Application of factorial design analysis to control bisphenol A MIP fabrication

M. Sharif Shareena and Che Ku M. Faizal

Faculty of Chemical and Natural Resources Engineering,
Universiti Malaysia Pahang
Lebuhraya Tun Razak,
26300 Gambang, Pahang, Malaysia
eeneight@yahoo.com

ABSTRACT: A key issue in the synthesis of molecular imprinted polymer 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 which are approximately 60^{o}C, 100rpm, 65% solvent ratio and 1% initiator, respectively. Generally, it can be concluded that MIPs should be synthesized using enough heating, adequate agitation, low concentration of initiator and with a bit 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 MIP NPs for different target molecules.

Key words: Bisphenol A, Molecular imprinted polymers, Nanoparticles, Full factorial design

1. INTRODUCTION

Normal functioning endocrine system is important to ensure normal follicle development and to generate good quality of oocytes [1]. Thus, it is crucial to take into consideration concerns about adverse effects threats of environmental contaminants, especially endocrine disruptors (EDC).

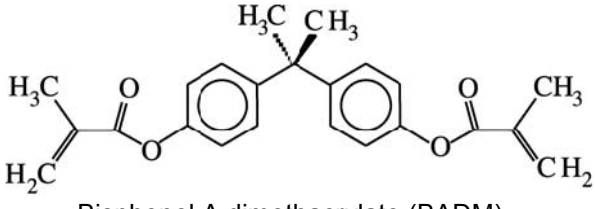
One of these EDCs, particularly Bisphenol A (BPA), is reported having an extensively used in industrial chemical, as an intermediate to manufacture epoxy, polycarbonate, polysulphone, polyester resins, and numerous plastic articles [2,3,4]. BPA is present in a multitude of products, including the interior coatings of food cans, milk containers, and baby formula bottles, as well as in dental sealants. Though toxicity of BPA towards humans is fairly low, the large amounts usage for manufacturing of polycarbonates and such product, also with having characteristic of highly water soluble, has shown that it is easily eluted and can leach out of those products mentioned before [2,5].

Newly preferred used approach, known as molecular imprinting, is a technique for preparation of synthetic polymers that can recognize a target molecule [5,6,7,8]. Poma et al reported that MIPs are stable at low and high pH, pressure and temperature (<180 ^{o}C) [9]. They are also less expensive than antibodies, easier to obtain and can be synthesized for a wide range of substances. Attempts have been made to prepare BPA or BPA analogue-imprinted bulk polymers by imprinting techniques [3,10,11]. In contrast to bulky polymers, molecular imprinted polymer (MIP) nano-size particles have higher surface area-to-volume ratios; thus, imprinted pores are easier to access by the templates and the binding performance are improved [12,13].

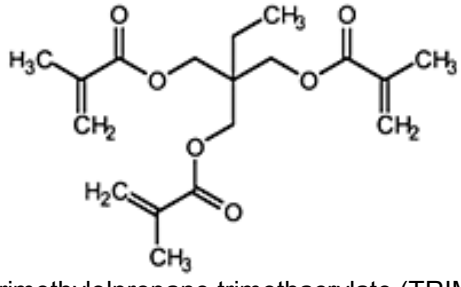
Although MIP nanoparticles have been successfully developed, they still suffer from lack of an automated standard manufacturing process. Adequate selection of factors influenced the molecular recognition properties of the tailor-made polymers, including the amount of template, the type and amount of functional and cross-linking monomers, the polymerization approach (UV/thermal), and porogen used, marked the successfulness of the imprinting process [9,14,15].

Screening and optimization of these parameters has been carried out using the traditional univariate method, which is by changing one factor at a time over the investigated range while the others are held constant at a selected point, or nowadays, with the assistance of chemometrics analysis. The complexity of the problems resulted from crucial selections of key factors makes the application of chemometric methods an ideal prospect for the design and the evaluation of the MIPs [16,17]. This methodology can open a new approach for the synthesis of tailor-made polymers, and thus later will develop a sophisticated approach to control the fabrication of MIPs.

In the present work, a novel approach to control the fabrication of BPA MIP nanoparticles through application of an experimental design and multivariate analysis methodology is described. Four recognized parameters are selected and their relations will be linked together by the chemometric approach. This cost-effective procedure can be extended for many other analytes and it will be a valuable general tool for future developments of MIP materials.



Bisphenol A dimethacrylate (BADM)



Trimethylolpropane trimethacrylate (TRIM)

Scheme 1: Chemical structure of BADM and TRIM

2. MATERIALS AND METHOD

2.1 Chemicals

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) as eluant, and acetonitrile (ACN) as polar solvent were of HPLC grade.

2.2 MIP nanoparticles preparation

In this study, covalent precipitation polymerization approach was employed, as stated to be simple and straight-forward nano-size molecular imprinting procedures [9,18]. Several sets of imprinted polymers were synthesized using thermal initiation polymerization with variation on four selected factors which are cross-linker to solvent ratio, temperature, stirring rate and initiator percentage. Other factors are set to be fixed throughout the experiment and they are functional monomer to cross-linker ratio and time. In the presence of 2,2'-azobis(isobutyronitrile) under nitrogen atmosphere, the BPA MIP was prepared with BADM and TRIM (1:10 mol ratio) according to previous report [10]. Samples were allowed to polymerize in ACN according to the experimental design condition for 12 hours. The resulting precipitate was centrifuge and the supernatant was filtered under vacuum/was left in nanocolloid condition. Resulting polymer were washed with THF then dried under vacuum. Template removal was carried out in aqueous solution containing 1.0 M sodium hydroxide (NaOH) at 50 ^{o}C for 12 h with agitation. Then, the hydrolyzed polymer was washed with excess water until neutral pH and they are set to be characterized and analyzed. Non-imprinted polymers (NIPs) were then prepared following the optimum condition obtained from chemometric analysis.

2.3 Experimental design approach

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 the 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) [19]. 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, indicating which effects are significant [20]. In the experimental design, four factors were selected as potentially affecting the rebinding efficiency. These factors can be the compositional variables, such as the amounts of porogen of polymerization, as well as the operational variables such as the initiator,

polymerization temperature and stirring 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*; ^{o}C), stirring rate (*B*; rpm), amount of porogen (*C*; %), and amount of initiator (*D*; %) on affecting the fabrication of resultant polymer. In this case particle size was chosen to represent the quality characteristic performed by the polymerization as optimal configuration of operating conditions was capable of controlling the nanoparticle fabrication. The range and levels of the variables investigated in this study are shown in Table 1. Range settings for variable factors were adjusted based on previous findings and literature.

2.4 Characterization

Transmission electron microscopy (TEM) was performed using a Hitachi H7100 transmission electron microscope operating at 80 kV. Nanoparticle (NP) suspensions were dropcast onto 300-mesh copper grids coated with Formvar and carbon and airdried before viewing.

Particle distribution was analyzed by using Mastersizer 2000 (Malvern).

3. RESULTS AND DISCUSSIONS

Study on the control of BPA MIP-NP formulation was conducted by investigating the interactions of four thermal initiation polymerization major factors which were polymerization temperature, agitation rate, solvent to cross-linker ratio and initiator percentage, as summarized in Table 1.

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

Run	Factor A: Temperature	Factor B: Agitation	Factor C: Solvent ratio	Factor 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

Results from the data collected were expressed as particle size. The ANOVA (Fisher) statistical test was employed to determine the significant and most contribute factors where they were ranked based on degree of *F*-ratio. Also, the higher *F*-value correspond with smaller "Prob>*F*" value, the more significant are the resultant model and individual coefficient [20]. Table 2 shows the reading of ANOVA analysis where F-value and P-value of the model were 4.67 and 0.0362 respectively, demonstrating that the estimated model fits the experimental data satisfactorily. And also, the R^2 value was nearly close to 1 (0.9), which means that 90% of the data variability was successfully explained by the model. It also showed that the most significant among all four factors was polymerization temperature with confidence level higher than 95% (P>0.05). This means that with slight change of temperature difference during polymerization, the polymers form will be affected thus, the size and distribution will be different. Hence, from this data generated by the model, it explained that polymerization temperature is crucial to be compared with other factors. It is also said by previous study, that when increase the polymerization temperature, it could gives negative effect on the complex formation [21].

Table 2
ANOVA for 2⁴ full factorial design: response: particle size (μm)

Source	Sum of Squares	DF	Mean squares	*F*-value	Prob>*F*
Model	157.95	10	15.79	4.67	0.0362 [a]
A	85.41	1	85.41	25.27	0.0024
B	3.71	1	3.71	1.10	0.3353
C	0.95	1	0.95	0.28	0.6155
D	5.26	1	5.26	1.56	0.2587
AB	0.12	1	0.12	0.036	0.8559
AC	2.58	1	2.58	0.76	0.4162
AD	3.6E-004	1	3.6E-004	1.078E-004	0.9921
BC	1.70	1	1.70	0.5	0.5048
BD	27.33	1	27.33	8.09	0.0294
CD	7.91	1	7.91	2.34	0.1769
Residual	20.28	6	20.28		
Lack of Fit	19.12	5	19.12	3.31	0.3937 [b]
Pure Error	1.15	1	1.15		
Cor Total	178.23	16			
Std. Dev.	1.84		R^2		0.8862
Mean	0.68		Adjusted R^2		0.6966

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

The size and structure of molecular imprinted polymer was investigated using transmission electron microscopy procedure and the particle distribution was observed using mastersizer. Figure 1 show the TEM images where 1(a) was result from Run 9 while 1(b) was result of Run 12. Images, 1(a) and (b) show particle sizes of 110 nm and 20 nm, respectively where both differ on agitation and temperature value. It can be said that agitation helps the particles disperse better and a higher temperature contribute in the formation of polymers but too much heat would disrupt the complex formed between template and monomers, thus it impacted on the polymer rigidity and structure, explaining the agglomerate structure of Run 9 [21]. From mastersizer results (data not shown), Run 1 and Run 16, resulted in 0.1 to 100 μm, showed most irregular particle distribution where both sample presented broad range of particle sizes. It is assumed that a low ratio of porogen affect the particles to distribute uniformly. Previous study also reported that the particle pores diameter and surface areas was affected by the amount and properties of the porogen, since surrounding medium contribute to swelling of polymers [22].

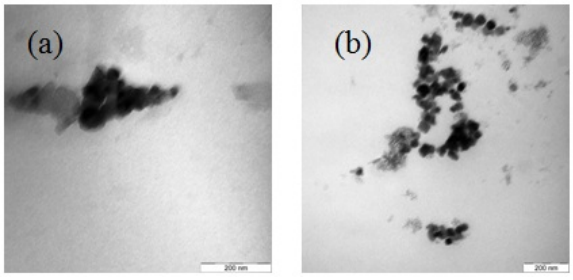


Fig. 1: TEM images of (a) Run 9, (b) Run 12

Interactions of factors were further shown in the graphically illustrated in the half normal plot (Figure 2). Results of most contributing effects likely represent the significant and influential were found consistent with the ANOVA analysis results.

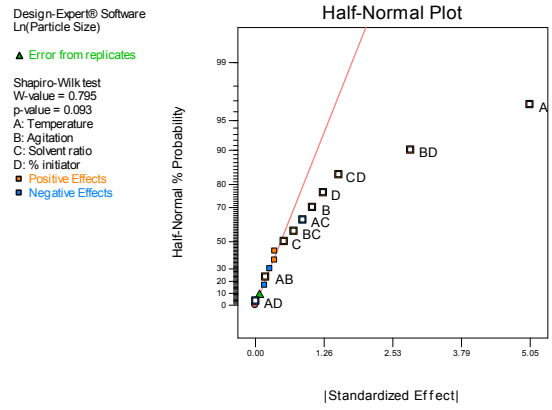
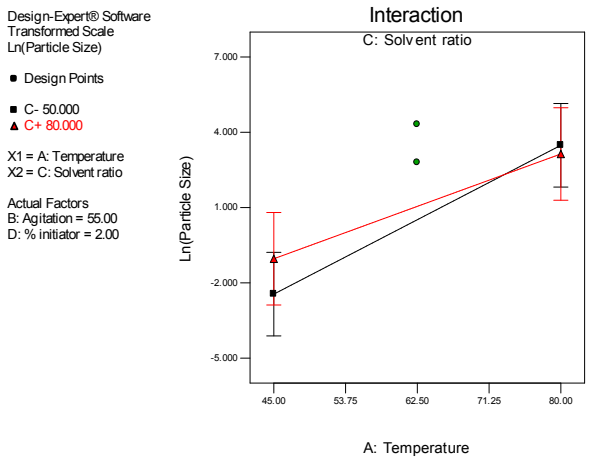


Fig. 2: The half normal plot for 2^4 full factorial designs

Interactions between factors that affecting the particle size and distribution can be better observed in Figure 3. It has been observed that the interaction between polymerization temperature and solvent ratio gives a significant change in particle size and distribution where bigger particle size will be obtained when temperature increases with increasement of solvent used until when polymerization is more heated, polymerization took place faster even at low amount of solvent thus resulted in low reproducibility of molecular imprinted polymer since to obtain good and rigid polymer bonding should consider adequate temperature and porogen. Similar trend was reported where it stated that using high temperature initiation generating fast reaction but it was hard to control at that temperature [22]. Also, a research done by Villoslada and colleagues studying the porogen effect on MIP performance reported that polymer synthesis by low temperature yield higher affinity [17]. Thus, the relatively low temperatures with a considerable amount of reaction medium are preferred in order to yield a more reproducible polymerization.

(a)



(b)

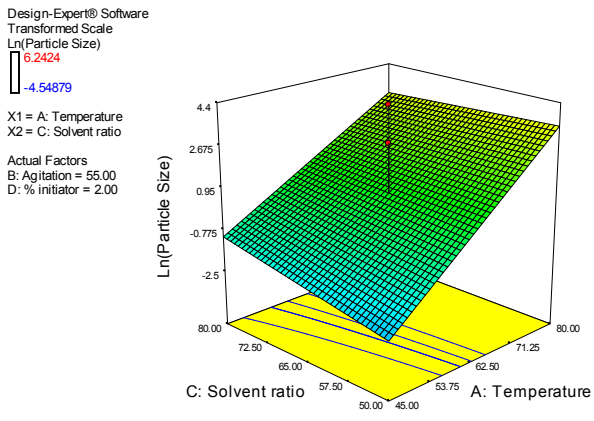


Fig. 3: Plot of interaction effect for particle size (μm): (a and b) effect between temperature and solvent ratio

4. CONCLUSION

An experimental design approach has been used to control the fabrication of molecular imprinted polymer aimed at analysis of the interaction of parameters such as polymerisation temperature, agitation, cross-linker to solvent ratio and percentage of initiator. The results showed the thermal polymerization condition to get optimum size and good distribution of BPA MIP-nanoparticle should be approximately 60^{o}C, 100rpm, 65% solvent ratio and 1% initiator, which demonstrate the importance of keeping the right balance between various parameters of polymerisation conditions. It can be said that very high temperatures during polymerization worsened the quality of the imprints formed. Effect on solvent amount, agitation and percentage of initiator also gives significant change in particle size distribution and should be used considerably adequate for producing a high yield uniform nanoparticles.

Future works will be furthering this analysis in the performance of MIP-NP and later will discuss on the correlation between particle size and binding performance. The early hypothesis should be the nanoparticles will enhance the BPA binding capacity because of its small and unique properties, as reported by previous study [13].

ACKNOWLEDGEMENT

The authors acknowledge financial supports from the Faculty of Chemical and Natural Resources Engineering of University Malaysia Pahang. The authors would like to thank everyone on the technical department and they who involved directly or indirectly throughout the research study.

REFERENCES

[1] F. Grasselli, L. Baratta, L. Baioni, S. Bussolati, R. Ramonib, S. Grolli, G. Basini, “Bisphenol A disrupts granulosa cell function,” *Domestic Animal Endocrinology*, vol. **39**, pp. 34–39, 2010.

[2] Gwynne Lyons, “Bisphenol A – A known endocrine disruptor,” *A WWF European Toxics Programme Report*, April, 2000.

[3] Jiang-hua Zhang, Ming Jiang, Lijun Zou, Dan Shi Su-rong Mei, Ye-xiang Zhu, Yun Shi, Kang Dai, Bin Lu, “Selective solid-phase extraction of bisphenol A using molecularly imprinted polymers and its application to biological and environmental samples,” *Anal Bioanal Chem,* vol. **385**, pp. 780–786, 2006.

[4] Dong Bing-zhi, Chu Hua-qiang, Wang Lin, Xia Sheng-ji, Gao Nai-yun, “The removal of bisphenol A by hollow fiber microfiltration membrane,” *Desalination*, vol. **250**, pp. 693–697, 2010.

[5] Takashi Ikegami, Takashi Mukawa, Hiroyuki Nariai, Toshifumi Takeuchi, “Bisphenol A-recognition polymers prepared by covalent molecular imprinting,” *Analytica Chimica Acta*, vol. **504**, pp. 131–135, 2004.

[6] Sergey A. Piletsky, Susan Alcock, Anthony P. F. Turner, “Molecular imprinting: at the edge of the third millennium,” *TRENDS in Biotechnology*, vol. **19**, pp. 9–12, 2001.

[7] Che Ku M. Faizal, Yasuaki Kikuchi, Takaomi Kobayashi, “Molecular imprinting targeted for α-tocopherol by calix[4]resorcarenes derivative in membrane scaffold prepared by phase inversion,” *Journal of Membrane Science*, vol. **334**, pp. 110–116, 2009.

[8] Yuqing Zhang, Ling Xiang, Yahui Zhang, Xiaoquan Gao, “Study on preparation of composite membrane with molecular recognizing property and its selective

permeance mechanism," *Separation and Purification Technology*, vol. **65**, pp. 130–136, 2009.

[9] Alessandro Poma, Anthony P.F. Turner and Sergey A. Piletsky, "Advances in the manufacture of MIP nanoparticles," *Trends in Biotechnology*, vol. **28**, no. 12, 2010.

[10] Kohei Takeda, Takaomi Kobayashi, "Bisphenol A imprinted polymer adsorbents with selective recognition and binding characteristics," *Science and Technology of Advanced Materials*, vol. **6**, pp. 165–171, 2005.

[11] Claudio Baggiani, Patrizia Baravalle, Cristina Giovannoli, Laura Anfossi, Gianfranco Giraudi, "Molecularly imprinted polymer/cryogel composites for solid-phase extraction of bisphenol A from river water and wine," *Anal Bioanal Chem*, vol. **397**, pp. 815‑822, 2010.

[12] Daming Gao, Zhongping Zhang, Minghong Wu, Chenggen Xie, Guijian Guan, Dapeng Wang, "a surface functional monomer-directing strategy for highly dense imprinting of TNT at surface of silica nanoparticles," *J. Am. Chem. Soc.*, vol. **129**, no. 25, 2007.

[13] Shiho Tokonami, Hiroshi Shiigi, Tsutomu Nagaoka, "Review: Micro- and nanosized molecularly imprinted polymers for high-throughput analytical applications," *Analytica Chimica Acta*, vol. **641**, pp. 7–13, 2009.

[14] Matthew P. Davies, Vern De Biasi, David Perrett, "Approaches to the rational design of molecularly imprinted polymers," *Analytica Chimica Acta*, vol. **504**, pp. 7–14, 2004.

[15] Kal Karim, Florent Breton, Regis Rouillon, Elena V. Piletska, Antonio Guerreiro, Iva Chianella, Sergey A. Piletsky, "How to find effective functional monomers for effective molecularly imprinted polymers?," *Advanced Drug Delivery Reviews*, vol. **57**, pp. 1795–1808, 2005.

[16] A.G. Mayes, M.J. Whitcombe, "Synthetic strategies for the generation of molecularly imprinted organic polymers," *Advanced Drug Delivery Reviews*, vol. **57**, pp. 1742–1778, 2005.

[17] F. Navarro-Villoslada, Blanca San Vicente, Maria C. Moreno-Bondi, "Application of multivariate analysis to the screening of molecularly imprinted polymers for bisphenol A," *Analytica Chimica Acta*, vol. **504**, pp. 149–162, 2004.

[18] Keiichi Yoshimatsu, Kristina Reimhult, Anatol Krozer, Klaus Mosbach, Koji Sode, Lei Ye, "Uniform molecularly imprinted microspheres and nanoparticles prepared by precipitation polymerization: The control of particle size suitable for different analytical applications," *Analytica Chimica Acta*, vol. **584**, pp. 112–121, 2007.

[19] A.R. Koohpaei, S.J. Shahtaheri, M.R. Ganjali, A. Rahimi Forushani, F. Golbabaei, "Application of multivariate analysis to the screening of molecularly imprinted polymers (MIPs) for ametryn," *Talanta*, vol. **75**, pp. 978–986, 2008.

[20] A.W. Zularisam, A.F. Ismail, M.R. Salim, Mimi Sakinah, T. Matsuura, "Application of coagulation–ultrafiltration hybrid process for drinking water treatment: Optimization of operating conditions using experimental design," *Separation and Purification Technology*, vol. **65**, pp. 193–210, 2009.

[21] Irene Mijangos, Fernando Navarro-Villoslada, Antonio Guerreiro, Elena Piletska, Iva Chianella, Kal Karim, Anthony Turner, Sergey Piletsky, "Influence of initiator and different polymerisation conditions on performance of molecularly imprinted polymers," *Biosensors and Bioelectronics,* vol. **22**, pp. 381–387, 2006.

[22] Hongyuan Yan, Kyung Ho Row, "Characteristic and Synthetic Approach of Molecularly Imprinted Polymer," *Int. J. Mol. Sci.*, vol.**7**, pp. 155-178, 2006.