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Synthesis and Evaluation of Bis(2-Ethylhexyl) Phthalate-Imprinted Polymers in an Aqueous Medium

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A synthetic molecular imprinted polymer (MIP) as a sorbent material for bis(2-ethylhexyl) phthalate (DEHP) was prepared using bulk polymerization method. This preliminary study aimed to investigate the possibility of the complex formation of MIP by studying its physical characteristics and adsorption performance via isotherm study. The polymer was synthesized by a non-covalent approach using 4-vinylpyridine, ethylene glycol dimethacrylate and benzoyl peroxide as functional monomer, cross-linker and initiator, respectively. The non-imprinted polymer (NIP) was prepared as controlled samples, using the same protocol in the absence of DEHP molecules. The characterization of the polymers was carried out by means of Fourier transform infrared spectroscopy, scanning electron microscopy and surface area and porosity analyses. The performance of the synthesized polymers was evaluated through batch rebinding experiment. Therefore, the kinetic spectrophotometric method was used to describe the determination of DEHP molecule based on its adsorption effect onto the polymers. The effect of initial concentration and time taken were investigated to reveal the possible mechanism through the adsorption isotherm studies. The result revealed that the adsorption capacity of MIP was 34.42 mg/g with 80% of percentage removal and showed that MIP exhibited good adsorption performance toward DEHP compared to NIP. Meanwhile, the adsorption isotherm successfully described that the polymers followed Langmuir isotherm model and displayed the Pseudo-second-order as the best kinetic model. As a conclusion, the present study revealed that the MIP is a promising adsorbent for DEHP molecules.

Key words: Bis(2-ethylhexyl) phthalate, molecularly imprinted polymers, adsorption isotherm, adsorption kinetic

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Bis(2-ethylhexyl) phthalate (DEHP) is a group of phthalate esters used worldwide as plasticizers (plastic flexible) in industries. They have been used in large scale especially in polyvinyl chloride (PVC) products. They have been found in many industrial sites, landfills and disposal sites. DEHP can enter the environment predominantly through disposal of industrial products from industries such as medical device industry, electronics, plumbing and other industries related to DEHP uses. DEHP has been categorized as a harmful chemical based on factors such as dosage, duration and the way of exposure. A study has proven that exposure to plastic additives including phthalate may cause harm and has the potential in altering the endocrine functions in humans [8]. According to toxicological study of DEHP by U.S. Department of Health and Human Services, people can be exposed to DEHP by air, water, or skin contact with plastics that have DEHP in them [9]. Furthermore, DEHP also can enter the body by eating food that contain leachate from food packaging made of materials which contain DEHP molecules [9]. This is possibly carcinogenic to humans because it can enter the bloodstream and the health effect may lead to more severe consequences if no actions are been taken. The development of reliable methods for the detection of DEHP is required in order to minimize the exposure towards the environment. Therefore, the detection and monitoring of DEHP exposure has been studied in a variety of advanced technologies such as biological methods [7], chromatography [2,4,5], catalysis and many more. An alternative technology called molecularly imprinted polymer (MIP) attracts a great interest over the past two decades [10] because of its selectivity, stability, relatively cheap and undemanding preparation. Molecular imprinting is a technique creating recognition entities into polymer networks. The analyte of interest or known as template is introduced during synthesis and undergoes polymerization process in the presence of functional monomer and cross-linker. The extraction of template molecules leaves specific cavities that will be used for recognition purposes. MIP has many crucial roles in many systems and it is a sensitive analytical method that could analyze substances at trace levels of concentration.

This work focused on the synthesis of non-covalent imprinted polymers for DEHP molecules, followed by characterization and utilization as an adsorbent material for the removal of DEHP molecules. Figure 1 shows a schematic diagram illustrating the imprint formation and molecular recognition process towards DEHP molecules by molecular imprinting technique. Bulk polymerization method was employed for the formation of DEHP-imprinted polymer assisted by ethylene glycol dimethacrylate (EGDMA) as a cross-linker, creating poly(4-vinylpyridine). The reaction underwent free radical polymerization, initiated by benzoyl peroxide (BPO). Removal of DEHP molecules left specific binding sites that are chemically complementary to DEHP. The two carboxyl group in DEHP can form electrostatic interaction with hydrogen atoms on the 4-vinylpyridine (4-VP). Adsorption study was monitored by a UV-Vis spectrophotometer as a detection technique. This method measured the changes of absorption over time as the reaction progress took place.

EXPERIMENTAL SECTION

Materials

The chemicals used were analytical grade. DEHP (>98% purity), BPO and acetonitrile were obtained from R&M Chemicals. 4-VP and EGDMA were obtained from Sigma Aldrich. Some of the chemicals were purified before use and BPO was recrystallized from chloroform to methanol in 1:1 v/v ratio.

Preparation of Imprinted Polymers

The MIP for DEHP was prepared using bulk polymerization method [14]. The template, DEHP (1 mmol) and monomer, 4-VP (4 mmol) were dissolved in 6.7 ml of acetonitrile in a screw cap glass tube. Subsequently, the cross-linker (EGDMA, 22 mmol), initiator (crystal BPO, 1.5 mmol) were added to the tube. The reaction tube then underwent nitrogen purging for 3 to 5 minutes, sealed and heated in silicone oil bath at 60°C for 24 hrs to complete the polymerization. The non-imprinted polymer (NIP) was prepared using the similar method as MIP-DEHP except with the absence of the template molecule. After the polymerization reaction was complete, the polymers obtained were crushed, ground and sieved prior to storage. For the template removal process, the polymers were treated by Soxhlet extraction method with methanol to acetic acid in 9:1 v/v ratio for 24 hours.

Characterization Techniques

The MIP and NIP were characterized by Fourier Transform Infrared (FTIR) spectrometer using Perkin Elmer spectrum 100 software and scanning electron microscopy (SEM) model JEOL JSM-6360L. The surface and porosity properties were determined by nitrogen gas adsorption method using surface area and porosity analyzer (ASAP 2020 V4.02) manufactured by Micromeritics.

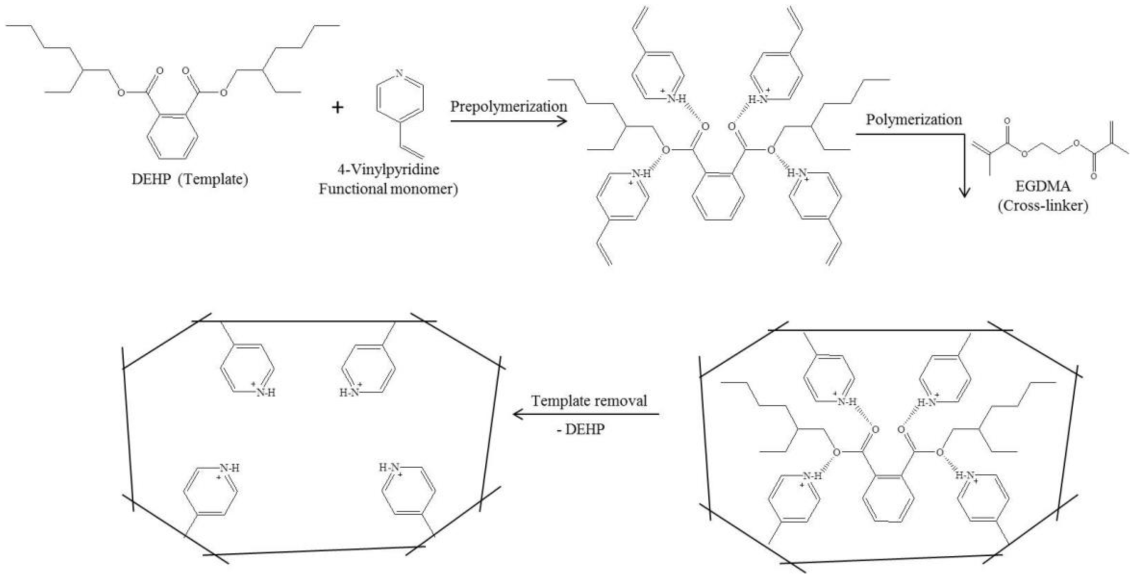

Figure 1. Schematic diagram of imprinting formation and molecular recognition process towards DEHP molecules by molecular imprinting technique.

Adsorption Studies

The adsorption study was conducted by batch experiments using kinetic spectrophotometric method by UV-Vis spectrophotometer (Shimadzu). This method can determine the reaction progress directly in specified time set by monitoring the concentration of DEHP. Various concentrations of DEHP solutions were prepared (100 - 1000 mg/L) and each was mixed with 20 mg of MIP or NIP in a quartz cell. The time and wavelength were set for 180 minutes and 274 nm, respectively. The results obtained were optimized into the effect of initial concentration of DEHP and time taken to reach equilibrium. The percentage removal efficiency of DEHP molecules was calculated using equation (1):

$$\text{Removal efficiency} = \frac{C_i - C_f}{C_i} \times 100\% \tag{1}$$

where C_i and C_f are the initial and final concentrations (mg/L) of DEHP solutions, respectively. The time point at which the equilibrium concentration achieved for each initial concentration tested was selected. The amount of DEHP adsorbed at equilibrium point or experimental adsorption capacity, Q_e (mg/g) was calculated using the following equation:

$$Q_e = \frac{(C_i - C_f)\ V}{W} \tag{2}$$

where V is the volume of DEHP solution (L) and W is the weight of the polymer sample applied to the solution (g).

Adsorption Isotherm and Kinetic Study

The relation of the equilibrium concentration and adsorbed analyte was then described by adsorption isotherm and kinetic study. The equilibrium data were fitted into Langmuir and Freundlich isotherm models. The Langmuir and Freundlich equations can be written as in equations (3) and (4), respectively.

$$\frac{C_e}{Q_e} = \frac{1}{Q_o} C_e + \frac{1}{Q_o\, K_L} \tag{3}$$

$$\ln Q_e = \ln K_F + \frac{1}{n} \ln C_e \tag{4}$$

where C_e is the equilibrium concentration of template (mg/L), Q_e is the adsorption capacity (mg/g), Q_o is the maximum adsorption capacity (mg/g), K_L is the Langmuir isotherm constant, K_F is the Freundlich constant and n is the value of the heterogeneity parameter. Linearized forms of the isotherm models were plotted and the relation coefficient (R^2) was compared.

The kinetic consideration was further investigated through the Pseudo-first-order and Pseudo-second-order equation models to fit the experimental data and investigate the reaction rate of DEHP adsorption towards MIP and NIP. The Pseudo-first-order can be calculated using the following equation:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \tag{5}$$

Meanwhile, the Pseudo-second-order can be defined according to the following equation:

$$\frac{t}{q_t} = \frac{k_2}{h} + \frac{t}{q_e} \tag{6}$$

where $h = k^2q^2_e$ is the initial adsorption rate, and q_e and q_t are the amounts of molecules adsorbed at equilibrium at any time *t* (mg/g), respectively. k_1 and k_2 are the adsorption rate constants for Pseudo-first-order and Pseudo-second-order, respectively. Parameter *t* is the contact time between adsorbate and adsorbent (min). The obtained parameter values were then calculated by normalized standard deviation value, Δq (%) and relative error, RE (%) as shown in equations 7 and 8, respectively. These equations were used to ensure the acceptability of the kinetic models [12]:

$$\Delta q\ (\%) = \sqrt{\frac{[(Q_{e,exp} - q_{e,cal})/Q_{e,exp}]^2}{N-1}} \times 100\% \tag{7}$$

$$\text{RE}\ (\%) = \frac{|q_{e,cal} - Q_{e,exp}|}{Q_{e,exp}} \times 100\% \tag{8}$$

where *N* is the amount of data points fitted to the plot, and $Q_{e,\ exp}$ and $q_{e,\ cal}$ (mg/g) are the experimental and calculated adsorption capacities, respectively.

RESULTS AND DISCUSSION

FTIR Spectra Analysis

Chemical structure determination of MIP and NIP before template extraction and DEHP are presented in the FTIR spectra as shown in Figure 2. MIP has a rather similar FTIR spectrum to NIP, as expected due to the formation of poly(4-VP) as the polymer backbone [15]. Therefore, the characteristic vibrations of pyridine ring can be seen at 1458, 1390 and 1630 cm^{-1} in MIP and 1458, 1389 and 1637 cm^{-1} in NIP, corresponding to C=C stretch overlap with N-H stretch. Furthermore, aromatic substitution of sp^2 C-H bend is also present in both samples; 754 cm^{-1} (MIP) and 756 cm^{-1} (NIP). In addition, the medium peaks observed at 2957 cm^{-1} in MIP and 2956 cm^{-1} in NIP were due to the sp^3 C-H stretching frequencies due to the formation of the alkyl group in the polymer chain.

The formation of intermolecular bond for O-H stretch can be seen at the broad absorption peak at 3567 cm^{-1} in the MIP spectrum due to the formation of hydrogen bonding as the proposed reaction in the imprinting process (Figure 1). However, this peak was observed slightly weakened for NIP (3447 cm^{-1}) as NIP was synthesized in the absence of DEHP molecules. Therefore, the peak might be due to O-H stretch from cross-linking process by EGDMA. Other peaks perceived, such as at around 1732 cm^{-1} (MIP) and 1732 cm^{-1} (NIP) are assigned as the C=O stretching.

The peaks present in the DEHP spectrum show characteristic peaks that coincide with the functional groups present within DEHP molecule. After DEHP was imprinted, most of the stretching peaks in the MIP spectrum clearly shifted towards the higher wavenumbers, thus proves the incorporation of DEHP template. The effect of neighbouring atoms and bond formations within the complex will affect the peak shifting.

Surface Morphology

The surface morphology of MIP and NIP (Figure 3) were observed under SEM at the magnification of 1500x. The particles possessed irregular shapes and sizes. The SEM micrographs revealed the morphological differences between MIP and NIP. MIP, with imprinting effects of DEHP molecules showed rough surfaces, whereas NIP has relatively smooth surfaces. This is due to the effect of template extraction, thus reveals the existence of binding cavities [13].

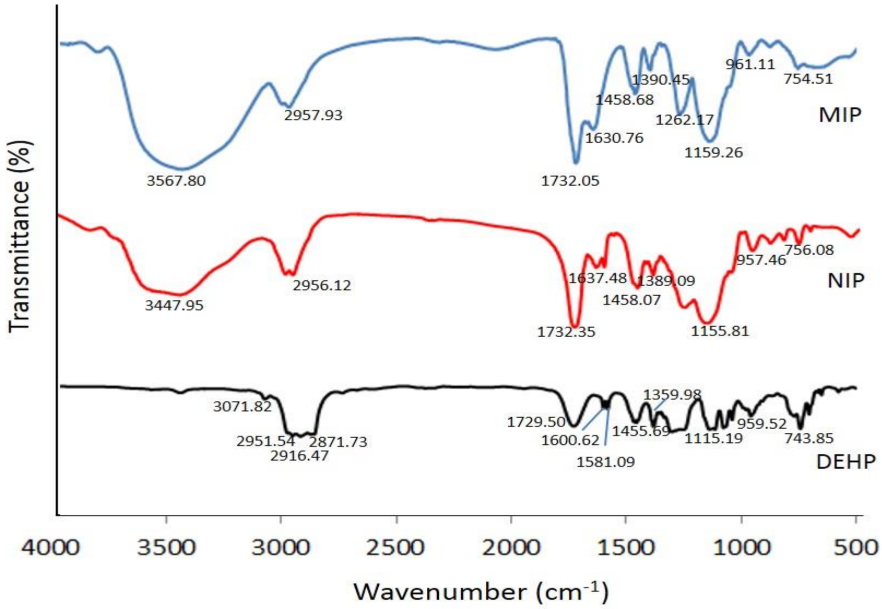

Figure 2. FTIR spectra for MIP, NIP and DEHP.

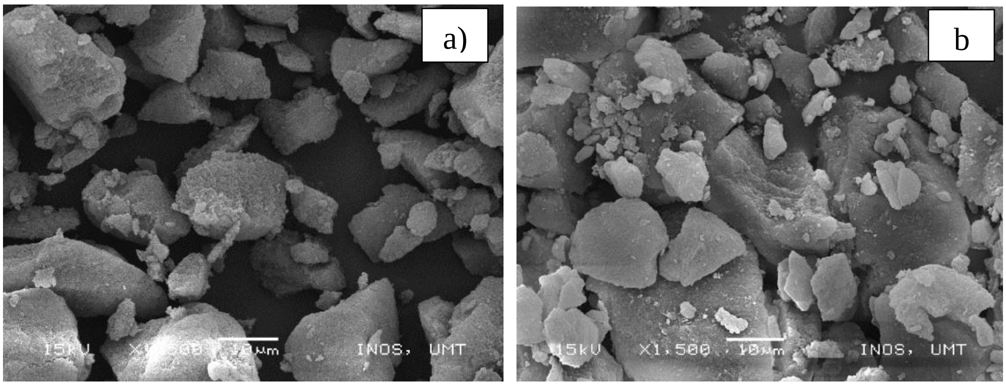

Figure 3. SEM micrograph for a) MIP and b) NIP.

Surface Area and Porosity Analysis

The polymer characterization was further investigated by Brunauer-Emmett-Teller (BET) method for surface area and Barrett-Joyner-Halenda (BJH) method for porosity analysis. Based on the summarized data in Table 1, the surface area of MIP was larger compared to NIP. This is because of the presence of more cavities in MIP, which are present on the polymer's surface. Furthermore, pore volume and pore size in MIP also showed larger values than NIP. These results were in a good agreement with the SEM finding which showed MIP has rougher surfaces than NIP due to the effect of the binding cavities [16]. Therefore, this could provide more accessible cavities and binding sites for target analytes than NIP [13].

Adsorption Studies

The Effect of Initial Concentration

The adsorption experiment was investigated under given sets of initial concentrations of the template molecule while keeping a constant mass of the polymers. Based on Figure 4, the percentage removal for MIP increased as concentration increased from 100 to 700 mg/L. This led to the increase in removal efficiency generally from 39% to 80% and nearly reached the equilibrium state after 700 mg/L, thus demonstrating that MIP has specific recognition sites for DEHP molecules. In comparison, NIP showed lower percentage removal than MIP and achieved saturation percentage removal of 29% considered at 900 mg/L. However, the pattern slightly decreased with the increase of concentration, indicating the releasing of DEHP molecules due to NIP not having the specific cavities.

In the imprinting process, the carboxylic group in DEHP molecules can form an electrostatic interaction with 4-VP monomers, as shown in the proposed mechanism in Figure 1. Furthermore, the π–π stacking interaction of pyridine rings in poly(4-VP) also contributes to the formation of polymer chains thus forming the cavities for DEHP [16].

In studying the adsorption properties of materials, Langmuir and Freundlich models are often employed in order to understand the binding behavior of imprinted polymers. Adsorption isotherms provide a relationship between concentration of analyte in the solution and the adsorbed on the polymers when both phases are in equilibrium state [1]. Figure 5 and Figure 6 show the data fitted into Langmuir and Freundlich model, respectively. The straight line equations were obtained and the highest linearity (correlation coefficient, R^2) was compared.

Table 1. Summary of nitrogen sorption surface area and porosity data.

Polymers	Surface area (m^2/g)	Pore volume (cm^3/g)	Average pore size(Å)
MIP	287.07	0.83	163.88
NIP	232.88	0.68	151.64

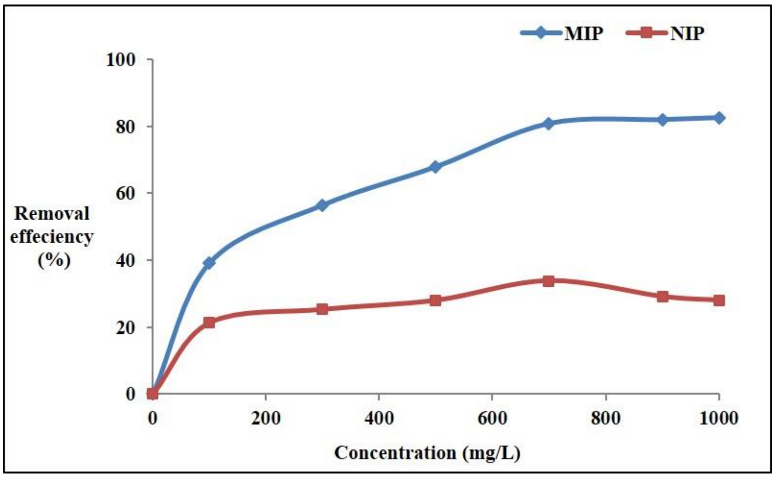

Figure 4. Removal efficiency for the effect of initial concentration (the curves were obtained from the point of optimum time taken; MIP= 80 min and NIP= 50 min).

Langmuir isotherm model was observed to have the highest R^2 value compared to Freundlich isotherm model and thus revealed that both MIP and NIP were following Langmuir isotherm model. Langmuir isotherm model describes the formation of monolayer adsorbate onto the homogeneous adsorbent surface [3]. Furthermore, the molecules are adsorbed at the adsorption sites that have uniform energy which there are no interactions that exist between molecules adsorbed to adjacent sites [6,11]. Based on theoretical calculation, all the parameters are tabulated in Table 2. The value of Q_o for MIP was 14.08 mg/g while NIP was 3.11 mg/g.

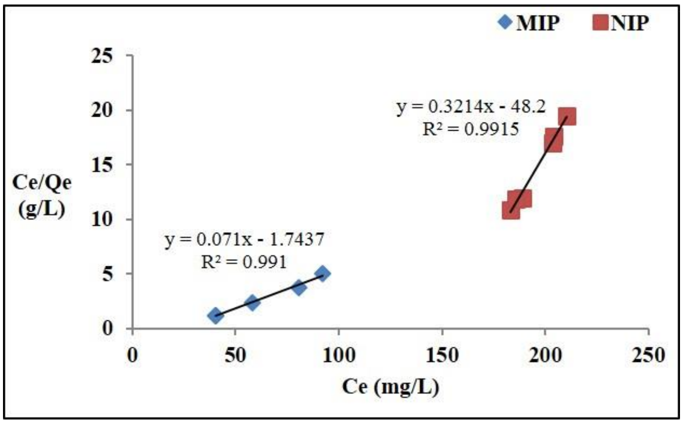

Figure 5. Langmuir isotherm plots.

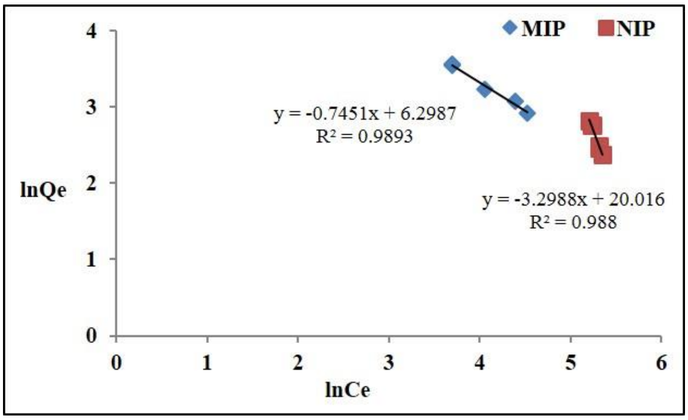

Figure 6. Freundlich isotherm plot.

Table 2. Langmuir and Freundlich isotherm parameters.

Polymers	Isotherm parameters						
	Isotherm model	Q_o (mg/g)	K_L (L/mg)	1/n	n	K_F (mg/g)	R^2
MIP	Langmuir	14.08	-0.04	-	-	-	0.9910
	Freundlich	-	-	-0.74	-1.34	543.86	0.9893
NIP	Langmuir	3.11	$-6.6x10^3$	-	-	-	0.9915
	Freundlich	-	-	-3.29	-0.30	$4.9x10^8$	0.9880

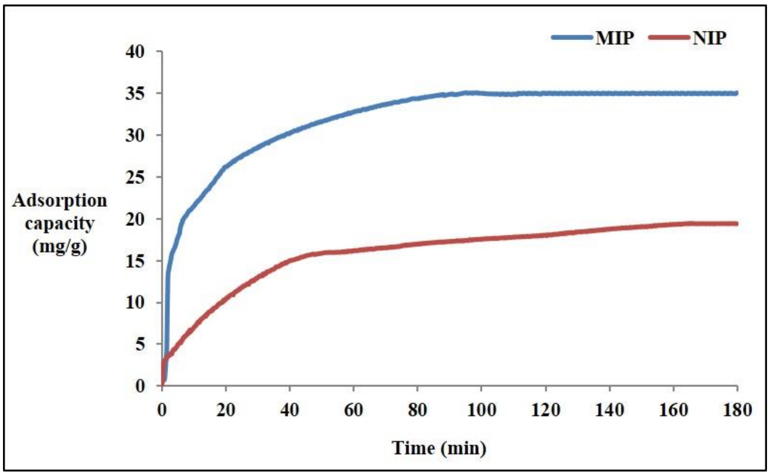

Figure 7. The effect of contact time of MIP and NIP in adsorption capacity (the curves were obtained from the point of optimum initial concentration; MIP= 700 mg/L and NIP= 900 mg/L).

The Effect of contact time

The experimental data at the optimum concentration was further investigated to analyze the time taken and rate of MIP and NIP in the process of removal. Figure 7 shows the adsorption curves of the adsorption capacity for MIP and NIP. Based on the curves, the adsorption rate rapidly increased at 0 to 5 min for both polymers. Afterwards, slow increases can be observed until they achieved the plateau state at around 80 min and 50 min for MIP and NIP, respectively. After the equilibrium time, no more binding sites were available for DEHP molecules. The adsorption capacity of MIP (34.42 mg/g) was higher than NIP (15.83 mg/g) because of there were no complementary sites present in NIP and the ability for holding DEHP molecules was limited [14].

Two kinetic models, Pseudo-first-order and Pseudo-second-order, were used to fit the equilibrium data. Linearized forms of both models were plotted, as shown in Figure 8 and Figure 9. Pseudo-second-order model was found to be the better fitting model than Pseudo-first-order model by providing the higher R^2 value for both MIP and NIP. According to this model, the rate limiting step of the adsorption of DEHP onto MIP or NIP was controlled by chemisorption. The calculated kinetic parameters for both models are summarized in Table 3. At the initial adsorption rate, h, MIP showed lower h value than NIP. It is possible because NIP have unspecific cavities for the target analyte, thus leads to the fast uptake rate.

In addition, the calculated adsorption capacity (q_e) was consistent with the experimental (Q_e). To validate the proposed model, Δq and RE values were calculated. By obtaining lower percentage values, it was concluded that Pseudo-second-order gave the best fitness to the kinetic adsorption of DEHP onto the polymers.

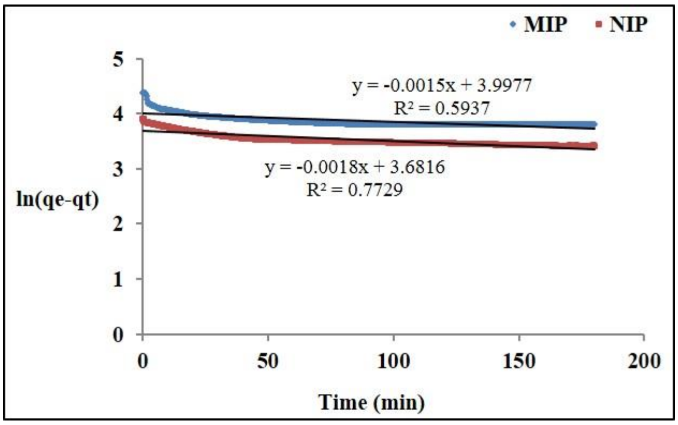

Figure 8. Pseudo-first-order plot.

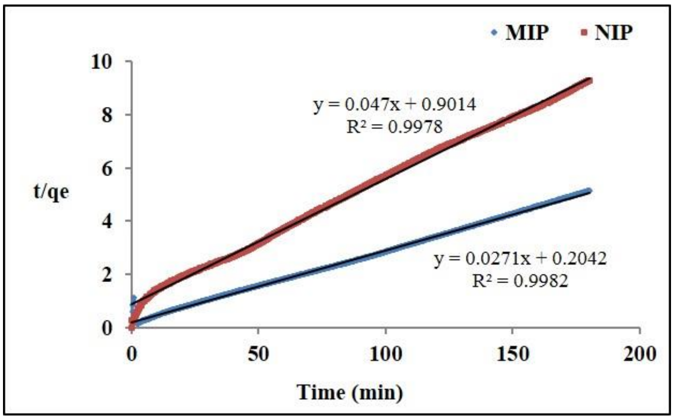

Figure 9. Pseudo-second-order plot.

Table 3. Pseudo-first-order and Pseudo-second-order kinetic parameters.

Polymers		Parameters					
		$Q_{e,}$ (mg/g)	q_e (mg/g)	h (g/mg/min)	Δq (%)	RE (%)	R^2
MIP	Pseudo-first-order	34.42	54.47	-	1.94	58.25	0.5937
	Pseudo-second-order		36.90	4.89	0.24	7.20	0.9982
NIP	Pseudo-first-order	15.83	39.71	-	5.03	150.81	0.7729
	Pseudo-second-order		21.27	1.11	1.15	34.38	0.9978

CONCLUSION

A new adsorbent material called molecularly imprinted polymer for DEHP removal was successfully synthesized for the purpose of environmental assessment for future investigations. Physical and chemical characterizations demonstrated the chemical interaction, surface morphology and cavities of polymer monoliths which are the key to the fundamental of imprinting study. The synthesized MIP showed an excellent performance towards DEHP template by giving the higher adsorption capacity in MIP compared to NIP. The DEHP removal was 80% within 80 minutes of time taken. The sorption study revealed that Langmuir isotherm and Pseudo-second-order kinetic were the best models to describe sorption behavior of MIP and NIP. The present study also revealed the imprinting technique as a relevant method in the determination of desired analytes.

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