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GLUCOSE SULFATE IMPRINTED POLYMER PREPARED BY SOL-GEL PROCESS ON SILICA MICROPARTICLES SURFACE: KINETIC MODELING AND ISOTHERM STUDIES

(Penyediaan Glukosa Sulfat Polimer Tercetak Melalui Proses Sol-Gel Pada Permukaan Mikro Zarah Silika: Kajian Model Kinetik dan Isoterma)

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Abstract

Determination of monomers or structures of Glucose sulfate (sulfated sugars) are prerequisite for understanding their biological roles. Here, surface molecular imprinted polymer (MIP) on silica gel particles was applied for recognition of the biologically relevant sulfated sugar substitution on sugar. The non-covalent surface MIP was prepared by a sol-gel process using three different functional monomers; amine, imidazole and methyl-imidazole functioned silane with glucose sulfate as template model. The sulfated sugar imprinted silica microparticles were characterized by Fourier Transform Infrared Spectroscopy (FT-IR). A batch adsorption experiment were carried out at constant temperature between these MIPs of three different monomers and shown that all MIPs presented the best fit to Langmuir isotherm model. The MIP-methyl-imidazole had showed highest binding capacity of 53.07 mg/g, whereas the MIP-amine possess high imprinting factor (IF; 2.24). Nevertheless, MIP with high imprinting factor was preferred due to the high affinity and more specific interaction of the template adsorption. The kinetic model behavior study was carried out on these MIPs. The results indicated that the MIP-amine and MIP-imidazole were best described of pseudo-second-order kinetic model while, the MIP-methyl-imidazole can be expressed as pseudo-first order kinetic model.

Keywords: glucose sulfate, kinetic modeling and isotherm, molecular imprinted polymer, sol-gel process

Abstrak

Penentuan monomer atau struktur glukosa sulfat (gula sulfat) adalah pra-syarat untuk memahami peranan biologinya. Di sini, permukaan polimer molekul tercetak (MIP) pada zarah silika gel telah digunakan bagi mengenalpasti sifat biologi yang berkaitan dengan penggantian gula sulfat pada gula. Permukaan MIP bukan kovalen telah disediakan melalui proses sol-gel menggunakan tiga monomer fungsian yang berbeza; amina, imidazole dan metil-imidazole berfungsi silana dengan sulfat glukosa sebagai model templat. Gula sulfat tercetak pada silika zarah mikro telah dikenalpasti oleh Spektrofotometer Inframerah Transformasi Fourier (FT-IR). Satu kumpulan eksperimen penjerapan telah dijalankan pada suhu malar di antara MIP yang mempunyai tiga jenis monomer berbeza dan ini menunjukkan bahawa kesemua MIPs menunjukkan ianya sesuai kepada Langmuir model isoterma. The MIP-metil-imidazole telah menunjukan kapasiti ikatan tertinggi 53.07 mg/g, manakala amina MIP- mempunyai faktor tercetak tinggi (IF; 2.24). Walau bagaimanapun, MIP dengan faktor tercetak tertinggi telah dipilih disebabkan oleh afiniti yang tinggi dan interaksi yang lebih khusus bagi penjerapan template. Kajian terhadap tingkah laku model kinetik telah dijalankan ke atas MIP ini. Keputusan menunjukkan bahawa MIP-amina dan MIP-imidazole telah mengikuti model kinetik pseudo-tertib kedua sementara, yang MIP-metil-imidazole boleh dinyatakan sebagai model kinetik pseudo-pertama.

MIP-amina

Kata kunci: gula sulfat, model kinetik dan isoterma, permukaan polimer molekul tercetak, proses sol-gel

Introduction

Glucose sulfate is sulfated sugars, which are naturally occurring compounds which serves as a central biological role in mammals. The sulfated sugar is a glycosaminoglycan (GAG) family of linear polysaccharide that includes heparin, heparan sulfate, chodroitin sulfate and dermatan sulfate. Heparan sulfate-like glycan, were shown to play an important roles in binding of growth factors to receptors and viral adhesion to the cell surface. Thus, it is shown that the sulfated sugar serves as the development, differentiation and homeostasis [1]. Therefore, determination of its monomer or structure of these sulfated sugars is a prerequisite for understanding their biological roles. There are various techniques for identification and purification of this fine structure level from biological extracts, such as sequencing, electrophoresis and chromatographic [2]. However, these techniques suffer from many drawbacks such as coextraction of matrix interference components with target analytes, hight cost, poor selectivity, extensive labor and time consumption [3,4,5]. One way to overcome the problems is by using a molecular imprinted polymer (MIP) which may offer good advantages.

MIP can be synthesized in situ by a copolymerization process between self-assemble of template and functional monomer in the polymeric forming mixture, enables the formation of discrete cavities to provide specific interaction with the template when rebinding [6]. However, the strength of interaction between the template and the functional monomer in the polymeric forming mixture is important in order to increasing the affinity recognition towards the specific molecule. Therefore, the choice of functional monomers is crucial in order to reach only the specific target molecule. According to previous study, there was a construction of sulfated sugar imprinted polymer using traditional bulk polymerization technique [7]. However, a chance of losing many cavity sites during the crushed steps was high, which influence the rebinding performance.

To overcome the problems, a non-covalent surface imprinted polymer was prepared by sol-gel process. Recently, surface molecular imprinted polymer has attracted much attention in order to reduce the undesired leakage from the traditional MIP technique. Moreover, the surface imprinting come with some advantage such as adequate selectivity, more accessible binding sites fast mass transfer rate and binding kinetic [8]. Here, the glucose sulfate as a template was imprinted over the amine functional silane modified silica gel microparticle via surface graft copolymerization. According to previous researches, the high binding were obtained using the amine-types group as a functional monomer towards the carbohydrate, phosphate and phosphate ester template through non-covalent interaction [9]. Therefore, in this study three different functional monomers; amine, imidazole and methyl-imidazole functioned silane were studied in order to examined the affinity and specificity of adsorption performance. The characterization of the polymeric material, adsorption isotherm and kinetic mechanism model were also being investigated.

Materials and Methods

Silica-gel (230-400 mesh) used as a support binder for surface imprinted polymer, NaH_2PO_4, Na_2HPO_4, NaCl, 3-chloropropionyl chloride (CPCl), Imidazole, N-methylimidazole and Triethylamine were purchased from Merck, Germany. Glucose Sulfate was provided by Easybuyer Ltd. Shanghai, China and 3 aminopropyltrimethoxysilane (APTES) and DMSO were obtained from Sigma, Germany. Tetrarthoxysilane (TEOS) was purchased from Acros (Sigma, Germany) and hydrochloric acid was obtained from Fisher, Germany. The analytical grade of ethanol and methanol were used as washer solution supplied from HmbG, Germany.

Preparation of Glucose Sulfated-Surface Molecular Imprinted Polymer by Sol-Gel Process

The silica-gel surface was activated before the imprinting using the following procedure: silica-gel was mixed with 120ml concentrated 6M HCl. The mixture was refluxed for 10h under stirring. Then the resulting mixture was filtered and washed thoroughly with deionized water. Each washing steps was repeated by centrifugation at the rotational speed of 10,000 rpm for 10 min until neutral, followed by drying in an oven at 70^{o}C for an overnight. The purpose of this treatment was to enhance the content of silanol group and to eliminate the metal oxide and nitrogen containing impurities.

Preparation of Glucose Sulfated-Surface Molecular Imprinted Polymer: Amine Functioned Silane

0.5 mmole of glucose sulfate was dissolved in a mixture of 15ml DMSO and 5ml water. Then 0.857 mmole of APTES was added in the mixture and stirred for 30 min and then 1.8048 mmole of TEOS was added into the solution with continues stirring for another 30 min. These steps ensure that all template, monomer and cross-linker are attached to each other.

Diazole Functioned Silane

15 ml of DMSO was mixed with equalmole 0.857 mmole CPCl and imidazole or N-methylimidazole into a round bottle flask. For preparation of imidazole functioned silane, 0.867 mmole of triethylamine was inducted into the round bottle flask. The function of the triethylamine to neutralize the HCl, which was liberated as quaternary ammonium salt [10]**.** However, no triethylamine was added to the flask of methyl-imidazole functioned silane, in order to obtain the triethoxysilylpropylimidazolium chloride ionic liquid [11]. Both diazole functioned silane preparation were refluxed at 100^{o}C in an oil bath for 12h. The color of solution was changed from light yellowish to dark brown. After the refluxing, glucose sulfate (0.5 mmole dissolved in 5ml water) was added and stirred until all monomer dissolved and attached to the template. Then, 1.8048 mmole of TEOS was added to the mixture solution and stirred for about 20min.

To these three solutions, 2g of activated silica-gel and 0.2ml of 0.012M HCl were added sequentially, under stirring and then the suspension was polymerized at 30-40^{o}C for 24h. After the polymerization, the product was filter and washed with ethanol in 50ml for 3 times and neutralized with water. Then the template was extracted by ultrasonic extraction for 30 min with 50ml MeOH:HCl (10% in water) in ratio (1:1), MeOH and water. Each extract, was repeated for 5 times until it could no longer be detected by UV in different solution. Finally, the imprinted polymer was collected by filtration and dried at 80^{o}C overnight. The NIP was also prepared using an identical procedure without template.

Batch and Kinetic Rebinding Experiment

10mg of imprinted polymer was placed into a 10ml vial. 5ml of glucose sulfate in water at different concentration was added in the range of 0.0626-1.0 mg/ml. The vials were in horizontal and the beaker was placed into the orbital shaker for several hours at room temperature. The supernatant was filtered by nylon syringe filter (0.45um) before it was measured by UV-Vis spectrometer (Varian Cary 50 UV-Vis Spectrometer, Agilent, US). An amount of glucose sulfate bound to the polymer was obtained by subtracting the glucose sulfate concentration in supernatant to the initial glucose sulfate concentration. Imprinting factor was calculated base on Q_{MIP} over Q_{NIP}.

Results and Discussion

Preparation and Characterization of Glucose Sulfated-Surface Imprinted Polymer

FT-IR spectra for LMWH-MIP and LMWH-NIP and silica-gel were conducted in order to prove the presence of amine groups in the silica-gel absorbents as shown in Figure 1. The FTIR analysis was performed using a Perkin Elmer Spectrum 100 Series FT-IR spectrometers using KBr pellet method. The observed feature around 1070 cm^{-1} was due to the Si-O-Si and Si-O-H stretching vibration. The peaks around 796 cm^{-1} was resulted from Si-O vibration. In all MIP sorbents, C-H peak and C-N peak were recognized at 2945 cm^{-1} and 1551 cm^{-1} respectively. Meanwhile for MIP-imidazole and methyl-imidazole, exhibited two characteristic peaks at 1540 cm^{-1} and 1460 cm^{-1}, which were due to C=N and C=C vibrations of the imidazole ring [12]. However, no peak at these IR was observed at activated silica-gel sorbent, which confirms the amine groups from functional monomers (amine, imidazole and methyl-imidazole functioned silane) was grafted into the silica-gel surface.

Batch Binding Capacity and Recognition Mechanism

Figure 2, shows the adsorption isotherm plots of binding amount of glucose sulfate on MIP with three different functional monomers. MIP-methyl-imidazole exhibits the highest adsorption capability compared to others MIP. The tertiary amine functioned silane formed an ionic liquid MIP, containing a cationic species in the polymeric MIP matrix. The cationic species causes strongest interaction towards the anionic species (sulfate group) in glucose sulfate, which explained the highest binding capacity of glucose sulfate.

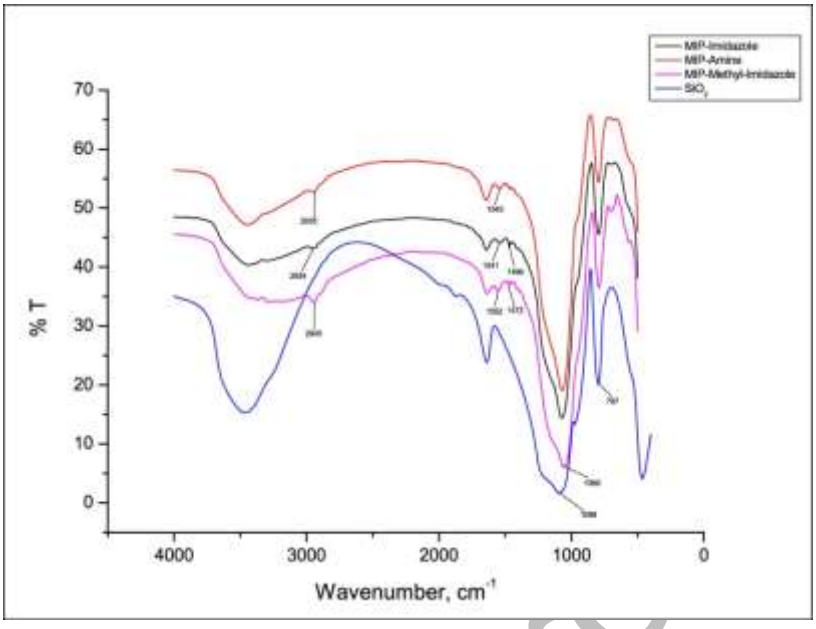

Figure 1. FTIR spectra for MIP with three different functional monomers.

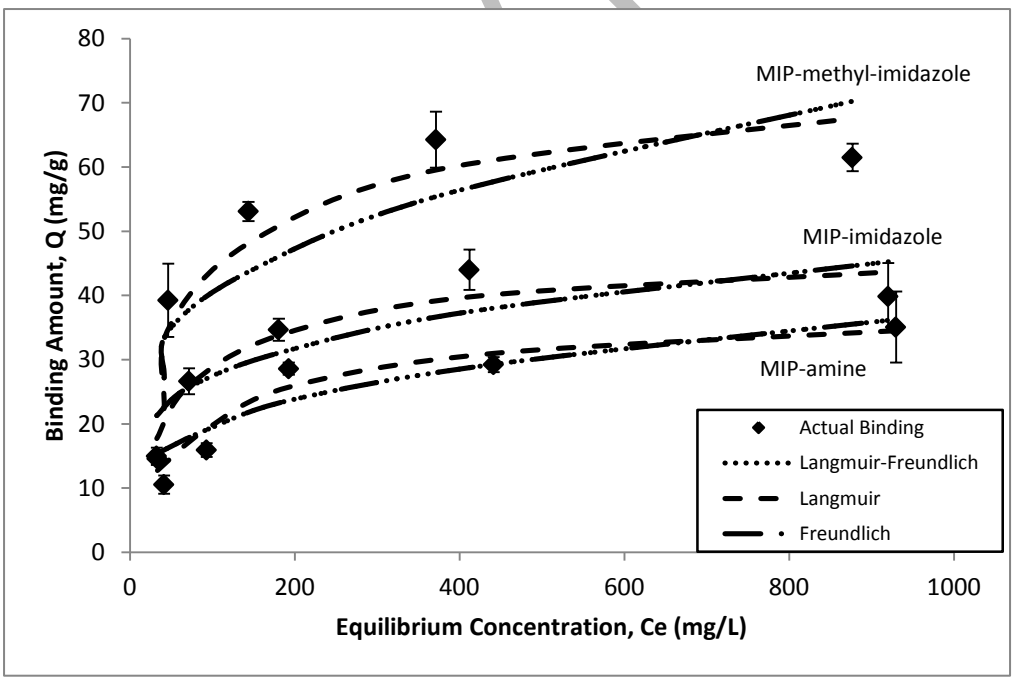

Figure 2. Adsorption isotherm and curve fitting of glucose sulfate on MIP with three different functional monomers.

The experimental data were fitted to the three isotherms models which are Langmuir (1), Freundlich (2) and Langmuir-Freundlich (3):

$$Q = \frac{q_{max} K_L C_e}{1 + K_L C_e} \quad (1)$$

$$Q = K_F C_e^{1/n} \quad (2)$$

$$Q = \frac{q_{max} K_{LF} C_e^n}{1 + K_{LF} C_e^n} \quad (3)$$

where, Q is a binding amount in adsorbent at equilibrium (mg/g), q_{max} is a maximum monolayer saturation capacity (mg/g), K_F, K_L and K_{LF} are dissociation constant of Freundlich (mg/g) (L/g), Langmuir (L/g) and Langmuir-Freundlich (L/g) respectively, while *n* is a heterogeneity parameter respectively. Meanwhile, Ce is a concentration in equilibrium (mg/L). The best fitted of the corresponding model were calculated and selected according to the minimum value of residual sum of square RSS (Eq. (4)) and largest value of F_{test} (Eq. (5)):

$$RSS = \sum_{i=1}^{n} (Q_{calc} - Q_{meas})^2 \quad (4)$$

$$F_{test} = \frac{n-l}{n-1} \frac{\sum_{i=1}^{n} (Q_{meas} - \bar{Q})^2}{\sum_{i=1}^{n} (Q_{meas} - Q_{calc})^2} \quad (5)$$

where, *n* denotes the number of experimental data, Q_{calc} is calculated equilibrium solid phase concentration while Q_{meas} is measured equilibrium solid phase concentration, meanwhile $\bar{Q}$ is the average of the experimental data points and *l* is the number of model adjustable parameters. These parameters were calculated according to the nonlinear regression methods using a Microsoft Excel solver function by minimizing the sum of square errors. According to previous study, nonlinear provides a mathematical rigorous method for determination of isotherm parameters using the original form of the isotherm equation. Through these nonlinear regression methods, experimental errors could reduce [13, 14]. The parameters calculated according to the nonlinear regression were listed in the Table 1.

Table 1. Adsorption isotherm parameters by nonlinear regression for the MIP with three different functional monomers

Fitting Parameters	**Isotherm Model**								
	MIP- amine			**MIP-imidazole**			**MIP-methyl-imidazole**		
	Langmuir-Freundlich	**Langmuir**	**Freundlich**	**Langmuir-Freundlich**	**Langmuir**	**Freundlich**	**Langmuir-Freundlich**	**Langmuir**	**Freundlich**
q_{max}	9.46	38.93		11.77	48.37		7.28	78.06	
k_L		8.00E-03			9.00E-03			6.42E-03	
n	0.31		0.31	0.27		0.27	0.34		0.34
k_F			4.3			6.92			6.58
k_{LF}	0.06			0.11			0.51		
∑RSS	36.71	5.13	7.34	125.75	8.25	25.15	108.64	48.77	108.64
F_{test}	23.55	34.97	23.55	9.82	37.15	9.82	7.86	23.52	7.86

Table 1 show these MIP with three different functional monomers were fitted on three different isotherm models, according to the minimum ∑RSS and largest F_{test} values. All three MIPs followed Langmuir isotherm model. This model assumes that adsorption take place at specific homogeneous site within the adsorbent and it could not proceed beyond monolayer coverage [15, 16]. The sum of maximum adsorption capacities q_{max} for MIP-amine,

MIP-imidazole and MIP-methyl-imidazole were found to be 35.07, 43.97 and 64.25 mg/g respectively, which was close to the measured data.

Kinetic Rebinding Capacity and Mechanism Model

Kinetic rebinding capacity was investigated to study the performance and predicted the adsorption mechanism model in range from 15-240 min. Here, pseudo-first order and pseudo-second order model were used to fit the experiment data by applying the adsorption kinetics equations as shown in (Eq.(6)) and (Eq.(7)):

$$Q_t = Q(1 - e^{-k_1 t}) \quad (6)$$

$$Q_t = \frac{Q^2 k_2 t}{(1+Qk_2 t)} \quad (7)$$

where, Q and Q_t are binding amount in adsorbent at equilibrium (mg/g) and binding amount in adsorbent any time *t* (min) respectively. Meanwhile, k_1 and k_2 are pseudo-first (1/min) and pseudo-second (g/mg/min) order rate constant respectively. Similar to an, adsorption isotherm, the best fitted of the corresponding model were calculated and selected according to the minimum value of residual sum of square RSS (Eq. (4)) and highest value of F_{test} (Eq. (5)). According to nonliner regression model, the kinetic parameters was calculated and listed on Table 2.

Table 2. Kinetic parameters for the adsorption of glucose sulfate to the MIP with three different functional monomers

Fitting Parameters	**Isotherm Model**								
	MIP- amine			**MIP-imidazole**			**MIP-methyl-imidazole**		
	Langmuir-Freundlich	**Langmuir**	**Freundlich**	**Langmuir-Freundlich**	**Langmuir**	**Freundlich**	**Langmuir-Freundlich**	**Langmuir**	**Freundlich**
q_{max}	9.46	38.93		11.77	48.37		7.28	78.06	
k_L		8.00E-03			9.00E-03			6.42E-03	
n	0.31		0.31	0.27		0.27	0.34		0.34
k_F			4.3			6.92			6.58
k_{LF}	0.06			0.11			0.51		
∑RSS	36.71	5.13	7.34	125.75	8.25	25.15	108.64	48.77	108.64
F_{test}	23.55	34.97	23.55	9.82	37.15	9.82	7.86	23.52	7.86

Table 2 shows the MIP-amine and MIP-imidazole fitted to the pseudo-first order kinetic model, due to the minimum values of ∑RSS and largest F_{test} and $Q_{(meas)}$ values of pseudo-first order were almost identical to the $Q_{(calc)}$ values. However, the MIP-methyl-imidazole fitted to the pseudo-second order kinetic model with $Q_{(meas)}$ 61.15mg/g almost identical to $Q_{(calc)}$ 61.38 mg/g. The model described that the adsorption process was a chemisorptions process of the rate-limiting step, which may involve the coordination bonding between the sulfate ion and adsorbent active sites [5]. These nonlinear fitting results were illustrated by the regress curve of experimental and calculated model data as shown in Figure 3.

As shown in Figure 3, glucose sulfate kinetic adsorption towards the MIP-amine was highest (85.60 mg/g) at 240 minutes compared to with others MIP. However, the binding adsorption capacity of MIP-methyl-imidazole was highest and faster (40.23 mg/g) in the early 15 minutes and its kinetic adsorption was very low (60.38 mg/g) at 240 minutes of adsorption process. It implies that MIP-amine owns higher affinity and specificity for glucose sulfate than MIP-methyl-imidazole. This data was agreed to the imprinting factor data as shown in Figure 4.

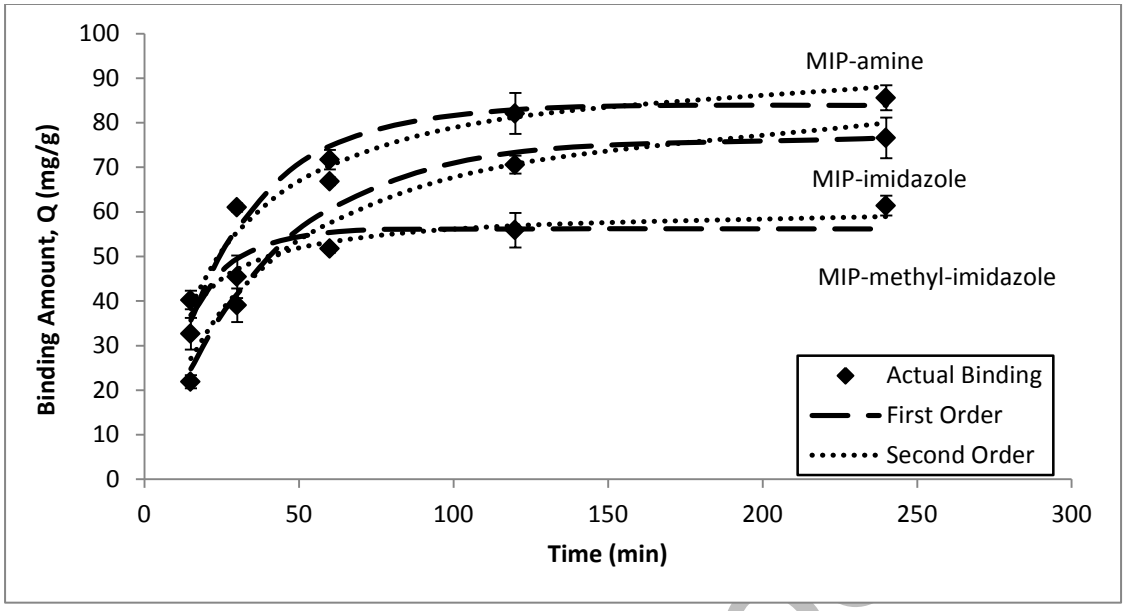

Figure 3. Kinetic adsorption and fitting curve of glucose sulfate on MIP with three different functional monomers

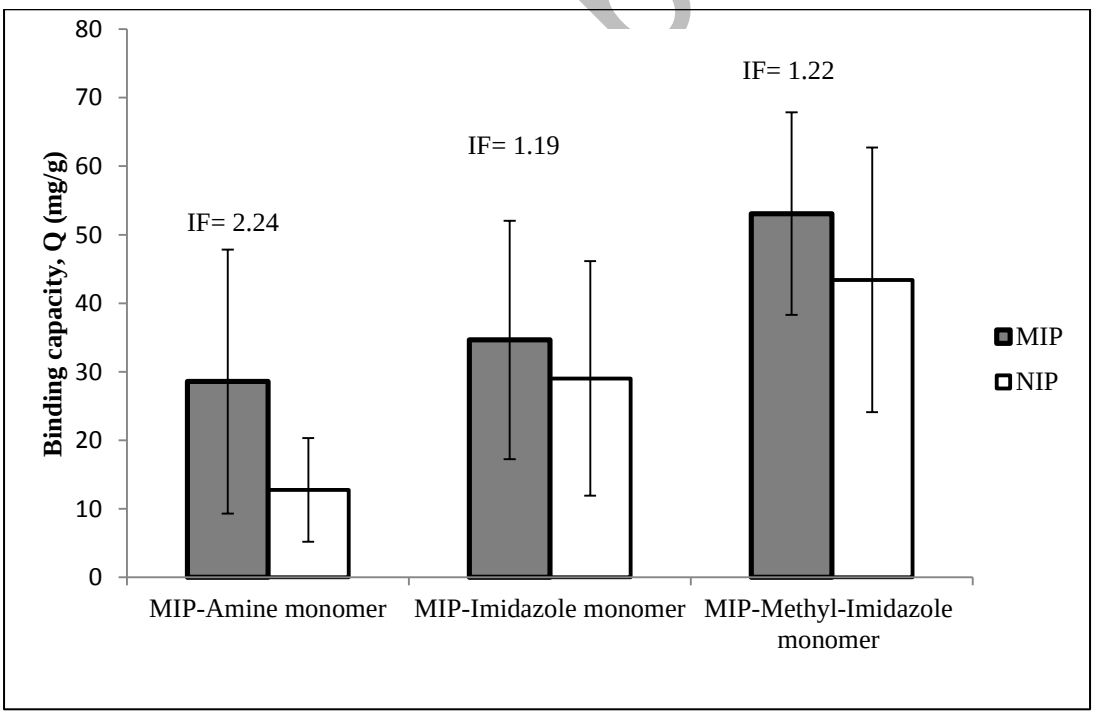

Figure 4. Imprinting factor of glucose sulfate on MIP with three different functional monomers (Conditions: 10mg of MIP into 5ml of glucose sulfate in water at concentration of 0.25 mg/ml)

The imprinting factor of MIP-amine was high (2.24) compared to others MIP (Figure 4). The highest values of the imprinting factor (IF) from MIP-amine possess the highest affinity to the template where, it is mainly due to the specific binding sites for glucose sulfate on the MIP-amine surface. The lowest values of IF from the others MIP showed that it preferred to a non-specific binding interaction. The non-specific of the interaction between glucose sulfate (template) and imprinting polymer was mainly due to the presence of anionic species at template side. The abundant anionic species might cause a strong ionic interaction to the MIP-methyl-imidazole and absorb more templates to surrounded the polymeric particle thus, block the access way into the imprinting cavities. That explains the highest adsorption capability and lowest kinetic adsorption of MIP-methyl-imidazole compared to MIP-amine.

Conclusion

The non-covalent surface molecular imprinted polymer was synthesized and grafted to silica-gel microparticles using three different functional monomers; amine, imidazole and methyl-imidazole functioned silane and glucose sulfate was used as a template model. An adsorption batch studies indicated that all MIPs followed Langmuir isotherm model with MIP-methyl-imidazole display high binding capacity. While the kinetic batch studies showed conflicting results, MIP-amine reported fast adsorption in homogeneous surface, which follow pseudo-first order kinetic model same as MIP-imidazole. Suppose the adsorption binding capacity and kinetic model should relate to each other. However, there were unconnected results between isotherm and kinetic model. The finding also agreed to the IF data where, MIP with high imprinting factor was preferred due to the high affinity and more specific interaction to the template adsorption. We hope that this novel molecular imprinted technology might efficiently be used in the identification and selection of glycosaminoglycan fragment for determines its biological activity role.

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