Bisphenol A MIP Fabrication using the Application of Factorial Design Analysis

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Abstract. Molecular imprinted polymer (MIP) has caught the attention of many researches in recent years as a great tool for molecule recognition and other applications. But the main issue in the synthesis of MIP nanoparticles is the identification and optimization of the main factors affecting the material structure and size. This paper describe an experimental design approach to synthesis bisphenol A molecular imprinted polymer nanoparticles (BPA-MIP NPs) aimed at analysis of the relationship of four selected parameters: the polymerisation temperature, agitation rate, cross-linker to solvent ratio, and percentage of initiator. The results presented demonstrate the importance of keeping the right balance between these various parameters of polymerisation conditions. Generally, it can be concluded that MIPs should be synthesized using enough heating, adequate agitation, low concentration of initiator and with a considerably higher amount of solvent. Such procedure is proven as time and cost effective, and also can be used as a general tool in the preparation of MIPs for many different target molecules.

Introduction

Scientific researches and developed technologies over the past decade has grown to release more chemicals that are prone to be toxics such as estradiol, nonylphenol, pharmaceuticals compound, and also some pesticides, also known as endocrine disruptor compound (EDC) mostly end up interfering the normal functioning endocrine system in living bodies [1].

One of them, called Bisphenol A (BPA) have been used widely in chemical industry as an intermediate to produce epoxy, polycarbonate, polyester resins, and various plastic articles [2,3,4]. This compound is easily eluted and can leach out of multitude plastic products including coatings of food cans, dental sealants, and also baby formula bottles.

Molecular imprinting polymer (MIP), which is a newly preferred adsorbent material, is prepared using synthetics polymers with the ability to specifically recognize any target molecule. It is reported as adequately stable at moderately any given pH, pressure and temperature, and also less expensive than antibodies. Attempts have been made to prepare BPA MIP bulk copolymers [1,5,6]. The polymers were ground in stages to form powders and the limitations on this procedure are irregular shape and inhomogeneous size of particles after grinding. To overcome this setback, MIP in nano-size was developed. Having the advantage of smaller size polymer, it also possesses higher surface area-to-volume ratios; thus, imprinted pores are highly accessible by templates and binding performances are improved [7,8]. Previously, MIP nanoparticles were synthesized by various polymerizations involving stabilizers, surfactants and additions that can contaminate the final products [9]. Others have applied surfactant-free dispersion technique [10,11,12] which produced comparable uniform submicron particles. Even though MIP nanoparticles have been successfully developed, they

suffer from lack of automated standard manufacturing process. Adequate selection of factors affecting the recognition properties of the tailor-made polymers, including the selection of template, monomers and cross-linkers, the initiation approach, and porogen, marked the successfulness of the imprinting process [13,14,15].

In the present work, a simple surfactant-free precipitation polymer was employed [12] with a novel approach through the application of an experimental design and multivariate analysis methodology considering four selected parameters. Studying polymerization in the high and low range of each parameter, we were able to synthesize different sizes and dispersion of MIP spheres. As shown in Scheme 1, BPA imprinted spheres were formed by copolymerization of bisphenol A dimethacrylate (BADM) and trimethylolpropane trimethacrylate (TRIM). The spheres were then hydrolyzed before investigating their binding performance. The resultant copolymers were characterized by Fourier Transform Infra-Red (FTIR), Scanning Electron Microscopy (SEM) and Mastersizer. Binding analysis confirmed the successful template adsorbent. The application of factorial design gave a statistically systematic approach for the formulation and optimization of nanoparticles with desired particle size and high binding efficiency [16].
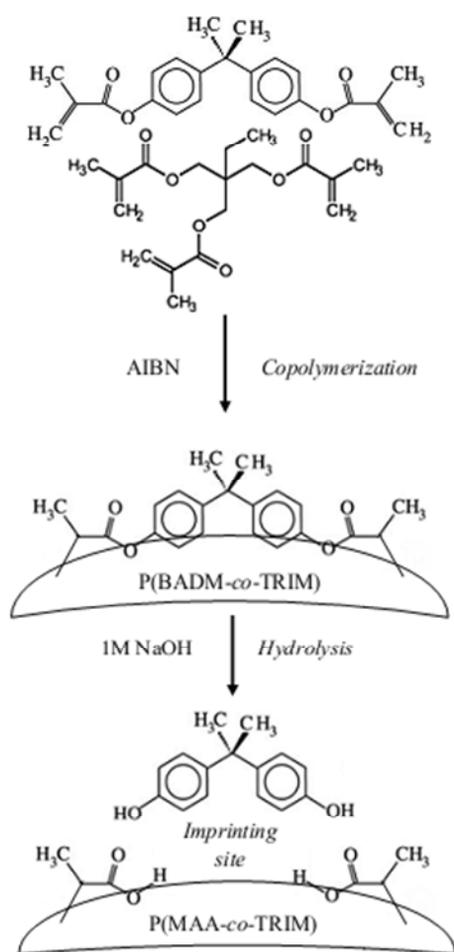
Scheme 1. Formulation of BPA imprinted copolymer.

Experiments

Materials. Bisphenol A dimethacrylate (BADM) as the functional monomer, 2,2'-Azobis(2-methylpropionitrile) (AIBN) as the initiator, and trimethylolpropane trimethacrylate (TRIM)

as cross-linker were purchased from Aldrich. Tetrahydrofuran (THF) a seluant, and acetonitrile (ACN) as polar solvent were of HPLC grade.

Procedure of Copolymerization. In this study, nano-size MIP spheres were synthesized by covalent precipitation polymerization approach, as stated to be simple and straight-forward procedure [13,12]. Several sets of MIP runs were conducted using thermal initiation copolymerization with variation on four selected factors which were temperature, agitation, cross-linker to solvent ratios, and initiator percentage, as listed in Table 1. Other factors such as monomer to cross-linker ratio and copolymerization time were fixed throughout the experiments.

The monomer solution was prepared by introducing BADM and TRIM (1:10 mol ratio) [5] into 250 mL three-neck flask in the presence of AIBN under nitrogen atmosphere. Samples were allowed to copolymerize in ACN following the sequence in Table 1 for 12 hours. The resulting precipitate was centrifuged and the supernatant was filtered under vacuum/was crushed with pestle and mortar. These copolymers were washed with ACN and then in THF. Finally, they were filtered under vacuum and store in desiccator for further use.

Template removal was carried out via hydrolysis reaction in aqueous solution containing 1M Sodium Hydroxide (NaOH) at 50 $^{\circ}$C with agitation until BPA concentration reached constant which the concentration reading were taken using UV-Visible Spectrophotometer (UV-Vis). The hydrolyzed polymers were washed with excess water until neutral pH. Bulk copolymers were prepared with the exemption of agitation. Non-imprinted polymer P(TRIM) were prepared following the same condition but without the functional monomer.

To examine the copolymers characteristics, FTIR spectra and SEM images were measured using FTIR, thermo Nicolet and SEM microscope (ZEIZZ), respectively. Particle distribution was analyzed using Mastersizer 2000 (Malvern).

Experimental Design. Design Expert Software (Stat-Ease Inc., Statistic made easy, Minneapolis, MN, USA, version 7.0.0) was used for the experimental design throughout this screening process study. The full factorial design requires fewer measurements than the classical one-at-a-time experiment to give tha same precision. At the same time, it detects and estimates any interaction between the factors, which the classical experiment cannot do. The order of the running experiments was restrictedly randomized to eliminate the possible bias (restricted factor was the polymerization temperature) [17]. The standard approach to the analysis of the experimental design data is to evaluate a list of the main and interaction effects supported by an ANOVA table, indicationg which effects are significant [18]. In the experimental design, only four factors were selected as potentially affecting the rebinding efficiency. The factors include the compositional variable, which was the amount of porogen of polymerization, and the operational variables which were the initiator, polymerization temperature and agitation rate. Consequently, a two-level full factorial design of 2^4 was utilized following a linear and quadratic model, containing squared terms. A total of sixteen sets of experiments and three replicates at the center point were used to demonstrate the statistical significance of the temperature (A;$^{\circ}$C), agitation rate (B;rpm), amount of porogen (C;%), and amount of initiator (D;%) on affecting the fabrication and performance of resultant polymer. The dependant variables that were selected were particle size [μm] (Response 1), and binding capacity [μmol/g] (Response 2). The range and levels of the variables investigated in this study were shown in Table 1. Range settings for variable factors were adjusted based on previous findings and literature.

Table 1. The range and levels of the variables in the 2^4 full factorial design model

Run	Values of independent variables			
	A: Temperature [^{o}C]	B: Agitation [rpm]	C: Solvent to crosslinker ratio [%]	D: Initiator percentage [%]
1	80.00	100.00	50.00	1.00
2	80.00	0.00	80.00	1.00
3	45.00	100.00	80.00	1.00
4	80.00	0.00	80.00	3.00
5	80.00	100.00	80.00	3.00
6	80.00	0.00	50.00	1.00
7	45.00	0.00	50.00	3.00
8	45.00	100.00	50.00	3.00
9	45.00	0.00	80.00	1.00
10	80.00	100.00	50.00	3.00
11	62.50	50.00	65.00	2.00
12	45.00	100.00	80.00	3.00
13	45.00	100.00	50.00	1.00
14	45.00	0.00	80.00	3.00
15	45.00	0.00	50.00	1.00
16	80.00	0.00	50.00	3.00
17	80.00	100.00	80.00	1.00
18	62.50	50.00	65.00	2.00
19	62.50	50.00	65.00	2.00

Binding Experiments. Batch BPA binding experiments were done as follows. 20 mg of MIP was dispersed in 20 mL aqueous BPA solution with 100 μm concentration. The mixed solution was shaken in a glass flask at 30 ^{o}C and it was let incubated to a saturated binding process for 24 hours. The final BPA concentration in the solution was determined using UV-Vis (Hitachi) at 278 nm. The binding S [μmol/g] of BPA was calculated using

$$[S] = (C_o - C_t) V / W \tag{1}$$

Where C_o and C_t represent the initial and final BPA concentration respectively, V in the volume of BPA solution and W is the weight of MIP.

Results and Discussion

Characteristics of MIP Spheres. The IR spectrum of obtained MIP particles was measured using FTIR where hydrolyzed (P(BADM-co-TRIM)$_H$) MIP is compared with the unhydrolyzed (P(BADM-co-TRIM)$_B$) one as shown in Fig. 1. Strong peak of C=O group occurred at 1730 cm^{-1} showed that BADM segments exist in the copolymer. Symmetric and asymmetric C-O stretch ester bands at 1265cm^{-1} and 1159cm^{-1} is well observed represents the TRIM segments in the copolymers.

To explain BADM IR spectrum in comparison with the resultant MIPs, it is observed that peak near 953cm^{-1} was decreasing due to the out-of-plane alkene C-H bending vibration, and noticed the loss of C-O stretching peaks in conjugated ester at 1292cm^{-1} and 1321cm^{-1}. It is also noted in the FTIR data between hydrolyzed and unhydrolyzed MIP that the band near 3450cm^{-1} for OH stretch show changes, signifying the formation of –COOH group in the copolymers after hydrolysis.
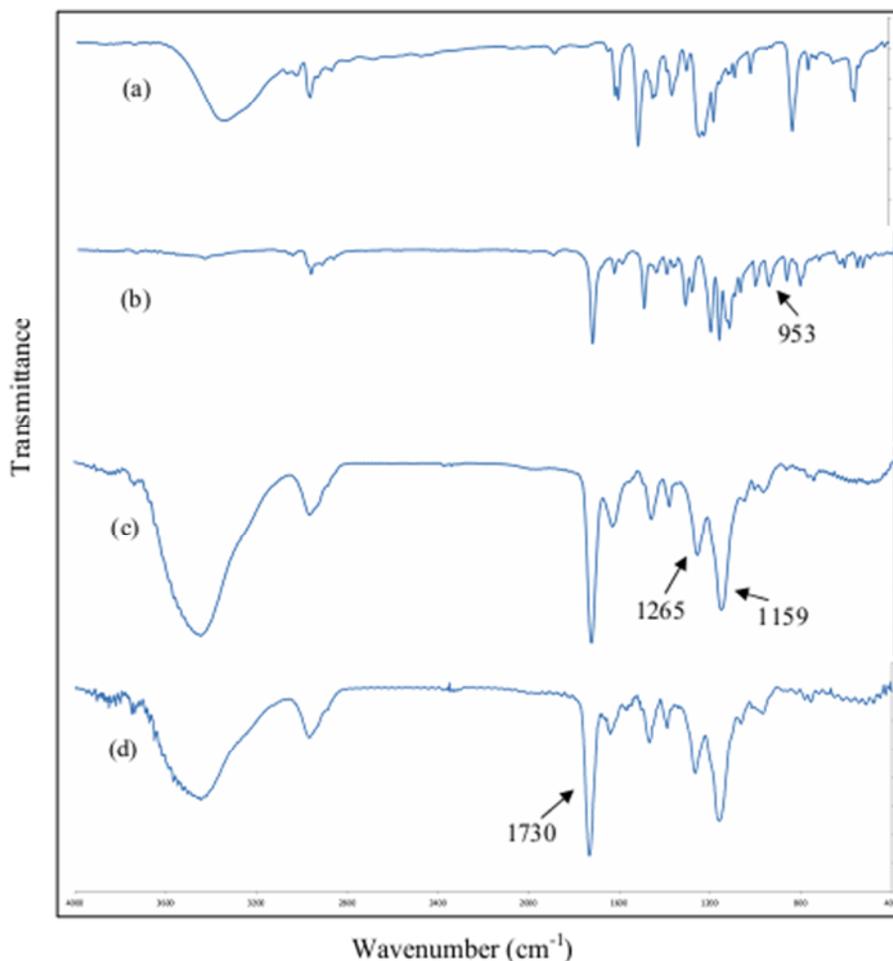
Figure 1. FT-IR spectra of (a) BPA, (b) BADM, (c) P(BADM-co-TRIM)$_H$, (d) P(BADM-co-TRIM)$_B$ by KBr pellet method.

Fig. 2 shows the TEM image of Run 12 and SEM image of Run 16 where sizes of MIP that were formed in powder showed 0.12 µm and 5 µm, respectively. From these images, it can be observed that Run 12 with parameters 45°C, 100 rpm, 80% solvent and 3% initiator, despite the small size, the polymer yield is less than Run 16 which parameters were 80°C, no agitation, 50% solvent and 3% initiator. This is the result of low temperature (45°C), which proved to contribute most in MIP synthesis.
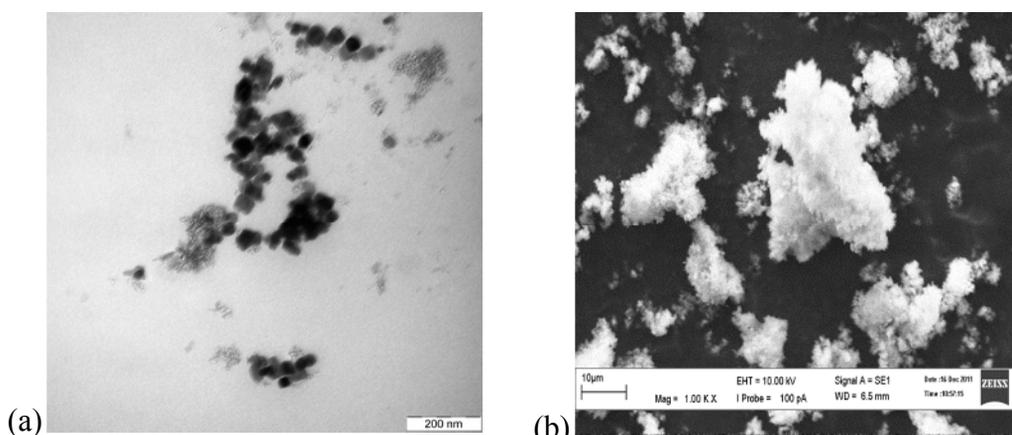
Figure 2 (a) TEM image of Run 12 and (b) SEM image of Run 16.

Fig. 3 shows the size and distribution of three resultant MIP Runs which is Run 10, Run 16 and Run 17 with sizes of 197 μm, 5 μm and 4 μm, respectively. Run 16 and Run 17 were more or less the same size but the Run 17 which experimental parameters were 80°C, 100 rpm, 80% solvent and 1% initiator, shows better dispersion and uniformity than Run 16. This proved that adequately moderate amount and usage of all factors affects the synthesis and formation of MIP spheres. For instant here, notice the unemployment of agitation in Run 16 may contribute to less homogenous polymers. However, Run 10 which parameters were 80°C, 100 rpm, 50% solvent and 3% initiator produce bigger size of MIP but showed good uniformity and particle dispersion. It can be said that, the use of agitation helped improve polymer formation.
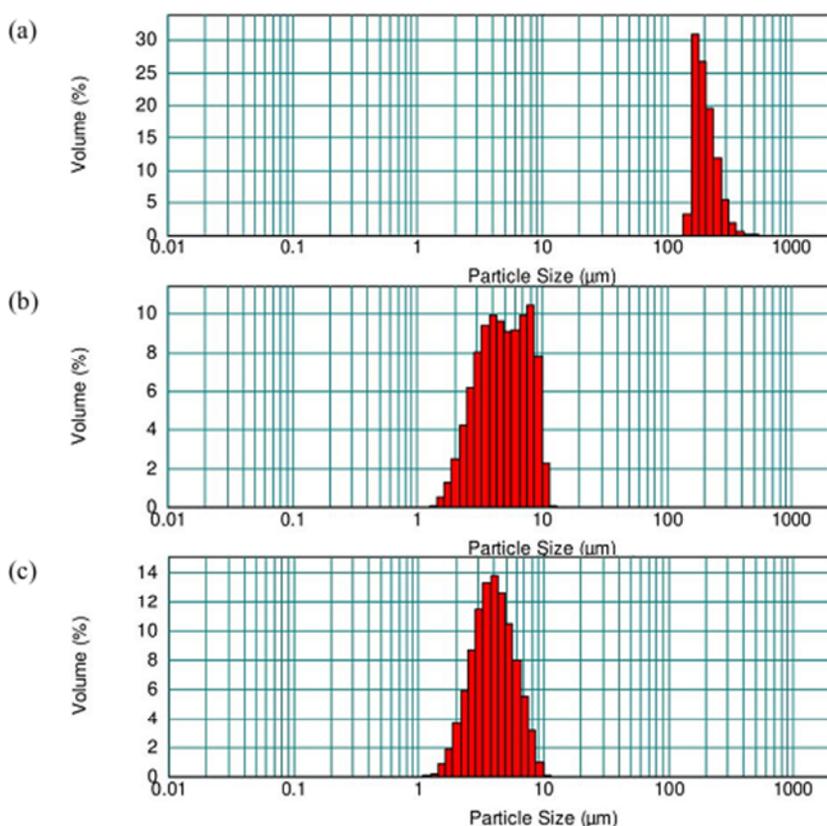
Figure 3. Particle size distribution of (a) MIP Run 10, (b) MIP Run 16, (c) MIP Run 17.

ANOVA Discussions. ANOVA test was employed to determine the significant and most contribute factors where they were ranked based on degree of F-ratio. Table 2 shows the ANOVA analysis reading where F-value and P-value of the model were 5.97 and 0.0223 respectively, indicating that the estimated model fits the experimental data fairly. The value of R^2 for this model was 0.8745, where it is stated that the R^2 value for a valid model is 0.6 or greater [14].

Table 2. ANOVA for 2^4 full factorial design; response: Binding Capacity [μmol/g].

Source	Sum of Squares	DF	Mean squares	F-value	Prob>F
Model	128.02	7	16.32	5.97	0.0223 [a]
A	41.99	1	41.99	13.71	0.0101
B	0.042	1	0.042	0.014	0.9110
C	0.17	1	0.17	0.055	0.8226
D	21.37	1	21.37	6.98	0.0385
AB	29.64	1	29.64	9.68	0.0208
BC	23.53	1	23.53	7.68	0.0324
CD	17.9	1	17.9	5.85	0.0520
Residual	18.38	6	3.06		
Lack of Fit	17.71	4	4.43	13.32	0.0710 [b]
Pure Error	0.66	2	0.33		
Cor Total	146.4	13			
R^2	0.8745				
Adjusted R^2	0.7280				

Values of "Prob >F" less than 0.0500 indicate model terms are significant.
[a] Significant.
[b] Not Significant

In order to analyze further on ANOVA analysis, the two-factor interaction was applied to improve the model equation despite that it will affect the main factors' p-values vaguely. Noticed that the significance of temperature stand out from the rest of the factors. It is reported that temperature produced a negative influence and it played an important role in the performance of the synthesized material. Temperature too responsible in affecting the polymerization process and the polymer structure hence would be the cause of the quality and quantity of the MIPs recognition cavities [17]. Contributing factors and parameter effects were also generated in Pareto Chart as shown in Fig. 4. From this chart, noticed two parameters fall below the t-Value limit, indicating that they were unlikely contribute much in the process, but nevertheless, both factor B (agitation rate) and C (solvent ratio) were selected to discuss their interactions with other factors. Other than that, all major contributing factors and relations go above the Bonferroni limit confirming their significance in explaining the model behavior.
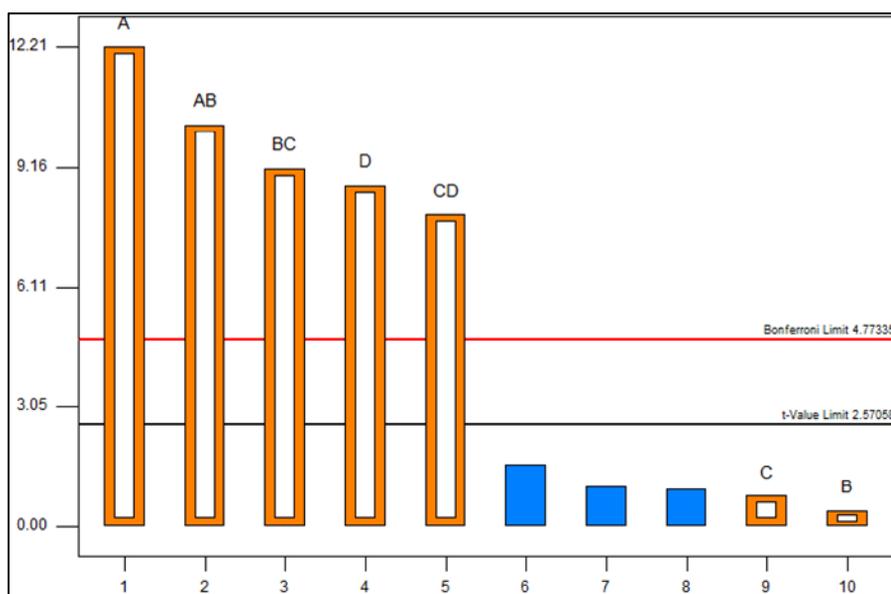
Figure 4. Pareto chart of main effect versus t-value of effects for binding capacity.

Binding Capacity. Fig. 5 showed the amount of binding capacity of selected MIP runs and Fig. 6 was the interaction graph generated by the design expert software where the main interactions factors shown were between AB, BC and CD. These factors correlate each other and will be explained thoroughly herein.

It is reported that high concentration of initiator added into the monomer mixture, would generate heat and thus, increasing the temperature and polymerization rate [19]. But be cautious if too much heat were used, it would disrupt the complex formed between the monomers and reduce the affinity and selectivity of MIPs. Nevertheless, high concentration of initiator added into the mixture would lead to the formation of a high amount of free radicals and thus generating large number of growing nuclei and globules which would result to smaller in particle size. These small sized globules would then produce a large number of small pores into the MIP spheres and possessed a large surface area all at once [20]. This behavior can be explained by monitoring MIP Run 12 and Run 14 which holds small submicron size polymer. These MIPs were produced at low temperature but high concentration of initiator. But it is also observed that they suffer a lower polymer yield to compare with other resultant polymers. In contrast with this, a low amount of initiator would result to higher amount of non-polymerisable double bonds remaining in the polymer thus lacking the rigidity of polymer, which is critically important for enantioseparation [21]. Moreover, the variation of average pores diameter and total pore volume was probably due to the porogenic ability of the solvent vapors released during the exothermic reaction [22].

Additionally, in order to control the average diameter of MIP produced, the used of agitation would caused an efficient dispersion of monomers in the solution mixture. The speeding mixture would cause decreasing of the complex polymer length chain and thus increasing the number of polymer particles because of the nuclei aggregation [23]. Because of this, smaller particle size with evenly distributed uniformity is produced. This phenomenon can also be observed in MIP Run 12 and Run 14. Both hold submicron size particles but have different value of binding capacity where Run 12 was higher than Run 14, probably due to uniformity and better dispersion of Run 12 particles acquired by utilization of agitation parameter during polymerization. Other researches also reported a similar result [11].

Furthermore, Koohpaei et al reported that the morphology was influenced by the properties of the porogen, since the swelling of the polymers is dependent on the surrounding

medium [17]. This swelling behavior most probably would cause in the alteration of the three dimensional configuration of the functional groups, taking part in the unique sites recognition resulting in poorer biding capacity. The binding efficiencies of the template in the diverse range forms if imprinted polymer were found to be very dependent on the type of solvent used, which indicative of hydrophobic and hydrogen bonding interactions [23]. Low binding amount observed could be probably due to the hydrogen bonding ability of acetonitrile. It can be said that some of the MIPs generate low binding capacity suggested that hydrophobic interactions were responsible for the adsorption of the solute in polar solution.
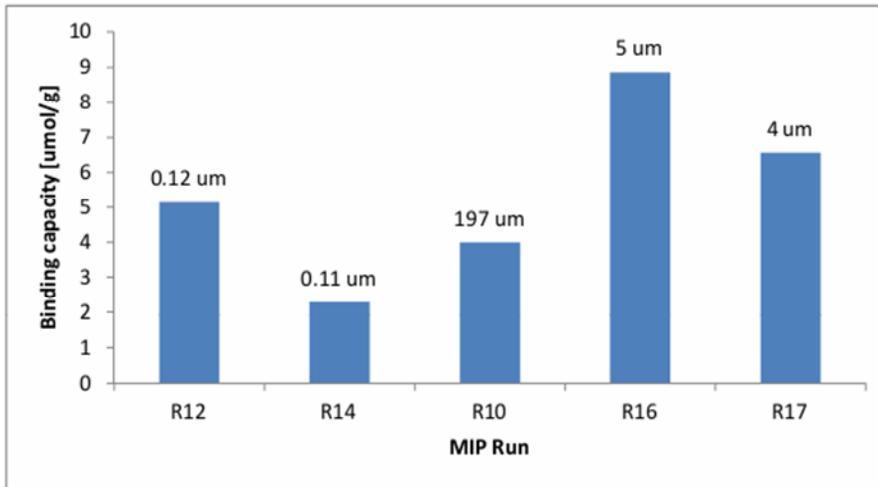
Figure 5. Binding capacity of selected MIP Runs and its particle size.
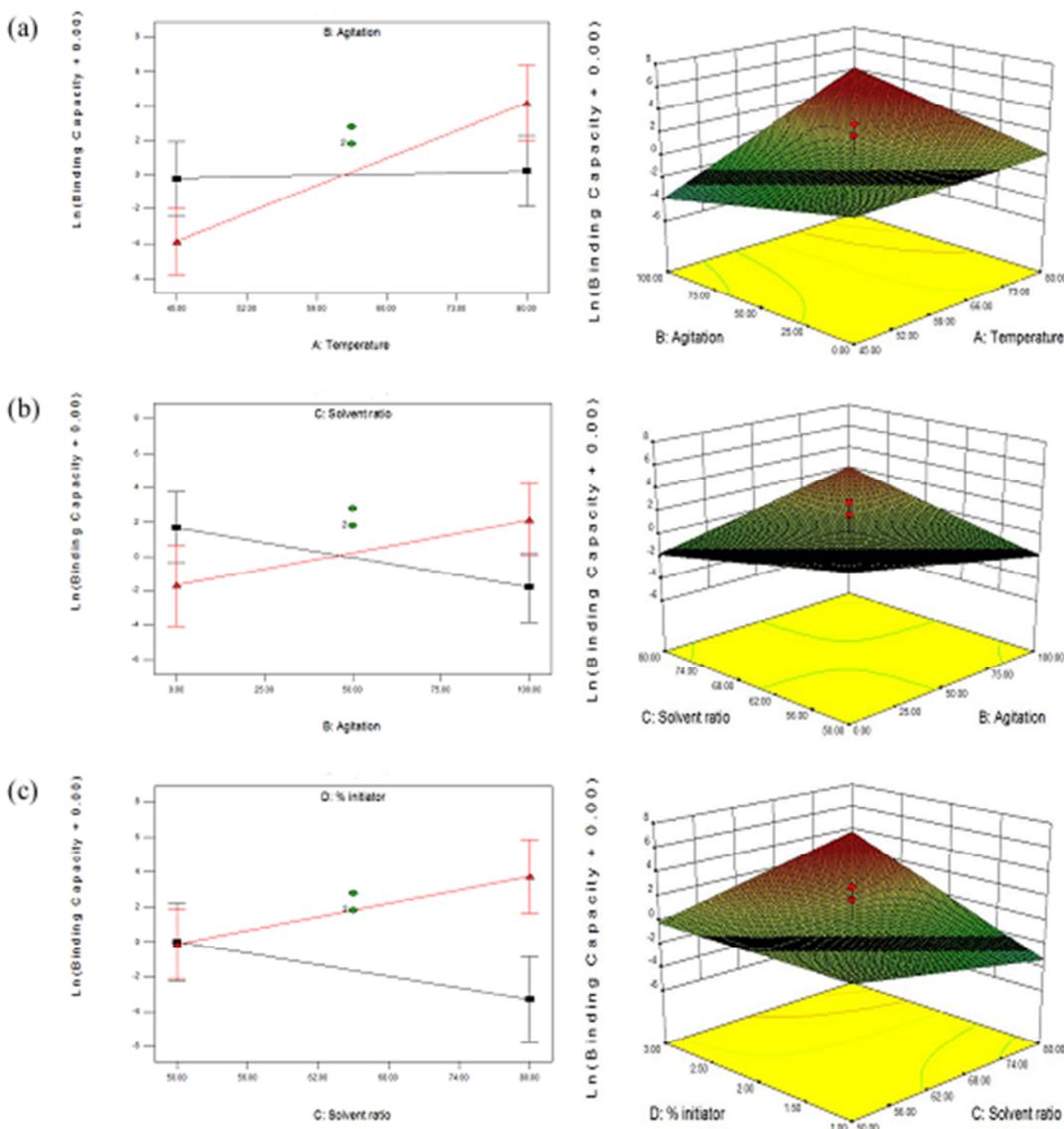
Figure 6. Plot of interaction effect for binding capacity (µmol/g): (a) Effect between temperature and agitation rate, (b) Effect between agitation and solvent ratio, (c) Effect between solvent ratio and initiator.

Conclusion

The application of experimental design was used in this work to control the size as well as to study the binding performance of the BPA molecular imprinting polymers. The results of ANOVA analysis led to a statistical model that described adequately the influence of the parameters at different levels on the responses. It can be said that we have developed a convenient method to gain the control of MIP nanoparticles and microspheres in the range of 100 nm to 200µm. temperature was the main factor contributed to the formulation followed by the amount of initiator, solvent and last but not least the agitation rate. An adequate size of MIP at approximately 5µm had the highest binding performance. This is due to the correlation between size and the amount of binding and as the result in this work, it can be concluded that the smaller the size of MIP doesn't necessarily improved its binding capacity and vice versa. The nano-size particles obtained could be because of the polymer swelling and incomplete copolymerization process. Hence, a sufficient usage of all parameters was highly recommended in formulating the synthesis of desired MIP. Although many factors and

interaction of factors affected the synthesis and performance of the resultant MIP, it can be said that this chemometric method using Design of Experimental software was capable to be an appropriate assist for controlling the size and distribution of MIP particles as well as selectively recognized BPA molecule.

Acknowledgement

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