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Surface Sites Distribution on Chlorogenic Acid Imprinted Polymers Based on Langmuir-Freundlich Isotherm Model by Frontal Liquid Chromatography Technique

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Surface sites distribution on chlorogenic acid (CGA) imprinted polymers (MIPs) was investigated under various temperature and mobile phase composition based on the best fitting parameters of Langmuir-Freundlich (LF) model to adsorption isotherms obtained by frontal chromatography technique. Curves of sites number towards sites energy distribution under different conditions were plotted by using the best Langmuir-Freundlich fitting values and total sites number, affinity constant and heterogeneity index for this MIPs. Results indicated that for various compounds, the most suitable sites occupied were of difference in sites energy distribution and total sites number and its energy distribution varied. For the template, total sites number on the MIPs decreased when measurement was performed at higher temperature or/and using higher concentration of acetic acid in mobile phase. Additionally, structure character of analytes like molecular size, shape, functional group type and number and mass transfer dynamic were shown to influence the binding capability of compounds on imprint sites.

Keywords: Molecularly imprinted polymers, Sites energy distribution, Frontal chromatography, Chlorogenic acid.

INTRODUCTION

Molecularly imprinted polymers (MIPs) are artificially synthesized material by using target compounds as template in the presence of excess functional monomer and cross-linker. After extracting the template out of polymers, some micro-cavities, also named imprint sites, in complementary to the template in size, shape and residual group were left. These sites can selectively rebinding the template molecules and its structurally analogues from complicated biological and environmental samples[1-5]. Theoretically, this selectivity recognition based on the complete complementary of analyte to binding cavities in molecular configuration (physical interaction) and chemical groups (chemical interaction) should be very satisfactory. However, the actual situation is far from ideal because of various factors, of which binding sites energy heterogeneity has sometimes been considered one of the most significant factors limiting its selectivity. Sites heterogeneity has been cited as a major source of peak broadening and asymmetry in HPLC applications using MIP as stationary phases[6]. When using MIPs catalysts as enzyme analogs, it has been regarded as a contributor to the low selectivity[7]. In addition, sites heterogeneity also leads to binding properties highly dependent on the concentration range of tested analytes, complicating the binding behaviour of molecularly imprinted polymers[8-10].

The main reason leading to binding sites heterogeneity in the MIPs matrix lies in inherent drawbacks in polymerization approaches utilized, such as the complicated interaction of template-template, monomer-template and template-solvent molecule in pre-polymerization system, the interaction strength between template and functional monomer and synthesis conditions, *etc*.[11,12]. Hence, many researches devoted themselves to developing new technique to prepare molecularly imprinted polymers with better rebinding behaviour or with increased chromatographic performance[13,14]. Herein, precipitation polymerization is an attractive and simple procedure for producing high-quality imprinted products as spherical particulates based on surfactant-free polymerization involving polymerization of monomers in dilute solution (typically < 5 % w/v)[15-17]. Particle growth occurs predominantly *via* entropic precipitation of gel (seed) particles followed by continuous capture of oligomers from solution. Nearly monodisperse, spherical particles can be routinely prepared in good yields *via* this method and this material has been applied in many fields such as solid-phase extraction, liquid phase chromato-

graphy, biosensor and so on[18-21]. Furthermore, characterization of binding sites on this imprinted polymer can offer information on binding mechanism so as to improve the quality of this polymer by optimizing preparation procedure and to increase the separation and application capacity[22].

Characterization of binding sites heterogeneity in MIPs often start from measurement of adsorption isotherm, followed by fitting suitable isotherm model to experimental data to obtain corresponding parameters which can be used to quantitatively describe the character of binding sites[23,24]. Among these isotherms models[25], Langmuir model, Freundlich model and Langmuir-Freundlich (LF) model, *etc*., are the more appropriate mathematical models and they are often used to describe adsorption process taken place on the surface of MIPs[26,27]. In present work, a precipitation polymerization technique was designed to prepare chlorogenic acid imprinted polymers and frontal analysis was utilized to measure adsorption isotherm of this MIPs column. Curves of sites number toward sites-energy distribution were constructed based on the best Langmuir-Freundlich isotherm parameters value obtained by fitting the Langmuir-Freundlich isotherm to experimental adsorption data. Sites distribution character of the chlorogenic acid MIPs for several structurally related compounds under various determination conditions was investigated.

EXPERIMENTAL

Chlorogenic acid, caffeic acid, vanillic acid, gallic acid, methacrylic acid and divinyl benzene were from Sigma company. 2,2'-Azobisisobutyronitrile, tetrahydrofuran, dimethyl sulfoxide, toluene and isooctane were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Glacial acetic acid, acetonitrile, acetone and methanol was from Hunan Normal University Reagent Factory (Hunan, China). All the reagents are analytical-grade. The monomers before use have been purified by distillation. Deionized water was filtrated through 0.45 m microporous membrane before use. Stainless steel columns (200.0 mm × 4.6 mm I.D.) were purchased from Beijing Jinouya Science & Development Co. Ltd. (Beijing, China).

Preparation of chlorogenic acid imprinted polymer by a precipitation polymerization method: Chlorogenic acid imprinted polymers were prepared by a precipitation polymerization method as follows: chlorogenic acid (0.375 mmol) as a template molecule, methacrylic acid, (1.5 mmol) as a functional monomer, divinyl benzene (7.2 mmol) as a cross-linker and 2,2'-azobisisobutyronitrile (0.48 mmol) as an initiator were dissolved in mixted solvent of 32 mL of acetonitrile-toluene (3:1, v/v) and 1 mL of DMSO. The mixture was degassed with argon for 15 min and then rotated slowly at 16 rpm using a rotor. Reaction temperature was increased from 25 to 60 ºC over 2 h and then kept at 60 ºC for 24 h. After polymerization, mixture was poured into 100 mL of methanol for three times, 100 mL of tetrahydrofuran for twice and 100 mL of deionized water for twice, sequentionally, then the supernatant was discarded after sedimentation of the particles. The obtained polymers particles were collected using a membrane filter and dried at 40 ºC. The preparation for non-imprinted polymer was carried out as the same procedure but without addition of template.

Rebinding experiments: Prior to measurement, 2.62 g chlorogenic acid MIPs polymers were packed into a stainless-steel column by a slurry packing technique using methanol-2-propanol (2:1, v/v) as the slurry solvent and methanol as the packing solvent. Then, the MIPs column was connected into liquid chromatography system for performing rebinding experiments.

To determine the adsorption isotherm of the MIPs column to compounds, frontal analysis technique was utilized as described in reference[9]. Before the determination, the volume of polymer was tested to be 0.47 mL. A series of tetrahydrofuran solution containing analyte with concentration ranging from 0.0005-1.2 mg mL^{-1} was independently used as mobile phase to perform frontal development on the MIPs column. The flow rate was at 0.2 mL min^{-1}. The detector wavelength was set at 280 nm. After producing a series of breakthrough curves for each analyte, the breakthrough volumes were obtained by the half height method[28]. Adsorption amount of analyte on the MIPs column was expressed as adsorbed mass of analyte on per mL polymer.

Competition adsorption of different compounds on the MIPs was performed by static method. 20 mg of polymer was, respectively added into 10 mL of tetrahydrofuran solution containing four compounds (chlorogenic acid, caffeic acid, vanillic acid and gallic acid) with the concentration ranging from 0.002-1.2 mg mL^{-1} for each substrate. After reaching adsorption equilibrium, the solid was separated by filtrating through a 0.45 μm microporous membrane and the liquid collected was analyzed by using HPLC method to determine the free concentration of each compounds. The adsorbed amount (B, mg g^{-1}) of each analyte was calculated as eqn. 1.

$$B = \frac{(C_0 - C_e)V}{W} \quad (1)$$

where C_0 and C_e (mg mL^{-1}) are the initial and free concentration of analyte, respectively; V (mL) is the volume of solution and W (g) is the mass of the polymers.

HPLC analysis: A LC-2010AHT high performance liquid chromatographer from Shimadzu Co., Ltd, (Japan), equipped with solvent delivery pumps, auto-injector, system controller, column oven, DAD detector and chromatographic workstation, was used for frontal analysis and chromatographic analysis. HPLC analysis of the content of analyte in solution was carried out on C_{18} column. Mobile phase used was a mixture of methanol-water-acetic acid (20.3:78.7:1.0, v/v) at a rate of 1.0 mL/min. Injection volume was 10 μL and UV detection was at 280 nm. Each analysis was repeated for three times. Standard curve method was used for quantification.

Isotherm models and data fitting: The Bi-Langmuir model (2), Freundlich model (3) and Langmuir-Freundlich model (4) describe a relationship between the concentration of bound (q_e) and free (C_e) guest in heterogeneous systems with different coefficients[29].

$$q_e = \frac{q_{m1}K_{L1}C_e}{1 + K_{L1}C_e} + \frac{q_{m2}K_{L2}C_e}{1 + K_{L2}C_e} \quad (2)$$

$$q_e = K_F C_e^{b_F} \quad (3)$$

$$q_e = \frac{N_t \alpha C_e^m}{1 + \alpha C_e^m} \quad (4)$$

where q_{m_1}, q_{m_2} are theoretically maximum adsorption amount for the first and the second type of binding sites and K_{L_1}, K_{L_2} are equilibrium dissociation constants for the first and the second type of binding sites at Bi-Langmuir adsorption model; K_F and b_F are Freundlich constants demonstrating adsorption capacity and intensity; N_t is the total number of binding sites, α is related to the median binding affinity constant K_0 ($\alpha = K_0^m$) and m is the heterogeneity index, which will be equal to 1 for a homogeneous material, or will take values within 0 and 1 if the material is heterogeneous.

Fitting process was carried out by using an Origin 5.0 non-linear fitter. Fitting accuracy was evaluated according to a parameter R^2 which is defined as eqn. 5

$$R^2 = \sum_{1}^{n} \left(\frac{x_{i,Theory} - x_{i,Experimental}}{x_{i,Theory}} \right)^2 \quad (5)$$

where $x_{i,Theory}$ and $x_{i,Experimental}$ are theoretically adsorption data and experimental values and n is the number of experimental points, respectively. In general, the smaller the R^2 values, the higher the fitting accuracy.

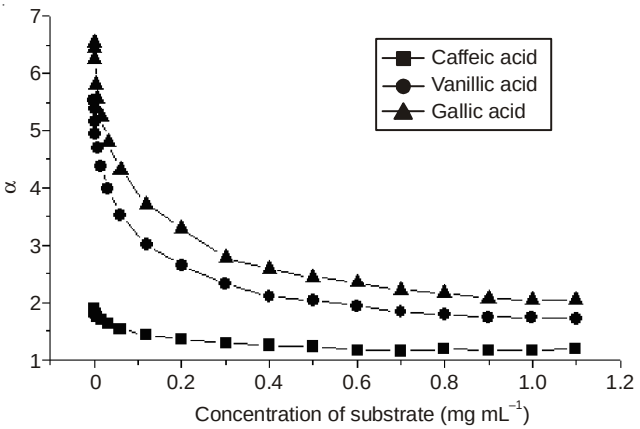

Fig. 1. Separation factor (α) of MIPs column for the template molecule toward structurally related compounds (SRC) caffeic acid, gallic acid and vanillic acid under various substrate concentration. The separation factor was independently measured by frontal chromatography technique and was calculated as: α = ([adsorbed chlorogenic acid]/[chlorogenic acid in mobile phase])/([adsorbed SRC]/[SRC in mobile phase]). Temperature: 298 K. Mobile phase: tetrahydrofuran

RESULTS AND DISCUSSION

Selectivity of chlorogenic acid imprinted polymers column: Firstly, selectivity test of the chlorogenic acid imprinted polymers column was carried out. Fig. 1 shows the separation factor of the MIPs column for the template toward caffeic acid, vanillic acid and gallic acid, respectively, obtained by using different concentration of substrate. Frontal analysis technique was for the determination of the adsorption amount. It can be found that at high analyte concentrations, separation factor is a relatively stable but low value for each of tested compounds. In contrast, α value increases rapidly at low analyte concentrations and, respectively reaches the highest value of 6.54, 5.52 and 1.89 toward gallic acid, vanillic acid and caffeic acid at a concentration of 0.0005 mg mL^{-1}. This behaviour results from binding site heterogeneity on the MIPs. While at high loading concentration, most of analyte located at the low affinity and low selectivity sites, analyte molecule preferentially sampled the high affinity and high selectivity sites at low substrate concentration. This concentration dependence of can yield very high separation factor but complicates the binding behaviour of the MIPs. This highlights the necessity for measurement and characterization of affinity heterogeneity on this MIPs.

Binding of different analytes on the chlorogenic acid molecularly imprinted polymers column: In order to investigate on the binding behaviour of the template and its structurally analogues on the MIPs column, it is of necessity of test adsorption isotherms for different analytes on the MIPs. For this purpose, tetrahydrofuran solutions of each analyte at concentration ranging between 0.0005 and 1.2 mg mL^{-1} was independently used as mobile phase to perform frontal analysis. Fig. 2 shows experimental adsorption isotherms of each analyte

TABLE-1
SUM OF DEVIATION FOR DIFFERENT FITTING EQUATION AND BINDING PARAMETERS OBTAINED BY LANGMUIR-FREUNDLICH FIT TO THE EXPERIMENTAL ADSORPTION ISOTHERMS DATA MEASURED UNDER DIFFERENT CONDITIONS FOR CHLOROGENIC ACID, CAFFEIC ACID, VANILLIC ACID AND GALLIC ACID ON CHLOROGENIC ACID IMPRINTED POLYMER COLUMN

Analytes	Temp. (K)	Mobile phase	N_t (mg mL^{-1})	α (mg mL^{-1})$^{-m}$	M	K_0 (mL mg^{-1})^a	$R_1^{2\,b}$	R_2^2	R_3^2
Chlorogenic acid	298	Tetrahydrofuran	15.93	4.842	0.8748	6.068	0.02245	0.02816	0.82069
Caffeic acid	298	Tetrahydrofuran	14.33	3.758	0.9430	4.071	0.03390	0.03983	0.49108
Vanillic acid	298	Tetrahydrofuran	11.41	2.024	0.9875	2.042	0.00967	0.01038	0.10702
Gallic acid	298	Tetrahydrofuran	9.947	1.804	0.9726	1.834	0.00807	0.00880	0.06670
Chlorogenic acid	298	Tetrahydrofuran	15.93	4.842	0.8748	6.068	0.02245	0.02816	0.82069
Chlorogenic acid	308	Tetrahydrofuran	7.8445	5.005	0.8802	6.232	0.00626	0.01346	0.20943
Chlorogenic acid	318	Tetrahydrofuran	6.819	5.055	0.8847	6.244	0.00623	0.01130	0.15957
Chlorogenic acid	328	Tetrahydrofuran	6.250	4.969	0.8774	6.217	0.00646	0.01142	0.13390
Chlorogenic acid	298	Tetrahydrofuran	15.93	4.842	0.8748	6.068	0.02245	0.02816	0.82069
Chlorogenic acid	298	Tetrahydrofuran + 1 % glacial acetic acid	11.19	4.935	0.8782	6.158	0.01860	0.03388	0.42307
Chlorogenic acid	298	Tetrahydrofuran + 3 % glacial acetic acid	10.19	4.733	0.8695	5.977	0.00574	0.00780	0.32172
Chlorogenic acid	298	Tetrahydrofuran + 5 % glacial acetic acid	8.310	4.903	0.8774	6.123	0.01045	0.01924	0.23134

[a]K is limited within 0.83-2000 mL mg^{-1} for all substrates according to the experimental maximum and minimum free analyte concentration (0.0005-1.2 mg mL^{-1}). [b]R_1^2, R_2^2 and R_3^2 represent the sum of deviation shown as eqn. 5 for LF, Bi-Langmuir and Freundlich fitting, respectively.

on the MIPs column and the corresponding fitting Langmuir-Freundlich isotherms. Table-1 gives the corresponding fitting coefficients from the best fitting of Langmuir-Freundlich isotherm model and the sum of squares of deviations (R^2) for Bi-Langmuir, Freundlich and Langmuir-Freundlich fitting. From the values of R^2 obtained by using different isotherm equations, it was found that the Langmuir-Freundlich model was the most suitable for describing the adsorption behaviour of this monolith to several compounds investigated.

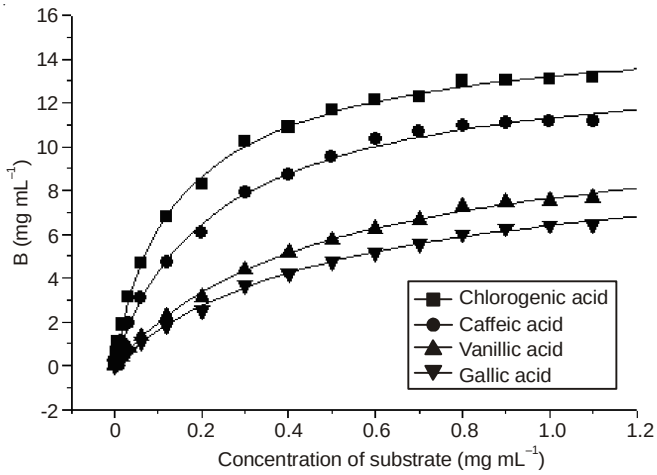

Fig. 2. Experimental adsorption isotherms data (solid dots) for MIPs column toward chlorogenic acid, caffeic acid, vanillic acid and gallic acid, obtained by frontal analysis technique using tetrahydrofuran as mobile phase at 298 K and the Langmuir-Freundlich fitting curves (solid lines) to experimental data

Several conclusions can be derived by comparing these parameters obtained for four analytes tested. One hand, the binding capacity of the MIPs column for the template chlorogenic acid is the highest, followed by caffeic acid, vanillic acid and gallic acid, with values of 15.93, 14.33, 11.41 and 9.947 mg mL^{-1}, respectively. These results can be attributed to the formation of a large number of binding sites in the MIPs matrix complementary to the template molecule in size, shape and functional group during polymerization. On the other hand, the MIPs possesses higher affinity constants for the template than that for its structurally related compounds. This is due to that those better-defined binding sites created by the template could better match with chlorogenic acid molecules. Additionally, by the comparison of m values, it was found that the heterogeneity index of the MIPs for the template (0.8748) is lower than that for structurally related compounds (0.9430, 0.9875 and 0.9726 for caffeic acid, vanillic acid and gallic acid, respectively), indicating a higher heterogeneity of binding sites toward the template molecule than toward other structurally related compounds.

In order to probe the relationship between sites energy distribution and rebinding behaviour for this MIPs, curves of sites number toward sites energy distribution for the binding of four analytes on the MIPs was plotted by following eqn. 6 using Langmuir-Freundlich fitting parameter values[30].

$$N(K) = 2.3N_t mK_0^m K^{-m} \times \frac{(1+2K_0^m K^{-m} + K_0^{2m}K^{-2m} + 4K_0^m K^{-m}m^2 - K_0^{2m}K^{-2m}m^2 - m^2)}{(1+K_0^m K^{-m})^4} \quad (6)$$

Fig. 3 shows semi-logarithm plot of the affinity distribution curves of the MIPs for four compounds. Boundary of log K, calculated from the maximum and minimum substrate concentration in mobile phase used in frontal analysis, was marked with two vertical lines corresponding to log K values of -0.08 and 3.30. It can be observed that these curves appear single peak and exponential decay formation within K limits. Obviously, the center of peaks for four compounds is misalignment. This suggests that the most suitable binding sites for different compounds are located at different energy distribution region. According to the area overlapped under curve, it should be probably deduced the similarity degree in energy distribution of binding sites occupied by different compounds. In this sense, that the binding capacity of caffeic acid on the MIPs is higher than that of vanillic acid and gallic acid is due to the greater area of caffeic acid overlapped with the template than that of vanillic acid and gallic acid. Whereas, the least overlapped area of gallic acid with the template might be responsible for its low binding capacity. Likewise, the overlapped area in high energy region may account for the difference in affinity selectivity of the MIPs for different analytes. Obviously, the bigger area of caffeic acid overlapped with the template clearly indicated the higher affinity of the MIPs for caffeic acid than for vanillic acid and gallic acid. As far as the difference in heterogeneity index, it can also be interpreted from the energy distribution curve. The template occupied the most sites number (*i.e.* the greatest area covered by distribution curve, log K borderlines and log K axis), whereas other three analytes inhabit on relatively less binding sites.

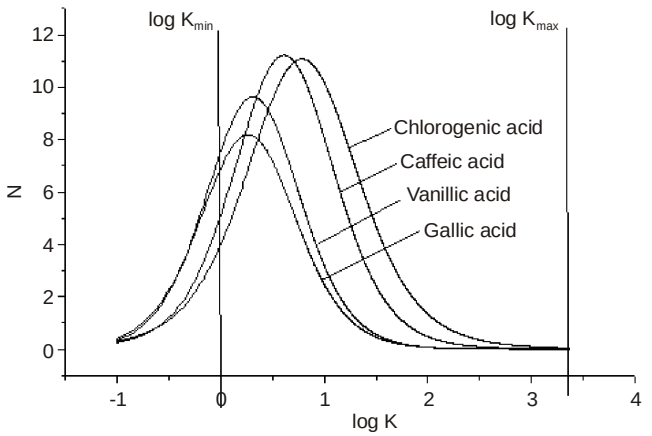

Fig. 3. Affinity distributions curves in semi-logarithm format for the binding of chlorogenic acid, caffeic acid, vanillic acid and gallic acid on the MIPs column based on the best Langmuir-Freundlich isotherm parameters

Influence of temperature on binding behaviour of the template on the molecularly imprinted polymers: The adsorption isotherm of the MIPs for the template was measured at different temperatures (298, 308, 318 and 328 K) and fitting these experimental data to Langmuir-Freundlich model carried out. Fig. 4 shows experimental isotherms data obtained by frontal analysis technique at different temperatures with tetrahydrofuran as mobile phase and the Langmuir-Freundlich fitting curves. It is observed that at the same substrate concentration, the adsorption capacity of the template on the MIPs decrease with the increase of temperature. Table-1 listed the corresponding isotherm parameters obtained by Langmuir-

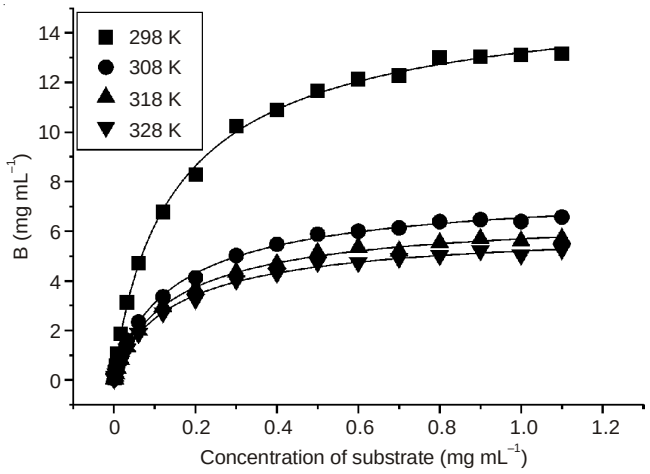

Fig. 4. Experimental adsorption isotherms data (solid dots) obtained by frontal analysis technique using tetrahydrofuran as mobile phase at 298, 308, 318 and 328 K, respectively, for the template molecule on the MIPs column and the Langmuir-Freundlich fitting curves (solid lines) to experimental ones

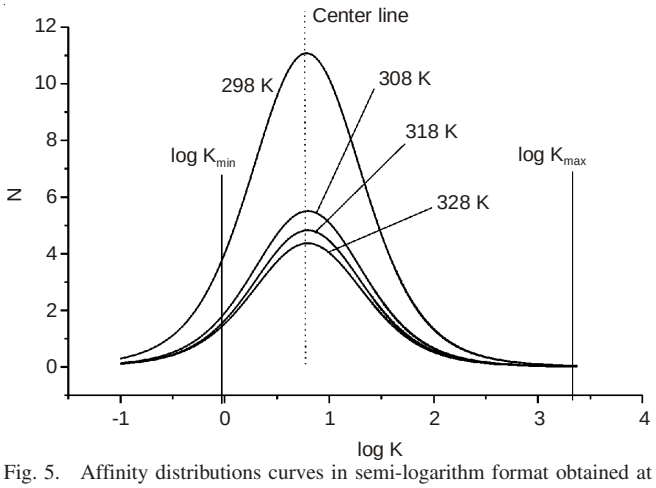

Fig. 5. Affinity distributions curves in semi-logarithm format obtained at different temperature, for the template on the MIPs column, based on the best Langmuir-Freundlich fitting parameters

Freundlich fitting. It can be found that the temperature can affect measurement of the total number of binding sites and the detected value reduces at increased temperature, whereas the influence of the temperature on the measurement of the affinity constant and heterogeneity index is relatively weak. In order to better understand the binding behaviour of the chlorogenic acid MIPs for the template at different temperature, sites number toward log K distributions curve tested at different temperature was also plotted in semi-logarithm formats based on Langmuir-Freundlich isotherm fitting parameter (Fig. 5). This plot indicates a signal peak and exponential decay formation of sites number toward continuous log K values for all curves obtained at different temperatures. Especially, the center of these peaks coincide with same log K value and the sites distribution toward log K values develops almost symmetrically along the same center line. Total affinity of the MIPs for the chlorogenic acid at different temperature can be calculated integrally within log K limits, *i.e*., the area between distribution curve and log K axis within log K limits. Visual comparison of these areas in Fig. 5 can also offer us information about total affinity of the MIPs for the template at different temperature. Following information can be extracted from this figure, (i) affinity constant of the MIPs for chlorogenic acid at 298 K is the highest within set temperature range but (ii) reduces with the increase of temperature; (iii) at different temperature, chlorogenic acid molecule might interact with all of binding sites within log K limits, however, (iv) the interaction strength with binding sites changed at various temperature. Additionally, the phenomenon that the binding site distribution measured under different temperatures all have the similar average binding site energy but have changed sites number might be in relation to variability in the shape of imprinted cavities and the flexibility of the polymer matrix.

Influence of mobile phase composition on binding behaviour of the template on the molecularly imprinted polymers: When mobile phase contains proton solvent, competition for adsorption sites on the MIPs between the proton solvent and substrate molecule occurs. However, this competition to some extent might better elution and resolution, beneficial for the application of molecularly imprinted polymer on chromatographic analysis and solid phase extraction[31,32]. Herein, tetrahydrofuran, tetrahydrofuran + 1 % glacial acetic acid, tetrahydrofuran + 3 % glacial acetic acid and tetrahydrofuran + 5 % glacial acetic acid are, respectively used as mobile phase for the measurement of adsorption isotherm of chlorogenic acid on the MIPs. Column temperature was fixed at 298 K. The experimental isotherm data and the Langmuir-Freundlich fitting curve are shown in Fig. 6, from which it is observed that the binding capacity of the MIPs for chlorogenic acid decrease with the increase of acetic acid amount in mobile phase under the same substrate concentration. This is easily understood for acetic acid molecules competitively occupy binding sites present on the MIPs. The corresponding fitting parameters based on Langmuir-Freundlich fitting are also listed in Table-1. As expected, total number of binding sites measured reduces with the increased amount of acetic acid, while affinity constant and heterogeneity indexes slightly change. This result might suggest that proton solvent may compete the adsorption sites on the MIPs but slightly influence the interaction strength between chlorogenic acid molecules and binding sites. To better understand the adsorption behaviour of chlorogenic acid on the MIPs when using mobile phase containing different amount of acetic acid, affinity distribution curves based on the Langmuir-Freundlich fitting parameters was also constructed as shown in Fig. 7. It was also observed that a single peak and exponential decay curve of sites number toward continuous log K values for all curves occurs. The area between the curve and log K axis within log K limits corresponds to the total affinity. The affinity based on the area analysis coincides with the binding capacity measured by experimentally frontal development method (Fig. 6). In addition, the center line of all curves peaks are also observed to be superposed and the binding sites distribute symmetrically along this line, indicating that acetic acid molecules could lead to a decrease in binding sites number measured within the same log K range. This homogeneous percents of decrease in the number of binding sites with the content of acetic acid might result from competitive occupation of proton solvent molecule on binding sites and shape change of imprinted cavities in the polymer matrix under different mobile phase.

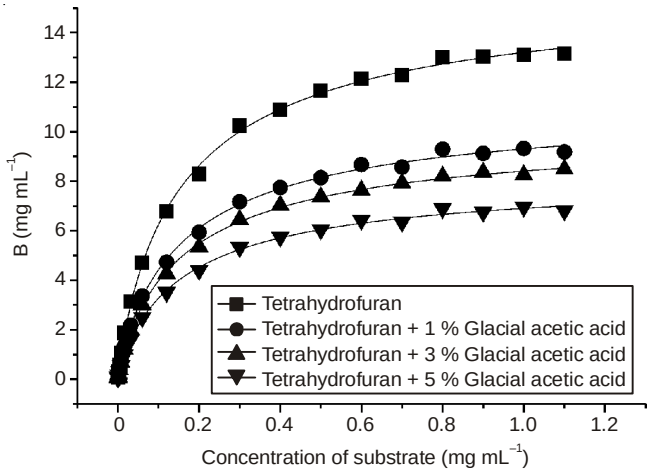

Fig. 6. Experimental adsorption isotherms data (solid dots) for the template on the MIPs column using tetrahydrofuran, tetrahydrofuran + 1 % glacial acetic acid, tetrahydrofuran + 3 % glacial acetic acid and tetrahydrofuran + 5 % glacial acetic acid as mobile phase at 298 K and the Langmuir-Freundlich fitting curves (solid lines) to experimental ones, respectively

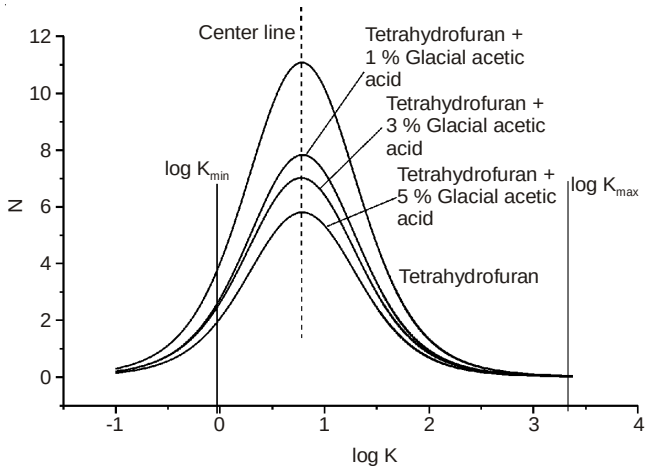

Fig. 7. Affinity distributions in semi-logarithm format measured by using different mobile phase composition for the template on the MIPs column, based on the Langmuir-Freundlich fitting

Rebinding of mixture of the template and structurally related compounds on the molecularly imprinted polymers: To explore how different analytes compete for the binding sites present in this polymer, rebinding experiments using mixtures containing all of the compounds tested were performed by static adsorption method. Fig. 8 shows competitive adsorption experimental isotherm data of each compound on the chlorogenic acid imprinted polymers and the Langmuir-Freundlich fitting curves. As is expected, the amount of each analyte adsorbed on the MIPs is lower than that obtained by independent loading (Fig. 2 and Table-1), due to competition adsorption between the different analytes for the binding sites on the MIPs. However, the reduction for the binding capacity did not occur at the same rate for all of the compounds. Comparatively, chlorogenic acid and caffeic acid suffered the lower diminishment, indicating higher competition capability for binding sites on the MIPs compared with vanillic acid and chlorogenic acid molecules. During competition adsorption, that gallic acid occupies less binding sites might mainly results

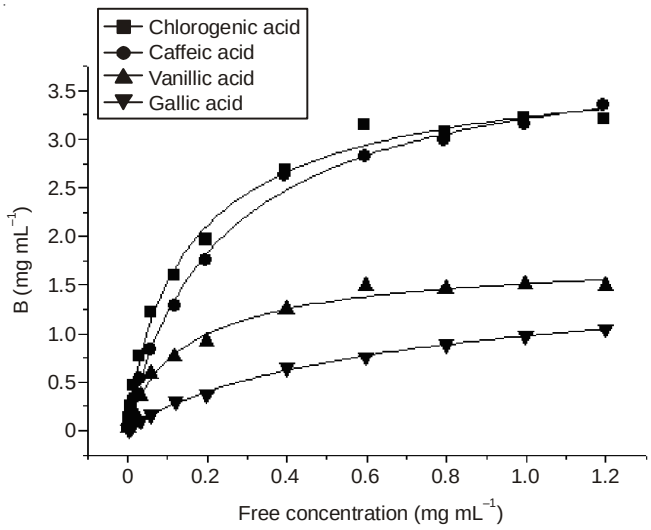

Fig. 9. Experimental adsorption isotherms data (solid dots) of chlorogenic acid, caffeic acid, vanillic acid and gallic acid on MIPs column, obtained by using mixture of four compounds and using tetrahydrofuran as mobile phase at 298 K and the Langmuir-Freundlich fitting curves (solid lines) to experimental ones

from low complementary of gallic acid molecular size to sites cavity, which obstructed the entrance of gallic acid molecules. vanillic acid molecules occupied more binding sites than gallic acid molecules, indicating that difference in number of functional group connected to molecule can also influence the interaction between the analyte and binding sites. When only three functional groups connected to benzene ring in vanillic acid molecule structure, this might lead to a higher complementary to binding cavities in size and functional group than gallic acid molecule. Template molecules possess the best complementary to binding sites in size, shape and functional group types and number. However, a little part of sites were still occupied preferentially by some smaller size molecules. This might be in relation to mass transfer dynamics.

Conclusion

This work focussed on investigation on the surface sites distribution of the chlorogenic acid imprinted polymer based on binding parameters obtained from the best fitting of Langmuir-Freundlich isotherm equation to adsorption isotherms obtained by frontal chromatography technique. Sites energy distribution for this chlorogenic acid MIPs towards various structurally related compounds was explored and the influence of temperature and mobile phase composition on surface sites distribution investigated. Curves of sites number on the MIPs towards sites energy distribution under different conditions was constructed by using the best Langmuir-Freundlich fitting parameter values. From these curves, it was found that the most suitable sites occupied by various compounds was of difference in sites energy distribution and total sites number for the MIPs decreased when the MIPs was measured at higher temperature or/and using higher concentration of glacial acetic acid in mobile phase. In addition, molecule size, shape, functional groups type and number and even mass transfer dynamics might affect retention of analytes on the imprint sites.

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