1. **Paralel Session XI: Phytochemistry Research**

Date / Time : Thursday, July 22th, 2010; 14.00 – 16.00					
Place : Auditorium Benedictus					
No	PIN	Paralel Session	Author	Institution	Title
8.	OPT-208	15.45 – 16.00	Iqmal Tahir	Gajah Mada University	Design of Molecularly Imprinted Polymer for Solid Phase Extraction of Sinensetin from *Orthosipon stamineus*

DESIGN OF MOLECULARLY IMPRINTED POLYMER FOR SOLID PHASE EXTRACTION OF SINENSETIN FROM *Orthosipon stamineus*

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ABSTRACT

Sinensetin extractions from *Orthosipon stamineus* (Misai Kucing) using an organic solvent are costly and need practical technique to get high yields. A Solid Phase Extraction (SPE) technique can be as an alternative using a novel solid material in the SPE column known as Molecularly Imprinted Polymer (MIP). In our paper, we reported computer aided design of MIP for sinensetin by molecular modeling approach to get the optimum procedure in MIP's synthesis. The modeling is done to select monomer that is usable to bind several active sites in sinensetin. Molecular modeling is performed using AM1 semi-empirical – quantum mechanics method to study the interaction between sinensetin as template and a functional monomer molecule. There are twenty functional monomer used in this work covering acidic, neutral and basic organic monomer. The result from the modeling showed that the acidic functional monomer is good for this purpose. Non covalent bonding has an important role to the interaction.

Keywords: sinensetin, molecular modeling, herb sensor, imprinted polymer.

INTRODUCTION

Computational technology in recently years has been growing very fast and it has been applied for many areas, including in chemistry. Chemist can use this technique known as molecular modeling to study theoretical aspect of molecular systems and it is also very useful to investigate materials that are too difficult to find or too expensive to purchase. It also helps chemists make predictions before running the actual experiments rather than 'trial and error' so that they can be better prepared for making observations (Leach, 2001). Molecular modeling has been used for material research and there are many papers that report its application to design of a novel prospective material for sorbents or sensors namely Molecular Imprinted Polymer or MIP (Karim *et al*, 2005, Spivak 2005, Yao *et al*, 2008, Piletska *et al*, 2005, Liu *et al*, 2007).

Molecular imprinting is a way of creating recognition sites in polymeric materials (Sellergren, 2001). The molecular imprinting technique involves a polymer, which has been synthesized in the presence of a target molecule, being used to separate a target molecule from other components. The polymers are constructed with ligands to contain cavities which closely match the shapes of various analyte molecules. The analyte molecules are incorporated into a pre-polymeric mixture and allowed to form bonds with the pre-polymer. The mixture is then polymerized with the analyte molecules in place. Once the polymer has formed, the analyte molecules are removed, leaving behind cavities with the analyte molecule's shape. In this way, a particular molecule can be identified since the shape of the cavity is specific to the molecule modeled. This method of identifying a particular molecule is attractive because of the simplicity of the preparation of the polymer and the simplicity and specificity of the identification of a target molecule (Kirsch *et al*, 2000). The product MIP is very useful for many applications, i.e., as an affinity material for sensors, adsorbents for solid phase extraction, binding assays, artificial antibodies, chromatographic stationary phase, catalysis, and others (Sellergren, 2001).

Sinensetin (fig 1) is a natural compound that has good effect against diurectic and hypuricemic to human metabolism process (Arafat *et al*, 2008, Khamsah *et al*, 2006,). Extraction of sinensetin from *Orthosiphon stamineus* leaves as herbs product could be done by chromatography separation after the bioactive extraction of dry leaf powder with 50% methanol (Hossain *et al*, 2005). In microscale, chromatography separation could be done using high performance thin layer chromatography (HPTLC), for example in purpose to determine sinensetin quantitatively. For macroscale separation, especially to produce sinensetin as herb product, the process is solvent extraction and it needs special techniques. The alternative of solvent extraction is a solid phase extraction (SPE) process using a specific sorbent material related to the target compound. Nowadays there is no special sorbent for sinensetin, therefore producing this material is interesting and we proposed sinensetin imprinted polymer for SPE application of sinensetin.

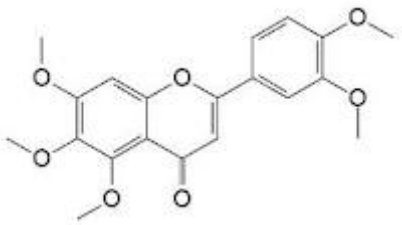


Fig 1. Structure of sinensetin

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. Nowadays it is important to design MIP by rational approach to select a functional monomer which gives suitable binding sites for a template (Piletsky *et al*, 2001). There are many paper reported a good MIPs that have been designed by application of experimental design (Zhu *et al*, 2007, Koohpaei *et al*, 2008) or using molecular modeling technique (Davies *et al*, 2004, Breton *et al*, 2007). Although a trial and error experimental could give an answer, molecular modeling approach is relatively more rational and more effective to study which a functional monomer that is good to build active site in polymer (Yao *et al*, 2008). The theoretical background was based on the interaction between monomers and template to form the stable complex that has a high binding energy. The most common approach of this interactions are in non-covalent interactions via hydrogen bonding, electrostatic or hydrophobic interactions (Karim *et al*, 2007). 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 (Hajnal *et al*, 1999) and homopolymerization of spiroorthocarbonate (Harris *et al*, 2000).

In this paper, the use molecular modeling to select functional monomer that can interact with sinensetin in MIP processes is described. The model is utilized to select and classify several functional monomers that could give a stable MIP for sinensetin.

METHOD

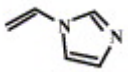
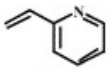
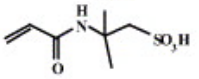
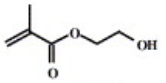
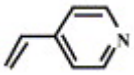
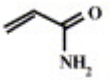
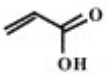
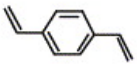
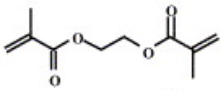
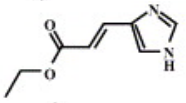
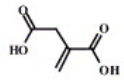
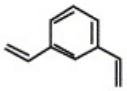
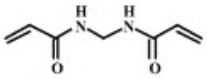
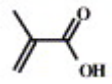
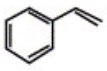
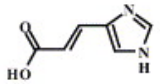
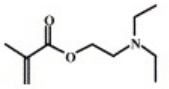
Materials

We used molecular models i.e. sinensetin (fig 1) and twenty monomers (table 1) that are followed of Karim's work (Karim *et al*, 2007).

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.

Table 1. Structure of the functional monomers used in modeling

No	Name	Structure
1	Vinylimidazole	
2	2-Vinylpyridine	
3	Acrylamido-2-methyl-1-propanesulfonic acid	
4	Hidroxyethylmethacrylate	
5	4-Vinylpyridine	
6	Acrolein	
7	Acrylamide	
8	Acrylic acid	
9	Acrylonitrile	
10	Allylamine	
11	p-Divinylbenzene	
12	Ethylene glikol dimethacrylate	
13	Urocanic acid ethyl ester	
14	Itaconic acid	
15	m-Divinylbenzene	
16	N, N’-methylene bis acrylamide	
17	Methacrylic acid	
18	Styrene	
19	Urocanic acid	
20	Nn-diethylaminoethylmethacrylate	

Procedure

The calculation started with geometry optimization and minimum energy calculation that were perform for the template structure i.e. sinensetin and the monomer individually. The obtain structure were applied to the complexes formed between sinensetin and the monomer. 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 complex exists as combination between sinensetin and a functional monomer (from table 1). The complex model was analyzed based on the most active site in sinensetin structure. The interaction energies of sinensetin with the monomer can be calculated from equation 1:

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

The calculation is employed based on similar works by Farrington & Regan (2007).

RESULT AND DISCUSSION

Firstly, we reported the electronic structure of sinensetin as the template. It is resulted by exploration using quantum mechanics calculations by applying of AM1 semiempirical level rather than ab initio or Density Functional Theory (DFT) levels. The result can show the availability of active sites in the compound naturally and it can guide the molecular interactions between sinensetin and the monomer. The electronic structure data covers several parameters i.e. total electronic charge density, electrostatic potential contour map and electric dipole moment,.

In figure 2, we could check the total charge density contour map of sinensetin. The shape of sinensetin 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 sinensetin.

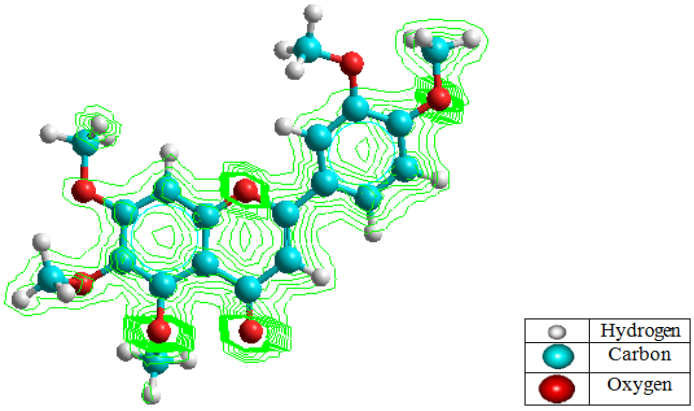


Fig 2. Total charge density of sinensetin

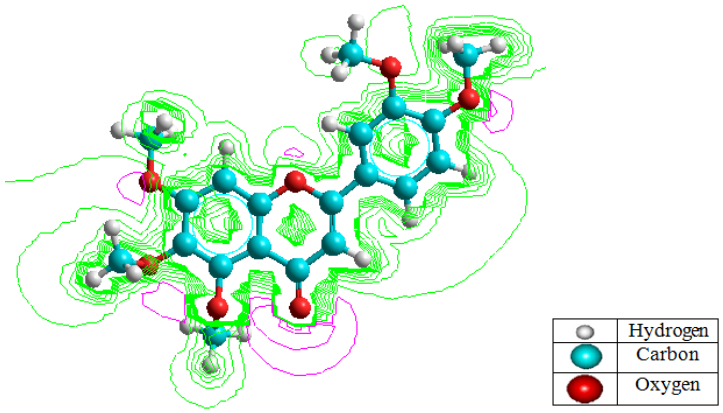


Fig 3. Electrostatic potential contour map of sinensetin

We can also check the active site in sinensetin by using the electrostatic potential contour map of sinensetin (fig 3). There are many active sites available in sinensetin 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 methoxy site. Site of atom O on methoxy site will give steric effect so it can make a barrier to the monomer molecules to interact easier.

Next data is dipole moment and it refers to the quality of a system to behave like a dipole. Dipole moment is the measured polarity of a polar covalent bond and is defined as the product magnitude of charge on the atoms ant the distance between the two bonded atoms. The calculated dipole moment (μ) of sinensetin is 6.330 Debye, indicating this template can interact with polar monomers. Table 2 shows calculated dipole moment of the twenty monomers. Several monomers that has dipole moment greater than 2 Debye (closer to the μ of sinensetin) could be considered as functional monomer to synthesize of sinensetin imprinted polymer. This monomer is polar or semi polar and according to the principle of solubility, like dissolving like, we can choose an appropriate solvent that can dissolve sinensetin and also the monomer. Monomers that have dipole moment less than 2, that indicate non polar molecules, will not be considered as the functional monomer for synthesis of sinensetin imprinted polymer. It is because in the process will need special optimization especially for selection of the polarity type of solvent. Therefore we can exclude monomers of vinylimidazole, allylamine, p-divinylbenzene, m-divinylbenzene and styrene respectively.

Data binding energy can show the stability of the complex. The more negative ΔE value is corresponding to the higher binding energy and it means the complex is more possible to be existed. On the other words, complexes with higher ΔE provide MIP with higher selectivity.

Table 2 shows that N, N'-methylene bis acrylamide can formed the most stable complex with sinensetin because it has the highest ΔE (-12.56 kcal/mole). Sinensetin can interact strongly with this molecule as the functional monomer. In reality the value is so high and it will make a problem in rebinding the template during washing process. It will need special treatment such as utility of strong solvent, extra energy by high temperature or longer time processing.

Table 2. Dipole moment (μ) of the monomer and the value of binding energy of interaction between sinenesetin and functional monomer. The value of ΔE was obtained from Eq 1.

No	Template	μ (Debye)	Energy (kcal/mole)			ΔE (kcal/mole)
			Sinensetin	monomer	Complex	
1	Vinylimidazole	0.839		-1,432.08	-6,482.49	-2.89
2	2-Vinylpyridine	1.850		-1,622.83	-6,673.27	-2.93
3	Acrylamido-2-methyl-1-propanesulfonic acid	5.336		-2,436.82	-7,491.81	-7.47
4	Hidroxyethyl-methacrylate	3.313		-1,850.83	-6,904.90	-6.56
5	4-Vinylpyridine	2.247		-1,625.43	-6,675.64	-2.70
6	Acrolein	3.064		-797.25	-5,848.55	-3.79
7	Acrylamide	3.954		-970.17	-6,024.67	-6.99
8	Acrylic acid	2.463		-916.60	-5,970.12	-6.01
9	Acrylonitrile	3.005	-5,047.51	-728.19	-5,784.96	-9.25
10	Allylamine	1.423		-979.07	-6,031.93	-5.35
11	p-Divinylbenzene	0.000		-2,174.89	-7,224.15	-1.74
12	Ethylene glikol dimethacrylate	1.950		-2,957.12	-8,008.82	-4.19
13	Urocanic acid ethyl ester	3.371		-2,371.82	-7,420.98	-1.65
14	Itaconic acid	0.897		-1,575.85	-6,628.46	-5.09
15	m-Divinylbenzene	0.011		-2,174.66	-7,223.79	-1.62
16	N, N'-methylene bis acrylamide	2.560		-2,088.14	-7,148.21	-12.56
17	Methacrylic acid	2.009		-1,198.20	-6,251.92	-6.21
18	Styrene	0.010		-1,745.43	-6,794.31	-1.37
19	Urocanic acid	8.605		-1,704.69	-6,756.57	-4.36
20	N,N-diethylaminoethyl-methacrylate	2.510		-3,011.38	-8,062.09	-3.19

The other monomer that can be considered as functional monomer are acrylamido-2-methyl-1-propanesulfonic acid, hidroxyethylmethacrylate, acrylamide, acrylic acid, acrylonitrile, allylamine, itaconic acid, methacrylic acid and urocanic acid. Their complex has a moderate value of ΔE. Based on the value of ΔE, characteristic of their interactions exist as non covalent interaction. Fig 4 shows model interaction between sinensetin and the monomer. Hydrogen bonding has an important role to keep the complex, especially for the complex formed between sinensetin-acrylamido-2-methyl-1-propanesulfonic acid, sinensetin-acrylic acid, sinensetin-itaconic acid, and sinensetin-methacrylic acid. For another complex, the interaction existed by dipole-dipole interaction and it is indicated by the value of ΔE is lower.

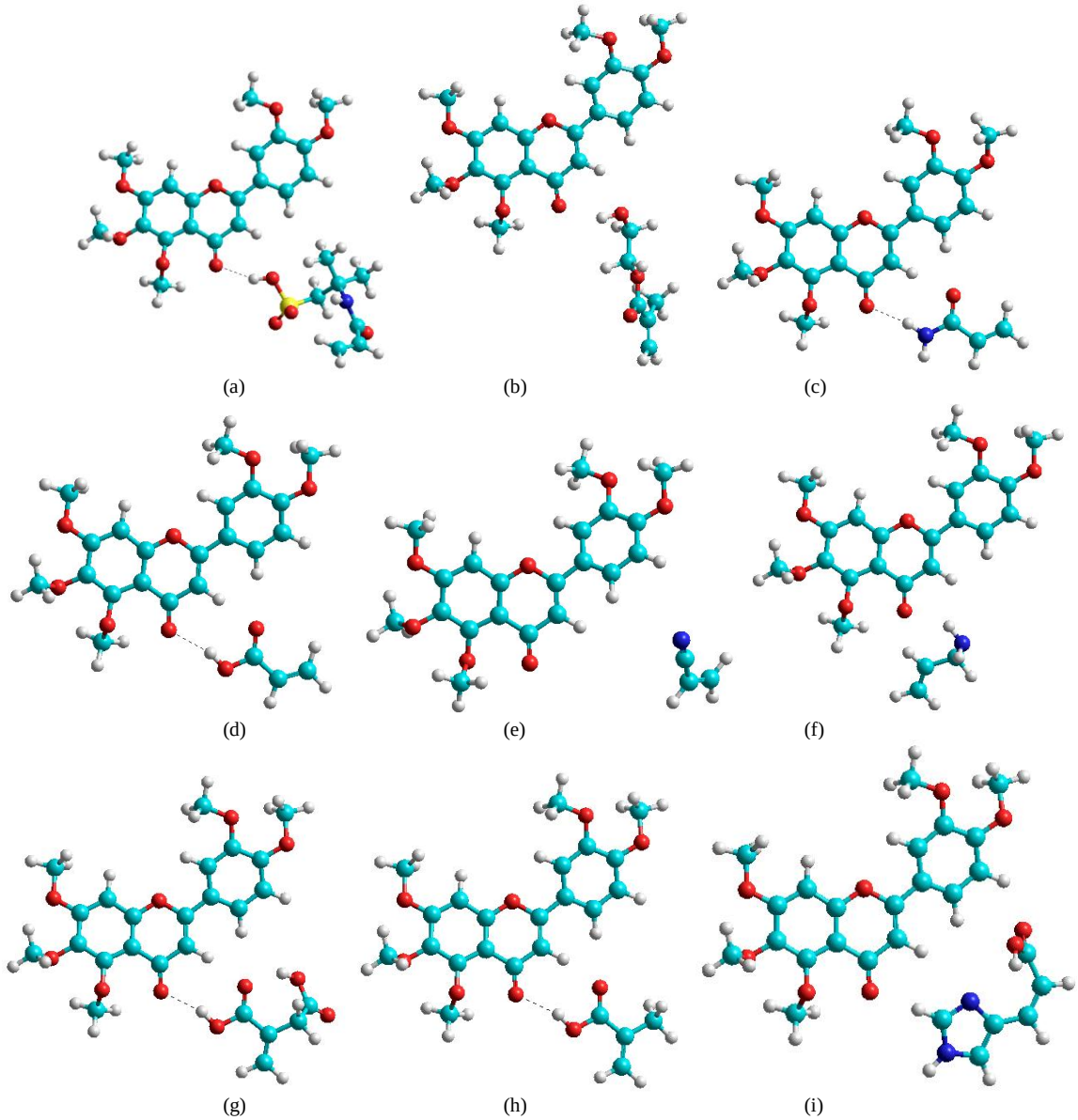


Figure 4. Complex model to illustrate the interaction between sinensetin and the functional monomer (a) acrylamido-2-methyl-1-propanesulfonic acid, (b) hidroxyethylmethacrylate, (c) acrylamide, (d) acrylic acid, (e) acrylonitrile, (f) allylamine, (g) itaconic acid, (h) methacrylic acid and (i) urocanic acid. Note: Dash line indicates hydrogen bonding.

CONCLUSION

We have shown that MIP for sinensetin can be designed by using molecular model to select a suitable functional monomer based on the interactions between sinensetin as the template and the monomer. The model developed for MIP showed that several functional monomers can be proposed to synthesize a good molecular imprinted polymer with high affinity MIP to sinensetin, such as acrylamido-2-methyl-1-propanesulfonic acid, acrylamide, acrylic acid, itaconic acid, and methacrylic acid. Next step modeling can be performed to calculate optimum mole ratio between sinensetin and selected monomer or synthesizing sinensetin imprinted polymer using variation of the mole of selected monomer.

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