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THE DEVELOPMENT OF MOLECULAR IMPRINTING TECHNOLOGY FOR CAFFEINE EXTRACTION

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ABSTRACT

Molecularly Imprinted Polymers (MIPs) is a type of macromolecule formed by application of molecularly imprinting technology, which creates cavities in synthetic polymeric matrices which are highly selective to an imprinted template. MIPs were synthesized experimentally using methacrylic acid (MAA), divinylbenzene-80 (DVB-80), azobisisobutyronitrile (AIBN) and methanol as a monomer, cross-linker, initiator and porogen, respectively. The Non-Imprinted Polymer (NIP) was produced simultaneously to serve as a control polymer. The rational design approach was theoretically conducted by Ab Initio Molecular Orbital Studies using Gaussian 09 computational software package at the theoretical level of DFT B3LYP/6-31 (d,p). The basis set is used to optimize the number of monomers and their binding site with the template. In both studies, MIPs were prepared with different ratios of template to monomer form a complex of 1:3, 1:4 and 1:5. Experimentally, the MIPs synthesized *via* precipitation polymerization technique produced homogenous spherical beads distribution where the complex 1:3 gave the best. Theoretical studies support this experimental finding where the complex 1:3 gave the highest interaction energy between caffeine and MAA, -45.29 kJ/mol followed by 1:4, -43.52 kJ/mol and 1:5, -43.11 kJ/mol.

Keywords: Caffeine; Computational study; Extraction; Molecularly Imprinted Polymers (MIPs); Monomer-template interaction

1. INTRODUCTION

Caffeine (1,3,7- *tri*methylxanthine) is a well-known compound which is naturally found in many plant and beverages, such as coffee, tea, cola and chocolate. German chemist, Friedrich Ferdinand Runge in 1819 was the first to isolate caffeine molecules from cocoa beans into its purest form, a white crystalline powder. Caffeine can be categorized as a drug that has addictive properties and uses as a stimulant to reduce physical fatigue. Excessive intake of caffeine can lead to insomnia (Chin et al., 2003; Farrington et al., 2006). Numerous studies have been carried out on the extraction of caffeine and most of the techniques can be somewhat complicated and expensive, such as organic solvent extraction (Nwuha, 2000), supercritical carbon dioxide extraction (Kim et al., 2008), and water extraction (Lou et al., 2012).

Therefore, a high degree of selectivity is desirable when extracting a single molecule in a complex matrix. Recently, molecular imprinting technology is a rapidly developing technique for the preparation of polymeric materials that are capable of molecular recognition for selective separation and chemical analysis, amongst other applications.

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MIPs are synthesized in the presence of a template (an interest molecule) and these will interact with functional monomers *via* non-covalent interaction before being cross-linked in the polymerization process. As the last step, removal of the template from the polymer network will leave a specific binding site or "binding pockets", which are complementary to the imprinted template in the terms of size, shape and functional groups.

To improve the efficiency of MIPs that occur at the molecular level, a series of molecular modeling systems have been studied, such as monomer selectivity (Pardeshi et al., 2012), template-monomer ratio interactions (Riahi et al., 2010; Tadi et al., 2012; Farrington et al., 2006; Liu et al., 2007) cross-linker selection (Muhammad et al., 2012) and the effect of solvents (Dong et al., 2007).

Therefore, in this research, preliminary studies are carried out using Gaussian 09 computational software package on the development of molecular imprinting technology for caffeine extraction. MIPs were prepared experimentally and theoretically with different ratios of template to monomer; 1:3, 1:4 and 1:5, respectively.

2. METHODOLOGY

2.1. Chemicals

MAA, DVB-80, AIBN, hydroquinone, acetonitrile, toluene and methanol were purchased from Merck and caffeine was supplied from Sigma Aldrich. All chemicals were purified before use and AIBN was re-crystallized from methanol.

2.2. Synthesis of Caffeine-Imprinted Polymer

The polymers were prepared by a precipitation polymerization method. Caffeine was dissolved in a mixture of acetonitrile and toluene (3:1 v/v, 57/19 mL) in a 250 mL reaction flask. This was followed by the addition of MAA, DVB-80 and lastly, AIBN (0.185 g). Table 1 showed precipitation polymerization conditions for the production of MIPs and NIPs. The solution was deoxygenated by bubbling oxygen-free nitrogen gas through the solution for about 20 minutes at ice-bath temperature, and the reaction flask was then sealed under nitrogen. Then the reaction flask was placed on a low-profile roller housed inside a temperature-controlled incubator. The temperature was ramped-up from room temperature to 60°C over a period of approximately 2 hours, and then kept constant at 60°C for 48 hours thereafter. After reaching 60°C, the monomer solution turned milky within two hours and the polymerization proceeded smoothly.

Table 1 Precipitation polymerization conditions

Polymer code	Mole ratio of T:M:X	Caffeine, T (mmol)	MAA, M (mmol)	DVB-80, X (mmol)
P1	0:3:20	-	2.62	17.5
P2	1:3:20	0.87	2.62	17.5
P3	0:4:20	-	3.39	17.0
P4	1:4:20	0.85	3.39	17.0
P5	0:5:20	-	4.12	16.5
P6	1:5:20	0.82	4.12	16.5

Notes : P1: NIP ; P2: MIP for ratio 1:3:20
P3: NIP ; P4: MIP for ratio 1:4:20
P5: NIP ; P6: MIP for ratio 1:5:20
T: template, M: functional monomer, X: cross-linker

After this time, the reaction flask was cooled to room temperature. A sample of the polymer was spotted onto a microscope slide and the bead size estimated under an optical microscope. The polymer was filtered off on a polyamide membrane (0.2 μm) filter, washed with acetonitrile (2×20 mL) and then washed with methanol (20 mL).

The caffeine-imprinted polymer, P2, was transferred to a pre-weighed vial and dried to a constant mass *in vacuo* at 40°C for 24 hours. A non-imprinted control polymer (NIP), P1, was prepared in the same manner as P2, but in the absence of caffeine. The MIPs and NIPs for the ratios of 1:4 and 1:5, respectively, were prepared in the same manner as 1:3.

2.3. Computational Methods

The molecular structure of caffeine, MAA and complex caffeine-MAA of 1:3, 1:4 and 1:5, respectively, were created using the Gauss View 5.0 program. All calculations were performed at the theoretical level of DFT B3LYP/6-31 (d,p) using Gaussion 09 computational software package in order to get the optimized molecular structure. The binding energy was then calculated as the complexes were formed between MAA and caffeine by using Equation 1.

$$\Delta E = E_c - [E_t + \Sigma E_m] \tag{1}$$

Where ΔE is the interaction energy between MAA and caffeine, E_c is the total Self-consistent Field (SCF) energy of the template-monomer complex, E_t is the SCF energy of template and ΣE_m is the accumulative SCF energy of the monomers.

2.4. Characterization Techniques of Caffeine-Imprinted Polymer

The beads size and morphological structure of MIPs and NIPs were characterized by using Scanning Electron Microscopy (SEM) model JEOL JSM-6360LA. Analysis for full porosimetry analysis included the specific surface area, total pore volume and average pore diameter of the samples, which were measured by the Brunner-Emmett-Teller (BET) technique. This technique brings forward the concept of adsorption of nitrogen gas on the surface of porous materials with monolayer molecular adsorption.

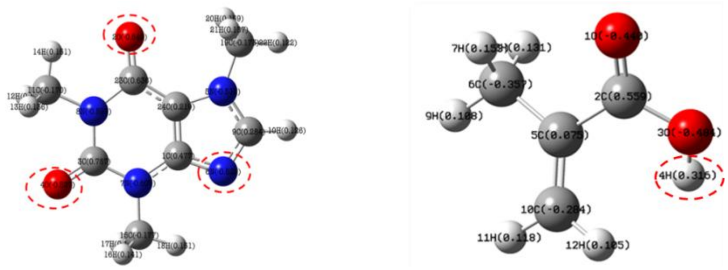
3. RESULTS

3.1. Synthesis of Caffeine-Imprinted Polymer

In this study, precipitation polymerization was conducted by using the 1:3:20 system. The amounts of polymerization reagents were calculated to produce 4 gram scale of each sample. The percentage yield for polymers produced are 85, 69, 87, 67, 98 and 96% for P1, P2, P3, P4, P5 and P6 respectively.

3.2. Computationally Study of Template-Monomer Interactions

Figure 1 shows the Mulliken partial atomic charges used to predict the binding site of hydrogen bonding between caffeine and MAA. There are three sites on the caffeine molecule that have a potential to form hydrogen bonding with a proton donor in the MAA molecule. Total Electronic Energy of caffeine and MAA were calculated to be −1786356.82 au and −807930.28 au, respectively.

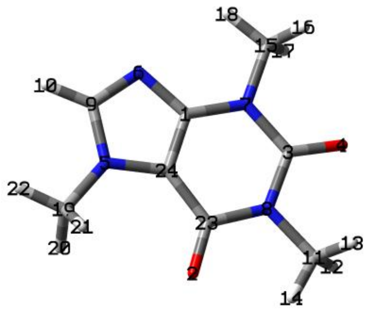
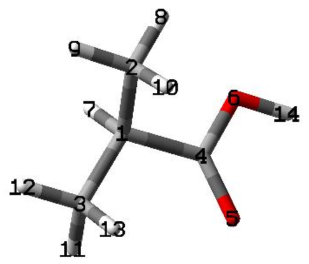
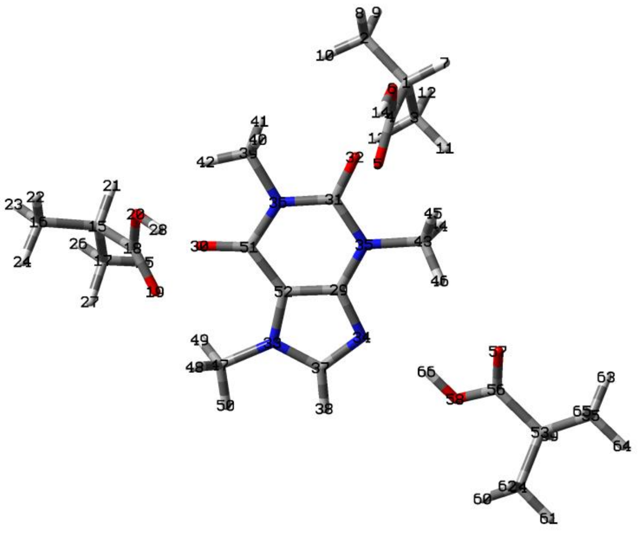


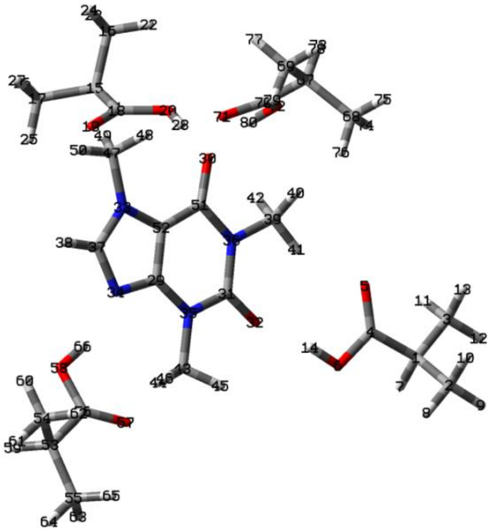
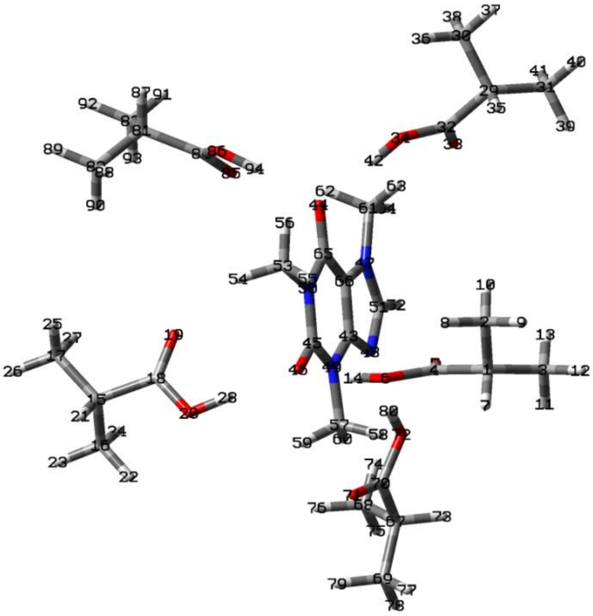
(a) (b)

Figure 1 Mulliken partial atomic charges of: (a) caffeine; (b) MAA

In order to assess the feasibility of template-monomer formation, the interaction energies (*ΔE*) of caffeine with different ratios of MAA (1:3, 1:4 and 1:5) were calculated and are shown in Table 2. It shows that all tested ratios may form a stable complex with the caffeine molecule, but MIPs with the ratio of 1:3 have been found to possess the strongest affinity towards caffeine when the binding energy value is -45 kJ/mol compared to other ratios. Hence, the molecular modeling calculated by the Gaussian 09 software predicted that a caffeine-imprinted polymer generated with a 1:3 system of template-monomer and there is a greater probability that it will possess greater binding and selectivity abilities.

Table 2 Calculated *ΔE* of caffeine per unit MAA for different ratios of MAA

Molecule	SCF Energy (au)	*ΔE* (kJ/mol)	Optimized Structure
Caffeine	−1786356.82	-	
MAA	−807930.28	-	
Complex (1:3)	−4210461.47	−45.29	

Molecule	SCF Energy (au)	*ΔE* (kJ/mol)	Optimized Structure
Complex (1:4)	−5018272.46	−43.52	
Complex (1:5)	−5826247.05	−43.11	

The optimized structure of the template-monomer complexes was further analyzed to determine the nature of interaction between caffeine and MAA. Table 3 shows the values of interaction energy with respect to bonding distance. The values of template-monomer interaction energy increased for the 1:3 systems, but decreased for 1:4 and 1:5 systems, respectively, as the bond distance increased for overall bonding in the complexes from 1:3 to 1:5 systems. Bond length is related to bond strength, which means the stronger the bond, the shorter the bonding distance.

3.3. Physical Properties of Caffeine-Imprinted Polymer

To verify the results from a computational approach, a few characterization techniques have been applied to observe the physical properties of the polymer samples.

3.3.1. Morphological structure by SEM

The size, shape and surface of MIPs obtained were analyzed by SEM (Figure 2). As expected for polymer particles produced by precipitation polymerization, the particles obtained were bead-shaped. From these images, MIPs for the complex 1:3 system are produced as well-shaped and homogeneous beads compared to polymers prepared in the complex 1:4 and 1:5 systems, respectively. The entire particles produced have diameter distributions from 5-10 µm.

Table 3 Calculated interaction energy for complexes caffeine-MAA

Interaction of O-H bond	Interaction energy (kJ/mol)	Hydrogen Bonding distance (Å)
Complex (1:3)		
C(31)=O(32)---H(14)-O(6)	−51.42	1.766
C(51)=O(30)---H(28)-O(20)	−48.17	1.765
N(34)---H(56)-O(58)	−49.42	1.824
Complex (1:4)		
C(31)=O(32)---H(14)-O(6)	−43.38	1.808
C(51)=O(30)---H(80)-O(72)	−50.35	1.786
C(51)=O(30)---H(28)-O(20)	−50.35	1.785
N(34)---H(66)-O(58)	−49.29	1.830
Complex (1:5)		
C(65)=O(44)---H(42)-O(34)	−49.18	1.802
C(65)=O(44)---H(94)-O(85)	−49.18	1.808
C(45)=O(46)---H(28)-O(20)	−46.01	1.786
C(45)=O(46)---H(14)-O(6)	−46.01	1.792
N(48)---H(80)-O(72)	−48.44	1.834

Note: Molecular structures with specific atoms number refers to Table 2.

3.3.2. Pore size distribution by BET surface area

Table 4 summarizes the data from nitrogen sorption porosimetry experiments for complex 1:3, 1:4 and 1:5 systems along with their corresponding controls. All the MIPs had similar properties in terms of surface area, total pore volume and average pore diameter, which suggested that the presence of a template during the polymerization process would not dramatically affect the morphologies of the resultant polymers. For all complexes, the caffeine-imprinted polymer has produced a type of mesopores polymer (2–50 nm).

Table 4 Nitrogen sorption porosimetry data for the caffeine-imprinted polymer

Polymer code	Surface area (m^2/g)	Total pore volume (cm^3/g)	Average pore diameter (nm)
P1	433.61	0.3085	2.8460
P2	448.13	0.3200	2.8561
P3	430.32	0.3026	2.8133
P4	445.40	0.3208	2.8808
P5	444.83	0.3120	2.8100
P6	448.16	0.3160	2.8174

4. DISCUSSION

The main objective of this study is to optimize the active binding sites between caffeine and MAA using quantum mechanical calculations performed in Gaussian 09 computational software package as a theoretical model that can be complemented with characterization techniques for an experimental work. Further optimization of template-monomer complexes was carried out in order to test the influence of the addition of a functional monomer in MIPs production. Theoretically, a caffeine-imprinted polymer produced through 1:3 ratio has a higher binding energy value when compared to 1:4 and 1:5 ratios. The result shown in Figure 2 is the comparison between theoretical and experimental. The result of a hydrogen bonding formation for the complex 1:3 caffeine-imprinted polymer is the best choice for the theoretical structure and in the experimental work by SEM it is preferable compared to P4 and P6 samples. The effective recognition between template and monomer are influenced by the strength and quantity of the interaction between monomer and template (Liu et al., 2007). The computational study by Gaussian 09 software has revealed that the hydrogen bonding formation for both heteroatoms, namely oxygen and nitrogen in caffeine will strengthen the bonding during recognition of caffeine-imprinted polymers. This is contrast with the study by Farrington and co-workers in 2006 using Hyperchem software. They reported that the complex of 1:2 ratio was the strongest complex formed between caffeine and MAA. The binding energy decreases in the further addition of the MAA molecule to the system. This suggested that the ideal energy of the system is reachable and the further addition of a functional monomer will not lead to greater level of complexion (Farrington et al., 2006). This also is in contrast to the concept of non-covalent imprinting in which by increasing the functional monomer, the self-assembly process is fully completed. It is actually in excess of the monomer, since non-specific binding will occur after only a specific number of monomer interacts with the template molecule.

Meanwhile, theoretically P4 and P6 samples are slightly decreased in binding energy value and increased in bonding distance. It can be concluded that in the excessive use of monomer, bulky structures are formed and a difficulty for interaction occurs between template and monomer. SEM images show beads obtained for P4 and P6 are not uniform in structure as for P2.

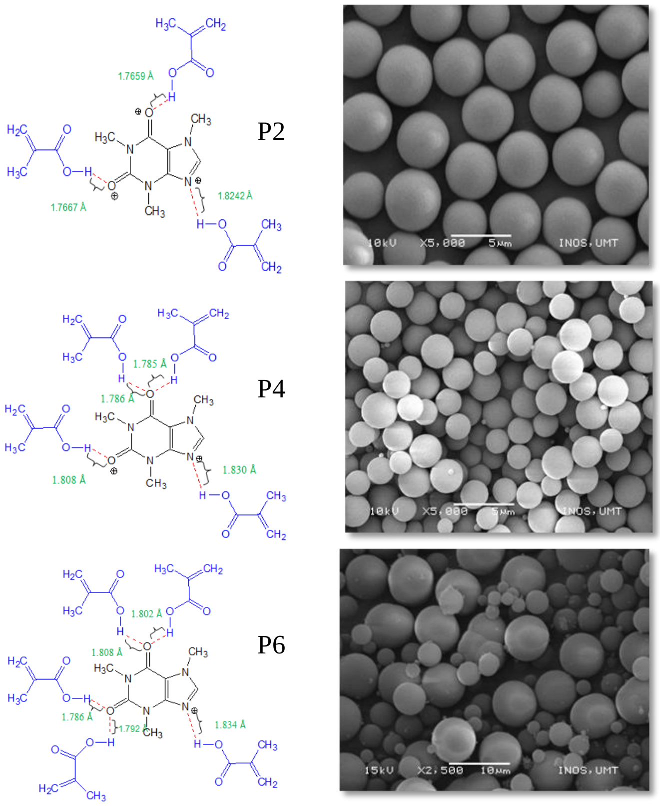


Figure 2 Molecular structures and SEM images for P2, P4 and P6

5. CONCLUSION

As a conclusion, it has been demonstrated that the caffeine-imprinted polymer was successfully produced by the precipitation polymerization method. By using the quantum mechanical software package Gaussian 09, the composition of active binding sites in the complexes was calculated. The result shows the caffeine-MAA complex of 1:3 is the best interaction by giving the highest binding energy, compared to 1:4 and 1:5 ratios. By increasing the binding energy, it will cause the distance of hydrogen bonding to be closer, thus making the interaction stronger and possible. The spherical beads for complex 1:3 are formed as a homogeneous type compared to 1:4 and 1:5 ratios either in MIPs or NIPs particles.

6. ACKNOWLEDGEMENT

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