

## Synthesis of Molecularly Imprinted Polymer for the Removal of Melamine

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In this article, molecularly imprinted polymer (MIP) of melamine was synthesized by non-covalent approach using microemulsion medium. In this study, melamine is used as a template molecule, acrylic acid as a functional monomer, N,N'-methylenebis(acrylamide) (MBAm) as a cross-linker and 2,2'-azobisisobutyronitrile (AIBN) as an initiator and microemulsion as a solvent. The produced polymers were characterized by Fourier transform infrared spectroscopy (FT-IR), transmission electron microscopy (TEM), Brunauer-Emmett-Teller (BET) and thermogravimetric analysis (TGA). The batch binding analysis was conducted for the evaluation of MIP and NIP rebinding efficiency with the melamine template. The adsorption studies including the adsorbent dosage of polymer, initial concentrations and pH of melamine solution were successfully carried out on both MIP and NIP. For selectivity test, 2,4,6-trichlorophenol was chosen as the competitive molecule against melamine template. A very good relative selectivity coefficient was achieved. The removal of melamine from different samples were conducted successfully by achieving a good removal efficiency in milk, river water and human blood serum 84.30, 94.26 and 93.32 %, respectively.

**Keywords:** Melamine, Molecular imprinted polymer, Microemulsion polymerization, Adsorption.

### INTRODUCTION

Melamine is a versatile compound used in the production of wide range of useful products such as adhesives, commercial filters, coatings, dishware, kitchenware, laminates, plastics, molding compounds, fertilizers and pesticides. Melamine is inexpensive and rich in nitrogen content and therefore, it has been exploited in food industries in order to increase the apparent protein content in food products. Melamine adulteration in infant food product formula in China have led to renal diseases in thousands of Chinese infants and children. In severe cases, at least six casualties of infants and children are reported in China due to melamine-doped infant formula [1]. Melamine itself is low in toxicity [2], but the accumulation of melamine with cyanuric acid will produce insoluble white crystals that can cause the formation of kidney stones, ultimately kidney failure [3]. Other than that, the extensive usage of fertilizers and pesticides containing melamine can contribute to water pollution to the nearby rivers that eventually have adverse effects to mankind. For these reasons, a convenient and effective method is required to combat melamine exploitation in any industries.

Molecular imprinting technology (MIT) is a promising technique which is broadly employed for various applications such as extraction/removal of a compound/analyte of interest in a sample by forming a robust polymer with high specificity and selectivity known as molecularly imprinted polymer (MIP). MIP is a cross-linked polymer containing specific binding sites highly selective towards its compound of interest (template molecule) [4].

Molecular imprinting techniques had been tremendously applied for the synthesis of MIPs because these methods are simple and inexpensive [5]. The generated MIPs have good storage stability as they can be kept for a long period of time while retaining the affinity for template molecule [6]. Besides that produced MIPs are not only capable to withstand extreme chemical conditions (highly acidic or highly basic), but also possess high stability and strength as well as durability to heat and pressure as a result of their highly cross-linked nature [7]. For these reasons, MIPs have been comprehensively employed in many crucial applications in various fields for their usage as adsorbents, stationary phases, reaction catalysts, chemical sensors or separation materials. MIPs have the ability to produce

binding sites within the polymer matrix similar to the interaction produced between antigen and antibody [8]. Several methods have been used for the production of MIPs such bulk polymerization, precipitation polymerization, suspension polymerization and emulsion polymerization. In the production of MIPs different approaches have been adopted to produce interaction between template and monomer such as covalent imprinting [9], non-covalent imprinting [10] and semi-covalent imprinting [11]. In this study, a non-covalent approach has been employed for the production of MIP and NIP. This study emphasized on melamine extraction/removal from three different spiked samples such as milk, river water and blood serum.

## EXPERIMENTAL

Melamine and N,N'-methylenebis(acrylamide) (MBAm), acrylic acid and potassium bromide were purchased from Sigma-Aldrich, USA. Acetone was obtained from Bendosen Laboratory Chemical, Malaysia. Acetic acid was supplied by J.T. Baker, USA. Dimethyl sulfoxide was provided by RCI Lab-Scan Ltd., Thailand. 2,2'-Azobisisobutyronitrile (AIBN), sodium dodecyl sulfate (SDS) and butan-1-ol were purchased from R & M Chemical, Malaysia. Methanol, ethanol and hexane were obtained from HmbG Chemical, Germany. 2,4,6-Trichlorophenol (2,4,6-TCP) was bought from Acrōs Organics, USA. Ultra-pure water was used throughout the study.

**Synthesis of MIP and NIP of melamine:** Initially, 0.5 mmol of melamine template, 1 mmol of acrylic acid functional monomer, 20 mmol of MBAm cross-linker, 0.030 g of AIBN initiator were added and dissolved in a conical flask containing 75 mL of microemulsion solution as the porogenic solvent. The pre-polymerization mixture was sonicated for 10 min followed by 15 min nitrogen gas purging. Then, the conical flask containing pre-polymerization mixture was sealed tightly and placed into a water bath. The polymerization was performed at constant temperature of 40 °C for 6 h. After that the generated polymers were filtered, collected and dried at 25 °C. The non-imprinted polymer (NIP) was synthesized in the similar procedure as MIP but without the addition of melamine template.

Finally, the removal of melamine template from the generated MIP was conducted by washing MIP with a mixture of MeOH and AcOH (7:3, v/v). This step was repeated until melamine template was not detected by RP-HPLC at 240 nm. The RP-

HPLC was conducted by using C<sub>18</sub> column (250 × 4 mm, 5 μm) with the mobile phase composed of MeOH, acetonitrile and ultra-pure water (70:20:10, v/v/v). The RP-HPLC condition such as flow injection, UV detection and injection volume were optimized at 0.5 mL/min, 240 nm and 20 μL, respectively.

Thermo Scientific Nicolet iS10 was used to provide IR spectra of the generated polymers. Transmission electron microscope (JEOL-1230) was carried out to determine the polymer morphology in term of size and shape. Brunauer-Emmett-Teller (BET) Quantachrome Autosorb was applied to measure the specific surface area, pore volume and average pore diameter of the synthesized polymers. Universal Analyzer 2000 with Universal V4.7A software is a TGA Instrument which was conducted for thermal analysis of the produced polymers whereby both MIP and NIP were examined in the temperature range of 30-900 °C, at a heating rate of 10 °C/min. Shimadzu LC-20A, a reversed-phase high performance liquid chromatography (RP-HPLC) instrument was employed to assess the batch binding of produced polymers.

**Rebinding experiment of the synthesized polymers with melamine:** The rebinding experiment was conducted to evaluate the MIP and NIP rebinding efficiency with the melamine template. A series of 100 mL conical flasks was used for both MIP and NIP. All the flasks were marked according to the time interval. Then, these conical flasks consisting mixture were placed on a shaker for agitation at 150 rpm and then were collected every after time interval 30 min. Melamine concentration after rebinding process was observed *via* RP-HPLC. The RP-HPLC was using C<sub>18</sub> column (250 × 4 mm, 5 μm) with the mobile phase comprising of MeOH, acetonitrile and ultra-pure water (70:20:10, v/v/v). The RP-HPLC condition such as flow injection, UV detection and injection volume were optimized at 0.5 mL/min, 240 nm and 20 μL, respectively. The MIP and NIP rebinding efficiencies were calculated according to the following eqn. 1:

$$\text{Binding efficiency} = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

where, C<sub>i</sub> is the initial concentration of melamine in the solution before rebinding; C<sub>f</sub> is the final concentration of melamine in the solution after rebinding.

**Adsorption studies of the synthesized polymers of melamine:** The adsorption parameters (Table-1) including polymer

TABLE-1  
ADSORPTION PARAMETERS

Parameter	Variation in parameter	Constant parameter(s)
Polymer dosage (g)	0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7	(i) Temperature: 25 °C (ii) Agitation speed: 150 rpm (iii) Agitation period: 240 minutes (iv) pH of melamine solution: pH 7 (v) Melamine concentration: 25 ppm
Initial concentration of melamine solution (ppm)	10, 15, 20, 25 and 30	(i) Temperature: 25 °C (ii) Agitation speed: 150 rpm (iii) Agitation period: 240 minutes (iv) pH of melamine solution: pH 7 (v) Polymer dosage: 0.5 g
pH of melamine solution	3, 5, 7, 9 and 11	(i) Temperature: 25 °C (ii) Agitation speed: 150 rpm (iii) Agitation period: 240 minutes (iv) Melamine concentration: 25 ppm (v) Polymer dosage: 0.5 g

dosage, different initial melamine concentration and the pH of melamine solution were performed on both MIP and NIP to determine the ideal condition for the polymer rebinding in order to attain the highest binding efficiency.

In each adsorption test, melamine concentration after the rebinding process was observed using RP-HPLC, whereby the HPLC conditions and mixture of the mobile phase used are similar to that batch binding experiment. Therefore, the binding efficiency of MIP and NIP were evaluated.

#### Selectivity test of the synthesized polymers of melamine:

Melamine template and 2,4,6-trichlorophenol (2,4,6-TCP), the competitive compound was used in selectivity test to determine the specificity of the generated polymers. Firstly, 5 mL of 15 ppm melamine was mixed with 5 mL of 15 ppm of 2,4,6-TCP to produce 10 mL solution. Then, the conical flask consisting 10 mL mixture of melamine and 2,4,6-TCP was added with 0.5 g of MIP. Similarly, NIP was treated in same way as MIP. After that both conical flasks comprised of 10 mL mixture of melamine and 2,4,6-TCP with MIP and NIP, respectively were placed on the shaker and agitated at 250 rpm for 240 min. The melamine and 2,4,6-TCP concentrations in MIP and NIP were observed through RP-HPLC. The distribution ratio for both MIP and NIP was calculated as follows:

$$K_D = \frac{C_i - C_f}{C_f} \frac{V}{m} \quad (2)$$

where,  $C_i$  is the initial concentration of melamine in the solution before adsorption;  $C_f$  is the final concentration of melamine in the solution after adsorption;  $V$  is the volume of solvent used; and  $m$  is the mass of MIP or NIP used.

The selectivity coefficients for melamine relative to 2,4,6-TCP, the binding competitors for MIP and NIP were evaluated as follows:

$$\text{Selectivity coefficient, } K = \frac{K_{D_{\text{melamine}}}}{K_{D_{2,4,6\text{-TCP}}}} \quad (3)$$

where,  $K_{D_{\text{melamine}}}$  is the batch binding assay of MIP or NIP for melamine;  $K_{D_{2,4,6\text{-TCP}}}$  is the batch binding assay of MIP or NIP for 2,4,6-TCP. Hence, the relative selectivity coefficient ( $k'$ ) was defined according to equation below:

$$K' = \frac{K_{\text{MIP}}}{K_{\text{NIP}}} \quad (4)$$

**Extraction/removal of melamine from milk, river water and blood serum:** The melamine extraction method from milk was conducted according to Qi *et al.* [7] with modification. In the extraction of protein from milk, 10 mL milk was mixed with 50 mL MeOH. Then, this mixture was centrifuged at 5000 rpm for 15 min and the milk extract was attained and kept for further procedure. About 5 mL of 25  $\mu\text{g/mL}$  melamine solution was prepared and spiked with 5 mL milk extract. Next, about 0.5 g of MIP and NIP were added to different conical flasks containing 10 mL of spiked melamine solution, respectively and the conical flasks containing mixture were put on a shaker and agitated at 150 rpm for 240 min. After that the samples were obtained and the melamine concentrations after adsorption were recorded using RP-HPLC. The melamine removal efficiency in milk by MIP and NIP were calculated using eqn. 1.

Prior to MIP application for the melamine removal from river water, the collected river water was first filtered using gravitational filtration in order to eliminate any suspended particles. Any presence of melamine in the collected river water was monitored using RP-HPLC.

Initially, 0.003 g of melamine powder was added to mixture of 24 mL river water and 94 mL EtOH to prepare 25  $\mu\text{g/mL}$  melamine solution. After this, same procedure was followed as applied in milk sample.

In the extraction of melamine from blood serum a drug-free human blood was required. About 10 mL of fresh human blood was collected and allowed to coagulate at 25 °C. The coagulated blood was centrifuged for 15 min at the speed of 5000 rpm and then, the blood serum was collected and kept in the refrigerator. Then, the collected blood serum was diluted using ultrapure water (UPW) in the ratio of 1:10 (UPW). About 5 mL of 25  $\mu\text{g/mL}$  melamine solution was prepared and spiked with 5 mL blood serum. After that same procedure was applied as followed in the removal of melamine from milk.

## RESULTS AND DISCUSSION

**TEM analysis:** In this study, TEM was employed for the study of morphology of the produced polymers [12, 13]. The TEM micrographs of MIP and NIP of melamine were analyzed under 100000X magnification. The MIP and NIP morphologies were shown in Fig 1a and 1b, respectively whereby the shape of the generated polymers were globular in shape and nearly constant in size. The MIP and NIP particles were produced in nanosized with the average diameter size of 54.33 and 63.33 nm, respectively.

Few possible reasons that majorly influenced the shape and size of the synthesized polymers were the kind of polymerization method, type of cross-linker and choice of porogenic solvent. Microemulsion polymerization is one of the emulsion polymerization method which was employed in this experiment for the formation of very small particles of MIP and NIP of melamine. Often, polymers synthesized by emulsion polymerization technique produces spherically uniform polymers with the size within micro to nanoscale particles [14]. Generally, the usage of cross-linker had a great effect on the physical properties of the generated polymers because one of its function is controlling the polymer morphology and therefore, the type of cross-linker was reported to have impact on the formation of MIP nanoparticles and its final size [15]. A proper choice of porogenic solvent such as microemulsion solution that composed of butan-1-ol, hexane, sodium dodecyl sulfate and ultra-pure water had contributed to the formation of nano-sized polymer particles. The amount of solvent was also responsible for the polymer morphology [16].

**FT-IR analysis:** The FT-IR spectra of the synthesized MIP and NIP are shown in Fig. 2. All the peaks were associated to the interactions between the melamine template and acrylic acid functional monomer as well as acrylic acid functional with the MBAm cross-linker. A broad peak was detected at 3430.21 and 3424.82  $\text{cm}^{-1}$  in IR spectra of MIP and NIP, respectively. This strong peak showed the presence of N-H bond stretching. This peak may be associated with presence of amide in MBAm cross-linker or amine in melamine template. However since there was no melamine template added in synthesis of NIP, so

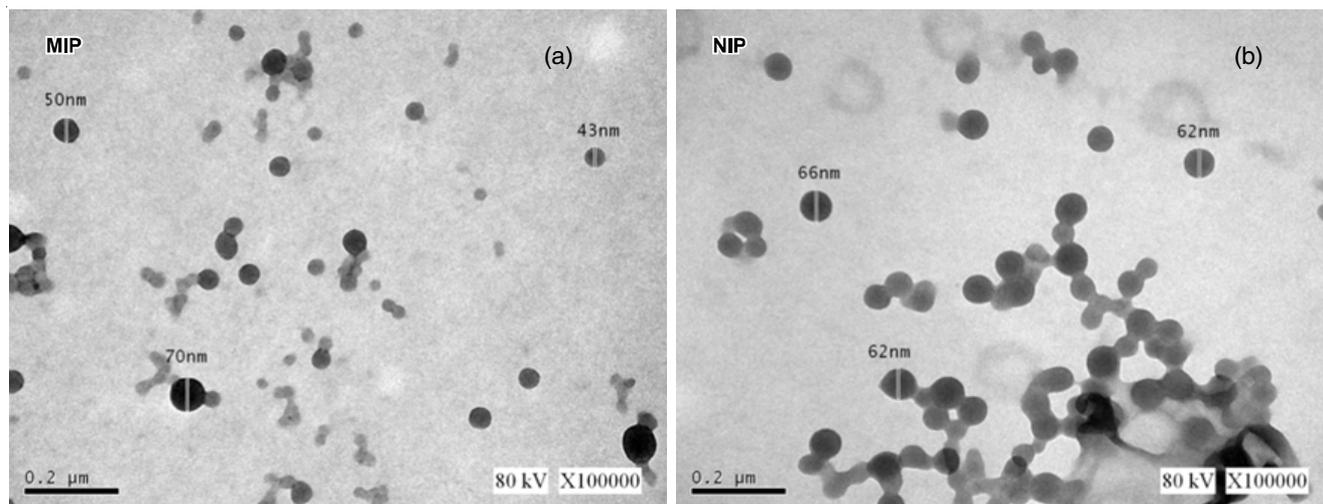


Fig. 1. TEM micrographs of (a) MIP and (b) NIP

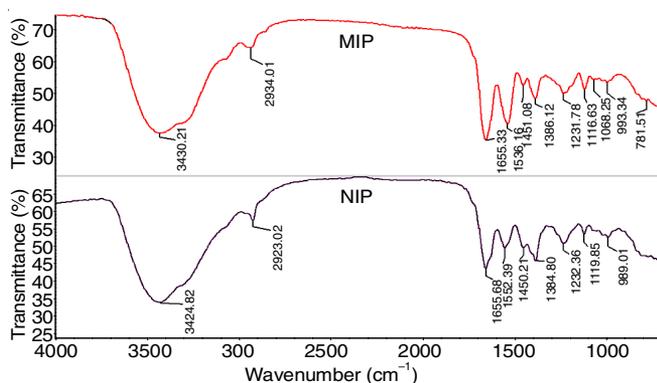


Fig. 2. FT-IR spectra of MIP and NIP

the peak representing amide in MBAm monomer. Besides that, the peaks existed at 2923.02 and 2934.01  $\text{cm}^{-1}$  in Fig. 2 were assigned as the intermolecular bonded OH stretching of carboxylic acid in acrylic acid monomer.

The absorption bands at 1655.33 and 1655.68  $\text{cm}^{-1}$  were observed for C=O bond stretching of either carboxylic acid in acrylic acid monomer or amide in MBAm cross-linker. Moreover, the N-H bending at 1536.16 and 1552.68  $\text{cm}^{-1}$  described the presence of either amino group in the melamine template or amide group in MBAm. Nonetheless, it can be deduced that both peaks representing amide group in MBAm cross-linker because MBAm was used in preparation of both polymers while melamine template was only added for MIP synthesis. The vibration mode of N-H bending at 1451.08 and 1450.21  $\text{cm}^{-1}$  were due to secondary amine group in MBAm cross-linker.

The peaks at 1386.12 and 1384.80  $\text{cm}^{-1}$  depicted the vibration mode of C-H in plane bending due to the presence of vinyl group in acrylic acid monomer or MBAm cross-linker. The peaks at 1116.62, 1231.78, 1119.85 and 1232.36  $\text{cm}^{-1}$  portray either the C-N bond stretching in MBAm cross-linker or C-OH bond stretching of carboxylic acid in acrylic acid monomer. The absorption in the region 1068.25-700  $\text{cm}^{-1}$  suggested the presence of =CH bending of vinyl group in either acrylic acid monomer or MBAm cross-linker. Based on Fig. 2, it is obvious that there were band shifting in MIP spectrum as compared to NIP due to the presence of melamine template in MIP which provides melamine template-acrylic acid monomer interactions.

**Brunauer-Emmett-Teller analysis of synthesized polymers of melamine:** The Brunauer-Emmett-Teller (BET) analysis was conducted by nitrogen adsorption experiments in order to evaluate the specific surface area, average pore radius and total pore volume of both synthesized polymers. Based on the results (Table-2), the synthesized MIP had larger specific surface area, average pore radius and pore volume as compared to NIP. This was due to the presence of template molecule that contributed for the imprinting effect in MIP [17]. The solvent used in the polymerization process played important role in the pore formation within the macroporous polymers. For this reason, the structure, concentration and volume of the porogenic solvent applied during the polymerization process are responsible for the morphology, quantity of the pore and surface area of the generated MIP and NIP [18].

TABLE-2  
SURFACE AREA, AVERAGE PORE RADIUS AND TOTAL PORE VOLUME OF THE SYNTHESIZED MIP AND NIP

Products	Surface area ( $\text{m}^2/\text{g}$ )	Average pore radius ( $\text{Å}$ )	Total pore volume ( $\text{cc}/\text{g}$ )
MIP	365.50	1.64	3.00
NIP	216.78	1.36	1.47

**Thermogravimetric analysis of imprinted polymers of melamine:** Thermogravimetric analysis of the synthesized MIP is shown in Fig. 3. The initial stage of MIP weight loss of about 18.98 % within the range of 30 to 156.81  $^{\circ}\text{C}$ . This may be due to the moisture content in the polymer matrix. Then, the second stage of weight loss occurred at temperature between 156.81 to 470.42  $^{\circ}\text{C}$ , whereby at this stage drastic weight loss of MIP which is about 79 %. This weight loss might be due to the evolution of moisture and decomposition of functional groups, such as CO, OH and  $\text{NH}_2$  on the imprinted polymer backbone. The weight loss of MIP continued up to temperature from 470.42  $^{\circ}\text{C}$  to 684.63  $^{\circ}\text{C}$  with about 23.65 % MIP weight loss. This may be due to the decomposition of remaining backbone of the polymer. The final stage of weight loss was continued until  $\sim 900$   $^{\circ}\text{C}$ .

According to this analysis, different endothermic curves were observed which can be seen from the DTA curve. First

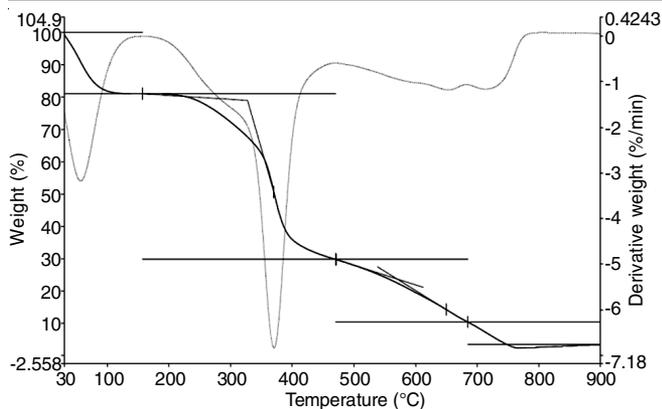


Fig. 3. TGA analysis of MIP

endothermic curve shows the removal of moisture present in the imprinted polymer. The second endothermic curve can be observed in the range of  $>350$  °C. This endothermic curve represents the breakage of crosslinking and thermal degradation of the molecular imprinted polymer.

**Rebinding experiment of synthesized polymers of melamine:** The rebinding results are presented in Fig. 4. Initially, the rebinding efficiency of MIP and NIP with melamine were increased as the contact time increases until 240 min and after that the rebinding efficiency remains almost constant until 300 min. At 240 min, MIP and NIP obtained their highest binding efficiency of 61.98 and 20.23 %, respectively. MIP had much higher rebinding efficiency as compared to NIP due to the presence of imprinting effect of melamine within the MIP cavity. Meanwhile, the deficiency of generated recognition sites for the target molecule within the NIP cavity resulting in lower percentage of rebinding efficiency [19].

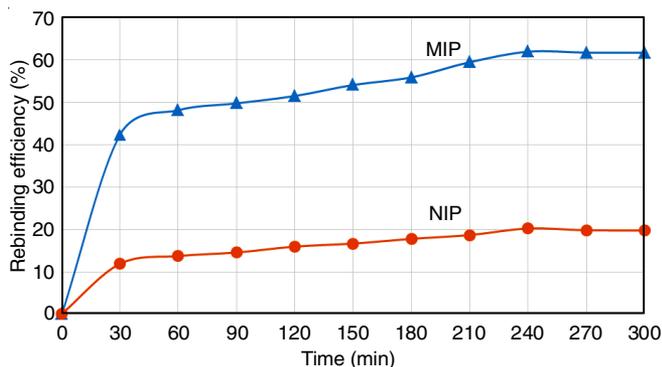


Fig. 4. Rebinding efficiencies of MIP and NIP at different time intervals

**Influence of melamine initial concentration on the rebinding efficiency:** In order to study the effects of melamine initial concentrations towards the synthesized MIP and NIP, various initial concentrations of melamine solution (15, 20, 25, 30 and 35 ppm) were applied, while other parameters including amount of polymer, agitation time, volume and pH of solvent were kept constant. As shown in Fig. 5, the adsorption efficiency of MIP and NIP increased as the melamine initial concentrations increased up to 25 ppm and then the binding efficiency of both polymers decreased as the initial concentrations of melamine was further increased (30 and 35 ppm). This indicated that 25 ppm melamine solution is the ideal melamine

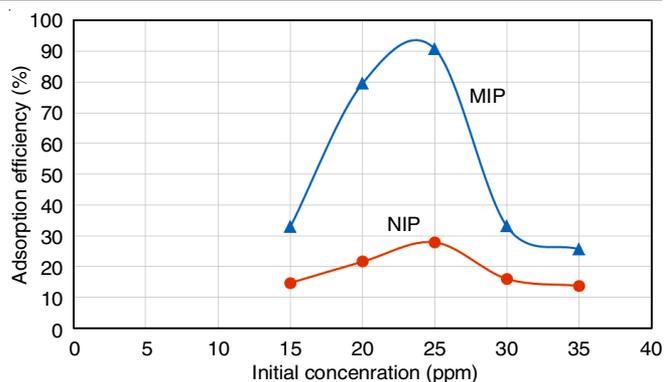


Fig. 5. Effect of initial concentrations of melamine on the rebinding efficiency of MIP and NIP

initial concentration to attain highest MIP rebinding efficiency. At this point (25 ppm), all the available binding sites have been saturated, while further increase in concentration have decreased the rebinding efficiency [20].

**Influence of polymer dosage on the rebinding efficiency:**

In this study, different dosage of polymer dosage (0.1 g to 0.7 g) of both MIP and NIP were employed while other parameters were maintained constant, in order to evaluate the amount of MIP needed to achieve its highest rebinding efficiency. The results (Fig. 6) showed that the adsorption efficiency increased as the MIP dosage is increased from 0.1 g to 0.5 g. MIP is known to have imprinting effects, so higher amount of MIP has increased the number of binding sites within the MIP matrix which provided MIP with more surface area for adsorption [21]. The highest adsorption efficiency was achieved when 0.5 g of MIP was used. This indicated that the optimum amount of available MIP particles for melamine molecule have fully occupied the imprinted polymer binding sites. Further increase in MIP dosage (0.6 g and 0.7 g) resulted in decrease in adsorption efficiency. This is due to excess amount of MIP particles that may have caused aggregation of polymer particles and thus interfere the binding process with melamine molecule. For comparison purpose, NIP was treated in similar way as MIP, whereby the trend of its adsorption efficiency was identical as MIP but in much lower efficiency.

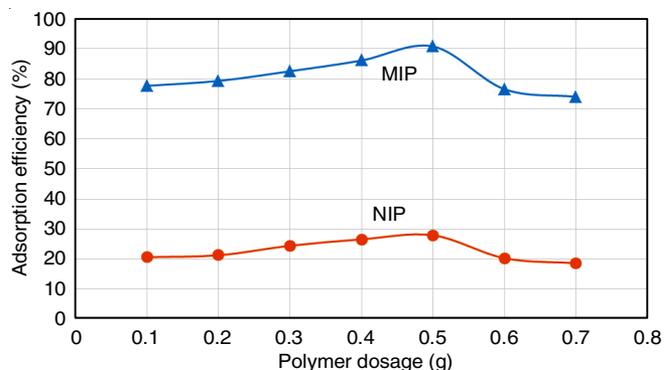


Fig. 6. Effect of polymer dosage on the rebinding efficiency of MIP and NIP

**Influence of pH condition on the rebinding efficiency:**

The effect of pH solution on both MIP and NIP were determine by applying five different pH conditions including acidic (pH 3 and 5), neutral (pH 7) and basic (pH 9 and 11), while other

parameters are kept unchanged. It was clear that the pH of melamine solution had influence on the performance of the synthesized polymers (Fig. 7). In acidic condition (pH 3 and pH 5), the melamine adsorption efficiencies were increased. At low pH condition, some functional groups within the chemical compound may be protonated which have affected the interactions between the compound and active MIP binding sites [22]. The same approach was observed in basic conditions (pH 9 and pH 11). A decrease in rebinding efficiency was achieved. This may be due to the alterations in functional groups available on melamine template. The neutral condition (pH 7) was considered best for the rebinding of melamine. This is because there were not any alterations in the functional sites available on both melamine and polymer. The NIP had much lower rebinding efficiency as compared to MIP because of the presence of non-specific interactions within the NIP matrix [23], as there is no imprinting effect within the NIP cavities.

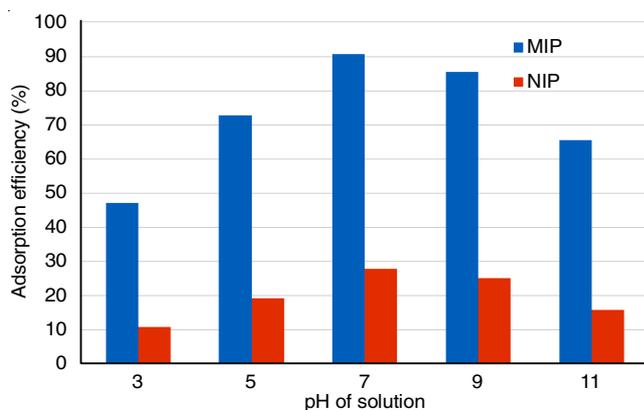


Fig. 7. Effect of pH on the rebinding efficiency of MIP and NIP

**Selectivity of synthesized MIP:** The selectivity test of produced MIP involving a template molecule and a competitive compound a structural analogue to the target molecule, whereby the binding efficiencies of MIP and NIP towards both compounds were compared [24]. In this experiment, the MIP selectivity towards melamine was demonstrated using 2,4,6-trichlorophenol as its competitive compound. Table-3 depicted the experimental results of MIP and NIP selectivity towards melamine and 2,4,6-TCP. It was observed that the distribution ratio of melamine compound in both MIP and NIP was higher as compared to the distribution ratio of 2,4,6-TCP compound in

both MIP and NIP. As a result, the selectivity coefficient of melamine compound in MIP and NIP was also higher than that 2,4,6-TCP. This is due to the presence of complementary recognition sites or cavities in the generated imprinted polymers that fit suitably with its target molecule than that competitive compound [25]. The relative selectivity coefficient ( $K'$ ) was 1.83, implying that the synthesized MIP has a good molecular recognition.

**Extraction/removal of melamine from milk, river water and blood serum:** The synthesized MIP has been employed in the removal/extraction of melamine from all the sample. A good removal efficiency was achieved by MIP in milk, river water and human blood serum (84.30 %, 94.26 % and 93.32 %, respectively). Meanwhile, the NIP has extracted much lesser melamine from the spiked samples such as milk, river water and human blood serum (33.37 %, 38.57 % and 36.91 %, respectively) (Table-4). This indicated that the synthesized MIP has been useful for the removal of melamine from different samples. From the results achieved, it is clear that the MIP contained complementarily binding sites with melamine. The presence of template during molecular imprinting process gave numerous interactions for the functional monomer to generate imprinted polymers with higher affinity and specificity towards the target molecule [26].

## Conclusion

The molecularly imprinted polymer of melamine was successfully synthesized by employing the non-covalent approach with microemulsion polymerization method. The shape of generated imprinted polymers was globular and in nano-sized. Generally, synthesized MIP had much higher binding efficiency than that NIP under several conditions including, different polymer dosages, initial melamine solution and various pHs of melamine solution. Through the selectivity test, it was proved that the synthesized MIP is more selective towards melamine in comparison with 2,4,6-TCP. The synthesized MIP have successfully extracted melamine from the spiked milk, river water and human blood serum.

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TABLE-3  
DISTRIBUTION RATIO, SELECTIVITY COEFFICIENTS AND RELATIVE SELECTIVITY COEFFICIENT OF THE SYNTHESIZED MIP AND NIP

	$K_D$ of MIP ( $\text{mL g}^{-1}$ )	$K_D$ of NIP ( $\text{mL g}^{-1}$ )	$K^{sd}$	$k'$
Melamine (template)	17.51	4.62	3.79	–
2,4,6-Trichlorophenol (competitive compound)	5.28	2.56	2.06	1.83

TABLE-4  
EXTRACTION/REMOVAL OF MELAMINE FROM MILK, RIVER WATER AND BLOOD SERUM

Samples	MIP				NIP		
	Amount of melamine added ( $\mu\text{g/mL}$ )	Amount of melamine found ( $\mu\text{g/mL}$ )	Recovery (%)	RSD (%)	Amount of melamine found ( $\mu\text{g/mL}$ )	Recovery (%)	RSD (%)
Milk	25	21.075	84.30	0.18	8.3425	33.37	0.55
River water	25	23.565	94.26	0.13	9.6425	38.57	0.74
Blood serum	25	23.330	93.32	0.16	9.2275	36.91	0.57

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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