separation and to determine the recognition properties of DHB, the concentration dependent removal of DHB was investigated. As shown in Fig. 7. It is obvious and evident that the ability of MIP to adsorb DHB was reasonably higher than that of NIP thereby confirming the existence of binding sites for DHB isolation on the MIP as compared to the NIP. As the concentration of DHB was increased from 100 mg/L to 300 mg/L, the adsorption capacity of the MIP was observed to increase from 47.45% to 86.07%, after which equilibrium was reached. On the other hand, the removal of DHB by the NIP was found to increase from 7.73% at 100 mg/L to 83.1% at 500 mg/L. Overall, it was observed that the adsorption percentage increases with increasing DHB concentrations. A plausible explanation for such observation might be associated to the fact that the increasing initial concentration could increase the touch of contact of surface liquid-solid, resulting in greater collision between the polymer (adsorbent) and DHB. The DHB binding by the NIP may be attributed to random binding due to physical adsorption and non-specific interaction of DHB molecules with functional groups in the polymer matrix [20].

4. CONCLUSION

This work has demonstrated that methacrylic acid based imprinted polymer is a promising adsorbent for removal of organic contaminants from aqueous media. The adsorbent exhibited good selectivity towards DHB. The amount of DHB adsorbed was found to vary with the initial concentration of DHB. It was also found that over 90% of the template was removed within 75 minutes. The elastic retractive force between the polymer and solvent was found to be more pronounced in chloroform compared to water and methanol. The imprinted sorbent had a well-defined morphology and crystalline orderly arrangements of atoms. The results also confirmed that the surface area of the MIP plays an important role in their binding properties. Based on the results, methacrylic acid-based MIP can be a feasible and effective sorbent for removal of phenolic compounds from aqueous media.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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History: http://www.sciencedomain.org/review-history/24025