Theoretical and experimental study of valine molecular imprinted polymer using sol-gel process on silica microparticles surface

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Abstract. Molecular imprinted polymer using sol-gel process on silica microparticles surface has been prepared using valine as a template, 3-aminopropyltriethoxysilane (APTES) as the functional monomer, and tetraethoxysilane (TEOS) as the cross-linker. The theoretical results of molar ratio 1:2 (Valine:APTES) showed highest interaction energy with -3.8479 kcal/mol. The molecular imprinted polymer (MIP) and non-imprinted polymer (NIP) were prepared followed by characterization and evaluation of the absorption binding performance of imprinted valine. Observation of MIP morphology on Scanning Electron Microscopy (SEM) showed that the surface of silica were coated with imprinted polymer. In Fourier-Transform Infra-Red Spectroscopy (FT-IR), the strong intensity peaks were observed at 2921.71 cm⁻¹ and 1500 cm⁻¹ represent the functional group of alkane due to presence of the C-H stretching and C-N vibration from APTES of functional monomer, respectively. Observations in MIP and NIP samples confirmed the existence of a monomer and cross-linker on the silica surface. However, the experimental binding capacity result contradict the theoretical computation, where the experimental binding capacity showed that the maximum absorption binding capacity was at molar ratio 1:1 instead of 1:2. In conclusion, this novel molecular imprinted technology might efficiently be used in the identification and selection of amino acid fragments such as valine on its biological action.

1. Introduction
Valine is a branched chain amino acid and classified as a group 1 non-polar amino acid. Valine is usually used to enhance normal growth, repair tissues, control blood sugar, stimulate the central nervous system and provide energy to the body. One of the method used to produce L-Valine is microbial fermentation, however, microbial fermentations produced impurities. Hence, extraction and purification are needed to reduce the by-products formed. Valine can be extracted using various methods, such as the enantioselective liquid-liquid phase extraction and distillation [1], however, there is no report on the extraction of valine using molecular imprinted polymer.

Molecular imprinting is a method used to make selective binding sites in synthetic polymers [2]. Molecular imprinting has high affinity and selectivity towards its template because the presence of the template mold formed in the polymer on recognition sites. A selected functional monomer are attached to the template and undergo polymerisation process in the presence of cross-linker. After imprinted polymer had polymerised, the template will be removed from the polymeric structure and a cavity complementary in shape and chemical properties is present in the structure. The imprinted polymer becomes available to bind molecules identical to the template [3]. Molecular imprinted polymers are
inexpensive, strong, easy to prepare and have high resistant towards high temperature and pressure. There are several known methods used in the polymerisation process including the sol-gel process, hydro-gel process, free-radical process and aerogel process [4,5]. Different techniques can be applied in each process such as the bulk polymerisation, precipitation polymerisation, surface imprinting, dispersion polymerisation and suspension polymerisation [6].

In this research, the polymerization process and technique employed were sol-gel process and surface imprinting, respectively, in which valine was used as the template, while 3-aminopropyltriethoxysilane (APTES) and triethoxysilane (TEOS) were used as the functional monomer and cross-linker, respectively, in the molecular imprinting process. Valine molecular imprinted polymer will be synthesized by applying sol-gel polymerization method. The research scope was to evaluate the theoretical and experimental criterion for molecular imprinted polymer design based on the interaction between the functional monomer and the template using computational modelling. The computational study will be implemented to optimize the ratio between the functional monomer and the template within prepolymerization complex. Next, the valine imprinted polymer was synthesized and characterized by batch sorption experiments in order to validate the computational study.

2. Materials and Methods

2.1. Materials
The silica-gel (230-400 mesh, 0.040-0.063 mm in size) used as the support material for surface imprinted polymer was purchased from Merck, Germany. Valine, 3 aminopropyltrimethoxysilane (APTES) and DMSO were obtained from Sigma Aldrich, Germany. Tetraethoxysilane (TEOS) was purchased from Acros (Sigma, Germany) and hydrochloric acid was obtained from Fisher Scientific, USA. The analytical grade methanol was used as washer solution supplied from HmbG, Germany.

2.2. Experimental Procedure
2.2.1. Computational Design of Polymer for Valine. The simulations was carried out by using a molecular modelling software program known as Hyperchem software (Hypercube Inc., Gainesville). Valine and APTES were drawn in 2D and converted to 3D structures. The geometry optimization of each molecular structure was carried out using Polak Ribiene algorithm until the energy gradient was below 0.01 kcal/mol. Computational study has been suggested as an alternative approach to seek rational MIP design, which is based on calculation of interaction energy (ΔE) between template molecule and the functional monomer. The interaction energy was calculated using the equation (1):

\[ \Delta E = E_{\text{template-monomer}} - (E_{\text{template}} + \sum E_{\text{monomer}}) \]  

(1)

Where, ΔE is the interaction energy between the template and functional monomer (kcal/mol), \( E_{\text{template-monomer}} \) is the energy between the template and functional monomer (kcal/mol), \( E_{\text{template}} \) is the template energy (kcal/mol), \( E_{\text{monomer}} \) is the monomer energy (kcal/mol).

2.2.2. Activation of silica microparticles surface (silica gel). Before the molecular imprinting process was carried out, the surface of silica-gel was activated. The mixture of silica-gel and 6M of HCl were first refluxed for 10 hours. Then, the resulting mixture was filtered and washed thoroughly with deionized water. The solid activated silica gel and water were saperated using centrifuge at 5,000 rpm and the process were continued until neutral. Lastly, the particles were dried overnight at temperature 70 °C in an oven. The purpose of silica activation was to enhanced the content of the silanol group. Moreover, by washing, it is able to eliminate nitrogen-containing impurities and metal oxide [7, 8].

2.2.3. Preparation of molecularly imprinted polymer. Valine, 3-aminopropyltrimethoxysilane (APTES) and tetraethoxysilane (TEOS) were used as a template molecule, functional monomer and cross-linker, respectively. The data obtained from computational study was used in the preparation of valine-molecularly imprinted polymer. In order to prepare template:monomer with the ratio of 1:1, 0.1172 g
of valine template was dissolved in 15 ml dimethyl sulfoxide (DMSO) together with 5 ml of water. After dissolving the template in the solvent completely, 0.234 ml of APTES was added follow by 2.238 ml of TEOS. Next, 2 g of activated silica gel and 0.2 ml of 0.012 M HCl were subsequently added into the dissolved valine template mixture under continuous stirring at temperature 40 °C. The mixture was then stirred continuously for 24 hours. Then, the imprinted polymer was filtered out and the imprinted polymer was washed using 50 ml of ethanol and 50 ml of water until it pH reaches neutral. The imprinted polymer was left to dry overnight at temperature 80°C. The steps were repeated in preparing template: monomer with the ratio of 1:2 where 0.468 ml APTES was used. Then, the template were extracted with the mixture of 50 ml MeOH and HCl (10% in water) in the ratio of 1:1, followed by methanol and distilled water. The mixture were shaken using an orbital shaker at 100 rpm for 30 minutes to remove the template on the imprinted polymer. The supernatant at every washing solution was tested using UV-Vis at 265nm until no longer template be detected in the washing solution. Finally, the imprinted polymer was collected by filtration and dried at 80°C overnight in oven. The NIP was also prepared using identical procedure without template.

2.3. Batch Rebinding.
Fifty miligrams of imprinted polymer was placed in a 50 ml centrifuge tube. Then, 5 ml of valine in water at different concentration ranged between 0.0625 mg/ml-0.25 mg/ml was added. The centrifuge tubes were placed horizontally in an orbital shaker at 100 rpm for 4 hours at room temperature. Then the centrifuge tubes were placed in the centrifuge and spin at 5000 rpm for 10 minutes to remove the imprinted residual from the supernatant. The supernatant was filtered using nylon syringe filter (0.45 μm), and was measured via UV-visible spectroscopy at 265 nm. The binding test was tested by calculating the binding energy, Q using equation (2):

\[
Q = \frac{V(C_t - C_i)}{W}
\]

Where, Q is the rebinding energy (mg/g), V is the volumes of standard valine solution (ml), C_i is the initial concentration (mg/ml), C_t is the final concentration (mg/ml), W is the weight of the imprinted polymer used (mg). Furthermore, the imprinting factor (IF) was used to evaluate the selectivity properties of molecular imprinted polymer and non-imprinted polymer towards valine the template. The imprinting factor, IF was calculated based on Q_{MIP} over Q_{NIP}.

3. Results and Discussion

3.1. Theoretical Optimization of Template: Functional Monomer Ratio
In this study, Hyperchem software was used for computational modelling on the study of valine as template while APTES as monomer. The imprinted molecule structures between valine and APTES with different molar ratio were optimized and an interaction energies were calculated. The interaction positions of the template-monomer was shown as well. The interaction energy between valine and APTES was calculated using equation (1). Molar ratio of 1:1 and 1:2 were chosen and to be used in this experiment because valine has only two active sites, which are carbonyl group and amine group that allow it to bind with the monomer.

Table 1 showed the energy of the imprinted molecules and the interaction energies, ΔE for both molar ratio of template: monomer at 1:1 and 1:2. The interaction energy, ΔE for molar ratio 1:2 shown higher ΔE -3.8479 kcal/mol compared to molar ratio 1:1 which, the value was calculated as - 1.4404 kcal/mol. Complex polymer with higher interaction energy is more stable compare to the polymer that has lower interaction energy [9]. This interaction energy also indicates that the molar ratio of 1:2 has greater affinity toward the template (valine) during rebinding test. However in a polymerization process, excess number of mole of functional monomer may cause non-specific binding and a reduction in the selectivity properties of the polymer [10].
Table 1. Energy and Interaction energies of valine with different ratio of 3-aminopropyltriethoxysilane (APTES).

| Compound | Energy (kcal/mol) | Molar ratio (Template:Monomer) | Energy (kcal/mol) | Interaction Energy, ΔE (kcal/mol) |
|----------|-------------------|--------------------------------|-------------------|----------------------------------|
| Valine   | -1769.2398        | 1:1                            | -5171.0855        | -1.4404                          |
| APTES    | -3400.4053        | 1:2                            | -8573.8983        | -3.8479                          |

The optimized potential interactions between template and functional monomers with different ratio complexes were illustrated in Figure 1. The geometric hydrogen bondings between valine and APTES at molar ratio 1:1 were presented in Figure 1 (A), where hydrogen atom from the carbonyl group on template was hydrogen-bonded with the nitrogen atom from amine group on monomer APTES.

![Figure 1](image1.png)

**Figure 1.** The optimized structures of valine and APTES with molar ratio 1:1 (A) and molar ratio 1:2 (B). Nitrogen [dark blue ball], Carbon [light blue ball], Oxygen [red ball], Silicone [white big ball] and Hydrogen [white small ball]. Hydrogen bond shown as dotted line.

Figure 1(B) showed the interaction of molar ratio 1:2, where two hydrogen bonds were obtained. The first hydrogen bonding was formed between hydrogen atom from carbonyl group on valine with nitrogen atom from amine group on monomer. In which, same hydrogen bonding as formed in molar ratio 1:1. The second hydrogen bonding was formed between hydrogen atom from amine group on valine with oxygen atom from monomer. Therefore, from the structure obtained, the valine and APTES in molar ratio 1:2 is more stable as compares to the structure of valine and APTES in 1:1 molar ratio. This is because molar ratio 1:2 had a greater molecule structure than molar ratio 1:1.

3.2. Characterisation of polymer

3.2.1. Fourier transform infrared spectroscopy (FT-IR). Perkin Elmer FT-IR Spectroscopy 100 was used to determine the possible functional group present in the polymer using KBr pellet method. The wave numbers of FT-IR analysis were collected at one data point per 2 cm$^{-1}$ and ranged from 400-4000 cm$^{-1}$. Figure 2 showed the FT-IR spectrum of silica gel, molecular imprinted polymer (MIP) and non-imprinted polymer (NIP).
The IR spectra (Figure 2) for both NIP and MIP showed similar peak. This observation may be due to the same functional group present in both polymers. The similar peaks for silica gel, MIP and NIP were observed at 3457.84 cm\(^{-1}\). These strong and broad intensity peaks correspond to the presence of H-N-H bond and alcohol (-OH) stretching. However, the stretching vibration slightly shift to 3445.51 cm\(^{-1}\) and 3441.12 cm\(^{-1}\) due to the removal process. The peaks observed at 2921.71 cm\(^{-1}\) and 2934.17 cm\(^{-1}\) represent the functional group of alkane due to the presence of the C-H stretching from the monomer that have strong intensity. Meanwhile, between 1400 cm\(^{-1}\) and 1500 cm\(^{-1}\) represent the presence of the C-N vibration from APTES of functional monomer. However, there was no peak on the silica gel curve, therefore, the template and monomer were successfully imprint on the activated silica gel. The peaks of stretching vibration Si-O-Si observed at 1063.84 cm\(^{-1}\), 1081.00 cm\(^{-1}\), and 1096.26 cm\(^{-1}\) were due to silica particles, whereas Si-O peak observed at 797.59 cm\(^{-1}\), 794.39 cm\(^{-1}\), and 795.22 cm\(^{-1}\) were due to the vibration from the interaction with the silica surface and APTES.

3.2.2. Scanning Electron Microscopy (SEM). Figure 3 showed the image captured using SEM (JEOL Scanning electron microscopy) for the activated silica gel and valine MIP. The activated silica gel exhibit a smooth surface as shown in Figure 3(A) while surface of silica coated with imprinted polymer (Figure 3(B)) exhibited a rougher surface morphology, which indicate that valine MIP have been successfully prepared.

Figure 3 (C) exhibited small agglomerate at the surface of the silica which may be due to the impurities from insufficient washing [7,8]. However, the overall surface was smooth compared to MIP in figure 3(D). The SEM scans verified that the imprinting polymer of siloxane leads to the formation of agglomerates with multiporous surfaces. The roughness on the surface of valine MIP can be clearly seen compared to activated silica gel. Moreover, the imprinting were spread homogenously around the silica microparticle surface, which proved that the silica gel had been fully coated with imprinting polymer [11].
3.2.3 Batch Rebinding Experiment

The adsorption binding capacities for MIP of molar ratio 1:1 was \(35.06 \pm 0.0007 \text{ mg/g}\) higher than NIP in which, was \(26.85 \pm 0.0005 \text{ mg/g}\) (Figure 4). The situation could be explained by the existence of recognition sites at the surface of silica microparticles which enhance the surface area of adsorption. The amount of valine bound to both MIP and NIP at molar ratio 1:1 increased sharply with increasing concentration of valine but the adsorption ability of NIP was lower than MIP. This showed that MIP has higher loading ability towards the template as compared to NIP. The results showed that the existence of imprinting cavities on MIP differentiated the binding ability of NIP. Similar observation was also observed in both MIP and NIP at molar ratio 1:2. The amount of valine bound to MIP and NIP at ratio 1:2 increased with increasing concentration of valine and then, the curve reached equilibrium 0.25 mg/ml (Figure 4). However, the adsorption ability on NIP (26.64 \(\pm\) 0.0012 mg/g) was lower than MIP (28.39 \(\pm\) 0.0018 mg/g) (Figure 4).

![Figure 3. SEM image of (A) activated silica gel and (B) valine MIP at 1000 magnification while, (C) activated silica gel and (D) valine MIP at 7000 magnification respectively.](image)

![Figure 4. Comparison for the binding test using valine on NIP and MIP of molar ratio 1:1 and molar ratio 1:2.](image)
MIPs of both molar ratio 1:1 and 1:2 had higher binding capacity, attributed to the presence of specific binding sites formed in the process of molecular imprinting and enhance the binding performance of template fits into the imprinted polymer cavity. The difference in binding capacity between both molar ratios showed that molar ratio 1:1 had higher affinity binding sites while molar ratio 1:2 had lower affinity sites. Whereas in NIPs, although functional monomer were present in the polymer, the monomer was ineffective for binding with the template due to the lack of recognition cavities [12]. Therefore, the binding capacity of NIPs for both molar ratio 1:1 and 1:2 were lower than MIPs. However, the template-monomer complexation was influenced after polymerization by the number of monomers attached and the availability of the functional groups on the monomers [13].

The efficiency of the molecular imprinting polymer could be measured by the imprinting factor (IF). The imprinting factor is a measure of the imprinted polymer towards the template molecule [14]. Imprinting factor is the ratio of the substrate obtained from the molecular imprinted polymer and the recovery yield obtained using the same substrate on non-imprinted polymer with the same monomer. Our calculation indicated that the imprinting factor for molar ratio 1:1 (1.306) was higher compared to the imprinting factor for molar ratio 1:2 (1.066), indicating molar ratio 1:1 having highest selectivity towards valine compared to molar ratio 1:2.

In term of stability, the structure of the polymeric complex for molar ratio 1:2 should be greater affinity toward the template compare to the imprinted polymer structure for molar ratio 1:1. Therefore, higher adsorption binding capacity should obtained. However, a contradictory observation was obtained. This may be due to the recognition sites were dependent only to the distribution of functional groups. Moreover, in solution only one valine interact with one of the monomer and after polymerization the oxygen atom attached to template may not be available for interacting with APTES, due to steric hindrance [13]. Another possibility could be due to damaged monomer on the cavities after rewashing, which lead to the decrease in the affinity for the template [14] and also an incomplete removal of the valine during washing step [15].

The binding capacity is dependent on the end application. MIP for environmental analysis or sensing application does not require a high capacity, but for long-term controlled release, higher polymer loading capacities may be required [16].

4. Conclusion
The theoretical study using Hyperchem software was performed to obtain the interaction energy between the template and the functional monomer using AM1 semi empirical. The study showed that molar ratio 1:2 (-3.8479 kcal/mol) have highest stability compare to molar ratio 1:1 (-1.4404 kcal/mol). The synthesized valine imprinted polymer according to the result from computational studies was characterization. Results from the characterization proved that animo silane was successfully imprint on the activated silica gel. The adsorption studies were performed by batch rebinding and the results indicated that for both molar ratio 1:1 and 1:2 of MIP have highest adsorption results compare to NIP. However, both adsorption binding capacity and IF for MIP on molar ratio 1:1 was higher than the molar ratio 1:2. The results showed that the molar ratio 1:1 has higher selectivity towards valine. The contradicting result found between theoretical and experimental were due to several factors inculding the distribution of functional group on the recognition site, steric hindrance and the damaged functional monomer on the cavities. Overall, our study revealed that high selectivity MIP can be prepared using a simple and fast sol-gel method.

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