Molecular Modeling And Experimental Study On The Interaction Between Quercetin And Methacrylic Acid

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Abstract. Design of Molecular Imprinting Polymer (MIP) for solid phase extraction of quercetin has been done by pre-complexation study using molecular modeling and spectroscopy technique. Goal of the study is to determine the suitable ratio of quercetin:methacrylic acid as functional monomer. The molecular modeling was run on AM1 semiempirical level using HyperChem Software to analyse the interaction based on non covalent interactions study in the gas phase and isolated system. UV spectroscopic analysis of several pre complexation solution containing quercetin with varying of methacrylic acid. The result showed that a MIP suitable for quercetin could be produced on the mole ratio between quercetin 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. UV spectroscopy result also supports the similar ratio 1:4 to this interaction.

Introduction

Quercetin is a flavonoid are potential drugs for many biological activity such as anti-oxidative, anti-inflammatory and vasodilating effects, and it has been proposed to be a potential anti-cancer agent [1]. Quercetin or 3,3',4',5,7-pentahydroxylflavone has been known as a typical flavonol-type flavonoid that is present in herbs, fruits and vegetables. According the need to supply quercetin stock as a nutraceutical, extraction of quercetin is very important. Until now quercetin extraction is produced by using solvent extraction, but recently there are many innovative technique of macroscale extraction to separate quercetin from raw material, i.e. ultrasonic assisted extraction [2], solid phase extraction [3], microwave assisted extraction [4], supercritical fluida [5] and other extraction technique.

Solid Phase Extraction (SPE) is a technique that supports green technology because it use less solvent and it can minimize of the chemical waste [6]. SPE is a column chromatography which is employed as a liquid–liquid extraction substitute and therefore follows almost the same basic principles of the HPLC [7]. It is considered one of the most powerful techniques currently available for rapid and selective sample preparation. Technically it can be use from microscale into large scale. Application of SPE for quercetin has been reported by Zhu and Row using amino-modified active carbon [3]. Nowadays there is a novel material namely molecular imprinted polymer that is very useful to apply in solid phase extraction. It has developed a popular technique namely Molecular Imprinted Solid Phase Extraction (MISPE). Xie *et al* [8] and Song *et al* [9] have published an application of MISPE for quercetin. Developing MISPE for quercetin is still important to find a good and selective adsorbent in the column of SPE especially for large scale application.

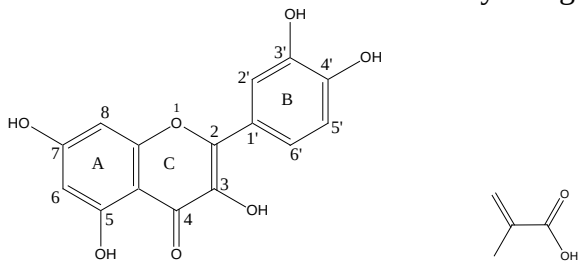
MIP is polymers prepared in presence of a template that serves as a mould for the formation of template complementary binding sites [10]. MIP can be programmed to recognize a large variety of

target structures with antibody-like affinities and selectivities. Recently the target structure are varied from small molecules i.e. bioactive, contaminant, ions etc, until macromolecule i.e. protein, virus, sacharides etc. Synthesize of MIP is worked 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 suitable binding sites for a template [11]. Utilization of the molecular modeling technique can be applied to aim this goal [12-14]. 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 [15]. So we could make a model of several possibilities of template with a number of monomers 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 semiempirical method. This technique has been successful to evaluate non covalent interactions in the case of organic crystal [16] and homopolymerization of spiroorthocarbonate [17]. In this paper, the use molecular modeling to design a novel adsorbent for MISPE of quercetin is described. Here methacrylic acid is used as a monomer model since it is known common functional monomer which gives a good MIP. The model is utilized to find a suitable mole ratio of template-monomers that could give a stable MIP for quercetin. It is combined with experimental determination of mole ratio quercetin-methacrylic acid using UV spectrophotometric analysis.

Methods and Materials

Materials. We used molecular models i.e. quercetin and methacrylic acid as shown in fig 1. The chemicals that we used are quercetin and methacrylic acid from Across, and the solvent is methanol that is purchased from HmBG Chemicals. All chemicals are analytical grade and used as received.



(a) (b)

Fig 1. Structure of (a) quercetin and (b) methacrylic acid

Instrumentation. All calculations were carried out using Hyperchem software (Hypercube). All of the calculations are worked on personal computer with specification of processor Intel® Core™2 quad CPU-Q9550 @2.83GHz, memory 4.00GB and 32-bit operating system. Experimental UV spectroscopic analysis uses Vary 20 Spectrophotometer.

Molecular modeling of the interaction. The calculation started with geometry optimization and minimum energy calculation that were perform for the template structure i.e. quercetin and the monomer individually. The complexes formed between quercetin and the monomer then were built and modeled to represent of the molecular ratio of quercetin-methacrylic acid. The complex exists as combination between quercetin and a functional monomer based on the most active site in quercetin structure. Figure 2 showed fifteen combinations between querectin and methacrylic acid that is possible to give a non covalent interaction mainly hydrogen bonding.

Each structures were drawn 2D and build in the 3D structure. The calculation was run using semi empirical AM1 within the restricted Hartee Fock (RHF) formalism. 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 (SCF) molecular orbital approach with

RHF. The root mean square (RMS) gradient was set to 0.001 kcal/(Å.mol) in the calculation. The electronic structure of quercetin is evaluated to give an explanation of the active site on the structure. Stability of the complex is determined by analysis of the interaction energies (ΔE). The value is calculated using equation (1) refer to works by Farrington & Regan [18] :

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

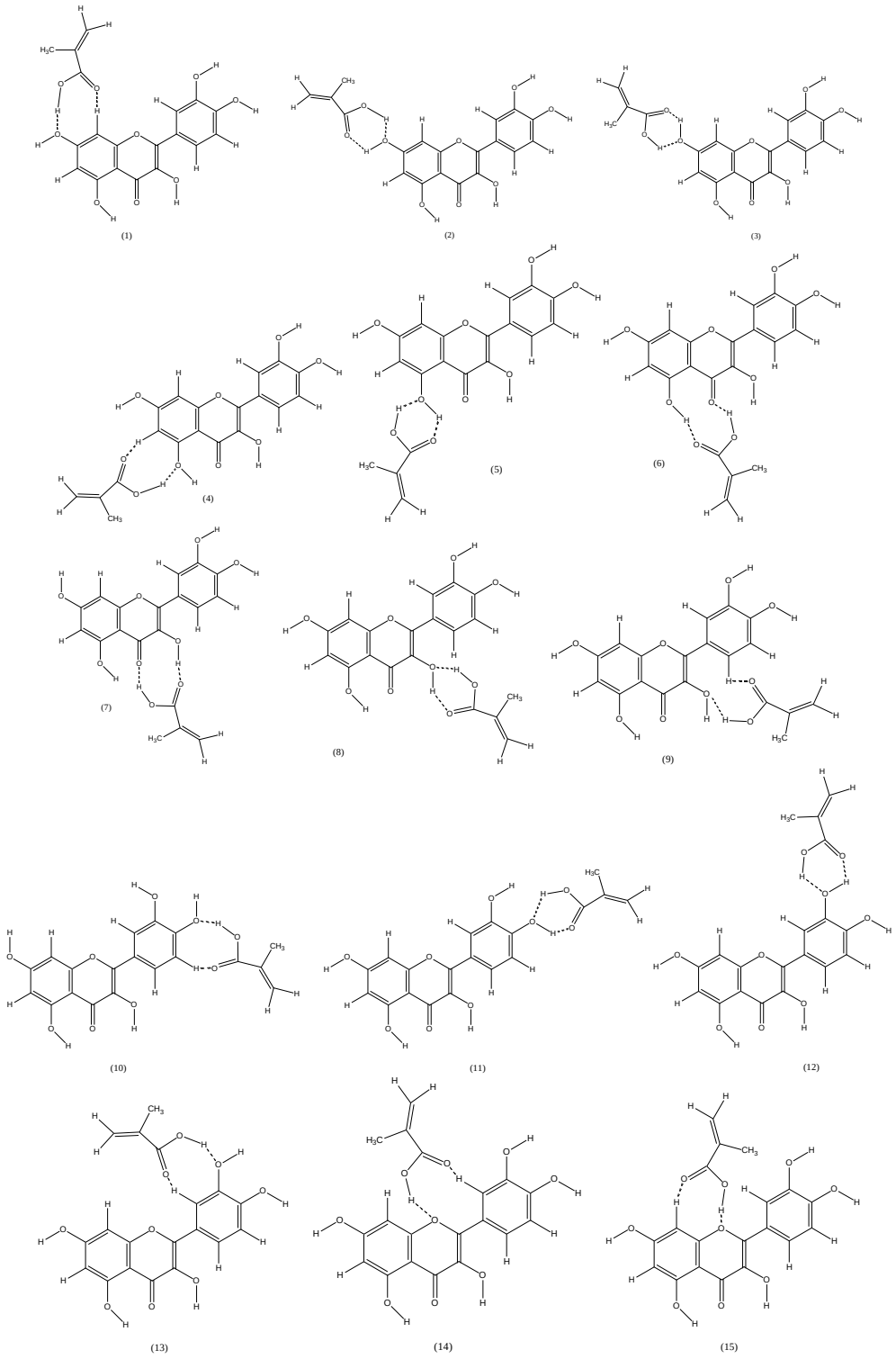


Fig 2. Variation of methacrylic acid position to the bind on quercetin

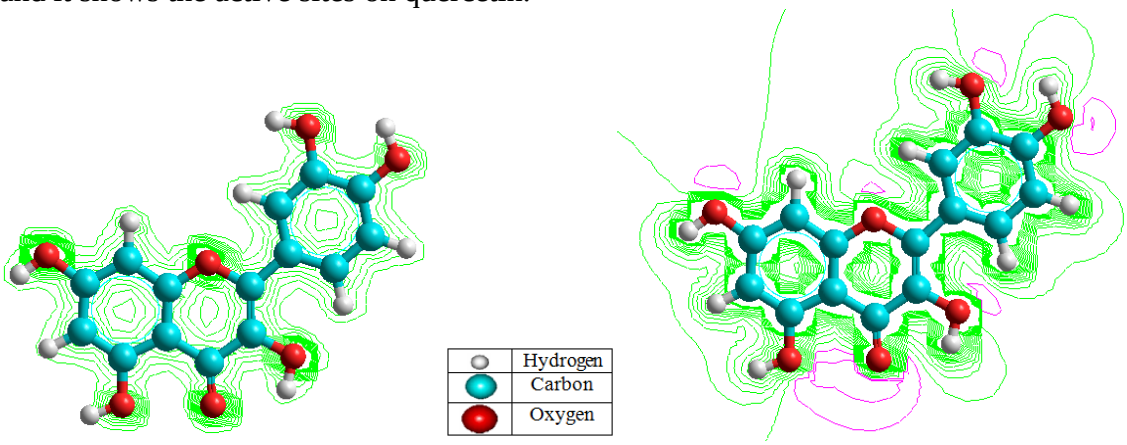
Spectrophotometric analysis of the interaction. Spectrophotometric analysis to determine the mole ratio is worked using titration method. Quercetin is prepared freshly as the template at 20 μM concentration and it was prepared from 10 mM quercetin stock solution to dilute 500 times.

Methacrylic acid solution as the monomer functional was prepared at 10 mM. Applying titration method, firstly 20 ml of 20 μM quercetin solution was add by 40 μL of 10 mM methacrylic acid solution that represent mole ratio of 1:1. Addition of methacrylic acid in a small volume ias assumed that there is no volume changes. The mixture was stirred and then kept for 5 minute to reach the equilibrium, then it scan in UV spectrophotometer in the wavelength range of 450-200 nm. Analysis is focused on the two maximum wavelength. Next ratio is done by adding 40 μL of 10 mM methacrylic acid solution until ratio 1:5 respectively.

Results and Discussions

Molecular modeling analisis. The first step in this study was to investigate the active site on quercetin using electronic descriptor resulted by molecular modeling. The electronic data is described by total electronic charge density and electrostatic potential contour map. Actually there are many other parameters resulted by quantum mechanics calculation such as HOMO-LUMO energy, but in MIP concepts, there is no bond breaking, thus this orbital energy is not necessary.

In figure 3 (a), we could check the total charge density contour map of quercetin. The shape of quercetin has a skeleton of flavonoid characteristics that is build two part connected by rotatable bonding. The skeleton is consisted by aromatic atoms that are possible to give conjugation of the electrons on carbons and oxygens. The high density of the atomic charges is indicated by green lines and it shows the active sites on quercetin.



(a) (b)

Fig 3. Electronic parameter of quercetin calculated by AM1: (a) total charge density and (b) electrostatic potential contour map

We can also check the active site in quercetin by using the electrostatic potential contour map of quercetin like given in fig 3(b). There are many active sites available in quercetin for suitable binding to the monomer molecule (indicated in red contour) i.e. in keton site, atom O on the ring and atom O on each hydroxyl site. There are five hydroxyl sites that is possible to support hydrogen bonding interaction with methacrylic acid.

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 quercetin-methacrylic complex. The calculated interaction energy of the fifteen complexes (fig 2) is given in fig 4.

As shown in fig 4, the highest negative score of ΔE is in complex (4) followed by complexes (6) and (5). If we check the existed complex, it can be known that complexes number (2), (3), (4), (5), (6), (7), (11), (12), (13) and (15) could be considered existed. The reason is based on the minimum of the energy to give hydrogen bonding that is about 5 kcal/mole. Complexes which have interaction energy less than 5 kcal/mol indicate the interaction are existed using van der Waals interaction and London force. In MIP synthesis this forces is not enough to keep the formation of

complex because there are many polar molecules that will attract methacrylic acid molecules from quercetin.

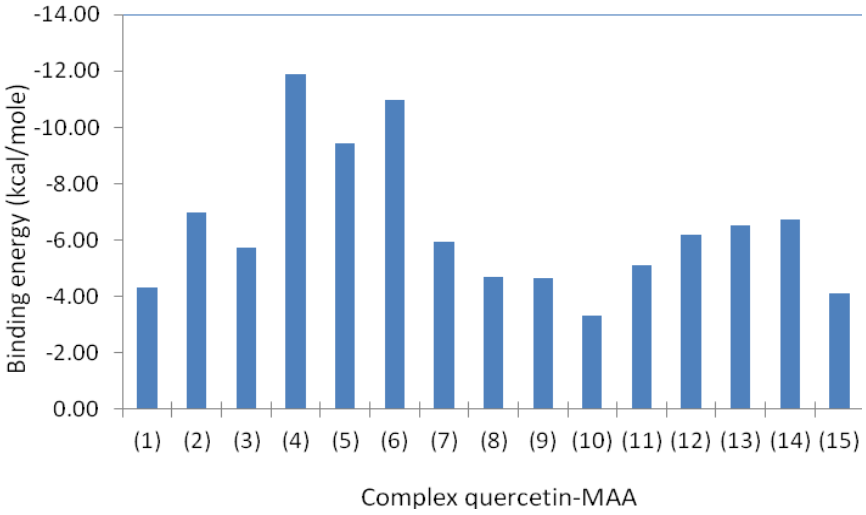


Fig 4. The interaction energy of the complex between quercetin-methacrylic acid at ratio 1:1. The complex number refers to the conformation from the fig 2.

Complex (4) indicates the interaction on hydroxyl site available on ring A (check fig 1). Next two complexes that are complex (6) and (5), indicates the interaction on the same hydroxyl site but different position. It assumes that this area should be involved to interact with methacrylic acid. There is interesting fact that the active site is in hydroxyl site on ring B but it is exist if quercetin will react to the other species. Justino and Vieira reported molecular modeling of quercetin by using four different semiempirical quantum mechanics level i.e. Modified neglect of differential overlap (MNDO), Austin model 1 (AM1), the derived Recife model 1 (RM1), and the Parameterized model 3 (PM3) [19]. All methods indicate the most acidic hydroxyl group is the 4' and that the H atom more easily abstracted from quercetin is also the 4' in the gas phase. As the antioxidant, it is expected from the structure of quercetin as both the radical and the anion formed from the 4'-OH group are the ones expected to be more stabilized by charge delocalization and resonance. If we refer this data complex (11) should be involved to the model. Complex (11) is also concerned to develelope the next combination, although in the total system it is possible to select out. In pre complexation during MIP synthesis, there is no bond breaking.

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 quercetin. Some compounds which are structurally similar to quercetin such as myricetin, kaempherol, galangin, luteolin, or other flavone compounds, could also be trapped in the cavity. Therefore the MIP will have low binding properties to quercetin and the selectivity of MIP as the goal is not accepted.

Based on the considered complexes, then we propose several new complexes of quercetin and n number of methacrylic acid in the ratio of 1:2, 1:3, 1:4 and 1:5. 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. These possibilities that could be existed depend on the position and bulky space relative to the active site in quercetin structure. Although the interaction is possible on ratio higher than 1:5, but it is not stable. The complete data will cover 91 complex models and the highest interaction energy resulted for each ratio is given in table 1.

Table 1. Interaction energy (ΔE) at several mole ratio of quercetin: methacrylic acid

Ratio	Complex	ΔE (kcal/mol)	Δ (ΔE) (kcal/mol)
1 : 1	(4)	-11.88	-11.88
1 : 2	(4)-(14)	-18.18	-6.30
1 : 3	(4)-(6)-(14)	-23.56	-5.37
1 : 4	(2)-(4)-(6)-(14)	-29.92	-6.36
1 : 5	(2)-(4)-(6)-(11)-(14)	-33.41	-3.49

Quercetin is a flavonoid that is characterized by 2 benzene rings (A and B) which are connected by an oxygen-containing pyrene ring (C). The three rings are planar and the molecule is relatively polarized. Three intermolecular hydrogen bonds are observed i.e. two with the carbonyl group and the other between the hydroxyl groups in ring B [20]. It is indicated that the mole ratio refer to this value of 1:3. Actually, if we check the conformation on next ratio from table 1, there is indication that complex is stil stable especially for complex 1:4. The highest interaction energy for complex establisher on ration 1:4 is -29.92 kcal/mole. It is not clear relatively to the other ratio to select which the highest stability complex. Using a modified parameter of Δ(ΔE), it can be more simplified. Start from ratio 1:1 until 1:4 the value of Δ(ΔE) is still high and it means that the complex is possible to exist. For ratio 1:5, then Δ(ΔE) decrease drastically almost half to previous Δ(ΔE). From this reason we assumed that ratio 1: 5 could not more stable than ratio 1:4. We choose ratio 1:4 to aim a high binding MIP to quercetin in order to get selective cavity hypothetically.

UV spectrophotometric analysis of the interaction. UV spectrophotometric measurements can be used to analyze the pre complexation between quercetin and methacrylic acid in order studying interaction between these molecules to select template-functional monomer ratio. In this case, there are many chromophore available both in the template and functional monomer, thus analysis using UC spectrophotometer is possible. Spectra of the pre complexation solution with variation of the mole ratio are given in fig 5.

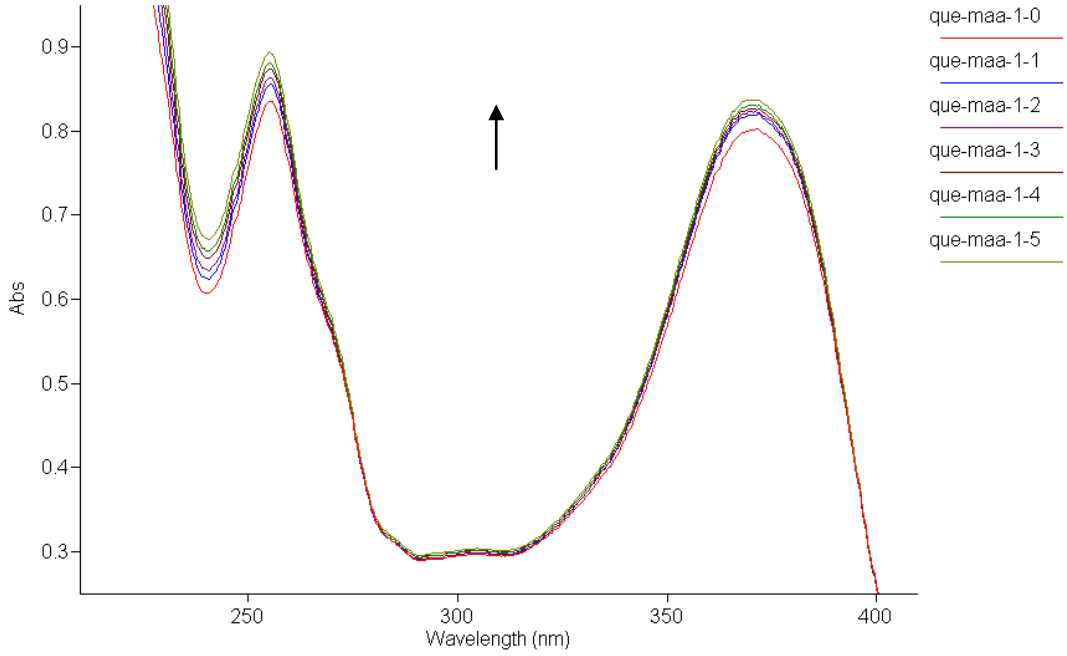
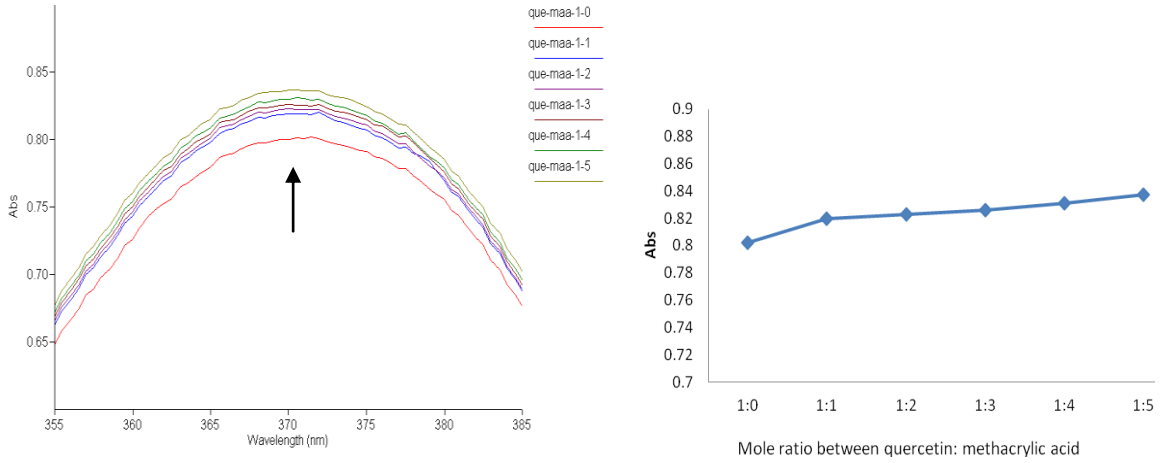


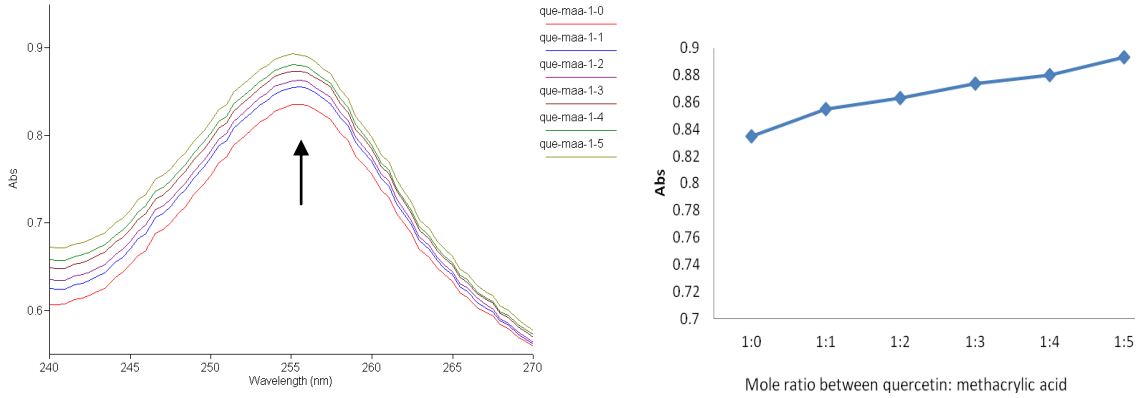
Fig. 5. Electronic absorption spectrum of titration quercetin with methacrylic acid in methanol (λ_{max} = 371.5 and 255.5 nm). The arrow shows the intensity increased with increasing mole ratio of quercetin-methacrylic acid (1→ 5).

As shown in fig 5 there are two distinctive bands, in a broad range of 240–400 nm, according to quercetin molecular structure. This is typical for flavonoid spectrum that is always consisted of two band I and II [21]. Quercetin in methanol exhibits band I absorption with maximum wavelength at 371.5 nm and it is considered to be associated with the absorption due to the B-ring system. Band II that has maximum wavelength at 255.5 nm is associated with the absorption involving the A-C ring system. Actually there is still a weak band with an absorption maximum around 300 nm, which is attributed to the C-ring only. Interaction effect of methacrylic acid to the active site is not clear detected that there is no significant shift in the data. It is contrast if comparing another case that there is accuring of a hypsochromic shift (to a shorter wavelength) if the hydroxyl group at position 3 of a flavonol is conjugated [22] or if the interaction involving an ionic interaction [23].



(a) (b)

Fig. 6. (a) Absorption spectra and (b) plot absorbance of complex solution of quercetin-methacrylic acid with variation of mole ratio for band I



(a) (b)

Fig. 7. (a) Absorption spectra and (b) plot absorbance of complex solution of quercetin-methacrylic acid with variation of mole ratio for band II

Analysing of the two main bands is clearer using separate bigger picture in fig 6 and fig 7. Increasing of the ratio between quercetin-metahcrylic acid will increase the absorbance and quantitatively it is plot in that figures. We plot the absorbance at the maximum wavelength versus mole ratio. As shown in fig 6(b), it indicates that there is minimum one methacrylic acid will interact to the ring B. This is because starting at 1:1 the plot is not increased, indicating that the active site in ring B had been bound with methacrylic acid. A different case is shown in Fig 7(b) that the plot begins to level off at mole ratio 1:3 although for 1:5 is increased. We obey a higher ratio because it will give excessing of methacrylic acid. An excess of functional monomers can cause more non-selective adsorption sites with random distribution in the MIPs and result in self-association of the monomers, which will reduce the selective adsorption sites and increase the

adsorption mass transfer resistance [24]. From this result, there is minimum three methacrylic acid molecules will interact to quercetin on ring A-C. Totally there is a suitable mole ratio of quercetin-methacrylic acid is 1:4 and it is comparable to the molecular modelling result.

Conclusions

The molecular modeling and UV spectrophotometric analysis of pre complexation between quercetin and methacrylic acid is useful to predict a suitable mole ratio of the template and functional monomer. We have shown that a suitable mole ratio is 1:4 that can produce molecular imprinted polymer with high stability. The verification of the MIP product will be reported on the other publication.

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