

Application of response surface methodology to synthesize appropriate molecularly imprinted polymer for diazinon

Mohsen Rahiminezhad ^a, Seyed Jamaledin Shahtaheri ^{b*},
Mohammad Reza Ganjali ^c, Abbas Rahimi Rahimi Forushani ^d

^a Department of Occupational Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

^b Department of Occupational Health, School of Public Health, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran

^c Center of Excellence in Electrochemistry, Endocrine & Metabolism Research Center, University of Tehran, Tehran, Iran

^d Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

* Corresponding author: Tel.: +98 91 21779019; e-mail: shahtaheri@tums.ac.ir

Introduction

Molecular imprinting technology has become an interesting research area to the preparation of specific sorbent material for environmental and occupational sample preparation techniques (1). In the molecular imprinting technology, specific binding sites have been formed in polymeric matrix, which often have an affinity and selectivity similar to antibody-antigen systems (2). In molecular imprinted technology, functional monomers are arranged in a complementary configuration around a template molecule, then, cross-linker and solvent are also added and the mixture is treated to give a porous material containing nono-sized binding sites. After extraction of the template molecule by washing, vacant imprinted sites will be left in polymer, which are available for rebinding of the template or its structural analogue (3). The stability, convention of preparation and low cost of these materials make them particularly attractive (4). These synthetic materials have been used for capillary electrochromatography (5), chromatography columns (6), sensors (7), and catalyze system (8). Depending on the molecular imprinting approach, different experimental variables such as the type and amounts of functional monomers, porogenic solvent, initiator, monomer to cross-linker ratio, temperature, and etc may alter the properties of the final polymeric materials. In this work, chemometric approach based on Central Composite Design (CCD) was used to design the experiments as well as to find the optimum conditions for preparing appropriate diazinon molecularly imprinted polymer.

EXPERIMENTAL

JASCO LC- 2000 series high performance liquid chromatography (HPLC) was used to analyze diazinon.

The instrument was equipped with PU-2080 pump, CO-2060 column oven, and UV/VIS 2075 detector set at 245 nm. The column was reversed phase-C₁₈ (250×4.6 mm i.d; Supelco, USA). The mobile phase was acetonitrile/methanol/water=60:20:20 (V/V/V) containing 5 µL H₃PO₄. The flow rate was set at 1.2 ml/min. The column temperature was fixed at 40 °C. The injection volume was 10 µL. In this study, CCD, as an approach in RSM, was used to design and analyze of experiments. This design composes of 2^K factorial points augmented by center points and 2K star or axial points. K represents the number of factors or variables being examined in experiment. CCD requires five levels for each experimental variable. CCD is made rotatable by the choice of axial pints ($\pm\alpha$). The value of α depends on the factorial points of the design ($\alpha=(2^k)^{1/4}$). CCD also must include center points to provide reasonably stable variance of predicted response. Five important variables as essential factors that extensively affect polymer properties were selected. The selected factors were the amounts of template, porogenic solvent, initiator, functional monomer/cross-linker ratio, and polymerization temperature. Thus, totally 52 MIPs (2⁵ factorial points plus 10 axial points plus 10 center points) were synthesized with different formulation. Diazinon bound per grams of MIPs was calculated as chemometric response. For each run, MIP was prepared via non-covalent imprinting method (Table 2). In run number 1, for example, diazinon

(1 mmol) and MAA (3 mmol) were mixed in a polymerization reactor. The mixture was shaken in an orbital shaker (100 rpm) at room temperature for 2 h. Then, EDMA (15 mmol), AIBN (30 mg), and porogenic solvent (chloroform: 4 ml) were added to the polymerization reactor. After 5 min ultrasonication, the mixture was bubbled with nitrogen for 5 min and sealed under the N₂ atmosphere. The polymerization process was carried out at 55 °C for 24 h. The bulk polymers were granulized, sizing from 53-106 µm, then diazinon was extracted with methanol, using Soxhlet extractor until no diazinon was detected in the effluent. Following the extraction procedure, particles were dried at 50°C over night. After washing with 10 ml methanol and 10 ml acetonitrile, particles were dried at 70°C. As mentioned in previous section, NIP for each experimental run was also synthesized using the same procedure without the addition of diazinon. Binding properties of the polymers were evaluated by equilibrium binding experiments. 500 mg of both MIP and NIP polymer particles was placed in 18 ml vials and mixed with 3 ml of diazinon in acetonitrile (10 µg/ml) at 27°C in a shaking water bath (100 rpm) for 5 h. Then, polymer particles were filtered using syringe driven filter unit (0.45 µm). After the filtration, the supernatants were analyzed by the HPLC system using the method described above. The amount of diazinon bound to the polymers was calculated by subtracting the final diazinon concentration from the initial diazinon concentration added to the polymer.

RESULTS AND DISCUSSION

Key important experimental variables in MIP preparation were selected for optimization via chemometric approach based on CCD. Following the polymer preparation, equilibrium binding experiments and response surface methodology were carried out to find appropriate diazinon-imprinted polymer. Table 1 demonstrates the obtained results from the equilibrium binding experiments. The results show different formulation of MIP pre-polymerization could extensively change MIP properties. Also, higher amount of diazinon bound per grams of MIPs versus NIPs reveals effectively imprinting template shape in MIP matrix.

Table 1. Diazinon bound per grams of MIPs and NIPs (ng/g)^a

Run	Diazinon bound by MIP	Diazinon bound by NIP	Run	Diazinon bound by MIP	Diazinon bound by NIP	Run	Diazinon bound by MIP	Diazinon bound by NIP	Run	Diazinon bound by MIP	Diazinon bound by NIP
1	4609	774	14	4572	921	27	2845	N.A.	40	5250	854
2	4338	754	15	3568	N.A.	28	3085	521	41	4697	1909
3	3558	485	16	2854	638	29	3854	N.A.	42	3278	717
4	2562	1352	17	2482	763	30	5524	N.A.	43	4017	982
5	2959	N.A.	18	1544	N.A.	31	3902	425	44	2641	1068
6	2101	552	19	2577	N.A.	32	2149	478	45	4457	704
7	2569	621	20	1254	532	33	3251	N.A.	46	2595	721
8	2384	391	21	531	N.A.	34	3254	661	47	1482	1021
9	3253	N.A.	22	2981	N.A.	35	2887	322	48	3325	452
10	3509	N.A.	23	5211	809	36	3095	881	49	3778	486
11	1982	N.A.	24	1159	755	37	5241	654	50	4221	754
12	3951	680	25	3541	833	38	4307	N.A.	51	2560	N.A.
13	4215	862	26	3216	N.A.	39	4397	624	52	4120	569

^a All experiments were carried out in triplicate and the results are expressed as mean. N.A.: no activity was observed.

The regression analysis was used to evaluate the individual and interaction effects of experimental variables on response. The estimated regression coefficients for the response are shown in Table 2. From the results, the effects of the amount of template, functional monomer/cross-linker ratio, the amount of initiator, and polymerization temperature on the response were statistically significant. Furthermore, the results showed that, in most cases, interactions exist between key important factors. The R² value for the results analysis was 87%. It means that, 87% of the variation in response is explained by the model. The results for the response were also analyzed with ANOVA. For this model, the R² was 96%, indicating that predictions would be reasonably accurate. It should be noticed that, R² value for a valid model is 60% or greater.

Table 2. Estimated regression coefficients for the response ^a

Term	Coefficient	SD Coefficient	T	P-value	Term	Coefficient	SD Coefficient	T	P-value
Constant	3704.17	161.99	22.867	0.000	A×B	-104.66	91.11	-1.149	0.259
A	-259.54	78.31	-3.314	0.002	A×C	-416.47	91.11	-4.571	0.000
B	-162.52	78.31	-2.075	0.046	A×D	-138.66	91.11	-1.522	0.138
C	-163.83	78.31	-2.092	0.045	A×E	472.03	91.11	5.181	0.000
D	-83.33	78.31	-1.064	0.296	B×C	454.78	91.11	4.991	0.000
E	-219.96	78.31	-2.809	0.009	B×D	-256.66	91.11	-2.817	0.008
A×A	254.95	67.37	3.784	0.001	B×E	106.16	91.11	1.165	0.253
B×B	-51.40	67.37	-0.763	0.451	C×D	210.03	91.11	2.305	0.028
C×C	-207.67	67.37	-3.083	0.004	C×E	-358.91	91.11	-3.939	0.000
D×D	-34.52	67.37	-0.512	0.612	D×E	104.78	91.11	1.150	0.259
E×E	-444.46	67.37	-6.597	0.000					

^a A: the amount of template; B: functional monomer/cross-linker ratio; C: the amount of initiator; D: the volume of porogenic solvent; E: polymerization temperature. The analysis was done using coded units. S=515.4, R²= 87%.

The result of the effect of the template and functional monomer/cross-linker ratio variables on response have been shown on three-dimensional response surface plot (Fig. 1). Also, the effect of the initiator and temperature variables on response can be seen in the Fig 1. For low amount of diazinon, the response increases with the functional monomer/cross-linker ratio.

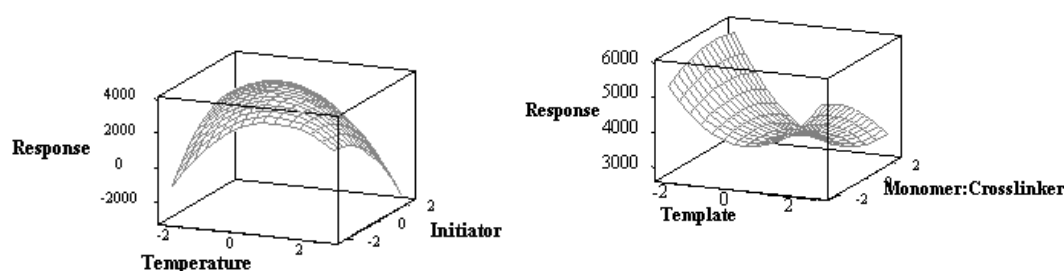


Fig.1. Three dimensional response surface graphs obtained by plotting the amount of functional monomer/cross-linker ratio versus template and amount of initiator versus temperature

The mean amount of diazinon bound by MIPs at the low level setting of the experimental variable is more than what is at their high levels. In the case of non-covalent imprinting, the amounts of template, functional monomer, and cross-linker must be determined empirically. If template-functional monomer complex formation as a reversible reaction is considered, fewer functional monomers, used in pre-polymerization mixture, can reduce complex formation between functional monomer and template. Thus, the number of binding sites will be limited in the final MIPs. On the contrary, the more the amount of functional monomer, the more the unspecific binding sites in polymer matrix. The amount of cross-linker is also important to prepare nono-sized cavities with proper mechanical stability in the polymer and sufficient flexibility as well. As mentioned earlier (9), increased temperature has a negative effect on the complex formation between template and functional monomer in pre-polymerization mixture. Also, higher amount of initiator decreases diazinon bound by the synthesized polymers. This may be attributed to the higher amount of initiator added to the pre-polymerization mixture could increase the polymerization rate and thus, increases the heat of reaction (10). Generally, in order to enhance the capacity of the MIPs, the materials should be prepared using low amount of initiator and low temperatures. The optimum values were calculated for each experimental variable by using Minitab response optimizer menu. For synthesizing appropriate diazinon MIP, the following optimum values have been obtained. The amount of template: 0.65 mmol; functional monomer/cross-linker ratio: 2:11.6; porogen volume: 7.38 ml; the amount of initiator: 16.21 mg; theoretical predicted temperature: 48.10 °C. The predicted optimum molar ratio among the template, functional monomer and cross-linker for diazinon was 1:3.07:17.85. It should be mentioned that, based on the model, the optimum predicted temperature was 48.10°C, however, the response for run 21 (polymerization temperature: 48.10) was

the lowest response among all runs. Beside of this, the literature value for proper AIBN thermal decomposition is around 60 °C. Therefore, it is concluded that, the model recommended using the lowest level value for temperature. It means that, the lowest possible level temperature must be used for polymerization. For this reason, the temperature was set at the lowest possible level of 55 °C.

CONCLUSION

Chemometric approach based on CCD was applied to design various MIPs with different formulation as well as to find the optimum conditions for preparing appropriate diazinon molecularly imprinted polymer. The obtained results showed the utility of the chemometric approach to determine factors with significant effect on response as well as interactions between the key important factors. Moreover, the approach has beneficial properties for MIP development via the prediction of an optimum amount of template, functional monomer/cross-linker ratio, porogenic solvent volume, and temperature. The approach will allow relative simple and fast opportunity to develop more MIPs for the other templates of interest.

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