The Synthesis and Characterisation of Retinol-molecularly Imprinted Polymers as a Selective Sorbent in Solid-phase Extraction

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Abstract

The aim of the work is to design and synthesize and evaluate the polymer recognition characteristics of molecularly imprinted polymers selective for trans-retinol (R-MIP). The optimal amount of template and methacrylic acid (MAA) functional monomer as well as ethylene glycol dimethacrylate (EDMA) acts as the cross-linker was examined toward imprinting process using radical polymerization in chloroform solvent. The FT-IR study indicated the interaction between hydroxyl group on trans-retinol and carbonyl group on MAA forms hydrogen bonding. From ^{1}H-NMR titration, the job's plot analysis was considered that trans-retinol interacted with MAA in a 1:1 molar ratio of the template-functional monomer complex. The binding isotherm of the imprinted polymer was fitted to the Freundlich model and the binding affinity constant was provided for 0.064 μM^{-1}. Cross-reactivity study revealed that the binding capability of the imprinted polymer was highest for the template when compared with other structurally related substances (cis-retinol, α, δ, γ-tocopherol, α-tocotrienol and β-carotene). The results proved that the obtained trans-retinol-imprinted polymer is appreciable as specific sorbents for separation of complex mixtures such as crude palm oils.

Keywords: Molecularly imprinting, *trans*-retinol, palm oil, molecularly imprinted solid phase extraction, complex matrix, molecular recognition

1. Introduction

Separation technology plays an important role in the antioxidant components in the palm oil industry as palm oil provides highly valuable antioxidant substances including carotenoids, tocopherol and tocotrienol. Development in adsorption technologies included identification and use of a number of new adsorbents. In the early period, molecularly imprinted polymers (MIPs) were used in the adsorptive separation as selective chromatrographic material for the chiral drug molecules (Kempe and Mosbach, 1994). Recently, MIPs have been applied to the field of solid phase extraction (SPE) as a candidate tool for separation. MIP based on solid phase extraction (MISPE) is a synthetic porous polymer with the selective and specific recognition ability of the binding cavities to target molecules by non-covalent interaction such as hydrogen bonding, electrostatic interaction, π-π and hydrophobic forces (Ramstroem, Ye and Mosbach, 1996). Besides target molecule, MISPE has then bound selectively to some target analogues. Moreover, it can be resistant to organic solvents and pH of the medium. Where as the conventional SPE shows a lack of the selectivity to the analytes of interest. A large amount of the interferences are also extracted together with the analyte through the sorbent material (Buszewski et al., 1986). Therefore, MISPEs have then received growing interest as a replacement for SPE.

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Palm oil contains a high amount of β-carotenes which can be cleaved by enzyme reactions in the intestinal mucosa and liver into two molecules of retinol (Whitney et al., 1990). Retinol plays an important role in many cellular metabolic processes and the visual functions of the eyes (Saari, 1999). They are important for reproduction system (Chung et al., 2005); the differentiation of epithelium cells (Olson, 1994); the regulation of cell divisions (Semba, 1994); bone remodeling (Bates, 1995); genetic regulation (Thurnham, 2000); enhancement of immune responses (Serghides and Kain, 2002); and the development of lung diseases (Sempertegui, 2001). Other antioxidant substances which include tocopherols and tocotrienols are considered found in crude palm oil (Bailey, 1995; Hamid and May, 1997).

MISPE is the alternative method for the replacement of the conventional adsorption in crude palm oil separations such as high pressure screw press, liquid-liquid extraction (Baryeh, 2000; Resa et al., 2002). supercritical fluid carbon dioxide (Birtigh, Johannsen and Brunner, 1995; Davarnejad et al., 2008; Saito, 1995; Shimoda et al., 1997) and membrane technology (Coutinho et al., 2009). This communication has then selected *trans*-retinol as a model system. A new potential adsorbent was prepared based on molecularly imprinted solid phase extraction (MISPE) applied to the fractionation of the *trans*-retinol in crude palm oil and characterization as selective adsorption material with a series of analyses of the imprinting effect and recognition properties, since the crude palm oil is often present as a mixture of several substances.

2. Experiment and methods

2.1. Chemicals and materials

Methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) were purchased from Fluka (Steinheim, Germany) and purified prior to removal of the inhibitor by vacuum distillation. 1,1'-Azobis-(cyclohexanecarbonitrile) (ABCN) was obtained from Fluka (Steinheim, Germany). *Trans*-retinol and (±) α-tocopherol were purchased from Fluka Analytical (Switzerland). The isomers of tocopherol, (±)-δ-tocopherol (purity 90%) and (+)-γ-tocopherol were purchased from Sigma (St. Louis, USA) and Fluka (Japan), respectively. β-Carotene was purchased from Fluka Analytical (USA). All chemicals and solvents were of analytical or HPLC grade. *Cis*-retinol is no available in commercial, it was prepare by heating all *trans*-retinol at 95°C for 15 min and determined by HPLC method.

2.2. Preparation of *trans*-retinol-molecularly imprinted polymer (R-MIP)

The synthesis of imprinted polymer was adapted from the procedure reported by Spivak et al.(Spivak, Gilmore and Shea, 1997). The molecularly imprinted polymers were prepared in a free-radical polymerization process by cross-linking MAA functional monomer with an EDMA cross-linker using all *trans*-retinol as a template molecule. Three polymer formulations were prepared with different molar ratios of template:functional monomer:cross-linker as 1:4:10, 1:8:25 and 1:16:30. In the typical preparation of R-MIP, 0.25 mg of *trans*-retinol was dissolved in 50 mL of chloroform in a glass vial with a screw cap and followed by the addition of monomeric mixture. The copolymerization process was then initiated with 0.7%wt of the initiator (ABCN). Then, the mixture solution was sonicated and purged with nitrogen gas for 10 min. The polymerization was carried out under a 365 nm ultraviolet lamp at 30 ± 1°C and heated in an oven at 80°C for 24 h. The rigid polymers were obtained, crushed and ground mechanically into powder. The extraction was used for the removal of template in a Soxhlet apparatus with 250 mL of a methanol:chloroform (8:2, v/v) mixture, and followed by 250 mL of the methanol:acetic acid (8:2, v/v) mixture for 48 h. The rinse was examined to confirm the absence of template residue by determination using spectroscopic method. The polymer particles were then dried in a vacuum at 15 mm Hg overnight. Non-imprinted control polymers (NIP) were prepared using the same procedure as the imprinted polymers but omitting the templates.

2.3 The characterization of the polymers

2.3.1 Morphology

The surface morphology of the polymer was observed by scanning electron microscope system (FEI Quanta 400, Phillips, USA). Prior to scanning electron microscopy (SEM) experiments, the dried specimen was coated under low vacuum with a thin layer of gold. The polymers were inspected for surface morphology and shape. The surface morphology of *trans*-retinol-imprinted polymer and control polymer (NIP) were compared.

2.3.2 Brunauer, Emmett and Teller (BET) analysis

The porous polymers were determined for the specific surface area, average pore diameter and pore volume by using nitrogen adsorption/desorption technique. The BET analysis was performed on a Coulter SA3100 (Coulter Corporation, Florida, USA). The amount of 0.5 g of polymers was degassed at 150°C under nitrogen flow for 1 h prior to the measurement to remove adsorbed gases and moisture. The data were recorded the characteristic of the porosity.

2.3.3 Particle size and size distribution

The particle size and size distribution of the polymers were measured using a Laser Particle Size Analyzer equipped with a wet sample unit (Coulter LS230, Connecticut, USA). The obscuration of the optical system was set at 11%. The polymer particles (20 mg) were suspended in a suitable solvent and left overnight to ensure saturation allowing binding of swollen polymers. The result were read at the size below 50% of size as the median of the particle.

2.3.4 Determination of swelling factor of the polymers

The swelling ratio was studied on three types of solvents, namely chloroform, acetonitrile and methanol. The degree of swelling was tested by incubating a known amount of dry polymer particles in each solvent for 24 h. The polymer particles were then rinsed through a filter paper (Whatman No.1). The weight of swollen polymers was quickly measured and calculated as swelling ratio which was defined as (Yavuz et al., 2007):

Swelling ratio = (wt. of swollen polymers – wt. of dry polymers)/wt. of dry polymers

2.3.5. FT - IR spectroscopy

The FT-IR spectroscopy of the imprinted polymers was recorded in order to identify the chemical structure of the obtained MIP particle. The spectra were conducted in neat sample (KBr disc) on a Bruker EQUINOX 55 (Bruker Instruments Inc., Germany).

2.4 Batch binding experiments

The recognition property to the template of the imprinted polymers was investigated using three different formulations of MIP polymerization prepared at template:MAA:EDMA of 1:4:10, 1:8:25, and 1:16:30 mole ratios. Twenty milligrams of polymer were dissolved in 2 μg/mL of *trans*-retinol standard solution (in chloroform) and incubated overnight at room temperature to assure saturation. The bound or free *trans*-retinol was determined by taking the solvent portion and analyzed by HPLC – FLD. The amount of bound *trans*-retinol was calculated by substracting the amount unbound (free) *trans*-retinol obtained from the polymer solution from the initial concentration of the *trans*-retinol. The control polymer (NIP) was studied in similar fashion to the imprinted polymer. Selectivity was determined from imprinting factor of the polymers which indicates the molecular recognition for the template and the functionality of the imprinted polymer. The imprinting factor is the ratio of bound *trans*-retinol in imprinted polymer and bound *trans*-retinol in the control blank polymer. The experiment was repeated five times and the standard error was within ±1%.

Furthermore, the effect of solvent was explored using three different types of solvent namely chloroform, acetonitrile and methanol. Twenty milligrams of polymer particles were suspended in 1 mL of *trans*-retinol standard solution containing 2 μg *trans*-retinol per mL solvent. Polymer solution dissolved with each solvent was incubated at room temperature overnight. The supernatant was filtered and the amount of free retinol in the filtrate analyzed by HPLC-FLD at excitation wavelength 320 nm and emission wavelength 470 nm. The resulting data were used to substract from the initial concentration of *trans*-retinol. The experiment was repeated five times and the standard error was within ± 2%.

2.5 Investigation of the interaction between the template and functional monomer by titration with ^{1}H-NMR spectroscopy

The interactions between template and functional monomer were determined by titrating the mole ratios of *trans*-retinol and MAA of 1:0, 1:0.25, 1:0.75, 1:1, 1:1.5, 1:2, 1:4, and 1:6. All ^{1}H-NMR titration was performed in CDCl$_3$ solvent (800 μL). Chemical shifts of the hydroxyl group of *trans*-retinol were observed as a function of MAA concentration.

The binding constants were calculated from fitted data to a 1:1 binding isotherm. ^{1}H-NMR spectroscopy of samples was recorded on a Varian 500 MHz instrument at 21°C (Varian Instruments Inc. Germany). Processing of spectra was performed by Fourier Transformation to obtain spectra of intensity versus frequency on a silicon graphics workstation operating off a Unix platform.

2.6 Molecularly imprinted solid phase extraction (MISPE)

In this experiment molecularly imprinted solid phase extraction was carried out using the template *trans*-retinol and the structurally related compounds, including *cis*-retinol, α-tocopherol, γ-tocopherol, δ-tocopherol, α-tocotrienol, and *β*-carotene as substances. These substances are compounds present in crude palm oil that may also bind imprinted polymers due to their molecular similarity to *trans*-retinol. An optimized assay configuration was used by mixing twenty milligrams of the polymer and the mixture of 1 µg/mL *trans*-retinol and 1 µg/mL each analogue compounds in a total volume of 1 mL chloroform. The mixture was then blended on a Vortex (Scientific Industry Inc., USA) for a few seconds. An aliquot (500 µL) was removed periodically over a 24 h period. The aliquots were then diluted with HPLC mobile phase and analysed by HPLC as described.

2.7 HPLC analysis

The HPLC system consisted of an Agilent 1100 series Hewlett Packard (Waldbronn, Germany) instrument. A fluorescence detector and an online vacuum degasser were used. The analytes were detected by fluorescence at the excitation wavelength of 330 nm and emission wavelength of 470 nm for *trans*-retinol. The sample volume was 20 µL. Chromatograms were recorded at 50°C. HPLC analyses were performed on a 250 x 4.6 mm ACE 5 C18 column, particle size 5 µm (Advanced Chromatography Technologies, Aberdeen, Scotland) using 100% methanol as the mobile phase. A flow rate of 1.0 mL/min was used.

Twenty milligrams of imprinted polymer were suspended in analogue compound solution (2 µg/mL in chloroform solvent) in batch binding condition. For the case of tocopherols and tocotrienol, the concentration of the unbound α-,γ-,δ-tocopherol and α-tocotrienol were measured by HPLC-FLD at the excitation wavelength of 290 nm and the emission wavelength of 335 nm. For the case of *β*-carotene, concentration of unbound *β*-carotene was detected by HPLC-DAD at the wavelength of 452 nm, using an individual calibration standard curve for each compound.
The HPLC conditions for the tocopherols and tocotrienol were as follows: ACE 5 C18 column, 250x4.6 mm, particle size 5 µm (Advanced Chromatography Technologies, Aberdeen, Scotland). Mobile phase for *trans*-retinol and structurally related substances was pure methanol, and column temperature 50°C. Mobile phase for the *β*-carotene was A (mixtures of methanol/*tert*-butylmethylether (MTBE)/water = 85:15:4, v/v) gradient to the 51% eluent B (6:90:4 (v/v) methanol/*tert*-butylmethylether (MTBE)/water mixture). The injection volume was 20 µL and the flow rate was 1 mL/min.

3. Results and Discussion

For HPLC analysis, method validation of *trans*-retinol was yielded a limit of detection (LLD) of 1.94×10^{-6} g/mL and limit of quantization (LLQ) of 3.01×10^{-6} g/mL. The calibration curves were linear with correlation coefficients of $R^2 > 0.998$. The intraday precisions of the relative peak areas were below 3.02% and the interday precisions below 5.79%.

3.1. The preparation and characterization of R-MIP

The *trans*-retinol-imprinted polymers and corresponding non-imprinted polymer were prepared by the method described in the experimental section. The reason for selection of *trans*-retinol, which is a breakdown product of *β*-carotene, as the imprint molecule instead of *β*-carotene is that *β*-carotene has no functionality to interact with the functional monomer of MIP.

The ratio of functional monomer to template is an important factor influencing the performance of resultant imprinted polymer. Figure 2 shows the differences in imprinting factor values for three protocols in mole ratio of 1:4:10, 1:8:25 and 1:16:30 (template:MAA:EDMA). The result showed that the mole ratio of 1:16:30 produced a higher selectivity of imprinted polymer than that of 1:8:25 and 1:4:10, respectively. It is evident that polymerization of monomer mixture at 1:16:30 mole ratio has a high selectivity when compared with other compositions.

Furthermore, the 1:16:30 mole ratio was shown to have a good imprinting effect indicating the selectivity of imprinted binding site of the polymer matrix in chloroform solvent. Higher concentrations of functional monomers provided higher numbers of binding sites, whereas higher concentration of cross-linkers increased the binding capacity. However, *trans*-retinol has a one-point interaction of hydroxyl group to form hydrogen bonding with carboxylic group of MAA. The strength of this interaction in the imprinting site is quite weak. The enhancement of interactions with either the optimized formulation of amount of functional monomers and template or an increase in the degree of the cross-linking could achieve higher selectivity. According to Buszewski et al., a short alkyl or oxyethylene chain between two methacrylate parts in the EDMA chemical structure increased the selectivity of the obtained polymer (Buszewski et al., 2010). The amount of functional monomer was relevant to that of cross-linker which is an important factor affecting MIP quality (imprinting effect). During investigation, the cross-linker amount was varied while the amount of template kept constant. The visualize observation was shown that at low amount of monomer:cross-linker ratio, corresponding to 20% of cross-linker molar ratio, the resulting polymers were soft and unstable and did not meet the requirements of SPE material. SEM photographs in Figure 3 revealed that MIPs prepared with 1:9 monomer to cross-linker ratio, corresponding to 90% of cross-linker, exhibited hard materials with non-porous and smooth surface. In addition, the amount of 1:4 monomer to cross-linker ratio gave porous, compact and adequate globule-size polymers (Buszewski et al., 2010).

Besides thermal-polymerization, photo initiation (UV radiation) is a way to provide the kinetic energy system to produce some free radicals (Cheong et al., 1997). Hence, the preparation of the imprinted polymer had to rely on the combination of photo and thermo-polymerization at ambient temperature, respectively to increase selectivity (Sellergren and Shea, 1993). During the photo-initiation and following by thermal polymerization, all-*trans*-retinol could isomerize to *cis*-retinol due to *trans*-retinol is unstable for heat and light. The *cis*-congener might be interacted with MAA in the template-functional monomer complex. Hence the existance of a part of *cis*-retinol imprint in the polymer matrix.

In the FTIR spectroscopy, MAA showed a carbonyl band at the wave number 1634.97 cm^{-1}. When the *trans*-retinol formed complex with MAA, the carbonyl band was shifted to the wave number 1632.94 cm^{-1}. In addition, the ^{1}H-NMR data, the methylene peak is adjacent to the hydroxyl group of *trans*-retinol. It is observed to be the doublet peak at the position of δ3.711 and δ3.696 ppm and shifted upfield to δ3.705 and δ3.644 ppm, respectively. The FTIR and ^{1}H-NMR data confirmed the presence of the intermolecular hydrogen bonding between *trans*-retinol and MAA during the polymerization process. By using job's plot analysis, the chemical induced shift of ^{1}H-NMR spectroscopy is multiplied by the mole fraction MAA indicated that the stoichiometry between template and functional monomer complexation of 1:1 (as shown in Figure 4). The binding strength of the complexes was considered from the dissociation constant value (K_d) to be 0.163 µM. The binding of template–functional monomer interacted in a one-point interaction, therefore the binding strength of the complexes was then weaker than the multi-point interaction.

3.2 Pore size analysis

The BET adsorption data obtained the surface consisted of a number of smaller pores which are 0.01 mL/g polymer of total pore volume value and 5.62 nm of average pore diameter value as shown in Table 1. The SEM micrograph confirmed that the polymer particle is a homogeneous monolith with m equals to 0.09 of the heterogeneity index (as shown in Figure 5b) which calculated by the Freundlich model (FI). However, the control polymer particles have the characteristics of globules, preferably being mesopores. The Freundlich isotherm (Equation 1) is an isotherm which establishes a wide range of heterogeneity of polymer particle as recognized by Freundlich (Freundlich, 1926). The Freundlich equation, presented a and m as constants was derived theoretically using the following approach (Hayward and Trapnell, 1964):

$$B = aF^{m} \qquad (1)$$
$$\log B = \log a + m \log F \qquad (2)$$
$$K_o = a^{1/m} \qquad (3)$$

where a means the binding affinity constant which provided from y-intercept of the linear curve. m represents the heterogeneity index which provided from slope of the linear curve. The value of m varies from 0 to 1. Nearly 1 means homogeneous and values approaching 0 mean increasing heterogeneity.

K_o means the average binding affinity. A plot of Log B (bound *trans*-retinol to imprinted polymer) versus log F (free bound *trans*-retinol) shows linear relationship (Figure 5b). The Freundlich model is most applied by plotting the experimental binding isotherm in log B versus log F format (Equation 2). In general, this model has been applied for the non-covalent MIP (Umpleby et al., 2001).

It was shown that the binding isotherm of the imprinted polymer was fitted well to the Freundlich model with R^2 = 0.993. The calculated binding affinity (K_o) was provided for 0.064 μM^{-1} whereas a and m values yielded 0.783 and 0.094, respectively (Figure 5b). The m value exhibited the heterogeneity of the imprinted polymer which the binding property tended to be dominated by predominant low-affinity binding site (Rushton, Karns and Shimizu, 2005).

In order to determine the effect of solvent media on binding affinity of R-MIP in adsorption process was carried by batch binding assay (Figure 6). The solvent effect on the swelling property and the particle size of R-MIP was investigated in different solvents; chloroform, acetonitrile and methanol. As shown in Table 2, there is no significantly change in the particle size and swelling property of R-MIP and control polymer (non-MIP) among three types of solvent. It is considered that the solvent has not influenced to the conformation change of the binding site in R-MIP. The conformation change of binding site can be caused by the specific binding of template to polymer. However, the particle size of R-MIP was slightly higher than non-MIP. There is a report suggested that the polymers are swollen or de-swollen depending on the nature of the polymer–solvent interaction as well as inter- and intra-chain interactions (Crowther et al., 1999).

3.4 The MISPE application of R-MIP

The molecular recognition property of *trans*-retinol-imprinted polymer is studied in adsorption process for MISPE application. The reason of choosing *cis*-retinol as an additional substance in recognition investigation is that *trans* isoform may be converted to *cis* isoform due to the exposition of UV radiation at ambient temperature during pre-polymerization step. Therefore the selectivity for both tocol (α, δ,γ-tocopherol and α-tocotrienol) and β-carotene which present in crude palm oil was studied for further analysis application.

The selectivity recognition of R-MIP against to the structurally related substances was studied as compared with the template molecule, which the solid phase extraction procedure was used as in the experimental section (Figure 7). The specific binding of these substances to R-MIP was estimated by the ratio of the amount bound of the structurally related substance and the amount bound of *trans*-retinol. It is noted that in each case, equilibrium binding was established within for 1 h through approximately 94% binding was achieved. The efficiency of extraction of the β-carotene by the R-MIP at thermodynamic equilibrium was lesser than the other related compounds due to the large size of β-carotene structure. Some moieties of β-carotene molecule could be present in the binding pocket and form van der Waal force. For the tocol structure, the number and position of the substituted methyl group on aromatic moieties of the chromane ring could affect to the affinity of R-MIP. The tocol contains a hydroxyl group on the chromane ring that can interact with the carbonyl group of the binding site in the polymer pocket to create H-bond. In the case of *cis*-retinol, the isomerized product of *trans*-retinol could appear in small quantity, it has an improper orientation of structure to binding affinity of the imprinted polymer.

The cross-reactivity of these structurally related substances from competitive binding results to the R-MIP was investigated by comparing the adsorption value obtained from MIP to NIP. The results revealed that *trans*-retinol had capability to compete these structurally related substances in binding to the polymer matrix as shown in Figure 8. However, β-carotene was bound with the lowest affinity to the MIP. Tocopherol and tocotrienol had also been the lower affinity due to the difference in the number and position of methyl group on the chromane ring. It could be meant the molecular recognition of these molecules into the imprinting sites of the polymer matrix.

The important molecular recognition of the non-covalent interaction of substances by the R-MIP is related to hydrogen bond and electrostatic hydrophobic interaction giving high recognition and selectivity to the template in competitive binding experiments, provides vast opportunities in MISPE application.

4. Conclusion

In this study, *trans*-retinol-imprinted methacrylic acid-co-ethylene glycol dimethacrylate polymers have been synthesized and characterization was carried by morphology testing, FTIR, ^{1}H-NMR and pore analysis. *Trans*-retinol-molecularly imprinted polymer exhibited the highest recognition ability of the template when the polymerization of optimized MIP formulation was done at 1:16:30 of template: functional monomer:crosslinker mole ratio. The imprinted polymer exhibited an excellent recognition ability to the template in chloroform over the other solvents due to the chloroform may not influence to the conformation change of the binding site. The results of this study demonstrate that the *trans*-retinol MIP as a selective sorbent in solid phase extraction protocols can be used to extract *trans*-retinol from the crude palm oil.

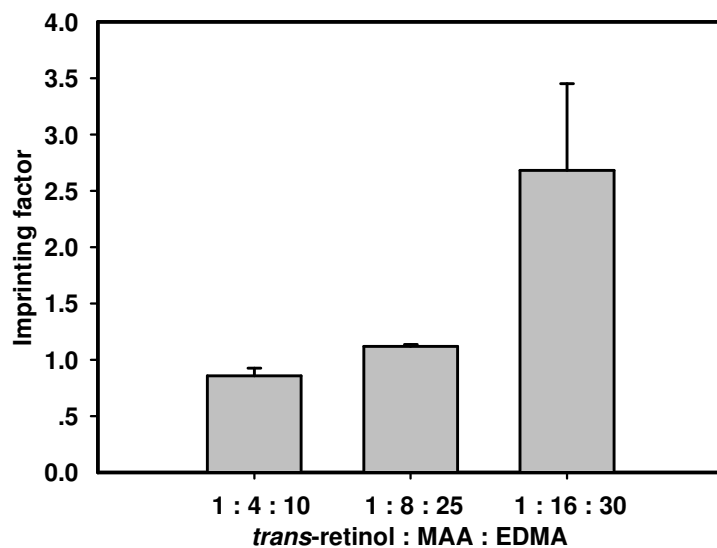**Figure 1 Chemical structures of various substances were used in this experiments**

Figure 2 Imprinting factor of three formula of *trans*-retinol-imprinted polymer prepared using template (*trans*-retinol):MAA:EDMA with different mole ratios 1:4:10, 1:8:25 and 1:16:30, respectively.
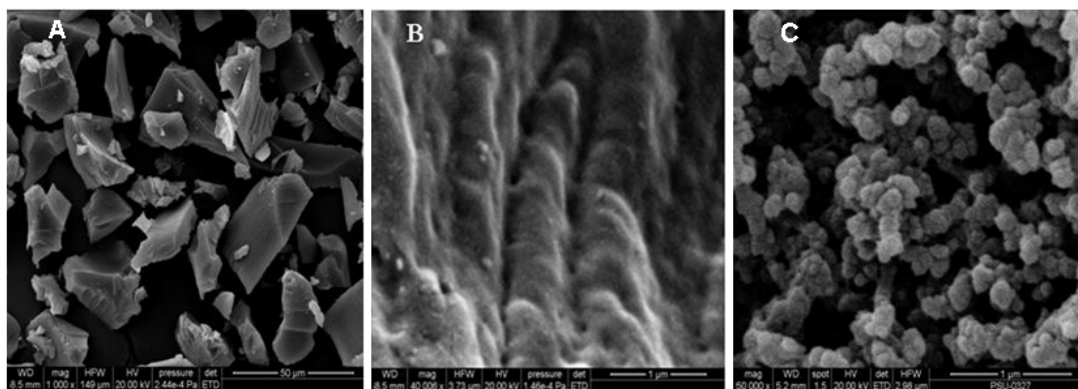

Figure 3 The SEM micrograph on surfaces of *trans*-retinol-imprinted polymer (A), 40,000 x magnification of *trans*-retinol-imprinted polymer (B) and 50,000 x magnification of non-imprinted polymer (C).
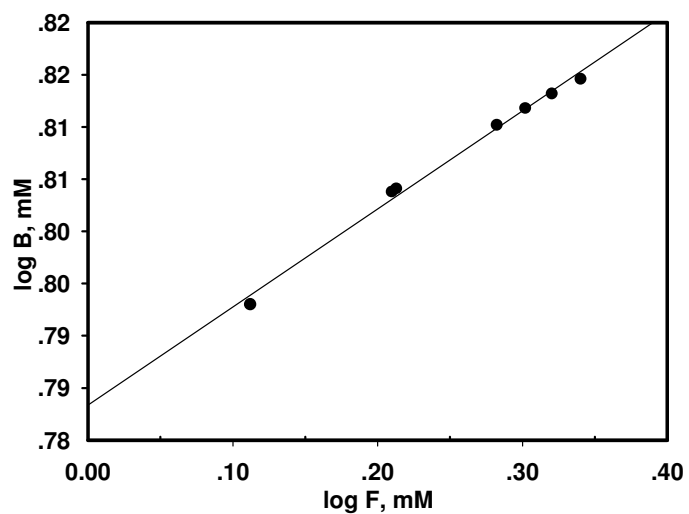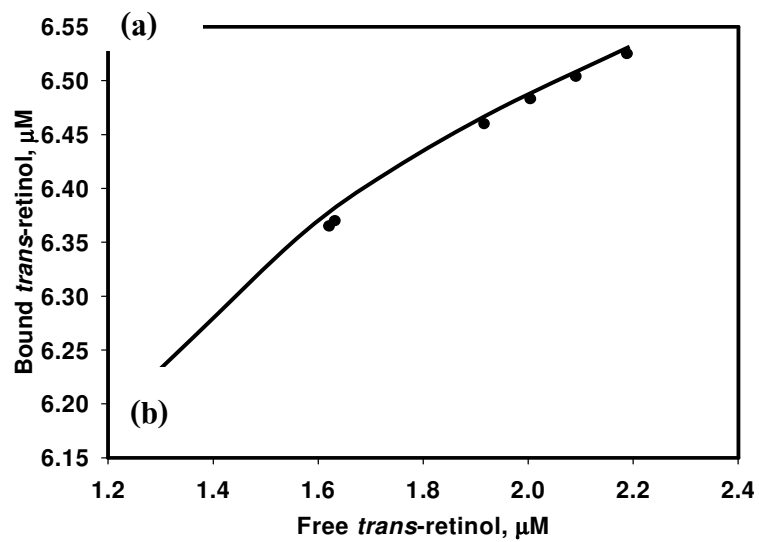
Figure 4 The job's plot of the complexation between *trans*-retinol and MAA is
formed hydrogen bond with the stiochiometry 1:1 which is indicated at 0.5
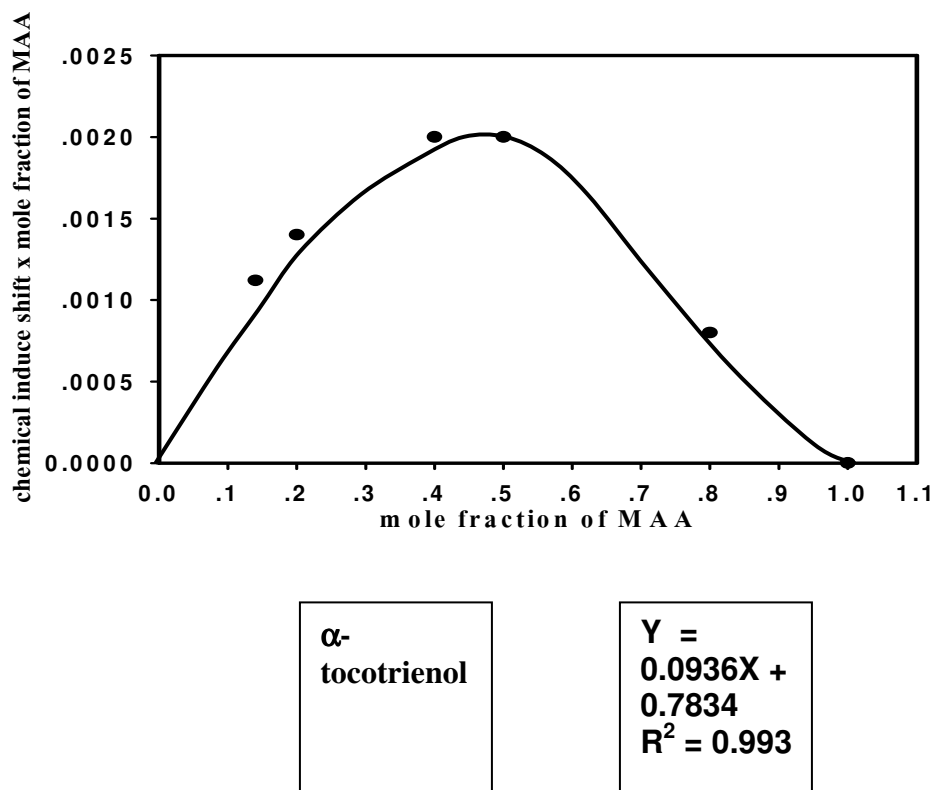of mole fraction MA

Figure 5 Binding isotherm for the *trans*-retinol standard solution and R-MIP,
Whereas figure (a) shows the plot of bound *trans*-retinol to R-MIP and free
bound *trans*-retinol, Figure (b) shows the plot of log-log format between
bound *trans*-retinol to R-MIP and free bound *trans*-retinol which the
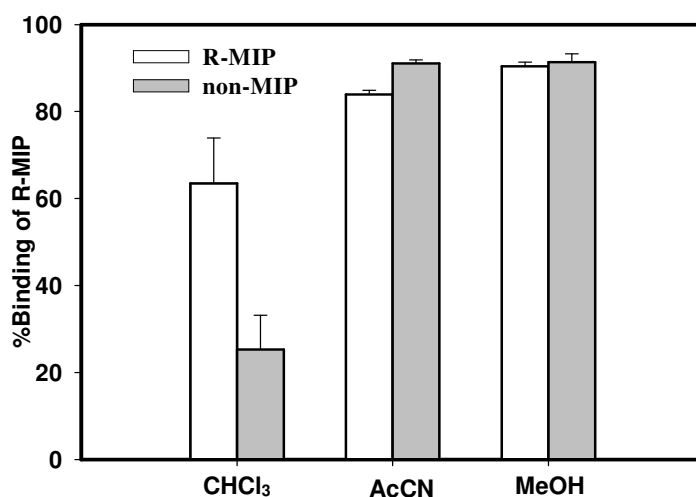equation of linear curve (b) is provided as Y = 0.0936 X+0.7834
where R^2 = 0.993.

Figure 6 The effect of binding of *trans*-retinol standard solution of 2 μg/ml on *trans*-
retinol-imprinted polymer (R-MIP) in different media including chloroform
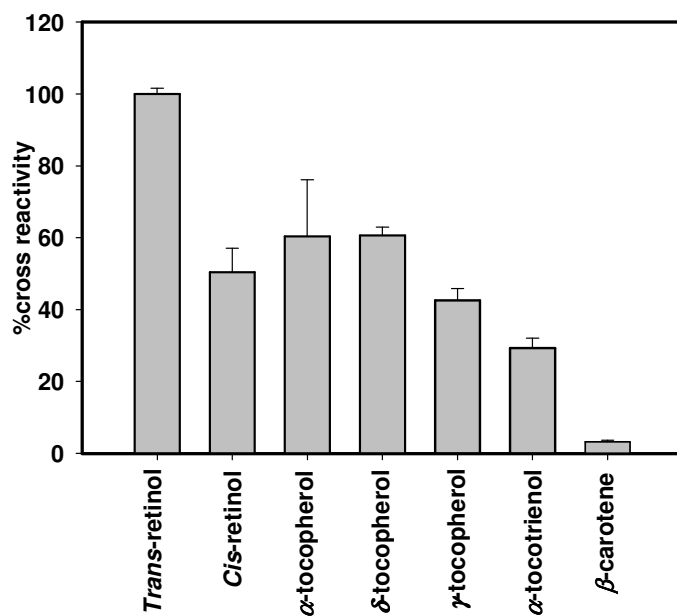(CHCl₃), acetonitrile (AcCN) and methanol (MeOH)

Figure 7 The binding affinity of *trans*-retinol and structurally related compounds to R-MIP. Structurally related compounds included *cis*-retinol, α-tocopherol, δ-tocopherol, γ-tocopherol, α-tocotrienol and *β*-carotene.
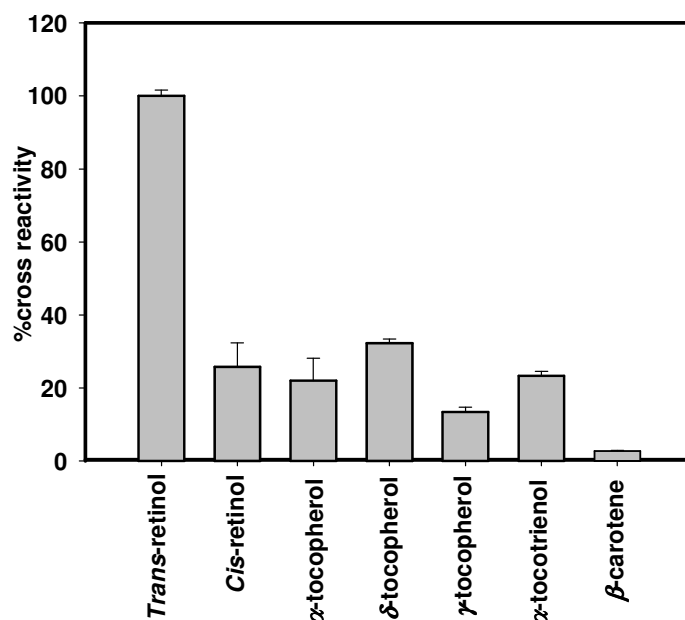

Figure 8 The competitive binding of the structurally related substances in presence of *trans*-retinol *to trans*-retinol-imprinted polymers is investigated by using the mixture of *trans*-retinol solution and structurally related substances at the concentration of 2 μg/ml competed into the imprinted polymer.

Table 1 Summary of BET adsorption analysis of *trans*-retinol imprinted polymer and
control polymer (non-imprinted polymer).

MIP / NIP	BET surface area (m^2/g)	Average pore dia. (nm)	Total pore volume (cm^3/g)
Trans-retinol MIP	12.34	5.62	0.01
NIP	125.24	23.44	0.16

Table 2 The correlation between particle sizes and swelling studies in three types of
solvent.

| Types of solvent | Particle sizes (μm)[a] | | Swelling ratio[b] | |
	Trans-retinol-MIP	Control polymer	*Trans*-retinol-MIP	Control polymer
Chloroform	25.85 ± 1.75	12.70 ± 0.95	0.84 ± 0.10	1.26 ± 0.32
Acetonitrile	29.92 ± 1.92	12.52 ± 0.79	0.90 ± 0.05	0.92 ± 0.05
Methanol	26.86 ± 1.44	11.98 ± 0.86	1.02 ± 0.09	0.96 ± 0.02

[a] Particle sizes and swelling ratios are measured in triplicates.
[b] Define as $(M_w – M_d)/ M_d$, where M_w is the mass of the wet polymer and M_d is the mass of the dry polymer.

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