Rational Design of Molecular Imprinting Polymer based on AM1 Semiempirical Study of Allopurinol-Methacrylic Acids Interactions

Iqmal Tahir[1,3,*] , Mohd Noor Ahmad[1], AKM Shafiqul Islam[2] and Dahyar Arbain[2]

[1]School of Material Engineering, University Malaysia Perlis, Malaysia
[2]School of Bioproces Engineering, University Malaysia Perlis, Malaysia
[3]Chemistry Department, Universitas Gadjah Mada, Yogyakarta, Indonesia

*Corresponding email: iqmal.tahir@yahoo.com

Abstract

A molecularly imprinted polymer (MIP) for allopurinol has been designed based on molecular modeling study using quantum mechanics-AM1 semiempirical calculation. Analysis was performed by studying non covalent interactions between allopurinol as template molecules and methacrylic acid as functional monomer to predict a stable MIP. A stable complex existed as one molecule of allopurinol interacts with several n number of methacrylic acid molecules via non covalent bonding. All of the calculation was run on AM1 semiempirical level using HyperChem Software. The result showed that a MIP suitable for allopurinol could be produced on the mole ratio between allopurinol and methacrylic acid at 1:4. This recommendation is based on the value of the binding energy to indicate the interaction that is enough to make a stable conformation.

Keywords: allopurinol, methacrylic acid, semiempirical AM1, molecular modeling, molecular imprinted polymer, quartz crystal microbalance

1. INTRODUCTION

Allopurinol or 1H-pyrazolo[3,4-d]pyrimidin-4-ol is widely known as a medicine for treating gout caused by a build- up of uric acid crystals in the joints. It is also used to treat certain types of kidney stones and to prevent high uric acid levels in chemotherapy. However, Allopurinol might bring a negative side effects such are nausea, flaking skin, sore lips or mouth, and drowsiness. It has also been observed that allopurinol can inflame the liver causing a type of hepatitis. Therefore, the dose of allopurinol needs to be properly determined by routinely monitoring its concentration in the blood [1].

Several methods had been developed for allopurinol analysis. These include conventional volumetric titration method [2], spectrophotometry [3], chromatographics technique [4], capillary electrophoresis [5], flow injection technique [6] and differential pulse polarography [7]. The two first techniques need large amount of chemicals and many steps to prepare standard solutions for pre treatment analysis. The other techniques are not always ideal for practical purposes, especially for routine analysis and a large number of samples. Accordingly there is a need for a simple, selective, sensitive and easy to use allopurinol sensor.

In the aim of developing allopurinol sensor, it is interesting to consider the use of Quartz Crystal Microbalance (QCM). This method is very sensitive and capable of sensing a change of mass within the nanogram of the sample. It utilises the piezoelectric properties of quartz crystals to measure changes in the attached surface mass [8]. Thus the QCM based sensor can be developed by immobilizing a novel material selective to the template such as molecular imprinted polymer (MIP). Applying MIP on crystal surface has been done by several researchers for examples for analysis of glucose [9], 8-hydroxy-2-deoxyguanosine [10], lysozyme [11], etc.

MIP is a material that can mimic the behavior (in terms of binding) of naturally occurring receptor sites to recognize a target compound specifically. MIP could be synthesized by polymerization of monomer, crosslinker, initiator and a suitable template with some common procedures.

To ensure its selectivity, it is important to design MIP by rational approach to select a functional monomer which gives sutable binding sites for a template [12]. Several reproted good MIPs have been designed by application of experimental design [13,14] or using molecular modeling technique [15-17]. Although a trial and error experimental could give an answer, molecular modeling approach is relatively more rational and more effective to try a predicted ratio [18]. The theoretical background was based on the interaction between monomers and template. The most common approach of this interactions are in non-covalent interactions via hydrogen bonding, electrostatic or hydrophobic interactions [19]. So we could make a model of several possibilities of template – n monomers (with n = 1, 2, 3… etc) and determine which the stable complex that have a highest binding energy. To study the interaction between template-monomer, we can apply a quantum mechanics molecular orbital calculation using AM1 semi empirical method. This technique has been successful to evaluate non covalent interactions in the case of organic crystal [20] and homopolymerization of spiroorthocarbonate [21].

In this paper, the use molecular modeling to design an allopurinol MIP is described. Here methacrylic acid is used as a monomer model since it is known common functional monomer which give a good MIP. The model is utlized to find a suitable mole ratio of template-monomers that could give a stable MIP for allopurinol.

2. METHODOLOGY

The molecular modeling software to evaluate the interaction in this study was Hyperchem 8.0 (Hypercube Inc.). Each structure of allopurinol (fig 1), methacrylic acid and the complexes were drawn 2D and subsequently build in the 3D structure. Allopurinol were modeled in its neutral structure corresponding to the (N1)-H/(N5)-H ketonic tautomeric form [22]. The structure was run using semi empirical Austin Model 1 (AM1) method within the Restricted Hartee Fock (RHF) formalism to do energy minimization and to get an optimum geometry of conformation. Geometry optimization was carried out by using Polak-Ribiere algorithm and the electronic structure of the system were calculated using the semi empirical self consistent field (SFC) molecular orbital approach with RHF. The root mean square (RMS) gradient was set to 0.001 kcal/(Å.mol) in the calculation.
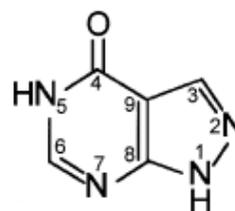

Fig 1. Two dimensional structure of allopurinol in ketonic tautomeric form. The number indicates atoms respectively that is used in this study

The complex exists as combination between allopurinol and n number of methacrylic acids, with n

equal to 1, 2, 3, 4 or 5. The complex model was analyzed based on its binding energies and electronic parameters. The calculation of the binding energy (ΔE) was based on equation 1:

$$\Delta E = [E_{complex} - E_{template} - E_{monomer}] \qquad (1)$$

The calculation is employed based on similar works by Farrington, et al [23] and Farrington & Regan [24].

3. RESULT AND DISCUSSION

Molecular interactions between allopurinol and various sites of monomer were analyzed based on their electric dipole moment, total electronic charge density, and electrostatic potential contour map.

Dipole moment acts importantly in molecular interaction, especially in non covalent interaction like in MIP formation during precomplexation steps. Intermolecular interactions that are resulted by forces between two molecules usually exist as non covalent interactions and they are consisted of hydrogen bonding, van der Waals interaction, London force (dipole–dipole) or ion-dipole forces. Physically these forces could be form when two molecules are sufficiently close to each other. Specifically intermolecular interaction will be formed when the dipole moments of the molecules are close relatively. The calculated dipole moment (μ) of allopurinol is 3.579 Debye, so it indicates this template could interact with polar monomers that has high dipole moment too. There are many polar monomers that could be selected for allopurinol imprinted polymer, i.e. acid monomer or basis monomer. Methacrylic acid is one example of acid monomer. It has moment dipole of 2.009 Debye, therefore it could be selected for this purpose.

Figure 2 shows total charge density of allopurinol in 2D perspective. Atoms of nitrogen and oxygen are classified as atoms to give the higher density that carbon or hydrogen. It is noticeable that the position of the charge density is distributed in several regions as indicated by the condensed repetitive green circle surrounding the corresponding atoms.
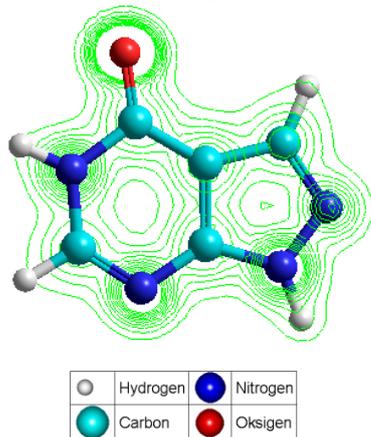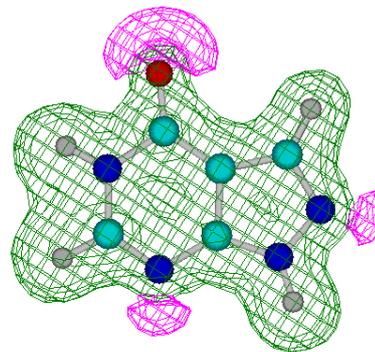

Fig 2. Total charge density of allopurinol

Fig 3. Electrostatic potential contour map of allopurinol

The active sites in allopurinol can be analyzed from, the electrostatic potential contour map of allopurinol depicted in figure 3. It can be seen from the figure that in allopurinol contains several active sites available for interaction with other molecules. There are three active sites available for suitable binding by methacrylic acid (indicated in red contour) i.e. in hydroxyl site, atom N7 and atom N2. The latter could give two interaction possibilities with methacrylic acid. Totally there are three possibilities and each of them has two alternative positions to form a suitable interaction. These interaction possibilities were then used to predict a complex model of allopurinol-methacrylic acid (fig.4)
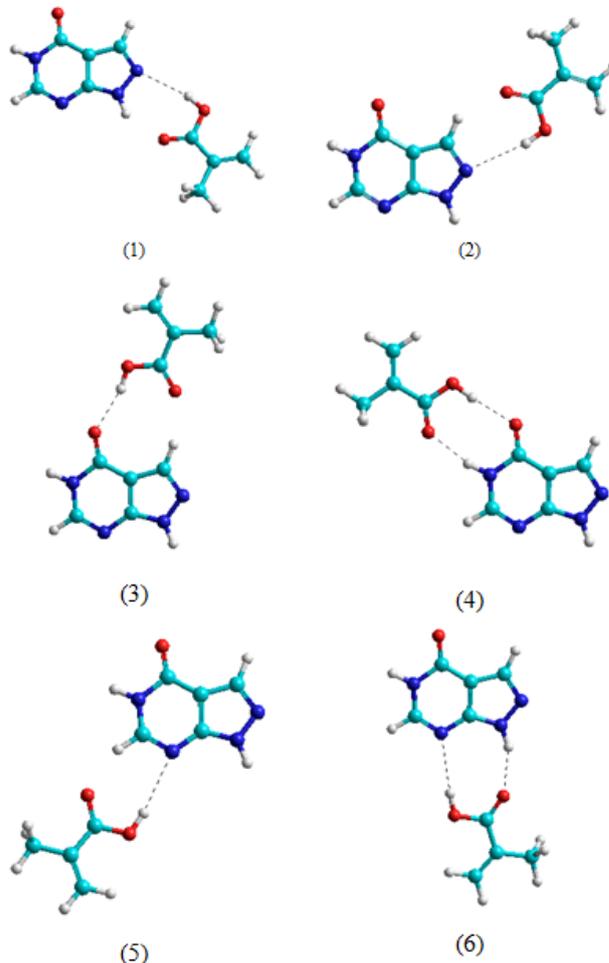

Fig 4. Complex model between allopurinol and methacrylic acid

Interaction between allopurinol and methacrylic acid involves one or more intermolecular attraction via non covalent bonding. These interactions can exist as hydrogen bonding, van der Waals interaction or only London force. Hydrogen bonding has binding energy in a range of 2 - 10 kcal.mol^{-1}, while van der Waals interaction and London force have binding energy in a range of less than 1.5 kcal.mol^{-1}.

In MIP, hydrogen bonding plays a significant role in pre polymerization process to give a stable complex. It is represented by the higher negative value of binding energy produced by the model, thus it can be used to evaluate the stability allopurinol - methacrylic complex.

We propose several complexes of allopurinol and n number of methacrylic acid in the ratio of 1:1, 1:2, 1:3, 1:4 and 1:5. For each ratio, there are many possibilities that could be existed, depending on the position and bulky space relative to the active site in allopurinol structure. We plot the binding interaction of methacrylic acid into the active site in allopurinol to form a possibility of the complex as illustrated in figure 4. The number in the bracket, (n), indicates a number of complexes that we used in this report. Table 1 shows several complexes that could be formed by one molecule of methacrylic acid with two or more monomers. The models are indicated by combination of number in the bracket as in fig 4.

Table 1: Binding energy at several mole ratio of allopurinol: methacrylic acid

Ratio	Complex	E$_{complex}$ (kcal.mol^{-1})	Binding energy, ΔE (kcal.mol^{-1})
1 : 1	(1)	-2722.23	-5.73
	(2)	-2718.97	-2.47
	(3)	-2721.63	-5.13
	(4)	-2725.03	-8.53*
	(5)	-2721.26	-4.76
	(6)	-2722.12	-5.62
1 : 2	(1)-(3)	-3925.63	-10.93
	(1)-(4)	-3928.81	-14.11*
	(1)-(5)	-3925.20	-10.51
	(2)-(4)	-3925.68	-10.98
	(2)-(5)	-3921.80	-7.11
	(3)-(5)	-3924.59	-9.90
	(3)-(6)	-3925.26	-10.57
1 : 3	(1)-(3)-(4)	-5132.03	-19.13*
	(1)-(3)-(5)	-5128.63	-15.73
	(2)-(3)-(5)	-5127.21	-14.32
	(2)-(3)-(6)	-5122.05	-9.15
	(2)-(4)-(6)	-5129.51	-16.62
1 : 4	(1)-(2)-(4)-(5)	-6330.28	-19.19
	(1)-(3)-(4)-(5)	-6334.14	-23.05*
	(2)-(3)-(4)-(6)	-6328.98	-22.10
1 : 5	(1)-(2)-(3)-(4)-(5)	-7537.72	-17.89*

Note : E$_{allopurinol}$ =1518.30 kcal.mol^{-1} and E$_{metahcrylic\ acid}$ = -1198.20 kcal.mol^{-1}. Star symbol (*) indicates the highest binding energy for each ratio.
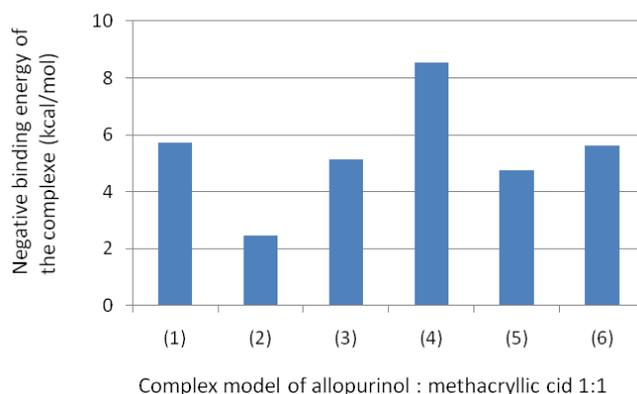

Fig 5. The highest negative of binding energy of allopurinol-methacrylic acid

The number of possible complex for each ratio is different relatively to the sterical effect. For low ratio, there are many possibilities of the complex that could be formed. For ratio 1:5, we proposed only one model because there is no chance for other complex to be exist. Recapitulation of binding energy for each studied complex is given in Table 1.

Analysis for complex that is formed by ratio 1 : 1, is illustrated in fig 5. It shows fig 5 that the highest negative score of ΔE is in complex in ratio 1:1. In this ratio we can see that complex (4) has a highest value of binding energy, indicated that carbonyl sites on allopurinol always gives priority to interact with methacrylic acid. The next possibilities are complexes (1), (6) and (3) respectively.

Actually the complex that is formed on ratio 1:1 is not suitable in reality because steric reason, the number of active site are more than the number of binding interaction in the model because all the rest of template molecule would be filled with crosslinker. In this case, mimicking of template in the polymer is still possible, but there is no specific site to bind to the active site in other region of allopurinol. Some compounds which are structurally similar to allopurinol such as xanthine or hypoxanthine could also be trapped in the cavity. Therefore selectivity of MIP as the goal is not accepted.
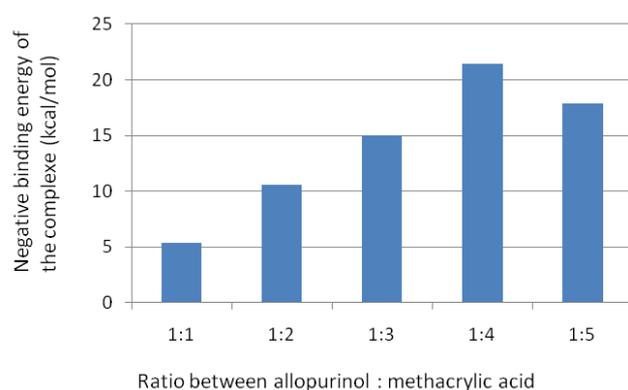

Fig 6. The binding energy (ΔE) of allopurinol-methacrylic acid in several ratios.
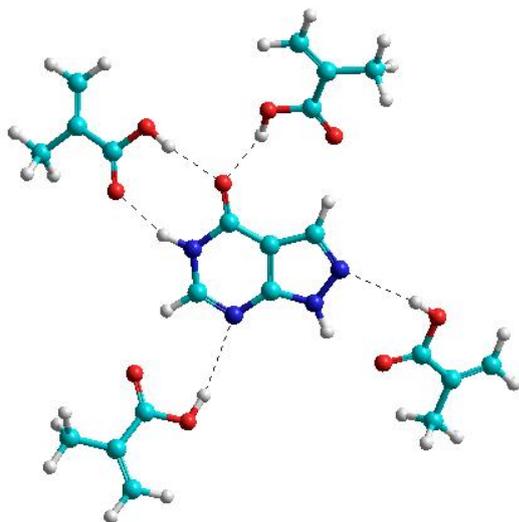
Fig 7. Model of complex resulted by mole ratio 1:4

The binding energy (ΔE) of allopurinol-methacrylic acid in several ratios is shown in fig 6. The complex with the highest binding energy indicates the stable complex that could be formed in each ratio. It is clear that the complex formed on ratio 1:4 will comparatively stable than others suggesting that a stable allopurinol imprinted polymer could be synthesized on ratio 1:4. Fig 7 shows a conformation of the complex of one allopurinol interacts with four methacrylic acid molecules.

4. CONCLUSION

We have shown that MIP for allopurinol can be designed by using molecular model based on the interactions between monomer and allopurinol. The model developed for MIP using methacrylic acid as the functional monomer and allopurinol as a template suggests that the theoretical mole ratio of 1:4 between monomer and template respectively will produce a stable complex with high affinity MIP to allopurinol. The verification of this model, as well as the application of the MIP for allopurinol QCM sensor is still in progress in our laboratory.

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