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PREPARATION, CHARACTERISATION AND APPLICATION OF MOLECULARLY IMPRINTED POLYMERS FOR THE SELECTIVE REMOVAL OF STEROLS FROM WATER

Kessy F Kilulya
University of Dar es Salaam: Chemistry Department, PO Box 35061, Dar es Salaam, Tanzania
kefidel@gmail.com

ABSTRACT

Water quality of the existing freshwater bodies in many countries has declined due to day-to-day human activities which result into discharging different pollutants. This study therefore, aimed at developing a selective technique based on Molecularly Imprinted Polymers (MIPs) for the removal of toxic sterols from water.

Cholesterol-Molecularly imprinted polymers and their corresponding Non-imprinted Polymers (NIPs) were prepared using a non-covalent method and their physical morphologies were characterised using Scanning Electron Microscope (SEM), Fourier-Transform Infrared spectroscopy (FT-IR) and surface analyzer [Brunauer–Emmett–Teller (BET)].

The surface area obtained after optimization of the necessary parameters was 180.26 m^2/g for MIP and 132. 18 m^2/g for the corresponding NIP at a 1:8 template to monomer ratio. The FT-IR spectra of MIP and NIP were similar indicating the similarity in the backbone structure. The TGA profiles of the imprinted and non-imprinted polymers showed that polymers were thermally stable up to about 250 ^oC. However, thermal stability was observed to vary with monomer to template ratios. In terms of binding capacity, MIPs were observed to have higher binding capacity of sterols as compared to their corresponding NIPs, and they were able to remove more than 98% of sterols from aqueous solution prepared at an optimal initial concentration of 40 mg/L.

Keywords: Molecularly imprinted polymers, Sterols, water quality, GCxGC-TOFMS. toxic plant extractives.

INTRODUCTION

Freshwater resources in most of African countries, such as lakes, rivers, dams and groundwater are faced with increasing pollution due to the growing population with the corresponding growing economical activities enhanced by the high growth rate of socio-economic undertakings (Thomas and Durham 2003). Thus, the water quality of the existing freshwater bodies has declined due to different factors such as industry, mining, agriculture, urbanization and afforestation (Ashton et al. 2008). As a result, the general environment and freshwater bodies in particular receive the massive pollutants leading to the limited water resource unfit for both human and animal consumptions (Savenije and Van-der-Zaag 2008, Msagati and Mamba 2011, Kilulya et al. 2012a). Among the activities which contribute to freshwater pollution, industrial activities are the main contributors through their by-products being released into the environment and water bodies by discharging waste effluents loaded with various toxic organic pollutants (Msagati and Mamba 2011).

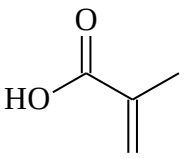
Plant extractives such as sterols and fatty acids are known for their different toxicities they exert on aquatic organisms (Orrego et al. 2010). These compounds normally find their way into water bodies once they are released from the plant cells as a result of any human activities that use plant as raw materials. These activities include agriculture, deforestation and industries. Pulp and paper industries are currently the major users of plants as raw materials for the production of paper and dissolving pulp. Literatures have reported that the process water highly loaded with plant extractives from pulp and paper industries are discharged into the environment and water bodies (Gutiérrez et al. 2001a, Gutiérrez et al. 2001b, Rencoret et al. 2007). It is very unfortunate that paper and pulp industries are mostly located close to water bodies for ease of water supply to the pulping process; as a result their effluents easily find their way to these water bodies (Gutiérrez et al. 2001b, Orrego et al. 2010).

Sterols are a component of plant extractives that have recently gained attention from different researchers due to the toxicities they exert on aquatic organisms (Orrego et al. 2010). Toxic sterols are among toxic plant extractives discharged into water bodies from different sources such as industries, agriculture, urbanization and municipal runoff. Sterols in aquatic environment are known to affect the development, growth and reproduction of aquatic organisms including fish (Kostamo et al. 2004, Meriläinen et al. 2006, Orrego et al. 2010). Although sterols are known to degrade in water bodies under aerobic conditions, they have an ability to bind to waterborne micro-particulates and hence deposit at the water-sediment boundary, and thus get into the anaerobic sediments where their degradation becomes very limited (Bartlett 1987, Sz´u´cs et al. 2006). Relatively higher levels of sterols in water bodies near pulp and paper industries and their effect on aquatic organisms have been reported (Meriläinen et al. 2006, Kilulya et al. 2012b). Moodley and co-workers (2003) reported on the existence of sitosterol among other organic compounds in effluents discharged from SAPPI Saiccor Pulp Industry, in South Africa (Moodley et al. 2003).

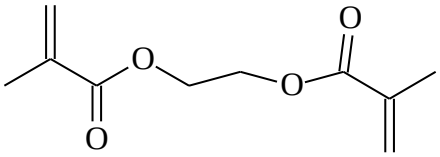
MIPs are a class of highly cross-linked polymer-based molecular recognition elements engineered to bind one target compound or a class of structurally related compounds with high selectivity (Alexander et al. 1999, Ramström et al. 2001). Selectivity is introduced during MIPs synthesis in which a template molecule, designed to mimic the analyte, is imprinted into the polymer matrix to guide the formation of specific cavities or imprints that are sterically and chemically complementary to the target analyte(s) (Hsu and Yang 2008). Extraction of the template after polymerization leaves imprints or binding sites in the polymer accessible to bind the analyte(s) of interest (Rachkov and Minoura 2001, Ramström et al. 2001, Cacho et al. 2008). MIPs have been successfully used in many fields such as separation processes (chromatography, capillary electrophoresis, solid-phase extraction and membrane separations), immunoassays, antibody mimics, sensors, catalysis, sorbents for removal of pollutants from contaminated water and artificial enzymes (Meng et al. 2005, Wang et al. 2006, Baggiani *et al.* 2007, Li et al. 2008, Schweiger et al. 2009, Yusof et al. 2013). MIPs can be prepared by self-assembly approach, where the prearrangement between the template and the functional monomers is formed by non-covalent interaction, and/or the pre-organized approach, where the aggregates in solution prior to polymerization are maintained by covalent bonds (Ramström et al. 2001). The non-covalent approach has

been mostly used, in which the template-monomer complex is formed by associating the target molecule with functional monomers such as methacrylic acid (MAA), acrylamide, vinylpyridines (**Figure 1**), dissolved in suitable solvent (porogen) (Lübke et al. 1998, Cormack and Elorza 2004, Mayes and Whitcombe 2005). MIPs have a number of advantages that include physical robustness, inertness towards organic solvents, acids and bases as well as easy preparation (Lin et al. 2008, Yusof et al. 2013).

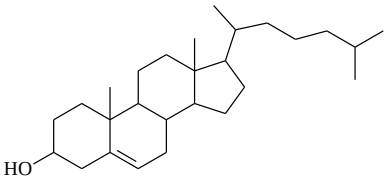
The characterisation of synthesized molecularly imprinted polymers is accomplished by investigation of the physical morphologies by using SEM to check the porous structure built up (Ulbricht 2004), FTIR to check the functional groups formed and surface analyzer (BET) to assess the surface area being built (Cormack and Elorza 2004, Shi et al. 2007).



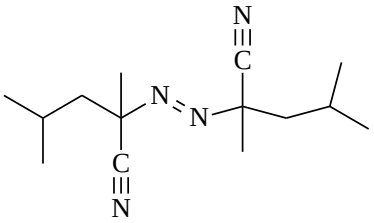
Methacrylic acid (MAA)



Ethylene glycol dimethacrylate (EGDMA)



Cholesterol



2, 2-Azobis(2, 4-dimethyvaleronitrile) (ABVN)

Figure 1: Chemical structures of the compounds used in polymerization

This paper therefore, reports the findings of a study designed to offer a solution to toxic sterols discharged into water bodies from industries. The study was designed to use MIP as a sorbent so as to specifically remove sterols from water avoiding the use of general sorbents which get saturated and deactivated easily due to the presence of non-targeted compounds and species that can be adsorbed. The prepared MIP is expected to be a more reliable sorbent for industrial effluent treatments before being allowed into water bodies due to their selectivity which enables the polymers to avoid competition with other occurring organic compounds and hence take longer time before they are saturated. Identification and quantification of the compounds was performed using two dimension GCxGC–TOFMS.

MATERIALS AND METHODS

Chemical Reagents and Standards

Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), 2,2-Azobis(2,4-dimethylvaleronitrile (ABVN), $C_{14}H_{24}N_4$), cholesterol (95%) (**Figure 1**), chloroform (99 – 99.4%), toluene (99.5 %), stigmasterol and HPLC grade methanol were all purchased from Sigma Aldrich (St Louis,

Mo, USA). Acetic acid (analytical grade quality 99.8 %) was supplied by Thomas Baker (Chemicals) (Mumbai, India). MAA was distilled under vacuum to remove the polymerization inhibitor (MHEQ) prior to use.

Experimental Procedures

Preparation of Cholesterol-MIPs

Initially two sets of imprinted polymers were prepared; one in chloroform and the other in the mixture of toluene/chloroform (7:1 v/v) as porogen solvents using cholesterol template. 0.0807g of cholesterol and 0.18 mL of functional monomer (MAA) were dissolved in 30 mL of porogen in round bottomed flask, then 1.87 mL of the cross-linker (ethylene glycol dimethacrylate, EGDMA) was added, and the mixture left to mix. Pre-polymerization solution was sonicated for 20 min and deoxygenated with nitrogen for 5 min, as free-radical polymerization is inhibited by the presence of oxygen. About 0.0612 g of 2,2-azobis(2,4-dimethylvaleronitrile) was added as initiator and the mixture allowed to polymerize thermally in an oil bath at 60 ºC for 24 h. The preparation protocol was that reported previously by Sellergreen, et al., 1998 with some modification. The resultant polymers were dried under vacuum at 50 °C for 12 h, then ground into powder and sieved using 45 µm sieves. Non-imprinted polymers (NIPs) were prepared in the same way as the imprinted polymers in the absence of template.

The template (Cholesterol) was then extracted using a soxhlet apparatus with methanol/acetic acid (80:20 v/v)) until no further template could be detected in the eluent by UV/Vis-spectrophotometer (**Scheme 1**). Finally, particles were washed with pure methanol to remove residual acetic acid, followed by distilled water to remove residual methanol, dried under vacuum at 50 °C and stored at room temperature in the desiccator before further use.

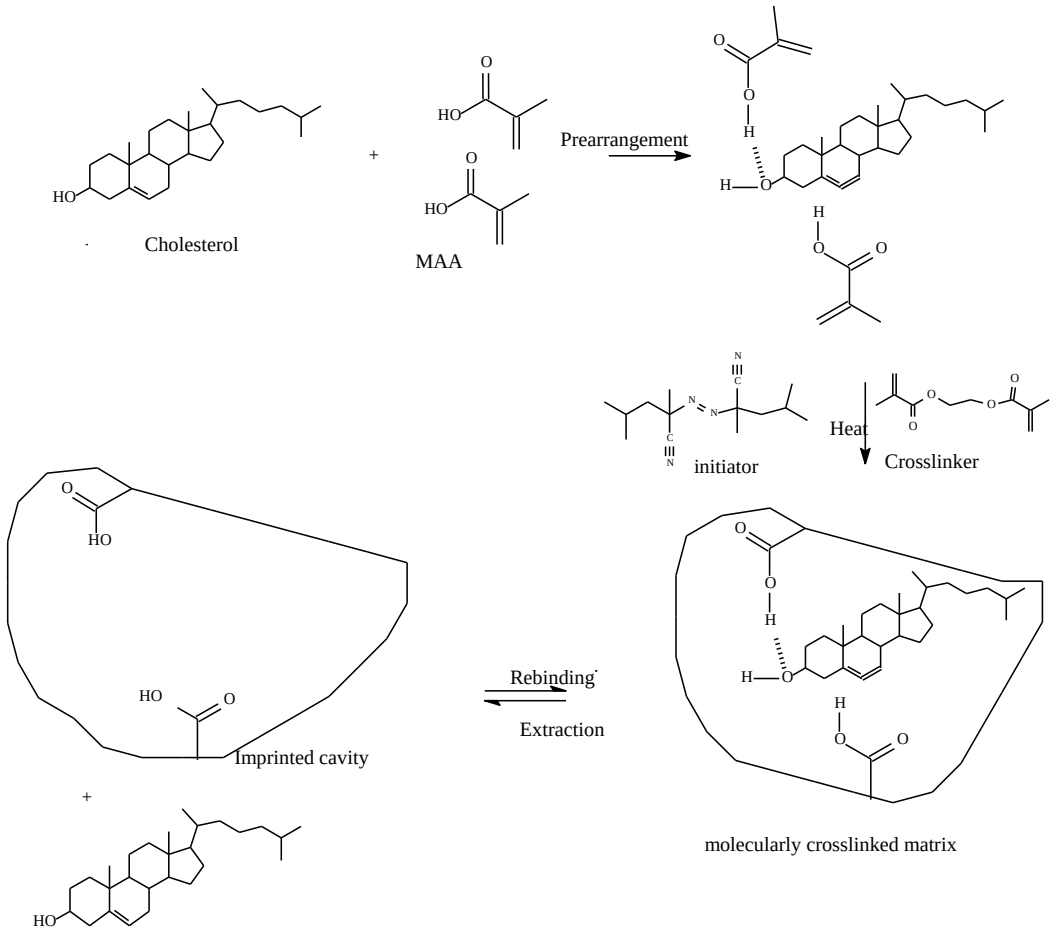
Characterization and Identification of the Polymers

FT-IR spectroscopy was performed using a PerkinElmer Spectrum 100 FT-IR spectrometer with attached diamond Attenuated Total Reflectance (ATR) accessory. Spectra were recorded from 4000 cm^{-1} to 650 cm^{-1} in transmittance mode with 4 scans per spectrum at a resolution of 4 cm^{-1}. The surface morphology of polymers was characterized by SEM Thermo Scientific, model 6658A-1NUS-SN, USA, whereas the determination of material's surface area was carried out using BET method.

Determination of the thermal stability of the produced MIPs and their corresponding NIPs was performed using TGA on a Perkin Elmer TGA 4000 Thermogravimetric Analyser. Sample aliquots of approximately 10 mg were placed in a platinum sample holder pan. TGA curves were recorded at temperatures ranging between 100 °C and 600 °C at a ramp-up rate of 10 °C/min under nitrogen flow rate of 20 mL/min.

Swelling Behaviour of the Prepared MIPs and NIPs

Swelling behaviour of the prepared MIPs and NIPs was determined using the method reported previously (Feás et al. 2009). Three solvents were used; water, methanol and dichloromethane (DCM) for swelling percentage determination in which about 40 mg of the polymers were immersed in the solvents at room temperature for 24 h. Then, the swollen polymers were first blotted with filter paper and weighed. The solvent uptake ratio (percentage of swelling (S(%)) of the polymers was then calculated.



Scheme 1: Preparation protocol of cholesterol-molecularly imprinted polymers.

Study on Template Rebinding Capacity of the Polymers

Evaluation of the binding capacity of the polymers (MIPs and the corresponding NIPs) was studied at 25 ºC in a thermostated water bath shaker by varying the concentrations (i.e., 0.2 to 1.6 mmol/L) of the template (cholesterol) that was exposed to 20 mg of polymers for rebinding in aliquots of aqueous solutions of 3 mL each prepared in duplicate.

Study on the Effect of Initial Concentration

Aliquots of aqueous solutions of 4 mL each were loaded with 100 mg of MIPs in duplicate and spiked with increasing concentrations of sterols (cholesterol and stigmasterol) in the following order; 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L and 60 mg/L. Aliquots were then placed on the auto shaker and shaken for 10 hrs at a speed of 185 rpm. Thereafter the samples were centrifuged at a speed of 7800 rpm for 10 min and the supernatant solution was decanted. The solution pH was adjusted to about 4 and extracted by 2 mL of hexane × 2

followed by 2 mL hexane:ethylacetate (2:1 v/v). The extraction was performed by vortexing and allowed the phases to separate followed by collecting the organic phases and combined. The organic phases were dried up using unhydrous sodium sulphate (2 full spatulas) per each aliquot. The solvents were evaporated to dryness. The dried extracts were then redissolved in 1 mL of HPLC grade hexane and filtered using syringe PTFE filters of 0.2 µm pore size for GC-TOFMS analysis.

Effect of Adsorbent Loading

After establishment of the optimal concentration then the effect of MIPs mass loading on the adsorption was investigated at a range of 20 mg – 140 mg by spiking the established optimal concentration of sterols (i.e., 40 mg/L) in 4 mL of aqueous solution and shaken for 10 h at an auto shaker at a speed of 185 rpm and then extracted as explained under the section of "study on the effect of initial concentration".

Investigation of Optimal Contact Time

After establishing the optmal loading mass and initial concentration, the effect of contact time was investigated using the template (cholesterol) at a time range of 1 – 8 h while keeping concentration and MIPs dosage constant (i.e., 40 mg/L and 100 mg, respectively).

GCxGC-TOFMS Conditions

The analyses of all samples were carried out using GCxGC-TOFMS (Pegasus 4D, LECO Corporation). Helium was used as a carrier gas whereas nitrogen, compressed air and liquid nitrogen for the operation of the quad-jet thermal modulator. The sample injector temperature was set at 280 ºC, and samples were injected at a volume of 1 µL with splitless mode. The flow of carrier gas was set at a rate of 1 mL/min. The GCxGC column set comprised of a 30 m, RXI-5Sil MS (0.25 mm internal diameter, 0.25 µm stationary film thickness) for the first column whereas the second column was 1.36 m, RTX-200 (0.18 mm internal diameter, 0.18 µm stationary film thickness). Temperature programme on the first column oven was set at 80 ºC held for 1 minute followed by an increase to 290 ºC at a ramping rate of 10 ºC /min and held for 5 minutes. The second dimension column oven temperature started at 90 ºC which was held for 1 min. then ramped to 300 ºC at a ramping rate of 10 ºC /min. and held for 5 minutes. The modulator interface was set at 15 ºC above the secondary oven temperature. The transfer line temperature was set at 250 ºC whereas the ion source temperature was set at 240 ºC. Electron impact ionization energy was set at 70 eV and the detector voltage was set at 1600 V. The analysis was carried out at mass range of 40 – 450 amu. The quantification of the data was performed using calibration curve in which cholesterol and stigmasterol standard solutions were used to obtain the calibration curve.

RESULTS AND DISCUSSION

Four sets of cholesterol imprinted polymers (MIPs) and non-imprinted polymers (NIPs) were prepared using non-covalent method (**Scheme 1**). The four polymers were synthesized by varying the template to monomer ratios and solvent composition, because these are the key factors for the formation of suitable MIP adsorbents for maximum binding capacity and selectivity of the analytes of interest.

Characterization of MIPs and NIPs

The physical appearance of MIPs formed by chloroform and toluene/chloroform solvents was a bit different. The one formed using chloroform was a rigid solid while that formed in toluene/chloroform was in a powdery form. This indicates that the role of solvents in the polymer synthesis process is

more than dissolving the components of polymerisation.

Differences in morphology between the MIPs and NIPs were observed using SEM as well as BET. Generally, NIPs had a smoother structure with small surface area than those of the MIPs (**Figure 2**), which indicates that the increase of surface area of MIPs was because of imprinting. This is consistent with the results reported by Shi, et al., 2007, who observed that MIPs tend to have higher surface area than that of the corresponding NIPs. Surface area analysis was used as a base for the selection of a suitable monomer to template through the comparison of the surface area as shown in Table 1. The template to monomer ratio is a vital factor on the number and quality of the resulting molecularly imprinted polymer recognition sites. From Table 1, it was observed that the template to monomer ratio has significant effect on the surface area of the prepared polymers. It was therefore, noted that the solvent composition used affects the morphology and the surface area of the polymers to a large extent as it was exemplified by the use of chloroform and the mixture of chloroform: toluene.

Table 1: Comparison of the morphological characteristics of the MIPs and NIPs prepared by varying template to monomer ratios.

Polymer	Porogen (Solvent)	Template to monomer ratio	Surface area (m^2/g)
MIP1	Toluene:Chloroform 7:1 % v/v	1:10	9.20
NIP 1	Toluene:Chloroform 7:1 % v/v	1:10	18.53
MIP 2	Chloroform	1:10	97.60
NIP 2	Chloroform	1:10	55.44
MIP 3	Chloroform	1:6	179
NIP 3	Chloroform	1:6	236
MIP 4	Chloroform	1:8	180.26
NIP 4	Chloroform	1:8	132.18

The MIPs and NIPs obtained from chloroform: toluene had very low surface areas. Based on the surface area values it was observed that MIP4 and NIP4 were suitable as sorbents since they registered a relatively higher surface area for the MIPs and good difference between the surface areas of MIPs and the corresponding NIPs which is the key factor for good performance of MIPs.

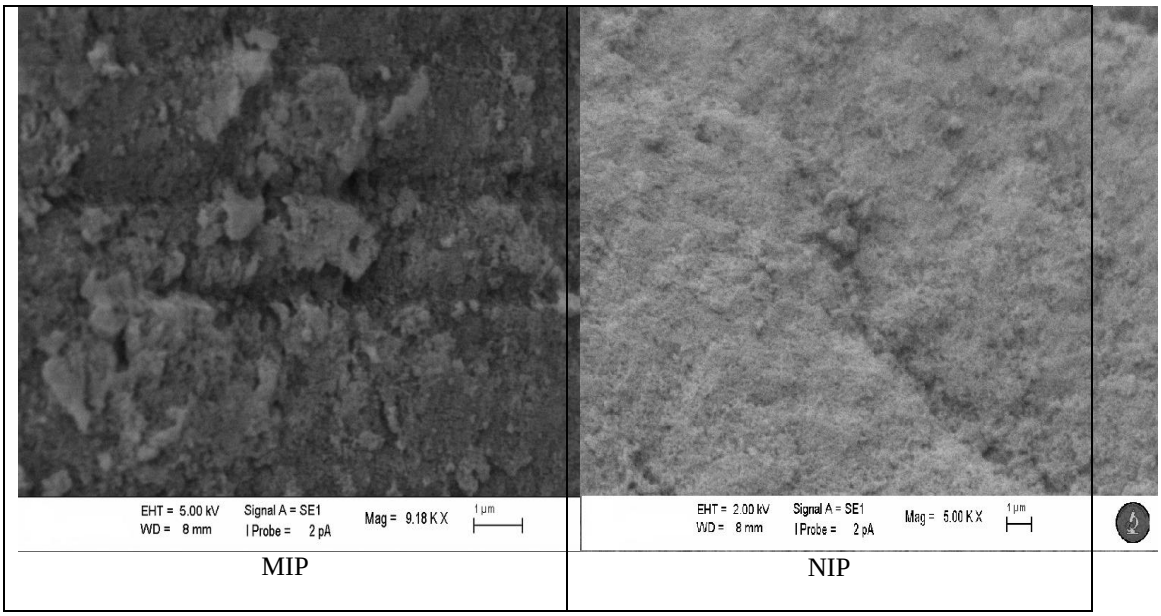


Figure 2: SEM micrographs MIPs and NIPs (represented by MIP4 and NIP4) showing morphology of the polymers

FT-IR spectroscopy analysis of imprinted, non-imprinted and template (cholesterol) materials were performed (**Figure 3**) to study the functional groups formed. The spectra of molecularly imprinted polymers and non imprinted polymers were similar and this indicated the similarity in the backbone structure. Absorptions due to carboxyl OH stretch was observed at about 3500 cm^{-1}, carbonyl group stretch at about 1721, C-O stretch at about 1249 while C-H vibrations were observed at 756, 1387, 1453 and 2956. The differences between the cholesterol (template) and that of molecularly imprinted polymer (MIP) FTIR spectra revealed that the template was fully removed from MIP during the extraction stage. Apart from the above information from FTIR spectra it was also observed that absorbance for C-H stretch of methylene group at about 2956, carbonyl group stretch at about 1721, C-O stretch at about 1249 and C-H bend of $–CH_2$ at about 1453 for the molecularly imprinted polymers were relatively stronger than those of the corresponding non-imprinted polymers,

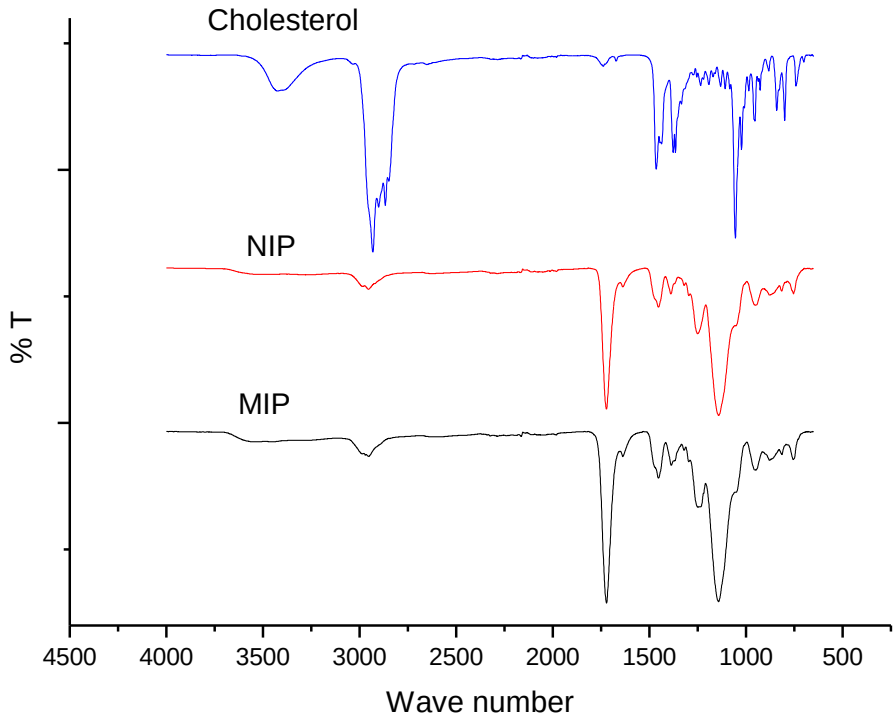


Figure 3: FT-IR spectra of NIP, MIP (represented by NIP4 and MIP4) and Cholesterol in the 650 – 4000 cm^{-1} range.

Thermal stability of the polymers was studied using a Thermogravimetric analyzer (TGA). The investigation of thermal stability of the polymers was important in order to establish the difference in decomposition stage between MIP and NIPs prepared at different monomer to template ratios as well as to establish the polymer stability when used at high temperatures. The TGA profiles of imprinted and non-imprinted polymers showed that the synthesized polymers were thermally stable up to about 250 ºC. Thermal decomposition started after 250 ºC with slight different trends based on the template to monomer ratio (**Figure 4**). This result agrees with the findings which were reported by Levchik et al., 2008, who reported that cross-linked polymers are thermally stable up to 247 ºC and serious decomposition begins at 274 ºC (Cummins et al. 2008).

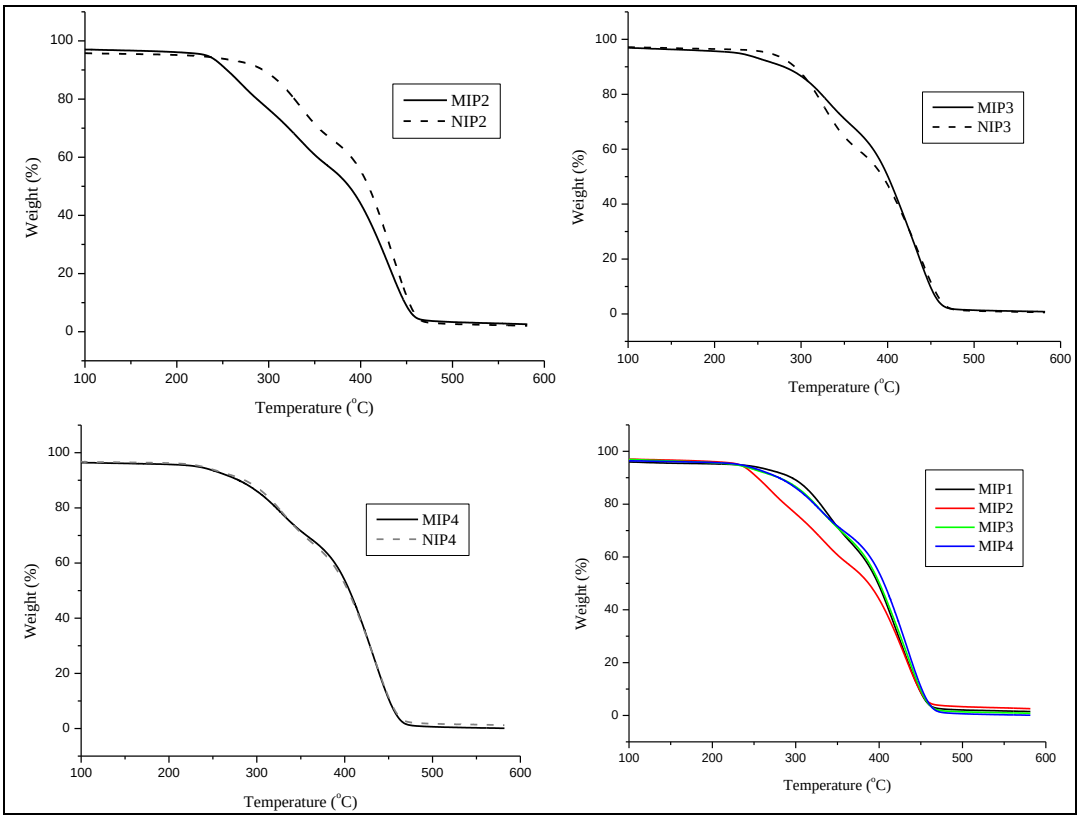


Figure 4: Comparison of TGA profile between MIPs and NIPs prepared in different template to monomer ratios

It was further observed that there was a variation in thermal stability as the ratios of monomer to template varied. For instance, it can be observed from Figure 4 that TGA profile of MIP4 and NIP4 was exactly the same throughout the temperature range unlike for MIP2 and NIP2 in which the decomposition of NIP2 was gradual while that of the corresponding MIP was rapid. This can be explained by the fact that MIPs have higher surface areas as well as a significant number of cavities which might have contributed to the rapid thermal decomposition. The thermal decomposition of NIP2 and MIP2, revealed a relative higher thermal stability of the NIP as compared to the corresponding MIP, in which for instance, NIP2 started decomposing at about 300 °C whereas the corresponding MIP2 started decomposition at 250 °C. This may be attributed to the particular monomer to template ratio as it was the only varied factor.

Swelling Behaviour

Solvent swelling factor, S%, describes the percentage of solvent held intrinsically by the polymers. Swelling is a vital parameter of molecularly imprinted polymers as it has influence on the polymers' binding capacity (Booker et al. 2011). This is due to the fact that the efficiency of the functional polymer is controlled by the accessibility to its reactive functional groups which is

influenced by the extent of swelling as it affects the diffusion speed of a reagent in the polymer (Lorenzo et al. 2011). Generally, the uptake of water by the polymers was higher compared to that of methanol and dichloromethane (DCM), where as the least uptake ratio was observed for DCM as shown in Table 2 and Figure 5.

Table 2. Swelling behaviour (n = 3)

	Water, S_r / %	**Methanol, S_r / %**	**DCM, S_r / %**
MIP3	59.57 ± 6.60	59.21 ± 9.94	20.93 ± 1.13
NIP3	72.03 ± 0.25	50.13 ± 2.58	20.44 ± 3.69
MIP4	49.16 ± 6.72	28.45 ± 3.08	14.29 ± 6.89
NIP4	51.34 ± 6.78	54.04 ± 4.78	12.86 ± 2.56

Swelling ratio, S_r (%) was calculated using the formula below:

$$S_r(\%) = \frac{(M_s - M_0)}{M_0} \text{ x } 100$$

Where, M_s is the mass of swollen polymer and M_0 is the mass of dry polymer (Feás et al. 2009) .

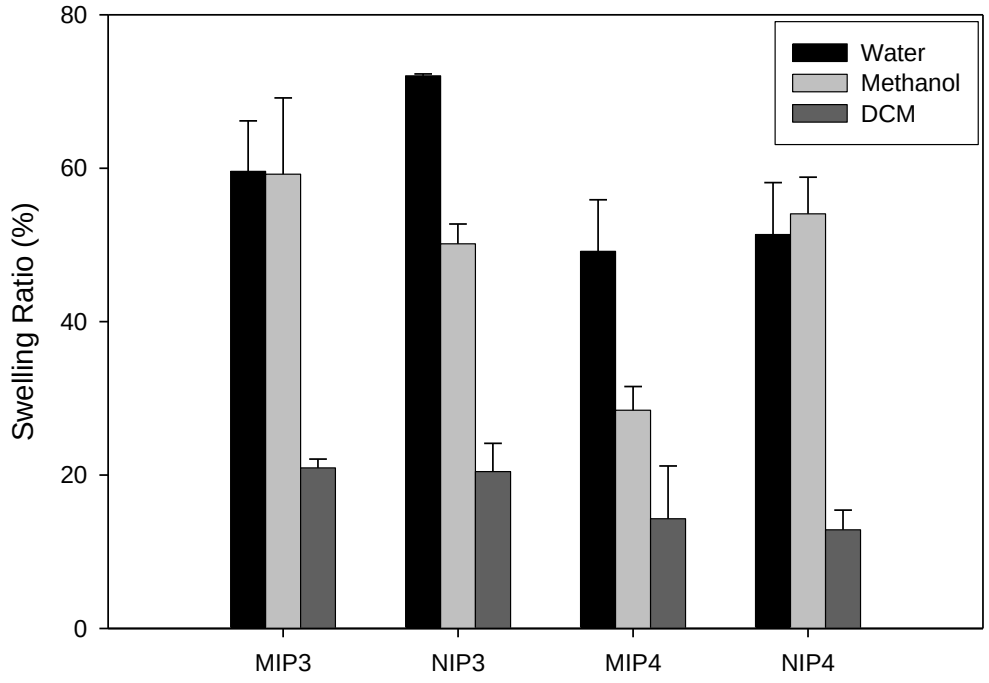


Figure 5: Swelling behaviour of MIPs and their corresponding NIPs as represented by MIP 3 & MIP4 whereas NIPs are represented by NIP3 & NIP4.

Evaluation of Template Rebinding

Evaluation of the binding capacity of the polymers (MIPs and the corresponding NIPs) and their saturation behaviour was studied at 25 ºC in a thermostated water bath shaker. The binding capacity of cholesterol by both MIPs and NIPs were observed to reach equilibrium with increase in concentration. The adsorption trend observed in NIPs indicated the occurrence of non-specific binding. However, comparing the binding capacity of MIPs and that of NIPs it was observed from Figure 6 that cholesterol imprinted polymers have higher affinity for cholesterol compared to the non-imprinted polymers (NIP). It is quite clear that the binding of cholesterol by MIPs was guided by the cavities formed by the imprinting process. The binding capacity (Q) of the polymers was calculated using equation 1 (Wizeman and Kofinas 2001, Trehan et al. 2013).

$$Q = \frac{(C_i - C_f)\, V_s}{m_{MIP}} \quad (1)$$

Where C_i (µmol/mL) is the initial cholesterol concentration, C_f (µmol/mL) is the free concentration (final concentration), V_s (mL) is the volume of test solution and m_{MIP} is the mass of dried polymer (g).

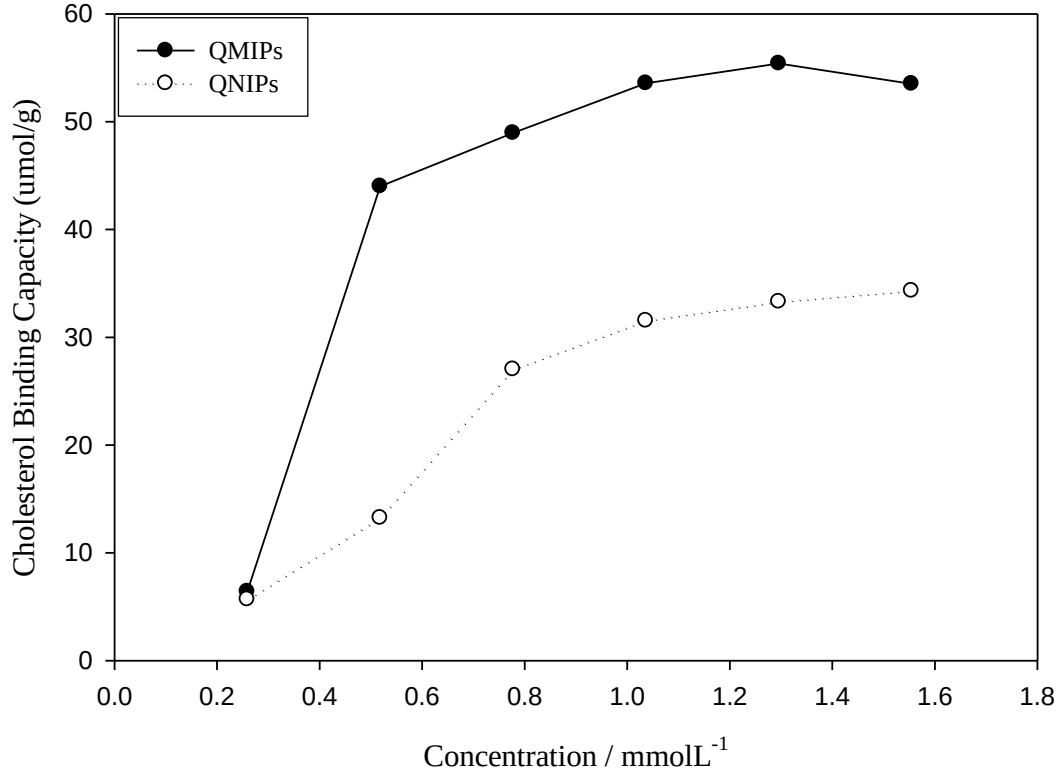


Figure 6: Binding profile of MIPs and NIPs for cholesterol from water

The evaluation of the rebinding capacity of MIPs was very important so as to establish the difference in adsorption capacity between the imprinted and non-imprinted polymers. However, the observed performance of rebinding was not conducted under optimal conditions and hence, after the establishment of the differences in cholesterol affinities between the polymers, then the investigation of optimal conditions for the important parameters such as initial concentrations, polymer loading mass and contact time was performed while simultaneously studying the ability of the polymer to remove sterols represented by stigmasterol and cholesterol from freshwater (prepared aqueous solution).

Effect of Initial Concentration on MIPs Adsorption of Sterols

The effect of initial concentration of sterols on the adsorption capacity of the prepared MIPs was performed to establish the concentration at which the polymer might work optimally. The observed performance of the polymer on the adsorption of cholesterol and stigmasterol indicated similar pattern with increasing concentration as shown on Figure 7. It was observed from the plot that the amount of sterols adsorbed increased up to 40 mg/L where it reached equilibrium. Thus, the prepared polymer materials can work efficiently at a concentration which does not exceed 40 mg/L. The attained equilibrium with increase in concentration of sterols indicates the saturation of the MIPs cavities by sterol molecules (cholesterol and stigmasterol). For adsorption studies the amount of adsorbed sterols (cholesterol and stigmasterol) to the polymers was obtained by subtraction of free concentration (C_f) from the initial concentration (C_i) of sterols (Spivak 2005).

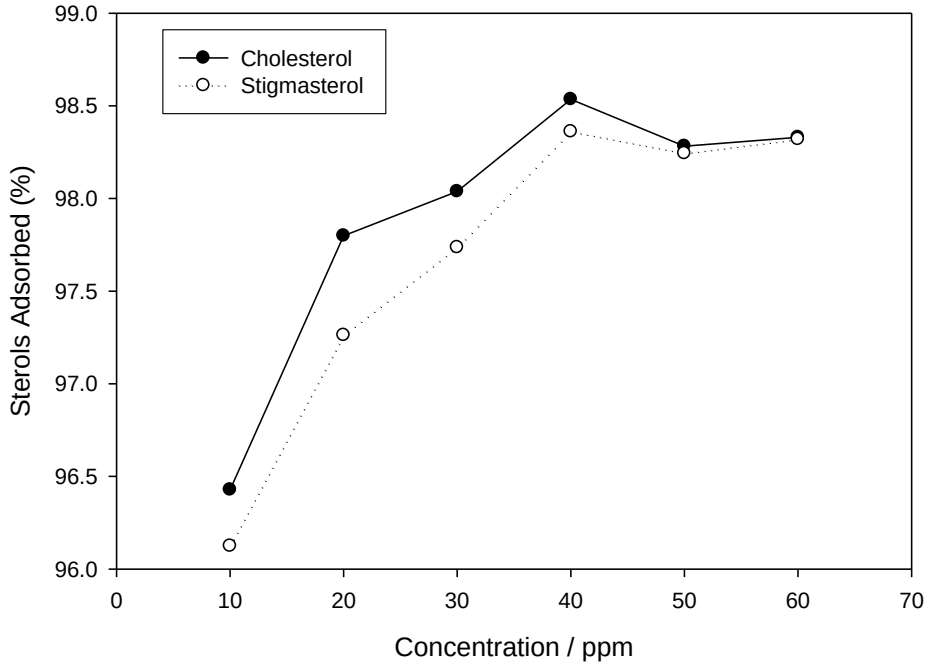


Figure 7: Effect of Initial Concentration

Effect of MIPs Loading Mass on the Removal of Sterols from Water

After obtaining the optimal concentration on the effective absorption of the sterols in the MIPs, the optimal loading mass on the adsorption was investigated by varying the amount of MIPs from 20 to 140 mg, while keeping concentration and contact time constant. Hence the optimum value for loading mass was found to be 100 mg as shown on Figure 8. Observing from the Figure, it was generally found that the binding affinity increased from low dosage to higher polymer dosage and that comparing the two sterols it was obvious that the affinity of MIP was relatively higher for cholesterol as compared to that of stigmasterol. This can be explained by the fact that cholesterol was used as a template for the preparation of imprinted polymers of which its structure fits exactly the formed cavities as compared to that of stigmasterol. Generally, the prepared MIPs have shown higher recognition capacity of sterol structures and hence suitable for removing sterols from freshwater. The observed higher affinity of prepared MIPs for cholesterol than that of stigmasterol is in agreement with what was reported by Kugimiya and co-workers in 2001 (Kugimiya et al. 2001).

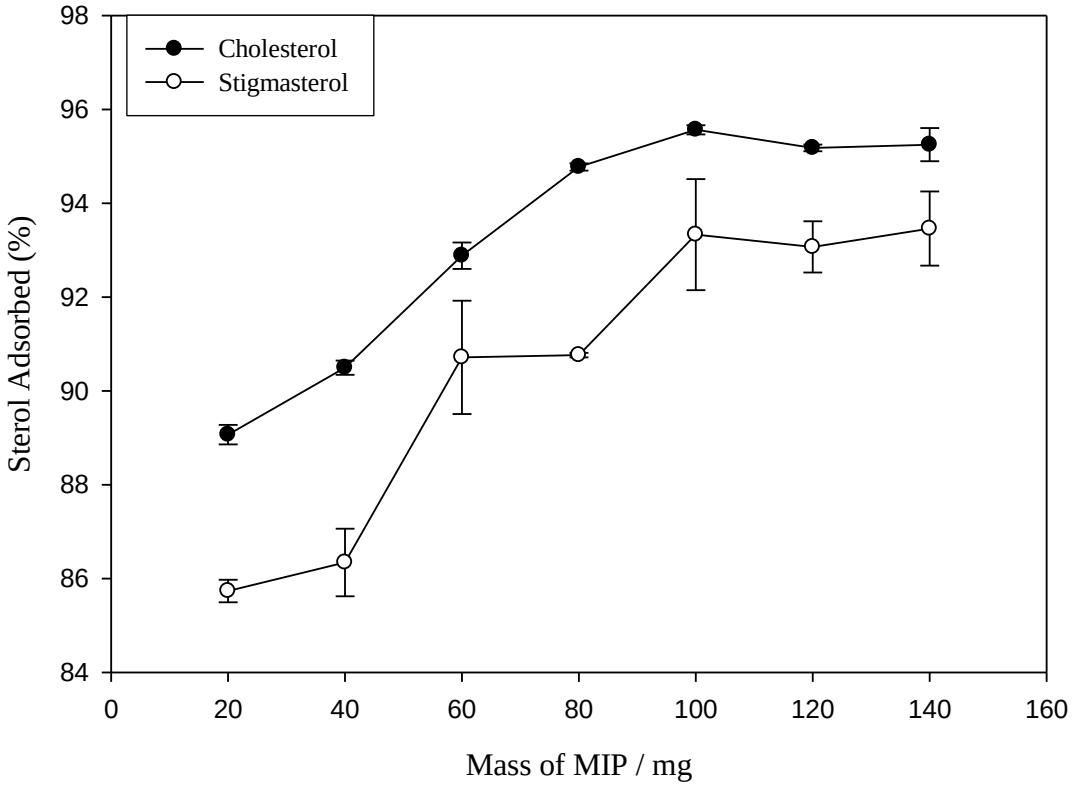


Figure 8: Effects of the amount of MIP on the adsorption of sterols

Therefore, the prepared cholesterol-molecularly imprinted polymers exhibited good performance in the removal of sterols from water which is a promising capacity.

Investigation of Optimal Contact Time

The effect of contact time was investigated using the template (cholesterol) at a time range of 1 – 8 h while keeping concentration and MIPs dosage constant. It was observed from Figure 9 that the adsoprtion of cholesterol by MIPs increased with an increase in adsorption/contact time up to 4 h at which an equilibrium profile was observed. Thus 4 h was identified as an optimum contact time. The observed initial higher adsorption rate exhbited by the polymers (MIPs) before 4 h can be explained by the availability of adequate binding cavities of the MIPs, and thus, as these cavities progressively got saturated with cholesterol molecules, the adsorption of cholesterol slowed down before reaching the equibrium at 4 h.

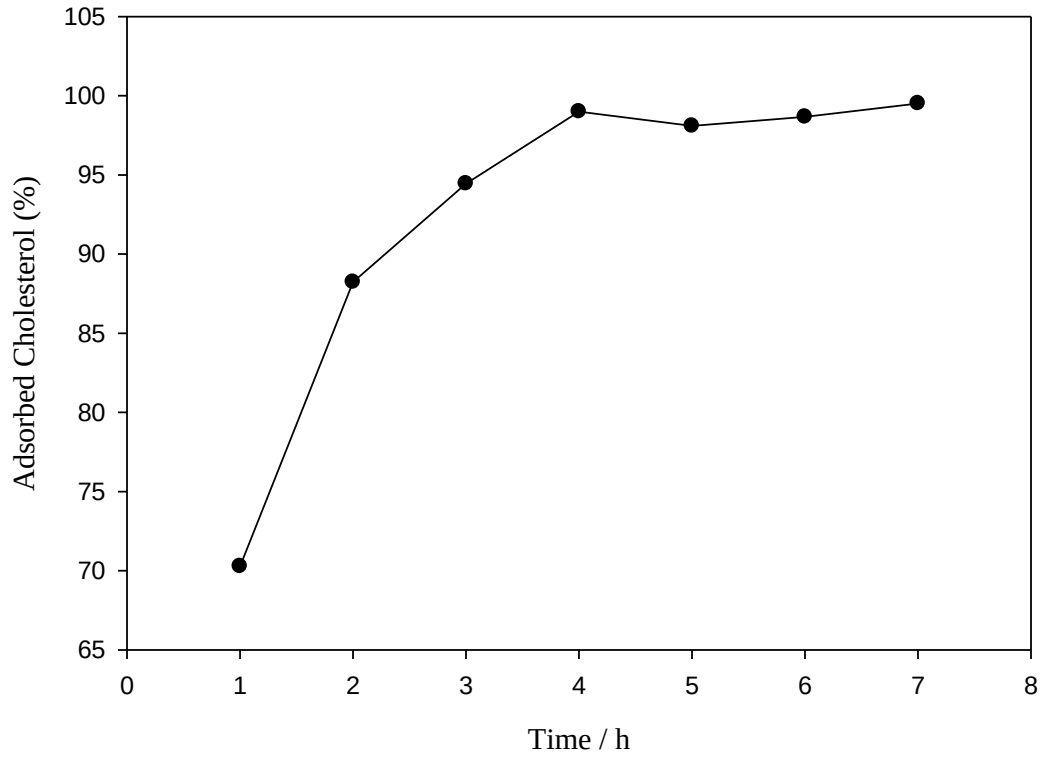


Figure 9: Effect of Contact Time for the adsorption of cholesterol on MIPs.

CONCLUSIONS

This study has successfully prepared and characterised MIPs for the removal of sterols from water. MIPs were synthesized and characterised using different techniques, from which it was observed that the formed NIPs were smoother than the corresponding MIPs. The results from FTIR characterization revealed a similarity in NIPs and MIPs with a clear difference observed from the FTIR spectrum of the template (Cholesterol), indicating that the

template removal procedure successfully removed all the templates from the polymer. The prepared MIPs were then tested for the removal of sterols from aqueous solution, and it was observed that at a loading mass of 100 mg the adsorbent was able to remove more than 98% of sterols for the solution of sterols at an optimal initial concentration of 40 mg/L and the optimal contact time was established to be 4 h.

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