Full Length Research Paper

Optimization of the process parameters of synthesis of oligomeric procyanidins imprinted polymer

Yuqing Duan¹, Yu Qin¹, Feifei Xu¹, Haihui Zhang¹*, Yongsheng Yan², Can Zhang¹ and Haile Ma¹

¹School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China.
²School of Chemistry and Chemical Engineering, Jiangsu University, Zhenjiang 212013, P.R. China.

Accepted 30 September, 2010

Molecularly imprinted polymers (MIP) are tailor-made polymers with high selectivity for the template molecule. This selectivity arises from the synthetic procedure followed to prepare the MIP. In this work, the influence of process parameters on the preparation of oligomeric procyanidins (OPC) imprinted polymers was presented. In the procedure of polymerization, OPC (90 mg) was used as the template molecule, azobisisobutyronitrile (AIBN), was employed to initiate the reaction with 360 nm UV light at 4°C. The influence of the following parameters was investigated: the moles of functional monomer (MAA, 0.2 – 0.8 mmol), the moles of cross-linker (EDMA, 2.0 – 4.0 mmol) and the porogenic solvent (acetonitrile, acetone or ethyl acetate). A mathematical method of response surface methodology (RSM) was applied to optimize these selected parameters in order to increase the selectivity of MIP for template molecule. The MIP was synthesized under the optimal conditions that 0.68 mmol of MAA and 3.3 mmol of EDMA copolymerized in ethyl acetate by regression analysis in the presence of 90 mg of OPC. After removal of the template molecule, the obtained MIP was then employed as the sorbents of solid-phase extraction (SPE) to separate OPC from lotus seedpod extracts. The results showed that the polymer exhibited high affinity to the template molecule and could separate and enrich OPC from lotus seedpod extracts effectively. A selective recovery of OPC on the optimal MIP was obtained 85.58%. Therefore, the nature of the polymer can be improved by optimizing the polymerization parameters with the method of RSM.

Key words: Molecularly imprinted polymer, polymerization, response surface methodology, parameter optimization, oligomeric procyanidins.

INTRODUCTION

Molecularly imprinted polymer (MIP) was synthetic materials with artificially generated recognition sites able to specifically rebind a target molecule (template molecule) in preference to other closely related compounds. The advantages that MIP hold over natural antibodies, including stability, ease of preparation, low cost and reusability have led to their wide application in chromatography, catalysis, chemical sensors and solid-phase extraction (SPE) (Jiang et al., 2009; Caro et al., 2005; Xu et al., 2010).

The selectivity of MIP arises from the synthetic procedure. First the functional monomer and template were to form the template-functional monomer complex by covalent or non-covalent bonds. Then the obtained complex is copolymerized with an excess of cross-linker in the presence of an initiator under thermal or photochemical conditions. Last the template molecule on the polymers is removed and then the vacant imprinted sites of a complementary shape and functionality to the template molecule were stayed on the polymers. Thus MIP show high affinity for the template and will rebind to the template specifically. It is known that several factors are concerned with the polymerization process, such as the kind and amount of functional monomers, MAA, Methacrylic acid.
cross-linkers, porogenic solvents and presence of components causing linking (Karim et al., 2005).

When many factors and interactions affect desired response, response surface methodology (RSM) is an effective tool for optimizing the process (Triveni et al., 2001). As the needed information about the shape of the response surface is applied, RSM is an effective statistical method that uses a minimum of resources and quantitative data from an appropriate experimental design to determine and simultaneously solve a multivariate equation (Kalaimahan and Tapobrata, 1995). Response surface experiments attempt to identify the response that can be thought of as a surface over the explanatory variables experimental space. It usually uses an experimental design such as central-composite experimental design (CCED) to fit an empirical, full second-order polynomial model. A central-composite experimental design, coupled with a full second-order polynomial model, is a very powerful combination that usually provides an adequate representation of most continuous response surfaces over a relatively broad factor domain. Therefore, it should be especially suitable for the present experiment investigation of polymerization process involving several parameters.

Procyanidins are a class of polyphenols widely present in the plant kingdom and our daily diet, are composed of chains of flavan-3-ol units, that is, (+)-catechin and (-)-epicatechin monomer, linked mainly through C4 - C8 or C4 - C6 bonds (Figure 1). The oligomeric procyanidins (OPC) was consisting of 2 – 4 monomer linked (Svedstrom et al., 2002). Recent studies have shown a number of promising pharmacological effects exhibited by OPC (Pinent et al., 2006), including antioxidative (Liu et al., 2010), antitumor (Duan et al., 2010; Gosse et al., 2005; Ito et al., 2000; Neuwirt et al., 2008), immunomodulatory (Kenny et al., 2007; Shibata et al., 2009), anti-inflammatory effects (Yoshioka et al., 2008; Zhang et al., 2005). But the content of OPC in the natural plants is lower than 0.1% and it is quite difficult to isolate OPC from natural plants by the conventional separation materials (Sun et al., 1999; João et al., 1999; Xiao et al., 2008; Monagas et al., 2010).

In this paper, the OPC from grape seeds was used as the template and OPC imprinted polymer was prepared by non-covalent imprinting method. The process parameters influencing the polymerization, the moles of functional monomer and cross-linker, the selection of solvent, were optimized by central-composite experimental design (CCED) of experiments in order to obtain OPC imprinted polymer with high separation capability to the template.

MATERIALS AND METHODS

Materials

The lotus (Nelumbo nucifera Gaertn.) seedpod sample obtained from a Zhenjiang (Jiangsu, China) was used in this study. The sample was milled and through 20-mesh screen, freeze-dried. All samples were stored under vacuum in the dark at a temperature of −20°C. (+)-Catechin and (-)-epicatechin were from Sigma (St. Louis, MO). Procyanidin B2 standard (97%) was from Nakahara Science Co., Ltd. (Japan). Procyanidins B1 and C1 prepared from grape seeds were donated by Professor Victor A. P. Freitas (Porto University, Portugal). Ethylene glycol dimethacrylate (EDMA) was purchased from Guangzhou Shuanjian Trading Co. Ltd. (Guangzhou, China). Methacrylic acid (MAA) was purchased from Tianjin Yongda Reagent Development Center (Tianjin, China). EDMA and MAA were purified by general distillation in a vacuum to remove the polymerization inhibitor. Azobisisobutyronitrile (AIBN) was purchased from Shanghai No. 4 Reagent and H.V. Chemical Co., Ltd. (Shanghai, China) and purified by recrystallization prior to use. Chromatography grade solvents (acetonitrile, methanol) were from Merck (Darmstadt, Germany). Water was double deionized and filtered through a 0.45 µm filter membrane.

All chromatographic evaluations were performed by reversed-phase high pressure liquid chromatography (RP-HPLC) on an Agilent 1100 HPLC system. The system consisted of a quaternary pump, a variable-wavelength detector, an on-line vacuum degasser and an automatic sample injector.

Reversed-phase HPLC analysis conditions

The analysis of procyanidins by RP-HPLC (Agilent 1100 HPLC system) was performed on a varian liquid chromatograph and detection was carried out using a photodiode array detector. The column used was a Zorbax SB-C18 column (5 µm particle size, 250 x 4.6 mm, ID). The method utilized a binary gradient with mobile phase, that is, 2% v/v aqueous acetic acid (mobile phase A) and acetonitrile (mobile phase B). A 5 µL sample solution was injected and the elution conditions were as follows: 5 to 15% B for 10 min, 15 to 20% B for 5 min, 20 to 40% B for 20 min, 40 to 50% B for 10 min, 50 to 5% B for 5 min, re-equilibrated with 5% B for 10 min before the next injection. The flow rate was 0.8 mL/min, and the UV-absorbance of procyanidins was monitored with a diode array detector at 280 nm.

Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) analysis

HPLC/MS analyses of polyphenolic extracts were performed using an Agilent (Agilent Technologies, Palo Alto, CA, USA) 1100 series LC/MSD trap equipped with an auto-injector, binary HPLC pump, column heater and diode array detectors. The sample was loaded on a Zorbax (Agilent Technologies) SB-C18 column (250 x 4.6 mm ID, 5 µm particle size). The column was equilibrated in solvent A (0.2% v/v aqueous acetic acid) and procyanidins (5 µL injected) were eluted with a gradient of solvent B (5% v/v aqueous acetic acid) from 0 to 5%B between 0 and 10 min, from 5 to 20%B between 10 and 20 min, from 20 to 40%B between 20 and 40 min, from 40 to 50%B between 40 and 45 min, from 50 to 5%B between 45 and 50 min and held isocratic at 5%B between 50 and 60 min. The mass spectrometer was operated in negative mode for LC/ESI-MS and scanned from m/z 100 to 1500. A nebulising pressure of 20 psig and a gas drying temperature of 325°C were used. Data were collected on an HP Chemstation (Agilent Technologies). Peaks were detected at 280 nm and identified by comparison with retention times of standards.

Extraction of oligomeric procyanidin

Approximately 100 g of grape seeds were manually separated from the berries, ground and homogenised in 70% (v/v) aqueous ethanol.
three times (3 × 150 mL). The slurry was centrifuged (6000 × g, 5°C, 40 min) and the precipitate was re-extracted twice with the same solvent (30 min, 2 × 50 mL). The supernatants were pooled and the mixture was concentrated under vacuum until all the ethanol had been removed. The extract obtained was washed three times with petroleum ether to eliminate liposoluble substances. The residual sample was lyophilised to get crude procyanidins. The ethanol-extracted crude procyanidin powder was dissolved in a minimum amount of the 60% (v/v) methanol water solution and then applied to the pre-equilibrated Sephadex LH-20 gel column (50 × 2.6 cm). The column was washed sequentially with 2.5 L of deionized water, 2.5 L of 60% (v/v) methanol plus 0.2% (v/v) formic acid and 2.5 L of 60% (v/v) acetone plus 0.2% (v/v) formic acid at a flow rate of 0.4 – 0.5 mL/min. Polymeric procyanidins were eluted with 2.5 L of 60% (v/v) acetone plus 0.2% (v/v) formic acid. The acetone was subsequently evaporated off in a rotary vacuum evaporator at 42°C. The residual sample was lyophilised and stored in a desiccator at room temperature. A 100 mg quantity of purified grape seeds procyanidins eluting with 60% (v/v) methanol was equivalent to 98 ± 3 mg of gallic acid when assessed by the Folin–Ciocalteu method. The 60% (v/v) methanol fraction was used as oligomeric procyanidin standard for template molecule (Porter et al., 1986). Compositions of OPC by reverse phase HPLC/ESI-MS and compare with each procyanidins standard.

Determination of adsorption capacity

The 20 mg of dryed MIP or NIP particles placed in a 10 mL conical flask and mixed with 5 mL of a known concentration of OPC solution. The mixtures were mechanically shaken (200 times/min) for 12 h at 30°C constant temperature bath and then separated centrifugally (5000 rpm) for 15 min. The concentration of unextracted OPC in the supernatant was measured by spectrophotometer at 280 nm. The adsorption capacity $Q$ was calculated based on the difference of OPC concentration before and after adsorption, the volume of OPC solution and the weight of the beads according to:

$$Q = \frac{(C_0 - C_t) V}{W}$$

Where $C_0$ is the initial OPC concentration (µg/mL), $C_t$ is the rhein concentration (µg/mL) of different time, $V$ is the volume of OPC solution (mL), and $W$ is the weight of the MIP or NIP beads (g).

Preparation of MISPE columns

The 500 mg amount of dry particles of polymer was packed into a 6.0 mL polypropylene SPE column. The column was attached with a stop cock and a reservoir at the bottom end and the top end, respectively. The polymer was rinsed with methanol and then with ethyl acetate.
Molecularly imprinted solid phase extraction of lotus seedpod oligomeric procyanidins (LSOPC)

Frozen lotus seedpod (10 g) was extracted using ethanol concentration 60% (50 mL) at room temperature for 4 h. The extract was filtered through a membrane (0.45 μm) and the filtrates were concentrated under vacuum until all the ethanol had been removed by a rotary evaporator at 40°C. The extract obtained was washed three times with petroleum ether to eliminate liposoluble substances. Then the extract was extracted directly by ethyl acetate and it was diluted to 0.1 mg/mL with EtOAc before loading onto the MISPE column. The procyanidins consent of extracts were estimated by colorimetric assay, after acid catalysis in n-butanol solvent serves to bring all the components in the complex and thus the imprinting effect (O'Mahony et al., 2004). In an imprinted polymer the cross-linker should not generate materials with adequate mechanical stability.因此，在这个实验中，高柔性的单体可以允许无限的构象可能性和一定程度的非共价相互作用。

RESULTS AND DISCUSSION

Identification of procyanidins derived from grape seeds extract by reverse phase HPLC/MS

LC-ESI-MS analysis showed that m/z ratios of the peaks at 280 nm were 1 (577.2), 2 (289.2), 3 (577.2), 4 (289.4) and 5 (865.2) (Figure 2) and the peaks were identified as dimmer B1, (+)-catechin, dimer B2, (-)-epicatechin and trimer C1, respectively, by coelution with standards on RP-HPLC. This suggested that procyanidins be a good source for OPC. Therefore, this OPC used for the preparation of molecular imprint polymers as a template molecular.

Selection of parameters of molecular imprinted polymers

It is known that the formation of template functional monomer complex is a key step in the synthesis of MIP. The chosen functional monomer should match the functionality of the template and is normally used in excess relative to the number of moles of template to favor the formation of template-functional monomer complex and thus the imprinting effect (O'Mahony et al., 2005). In an imprinted polymer the cross-linker should not overlook since a high degree of cross-linking (70 - 98%) is necessary for achieving specificity. The functions of the cross-linker are to stabilize the imprinted binding site, control the morphology and influence the mechanical stability of the polymer matrix (Lehmann et al., 2004). The solvent serves to bring all the components in the polymerization into one phase and is also responsible for creating the pores in the polymers. In a non-covalent imprinting polymerization, the solvent governs the strength of non-covalent interactions in addition to its influence on the polymer morphology. The best imprinting porogens, for accentuating the binding strengths, are solvents of very low-dielectric constant such as toluene.

The use of more polar solvents will weaken the interaction forces resulting in poorer recognition. Thus the solvent must be chosen carefully and maximizes the potentiality of the formation of template-functional monomer complex (Sellergren and Shea, 1993). MAA has been by far the most widely and successfully used functional monomer because it has carboxylic acid and can interact with template in various ways, as H-bond donors, H-bond acceptors and through ion-pair formation, etc. In the OPC imprinting processes the carboxyl of MAA may form the non-covalent interactions with hydroxyl groups of OPC through hydrogen bonds and thus a stable complex between template and functional monomer was formed in the imprinting process. The existence of the complex will produce the specific binding sites in OPC-MIP. The functional monomers are normally used in excess relative to the number of moles of template to maximize complex formation and thus the imprinting effect. It was not remarkable that the ratio of OPC to MAA was 1:10 to favor the formation of the complex and produce a sufficiently selective polymer. EDMA is by far the most commonly used. This probably stems from the fact that it has a reactive methacrylate ester with a short spacer and can allow infinite conformation possibility and a degree of rigidity in the resultant polymer. In this experiment, high cross-linker ratio could not only decrease the swelling and increase the selectivity of the polymer but also generate materials with adequate mechanical stability. Apolar and non-protic solvents are preferred in the non-covalent imprinting process because they cannot compete with the binding sites between the template and the functional monomer and thus in favor of the formation of the template-functional monomer complex. It was demonstrated that the selectivity increased as the porogen decreased in hydrogen bond capacity (Lu et al., 2004). Therefore, in this work OPC was used as the template, MAA as the functional monomer, EGDMA as the cross-linker and a commonly used initiator, azobisisobutyronitrile (AIBN), was employed to initiate the reaction. Since OPC The polymerized for 24 h with 360 nm UV light at 4°C.

Response surface methodology (RSM) design

In experiments design method the trial numbers were usually suggested to be about three times over the numbers of selected factors in order to facilitate the regression analysis. According to the synthesis principle of the polymers and the results of the preliminary experiments, a central-composite experimental design,
with three main factors, was used to study the response pattern and to determine the optimum combination of variables. The three main factors $X_1$ (MAA, 0.2 – 0.8 mmol), $X_2$ (EGDMA, 2.0 – 4.0 mmol), and $X_3$ (porogenic solvent, 1 acetonitrile, 2 acetone, and 3 ethyl acetate) and an $L_3^3$ design (Table 1) were used in the RSM analysis, the polymerization process is shown in Table 2. By taking the OPC adsorption capacity as the response value ($Y$), experiments were designed, all extractions were performed for the same condition ($360$ nm UV light for 24 h at $4^\circ$C), in which 1 – 12 were factorial experiments, three replicates (treatments 13 – 15) at the centre of the design were used to allow for estimation of a pure error sum of squares and incorporated into fifteen
polymerization trials. Each experiment was performed in triplicate. Experiments were randomized in order to maximize the effects of unexplained variability in the observed responses due to extraneous factors.

**Fitting the models**

The application of RSM yields the following regression equation, which is an empirical relationship between protein yield and the test variable in coded units, as given in the following equation:

\[
Y = 7.12 + 1.74X_1 + 0.39X_2 + 0.31X_3 - 0.64 X_1 X_2 - 0.16X_1 X_3 + 0.10 X_2 X_3 - 1.26 X_1^2 - 0.18X_2^2 - 0.14X_3^2
\]  

(1)

Each of the experimental values is compared with the predicted value, predicted value calculated from the model, as depicted in Figure 3 we can see that experimental accords with predicted. The multiple coefficients of correlation \( R = 0.9985 \) indicated a close agreement between experimental and predicted values of the recovery of OPC. The coefficient of determination \( R^2 \) of the predicted model was 0.9958, suggesting a good fit and the predicted model seemed to reasonably represent the observed values, which meant that the model explained 99.58% of the variability in the data, namely MAA and EDMA concentration and porogenic solvent. This ensured a satisfactory adjustment of the model to the experimental data. The analysis of the variance (ANOVA) for the regression model is given in Table 3.

The statistical significance of the model equation was also confirmed by an F-test. The prediction precision of the regressed equation provided by the residual mean square reached an acceptable level and the calculated
values agreed quite well with the experimental results. The significance of each coefficient was determined by a F-test in Table 4.

**Optimization of the process**

The 3D surface plots were drawn to illustrate the main and interactive effects of the independent variables on the dependent one. These graphs were drawn by imposing a constant value (i.e., the central points of the interval taken into consideration to one independent variable). The effects of MAA concentration, EDMA concentration and porogenic solvent on response, that is, adsorption capacity are shown (Table 4) by the coefficients of second order polynomials. The response surfaces based on these coefficients are shown in Figures 4a - c, with one variable kept at the optimum level and the other two within the experimental range. In general, exploration of the response surfaces indicated a complex interaction between the variables.

Figure 4a shows the effect of MAA concentration and the EDMA concentration on OPC adsorption capacity. A quadratic effect of MAA concentration and a linear effect of EDMA concentration on the response were observed. Figure 4b shows the effect of MAA concentration and porogenic solvent; MAA concentration exerted a quadratic effect on OPC adsorption capacity, whereas porogenic solvent had a linear effect. Figure 4c depicts the influence of EDMA concentration and porogenic solvent; it can be seen as a linear effect for both EDMA concentration and porogenic solvent. Therefore, increases of EDMA concentration and porogenic solvent both resulted in a higher OPC adsorption capacity.

The mathematical optimization of the obtained regression equation (Equation 1) was performed with the application of uniform design version 2.2. The optimal results of process parameters were as follows. The moles of MAA and EDMA were 0.68 and 3.3 mmol, respectively, and the solvent was ethyl acetate. The model
predicted that the maximum response for the OPC adsorption capacity was 7.87 mg/g. To test the accuracy of the regressive model, experiments under the optimum conditions were repeated, the OPC adsorption capacity was 7.79 ± 0.23 mg/g. Based on one-way ANOVA analysis, no significant difference was observed between the RSM model and the verification model (p > 0.05), which meant that the conditions optimized by RSM could be applied to the synthetic OPC imprinted polymer.

### Table 4.
Significance of regression coefficient for OPC adsorption capacity.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Regression coefficient</th>
<th>Mean square</th>
<th>F overall</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>7.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$X_1$</td>
<td>1.84</td>
<td>27.05</td>
<td>2586.26</td>
<td>**</td>
</tr>
<tr>
<td>$X_2$</td>
<td>0.39</td>
<td>1.23</td>
<td>117.84</td>
<td>**</td>
</tr>
<tr>
<td>$X_3$</td>
<td>0.31</td>
<td>0.79</td>
<td>75.30</td>
<td>**</td>
</tr>
<tr>
<td>$X_1 X_1$</td>
<td>-1.26</td>
<td>5.90</td>
<td>564.21</td>
<td>**</td>
</tr>
<tr>
<td>$X_2 X_2$</td>
<td>-0.18</td>
<td>0.12</td>
<td>11.65</td>
<td>*</td>
</tr>
<tr>
<td>$X_3 X_3$</td>
<td>-0.14</td>
<td>0.072</td>
<td>8.84</td>
<td>*</td>
</tr>
<tr>
<td>$X_1 X_2$</td>
<td>-0.63</td>
<td>1.58</td>
<td>150.60</td>
<td>**</td>
</tr>
<tr>
<td>$X_1 X_3$</td>
<td>-0.16</td>
<td>0.096</td>
<td>9.19</td>
<td>*</td>
</tr>
<tr>
<td>$X_2 X_3$</td>
<td>0.10</td>
<td>0.042</td>
<td>4.02</td>
<td></td>
</tr>
</tbody>
</table>

** $f_{0.01} (1, 5) = 16.3$; * $f_{0.05} (1, 5) = 6.6$.

### Table 5.
Evaluation of the MISPE procedure for extraction of OPC from lotus seedpod compared to the NIPSE column.

<table>
<thead>
<tr>
<th>SPE step</th>
<th>Amount of the OPC</th>
<th>MISPE peak $^a$ (µg)</th>
<th>NISPE peak $^a$ (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Loading</td>
<td>300 µg lotus seedpod crude extract (OPC was 38.48 µg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Washing</td>
<td></td>
<td>1.91 2.29 0.00 0.35 0.00 5.82 6.11 14.61 1.04 3.28</td>
<td></td>
</tr>
<tr>
<td>Eluents</td>
<td></td>
<td>4.12 4.95 18.27 1.83 3.76 0.29 0.92 3.55 1.23 0.41</td>
<td></td>
</tr>
<tr>
<td>Total peak</td>
<td></td>
<td>6.03 7.24 18.27 2.18 3.76 6.11 7.03 18.16 2.27 3.69</td>
<td></td>
</tr>
<tr>
<td>Total OPC</td>
<td></td>
<td>37.48</td>
<td>37.26</td>
</tr>
</tbody>
</table>

$^a$The chromatograms peak 1, dimers B1; 2, (+)-catechin; 3, dimers B2; 4, (−)-epicatechin; 5, Trimmers C1.

**Molecularly imprinted solid-phase extraction of lotus seedpod extracts**

Real samples are complex chemical matrices and in the chemical identification of their components, an intensive pre-treatment of the samples is always required. The use of selective sorbents such as MIP can be very useful to obtain cleaner HPLC chromatograms (Caro et al., 2005).

A typical SPE procedure consists of four steps: conditioning of the column material, loading of the sample, washing to remove non-specifically bound molecules and finally elution of the analytes. In order to select the loading, washing and elution conditions, a large variety of solvents including EtOAc, acetonitrile, methanol, ethanol and water were compared. Suitable solvents were found for loading (EtOAc), washing (methanol) and elution (methanol- acetic acid, 8:2, v/v). In our experiments, 60% ethanol water extract of lotus seedpod was extracted directly by EtOAc to obtain crude procyanidins EtOAc extract and the content of OPC in the extract was calculated with OPC as an external standard and it was 15.6%. The crude procyanidins extract re-dissolved in EtOAc, after filtration, to obtain the loading solution and it was analyzed by HPLC (Figure 5a). From Figure 4a it could be found that there were plenty of components in the EtOAc extract. Minor peaks of isorhamnetin and other polyphenols in the EtOAc extract were not discussed in this paper.

Lotus seedpod EtOAc extract was loaded to the polymeric cartridges and the almost complete retention of OPC by the MISPE and NISPE column was raised. The effluent, the washing solution and the final eluent from either the MIP or the NIP cartridge at every applying, washing and eluting step were all analyzed by HPLC and the major analysis results at this extraction test are shown in the chromatograms (Figures 5b - e and Table 5). In these chromatograms, peak 1 - 5 was identified as dimers B1, (+)-catechin, dimers B2, (−)-epicatechin, Trimmers C1, respectively. Two column washing steps
indicated nonspecific binding took some weight in compound adsorption on the polymers. However, when the cartridges were washed for the 10 mL, an evident difference was observed. OPC (80.19%) was washed out from the NIP while it was little (11.82%) from the MIP because of the MIP's stronger affinity to OPC compared with the NIP (Figure 5b, c and Table 1). Finally, OPC was selectively eluted with methanol/acetic acid mixture (8:2 v/v). A selective recovery of OPC was obtained 85.58% of the loaded OPC, the content of peak 3 (dimers B2) was highest (18.27 μg) and it was 47.48% on MIP column (Figure 5d and Table 1). In contrast, NIP column was only 16.63% of the loaded OPC recovery (Figure 5e and Table 1). Therefore it could be concluded that a higher selectivity could be achieved by imprinting method. The selectivity of the prepared MIP was acceptable for trapping certain trace amount active compounds from herb or natural plant.

**Figure 4.** 3D graphic surface optimization of OPC adsorption capacity versus Interaction between the two factors. (A) MAA and EGDMA (B) MAA and porogenic solvent (C) EGDMA and porogenic solvent.

**Conclusion**

MIPs have been applied in several areas especially in the separation science due to the specific affinity to the target molecule (Zhu and Xu, 2003; Dong et al., 2005; Hu et al., 2005). The non-covalent imprinting method was the most widely used for the synthesis of MIP and several factors contributed to the process of polymerization. Therefore, it was necessary to select an efficient statistic experimental design for optimizing the technological conditions of polymerization. In this present study, the statistical methodology, response surface methodology design, was demonstrated to be effective and reliable in selecting the statistically significant factors and finding the optimal conditions of synthesizing the OPC imprinted polymer. The polymer prepared under the optimal conditions was used as the sorbents of SPE and showed high selectivity to the template. It could separate and enrich OPC from
Figure 5. HPLC chromatograms. lotus seedpod crude extract (A), washing solution chromatograms of NIPs column (B) and MIPs column (C), eluting solution chromatograms of NIPs column (D) and MIPs column (E). The each OPC peak is indicated with an number in the chromatograms. 1, Dimers B1; 2, (+)-Catechin; 3, Dimers B2; 4, (−)-epicatechin; 5, Trimers C1.

were performed with methanol, respectively. In the washing fraction not so relevant several peaks were observed at the retention time of OPC (Figures 5b and c) by chromatographic analysis. These peaks referable to other compounds of the lotus seedpod sample. This Lotus seedpod extract effectively and the recovery of OPC amounted to 85.58%. Hence, it is worthy of employing uniform design to optimize the parameters of polymerization process and obtain high efficiency MIP for extracting certain molecule from complex matrix.

ACKNOWLEDGEMENTS

The financial support was provided by grants from the National Natural Science Foundation (30970309 and
REFERENCES


