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Synthesis and Characterization of Pyrogallol-Imprinted Poly(methacrylic acid) *via* Precipitation Polymerization

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Abstract

An innovative approach for the selective recovery of pyrogallol by means of molecularly imprinted polymer (MIP) was developed. Pyrogallol has been reported to be found in natural herb plants such as *Kacip Fatimah* (*Labisia pumila*) and harmful to human when it is consumed beyond the permissible amount. In this study, pyrogallol-imprinted polymer (PIP) and non-imprinted polymer (NIP) were synthesized *via* precipitation polymerization on methacrylic acid (MAA) with divinylbenzene (DVB) and azobisisobutyronitrile (AIBN) cross-linker and initiator, respectively. The synthesized polymers were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and UV-Visible Spectroscopy (UV-Vis). Studies on adsorption isotherm were carried out by using Langmuir and Freundlich isotherm models. Results showed that NIP and PIP follows Freundlich and Langmuir isotherm models, respectively. In order to analyze the sorption kinetics of NIP and PIP, two kinetic models were applied; pseudo-first and pseudo-second order. Samples of NIP and PIP were found to follow pseudo-second order, indicating the rate-limiting step is the surface adsorption.

Keywords: adsorption isotherms; adsorption kinetic; precipitation polymerization; molecularly imprinted polymer

INTRODUCTION

Herbal medicinal products or traditional medicines are mostly formulated from crude extract of herb plants. The herbal extract contains phytochemicals such as flavanoids and alkaloids that can serve as effective anti-oxidant. However, unnecessary phytochemicals may present and it is almost unavoidable. It was reported that some plant alkaloids and flavonoids are harmful to human.[1] Among all the flavonoids and alkaloids, a phenolic compound known as pyrogallol was reported to be present in the extract of Kacip Fatimah (*Labisia pumila* Benth) [1]. Beyond the permissible amount, pyrogallol has been reported to be harmful to human. LD_{50} of pyrogallol for human is 28 ppm [2]. Pyrogallol may be exposed to human in many ways such as through hair cosmetic[3], photographic developing agent[4], and decoction drink such as tea and herb products[5]. Pyrogallol was shown to cause the inhibition of the cell growth, cell death and toxicity in most important human organs [4,6]. Pyrogallol was also capable to induce O_2 to mediate the death in several types of cells in human organs. In the past, there were several methods that have been used in the determination and detection of pyrogallol including gas chromatography, HPLC, and flow injection analysis. However, there were disadvantages, such as long time consuming and long laboratory pretreatment [7, 8].

Molecularly imprinted polymer (MIP) has been very favorable approach in detection of specific target molecules. It has been used in various fields such as solid-phase extraction [9-11], sensor [12-14], and chromatography [7]. MIP works as a porous material with specific molecular recognition sites for specific target molecules [15]. MIP is prepared by using template molecules that are capable to self-assemble with monomers during the polymerization. The polymerization is usually carried out in the presence of a cross-linker that allows the formation of imprinted polymer matrix. After the removal of the template molecules, molecular recognition cavities with high selectivity and specific binding capacity are obtained [16]. The principles of molecular imprinting are as follows: (1) formation of specific complex from non-covalent interactions between template molecules and monomers in polar or aprotic solvent by assembling the monomers around template molecules prior to polymerization; (2) in the presence of cross-linker and initiator, a rigid and porous copolymer is obtained and (3) the distinct cavities remain complementary to the target molecules in size, shape and functionality after the removal of target molecules [7, 17].

MIP have been prepared by various methods, including bulk polymerization [18,19], suspension polymerization [20], emulsion polymerization and precipitation polymerization [18]. However, precipitation polymerization is the most preferable approach since it does not require the addition of emulsifier or any suspension reagent. Precipitation polymerization produces spherical MIPs that have been developed and applied in various studies such as biomolecules (amino acids, antibiotics and herbicides) and also in analysis of environmental interest (toxic compounds) [8]. A narrow particle size distribution and more homogeneous binding site can be obtained from precipitation polymerization as the polymerization takes

place in a medium condition in which the polymer particles are not stable and tend to agglomerate into spherical precipitates [18].

In this study, pyrogallol imprinted polymer (PIP) and non-imprinted polymer (NIP) was prepared *via* precipitation polymerization using pyrogallol (Py), methacrylic acid (MAA), divinylbenzene (DVB), and azobisisobutyronitrile (AIBN) as template, monomer, cross-linker and initiator, respectively. This study was carried out to determine the capability of PIP and NIP to adsorb the target molecules (pyrogallol). The synthesized polymers were characterized by Fourier transform infrared spectroscopy (FTIR), and Scanning Electron Microscope (SEM). The rebinding capability of the synthesized polymers was studied *via* UV-Vis Spectrophotometer in term of dosage effect and time.

EXPERIMENTAL

Chemicals :

Pyrogallol (Py) ($C_6H_3(OH)_3$, MW: 126.11 g/mol), methacrylic acid (MAA) ($H_2C=C(CH_3)COOH$, MW: 86.09 g/mol), divinylbenzene (DVB) ($C_6H_4(CH=CH_2)_2$, MW: 130.19 g/mol), and azobisisobutyronitrile (AIBN) ($(CH_3)_2C(CN)N=NC(CH_3)_2CH$, MW: 164.21 g/mol) were obtained from Sigma Aldrich, USA. Other chemicals are of reagents grade and were purchased from Merck, Malaysia. All chemicals were purified prior to use and AIBN was re-crystallized from methanol.

Synthesis of Pyrogallol Imprinted Polymer (PIP) and NIP :

The pyrogallol-imprinted polymer was prepared *via* precipitation polymerization method by dissolving 1.40 mmol of pyrogallol (template) in 100 mL of acetonitrile in a 500 mL Nalgene bottle with screw cap. This was followed with the addition of 4.19 mmol of methacrylic acid, 27.95 mmol of DVB-80 and finally 1.80 mmol of AIBN. The solution was purged with nitrogen gas for 30 minutes in an ice-bath. The bottle was placed on a Stovall flat-bed roller at room temperature. After approximately 2 hours, the temperature was ramped to 60°C for over 48 hours. The solution was cooled to room temperature. The obtained precipitates were filtered and washed with acetonitrile and then with the mixture of methanol and acetic acid (9/1: v/v). The polymer precipitates were then dried to a constant mass at 40°C for 24 hours. The non-imprinted polymer (NIP) was synthesized in the same manner, except that the template was excluded.

Characterization:

The surface, porosity, particle size and shape of samples were studied by Scanning Electron Microscopy (SEM) model JEOL JSM-6360LA. The samples were also

characterized by Fourier Transform Infrared Spectroscopy (FTIR) model Perkin Elmer 100 Spectrophotometer Spectrum GX.

Adsorption Study:

A sample solution (10 ppm) was prepared by dissolving the template in 100 mL of methanol. 5 mL of solution was placed in contact with different amount of adsorbents (2, 4, 6, 8, and 10 mg). The samples were analyzed at interval times of 5, 10, 15, 30, 60, 120, and 140 min by using UV-Vis Spectrophotometer, Shimadzu. The applicability of isotherm models used was studied by obtaining the highest correlation coefficient, R^2 values in the isotherm plot.

Selectivity Study:

In order to measure the selectivity of the imprinted polymer, a compound with most similar structure with pyrogallol was studied. In this study, Gallic acid has been chosen. 5 ml of 10 ppm for each compound was added in 10 ml centrifuge. 8 mg of PIP was added into the solution. The mixture was centrifuged for 60 min. After 60 min of contact time, the solution was filtered and measured. NIP also was examined under the same manner in order to observe the differentiation between PIP and NIP selectivity towards pyrogallol.

RESULTS AND DISCUSSION

Synthesis of Pyrogallol Imprinted Polymer (PIP) and NIP :

PIP and NIP were successfully synthesized *via* precipitation polymerization and spherical beads were obtained. Yields of product obtained were good, with PIP (89%) higher than NIP (79%). Functional monomer used gives the main contribution in production of yield.

Characterization:

Figure 1 showed the morphology of the obtained polymers that were in spherical shape. PIP (a) samples were obtained in homogeneous spherical beads. Meanwhile, NIP (b) samples were obtained in heterogeneous spherical shape. Particles obtained were in the range of ~4 µm and ~16 µm for PIP and NIP, respectively. The uniformity obtained due to the influenced of DVB and acetonitrile-toluene mixture and different size of diameter between PIP and NIP was due to the presences of the template molecule [16,21]. Report had been suggesting that template molecule give an important influence to the particle growth or size. It was explain that in the absence of template, functional monomer (MAA) can form hydrogen-bonded dimer in non-imprinted polymer. Whereas in imprinted polymer, there were additional interaction

between template and MAA that leads to the growth of polymer nuclei which produced smaller polymer beads [22].

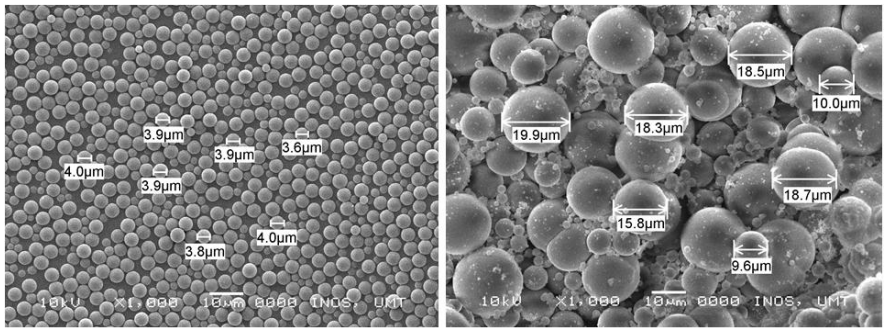

Figure 1: SEM Images of (a) PIP and (b) NIP

The spectra for Py, PIP and NIP were shown in Figure 2. PIP and NIP exhibit almost identical spectra due to the similar backbone from the same monomer used. The peak at 3399 cm^{-1} indicates the presence of O–H vibration bonds of phenol group in Py. The peaks at 1483 cm^{-1} and 1622 cm^{-1} in the spectrum of Py show the presence of aromatic (C=C) groups which is the characteristic of benzene aromatic ring. The C-O vibration group in Py is detected at 1247 cm^{-1} [23]. Peaks at 1792 cm^{-1} and 1796 cm^{-1} for NIP and PIP respectively show the presence of (C=O) groups while the peak at 1645 cm^{-1} was attributed to (C=C) and peaks at around 1170 - 1280 cm^{-1} were attributed with C-O stretching in esters. The absorption of hydroxyl groups was observed at 1220 cm^{-1} and it has been reported to be due to the conjugation with oxygen attached to the ring (C-O group). Corresponding peaks of the template (Py) molecules do not show up in the spectrum of PIP. This suggests that the template was successfully removed from PIP.

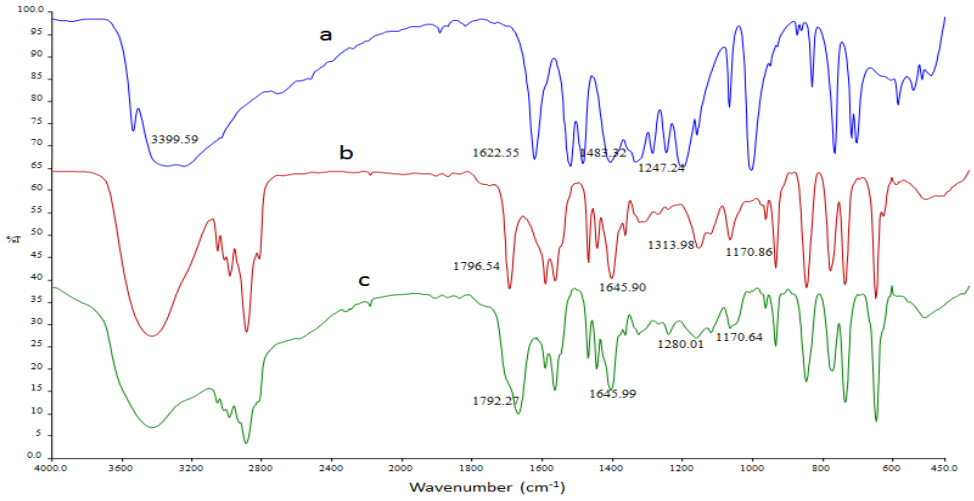

Figure 2: FTIR spectra for (a) Pyrogallol (Py); (b) Pyrogallol-Imrpinted Polymer (PIP) and (c) Non-Imprinted Polymer (NIP)

Adsorption Study:

Effect of Dosage and Time

Since pyrogallol was reported to be toxic and cause serious health problems, therefore this report was conducted in order to study adsorption activity of the PIP. From Figure 3 showed that the adsorption by PIP against several of dosage. Results showed that PIP reaches the equilibrium at 8 mg of dosage. Results also showed that PIP is capable to adsorb pyrogallol more than NIP. When 8 mg of samples was used, PIP was able to adsorb 77% of pyrogallol while NIP was able to adsorb only 31% of pyrogallol. The results shows that adsorption of pyrogallol by PIP was increased with increasing amount of polymer due to the increase of binding or imprinting sites of the PIP.

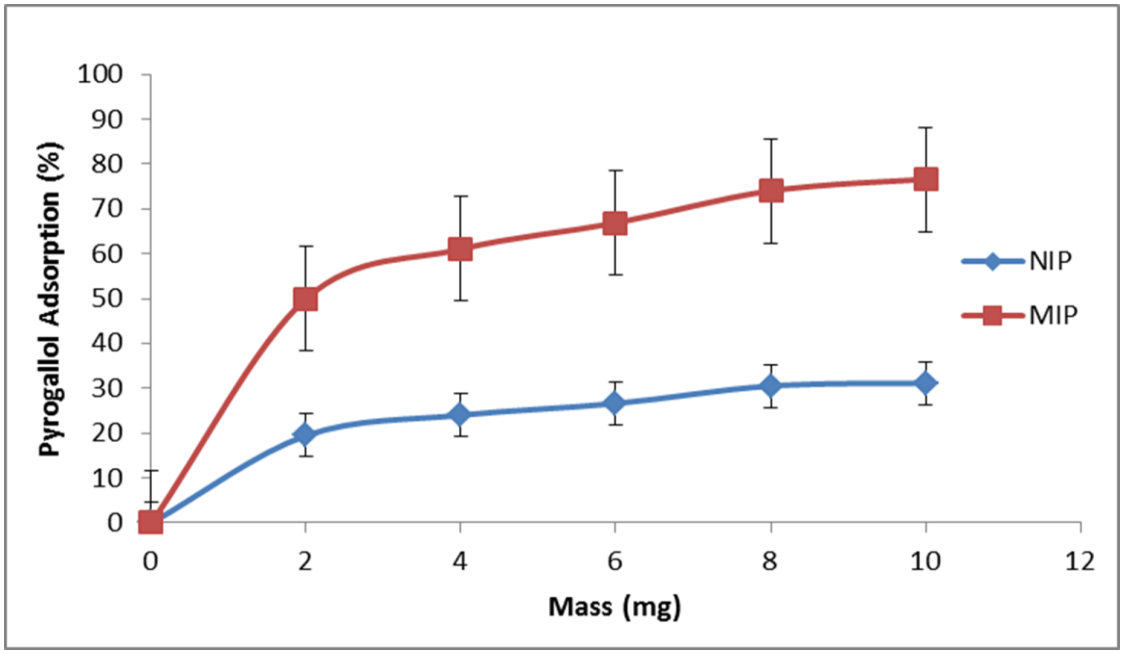

Figure 3: Pyrogallol adsorption of pyrogallol from pyrogallol solution by Non-Imprinted Polymer (NIP) and Pyrogallol-Imrpinted Polymer (PIP)

Whereas in Figure 4 shows the adsorption of pyrogallol by PIP and NIP towards effect of time. The adsorption of pyrogallol was increased for the first 45 minutes and achieved equilibrium at 60 minutes. Equilibrium was achieved due to the high complimentary between pyrogallol in the solution and binding sites in PIP [8,17]. At equilibrium, PIP and NIP adsorbed 77% and 31% pyrogallol, respectively. Equilibrium stage was established due to the resistance of the adsorption into the deep cavities when the surface cavities were occupied by target molecule [8].

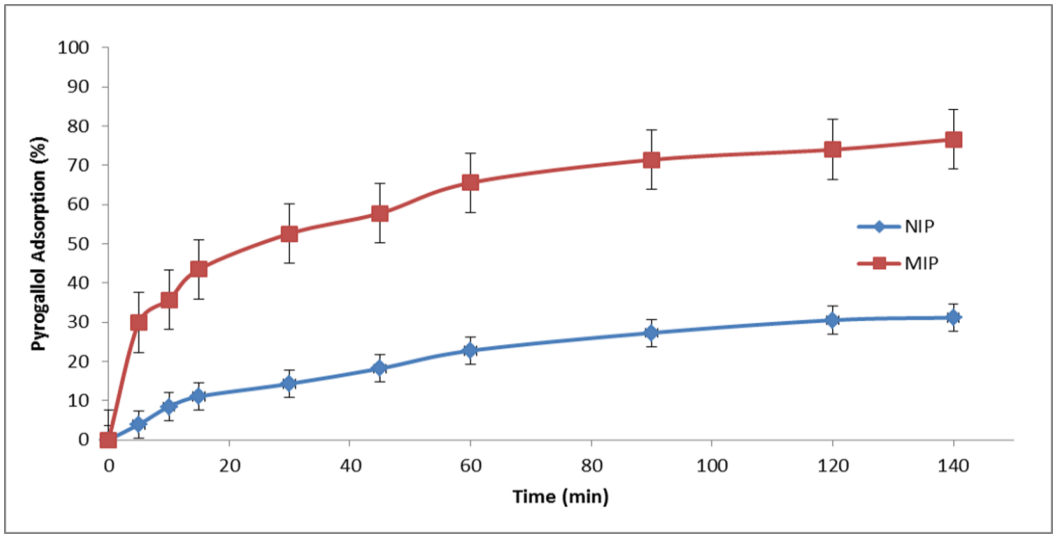

Figure 4: Effect of time on adsorption pyrogallol by Non-Imprinted Polymer (NIP) and Pyrogallol-Imrpinted Polymer (PIP)

There were rarely reports on pyrogallol imprinted polymer from past researcher but there were several reports on adsorption of pyrogallol using commercial active carbons. However, since pyrogallol molecule has the similar properties with Gallic Acid, this study was conducted according to the reports on adsorption of Gallic Acid imprinted polymer. Table 1 shows the comparison of the results obtained from the previous works on adsorption of Gallic Acid. It was also suggested that the higher the adsorption percentage, more selective the adsorbent material to extract or adsorb target molecule [24].

Table 1: The comparison of the results obtained from the previous works on adsorption of Gallic Acid.

Functional Monomer	**Porogen**	**Polymerization Type**	**Capacity (mg/g)**	**Ref**
4-VP	Methanol	Bulk	73	Zhu et al., 2009
Hydroxyethyl methacrylate	Dimethylformamide	Bulk	<70	Nicolescu et al., 2013
Acrylic Acid	Acetonitrile-toluene	Precipitation	200	Pardheshi et al., 2014
Dopamine	Phosphate buffer	Precipitation	634	Hu et al., 2015

Isotherm and Kinetic Study

Adsorption isotherm is the key to understand the adsorption mechanism [25,26]. Langmuir and Freundlich isotherm models were used to fit the adsorption mechanism of PIP and NIP. Basic assumptions of Langmuir adsorption isotherm theory are: (a) the sorption of templates takes place only on the monolayer surface; (b) the sorption rate is not related to the amount of the sorbate molecules that has been adsorbed. The linear form of Langmuir model is stated in the following equation [28]:

$$(l/q_e) = (l/Q_0) + (l/bQ_0)(l/C_e)$$

C_e is the equilibrium concentration of solute, Q_0 is the amount of solute adsorbed at equilibrium (mg/g), *b* is the constant related to the affinity of binding sites (L/mg). Table 1 shows the Q_0 and *b* that were calculated from the slope and intercept of the plotted graph. Freundlich adsorption isotherm is used to describe the heterogeneity of the surface and the continuous energy for sorption on different level of surfaces [29-31]. The linear form of Freundlich model is stated in the following equation [28]:

$$\log q_e = \log K_f + (l/n) \log C_e$$

K_f and *n* are Freundlich constants that are related to adsorption capacity and intensity. Both K_f and *n* can be determined from linear plot of log q_e versus log C_e and the value of K_f and *n* is shown in Table 2. According to Table 2, R^2 for PIP obtained for Langmuir and Freundlich isotherm are 0.9881 and 0.9804, respectively. Langmuir isotherm for PIP has higher R^2 value compared to Freundlich. These results prove that PIP fits the Langmuir isotherm implying that PIP is able to adsorb in monolayer surface. Whereas for NIP, it shows that R^2 for Langmuir and Freundlich isotherm are 0.9808 and 0.9915, respectively. From the results, it shows that NIP obeys the Freundlich isotherm model.

Table 2: Langmuir and Freundlich constants for adsorption of pyrogallol

Samples	**Langmuir**			**Freundlich**		
	R^2	**Q_o**	**b**	**R^2**	**K_F**	**n**
NIP	0.9808	1.5489	1.7978×10^{-5}	0.9915	29.1484	0.3385
PIP	0.9881	9.0171	0.6825	0.9804	4.6567	1.6132

Next is, in order to understand the behavior of the adsorption, kinetic models were used. The models of kinetic studies are pseudo-first-order and pseudo-second-order models. The pseudo-first-order is known as the Lagergern model [27], and can be represented by the following equation:

$$\log (q_e - q_t) = \log q_e - (k_1/2.303)t$$

q_e is the amount of adsorbate adsorbed at equilibrium (mg/g), q_t is amount of adsobate adsorbed at contact time (mg/g), t is time (min), k_1 is the rate constant of pseudo-first-order. The value of q_e and k_1 can be determined from the intercepts and slopes of log ($q_e - q_t$) versus t. The pseudo-second-order can be represented by the following equation [27]:

$$l/q_t = l/k_s q_e^2 + t/q_e$$

The initial sorption rate, h (mg/g min) as t approaches 0 can be defined as $h=k_2 q_e^2$, q_e is the equilibrium adsorption capacity, k_2 is pseudo-second-order can be determined from plot of t/q_t versus t. According to Table 3, the value for R^2 for pseudo-first-order and pseudo-second-order are 0.7437 and 0.9992, respectively for PIP and 0.6654 and 0.9969, respectively for NIP. Based on the results, adsorption kinetic of PIP and NIP obeys the pseudo-second-order as the value of R^2 is higher than the pseudo-first-order. Moreover, the q_e(calculated) values was more consistent with experimental q_e.

Table 3: Kinetic constants for NIP and PIP

Samples	$q_{e\ exp}$	**Pseudo-First Order**			**Pseudo-Second Order**		
		R^2	q_e	K_1	R^2	q_e	K_2
NIP	2.5217	0.6654	1.3611	0.0147	0.9969	2.4988	0.0115
PIP	5.0726	0.7437	2.0436	0.1180	0.9992	5.1975	0.0130

As an explanation, pseudo-second-order explains the rate-limiting step at surface involves chemisorption. Chemisorption proposed that the adsorption mechanism of PIP occur in two stages which follow: (1) pyrogallol is diffused towards the surface of adsorbent and (2) interaction between the molecule of adsorbate (pyrogallol) and the surface of adsorbent [32].

Selectivity Study:

Selectivity of PIP and NIP towards pyrogallol was studied in order to estimate the effect of imprinting factor. Gallic acid (GA) was selected to be used as analogue template to pyrogallol due to the similarity properties as stated earlier. Figure 5 shows that the adsorption of pyrogallol by PIP was higher than NIP. This was due to the complementary binding sites in PIP compared to NIP. Selectivity studies involved the distribution and coefficient of GA with pyrogallol which can be calculated by the following equation, where K_d is the distribution coefficient.

$$K_d = \frac{(C_i - C_f)}{M} \times V$$

Selectivity coefficient (*k*) can be calculated using the following equation:

$$k = \frac{K_d\ (Py)}{K_d\ (GA)}$$

The relative selectivity studies can be calculated from selectivity coefficient by the following equation:

$$k' = \frac{k_{imprinted}}{k_{control}}$$

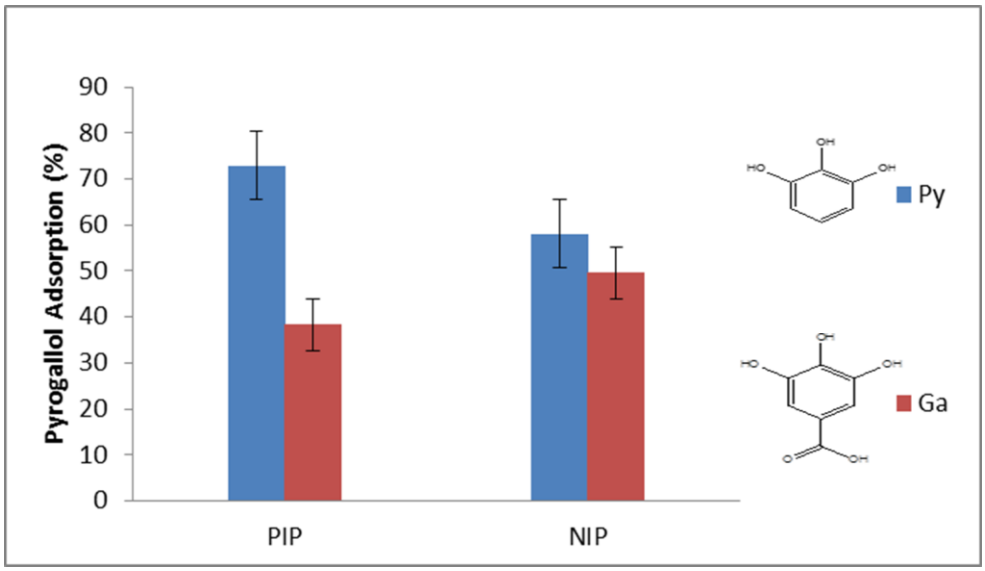

Figure 5: Selectivity study by PIP and NIP

Table 4 shows the summarized of K_d, *k* and *k'* for the selectivity study between GA and pyrogallol. The *k'* value for PIP is 1.6051. The value of *k'* must >1 to consider the imprinted polymer is acceptable and have good selectivity towards target molecule [25,26]. Results showed that the value of *k'* was >1 which representing a good selectivity towards pyrogallol and it can be concluded that the binding cavities has successfully imprinted in Py-IP.

Table 4: Selectivity study constant for PIP and NIP

System	**K_d**		***k* NIP**	***k* PIP**	***k'***
	Pyrogallol (Py)	Gallic Acid (GA)			
NIP	3.6651	3.0992	1.1826	1.8981	1.6051
PIP	4.5525	2.3984			

CONCLUSIONS

Spherical beads of PIP and NIP were successfully prepared by precipitation polymerization using pyrogallol as template. The maximum capacity of pyrogallol adsorption was 77% and 31% for PIP and NIP, respectively at 8 mg. Langmuir model was found to be more applicable for PIP compared to Freundlich model. In contrast, NIP was more applicable on Freundlich model. Adsorption and kinetic study indicated that the NIP and PIP obey the pseudo-second order.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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