Energetic heterogeneity of the surface of a molecularly imprinted polymer studied by high-performance liquid chromatography

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Abstract

The influence of thermal annealing on the surface homogeneity of a polymer imprinted against L-phenylalanine anilide (LPA) was examined using high-performance liquid chromatography (HPLC) for the measurement of the adsorption isotherms. The isotherms obtained for LPA and for its enantiomer, D-phenylalanine anilide (DPA) were fitted to the Freundlich (F) equation which accounts for the energetic heterogeneity of the surface with a separate parameter. Changes in the adsorptive properties of the polymer produced by thermal annealing were deduced by comparing the heterogeneity parameters given by the nonlinear regression. These changes were also illustrated by deriving the isosteric heats of adsorption as functions of the amounts adsorbed and by calculating the associated affinity distributions. This latter technique involves an application of the affinity spectrum (AS) combined with the F adsorption model. The plausibility and accuracy of the combination is discussed. It is shown that the derivation of the amplitudes of the affinity distributions from the F parameters is inaccurate, making difficult the proper estimate of the changes in the total population of adsorption sites. In contrast, the AS method gives correct estimates of the parameter that characterizes the slope of the affinity distributions. The results derived from the three sets of results (F model parameters, isosteric heats of adsorption, AS+F method) show consistently that annealing reduces the energetic heterogeneity of the polymer surface for both LPA and DPA. In practice, however, the improvement of the polymer performance in HPLC is relatively limited.   © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Molecularly imprinted polymers (MIP) are modern synthetic materials that are mostly used as highly selective adsorbents [1–4]. The principle of the design of MIPs allows such a degree of customization that they are often compared to natural receptors, such as antibodies or enzymes that are capable of recognizing complex biomolecules. The major advantage of MIPs compared to the traditional adsorbents commonly used in HPLC (e.g. modified silica) is the large diversity of the possible molecular structures against which these materials can be imprinted and the high selectivity of the imprinted polymer for the template. Furthermore, MIPs are much more chemically, mechanically and thermally stable than the fragile biomolecules that are sometimes used for separations but are unable to work in harsh environments. Recent notable examples include MIPs that are targeted to bind peptides and proteins [5,6], sugars [7], pesticides [8], small gas molecules [9] and steroids [10]. MIPs have found numerous applications in different branches of bio/ chemistry, such as solid-phase extraction [11–13], the design and preparation of sensors [8,14–16], immuno-analysis [17,18], catalysis [19] and chiral separations [20–23]. In particular, the development of the synthesis of new MIPs has made new chromatographic stationary phases available for analytical and preparative purposes. Among them, stationary phases synthesized for enantiomeric separations are the most intensely studied materials in this field [24]. The gathered experimental evidence suggests that chromatographic techniques using MIPs as enantioselective adsorbents are very promising for future drug production, for many applications in biotechnology and for the monitoring of some harmful or illegally used substances [25].

MIPs prepared as spherical particles [26–29], superporous monoliths [30–33], films or coatings covering the column wall [34,35] have been successfully used in HPLC and in capillary electrochromatography (CEC) [27–29,32,33]. Despite several advantages discussed above, experimental results have so far been rather disappointing, showing that, in general, MIPs display a most serious drawback, a strong energetic/structural binding-site heterogeneity [36–39]. The origin of the heterogeneity of the surface of MIPs lies in the nonuniformity of the polymerization (imprinting) process, which creates adsorption centers of widely diversified affinity. Thus, the polymer surface is not characterized by a single value of a binding constant (adsorption energy) but by a wide continuous distribution of the adsorption energy. From the microscopic point of view, the mechanism that leads to the formation of nonequivalent adsorption sites can be explained following the rational proposed by Katz and Davis [40] and directly related to the system studied in this work. In the synthesis of an LPA imprinted polymer, there are several sources of surface heterogeneity.

For example, since the imprinting process is based on entrapping the target molecules inside the polymer network, it is highly probable that clusters of molecules can be built into the matrix besides the occlusion of single molecules that takes place in the ideal case. In the next synthesis step, i.e. during the extraction of the template molecules, all of them should, in principle, be removed from the imprinted polymer. Unfortunately, this process is always somewhat incomplete and some residual molecules are left behind, bound in the network. Thus, several different kinds of adsorption sites can potentially be generated during the extraction process: (1) Cavities are formed after removal of a single molecule. (2) Single template molecules remain on the polymer surface. (3) Sites are formed after the cluster is extracted but this process may be incomplete, leaving one or several template molecules inside the cavity. Finally, (4) cavities are formed by the complete extraction of the entire cluster of molecules. Because the ideal (i.e. homogeneous) imprinting process assumes only the first situation, the three remaining are highly unwelcome. Fig. 1 summarizes in a schematic way all the cases described above.

The problem of the binding site heterogeneity of MIPs is difficult to handle both from experimental and theoretical perspectives. The major reason in this case is the lack of a simple method able to predict the changes in the adsorptive properties of MIPs that are induced by modifications of each of the various external factors that influence the imprinting process and the subsequent treatment of the polymer. Thus, most of the attempts aiming at modifying the chromatographic properties of MIPs are based on the old trial-and-error method. To improve the quality of
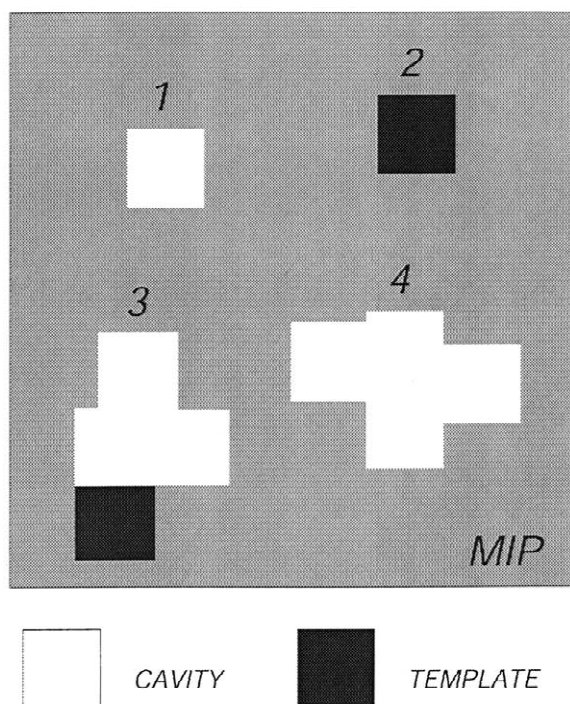
Fig. 1. Possible types of adsorbing sites created upon the imprinting process. See the text for details.

MIPs, i.e. to make their surface more homogeneous, various approaches have been proposed. They include manipulations of all the factors related to the preparation of the material, such as the choice and the relative concentrations of the monomers, the solvent and the reaction temperature. They also include the design of new functional monomers targeted toward specific structural groups of the template [19]. Another possibility is an application of a covalent imprinting protocol, which gives more homogeneous MIPs but is more difficult to carry out [41]. The quality of MIPs can also be influenced by different post-treatments, the most important of which are chemical modifications [38] and thermal annealing [20,22].

The main objective of this work is to study the influence of thermal annealing on the heterogeneity of an LPA imprinted chiral stationary phase. This investigation includes the application of HPLC as an experimental technique for isotherm determination and the subsequent interpretation of the collected data by means of the theory of adsorption on heterogeneous surfaces.

2. Theory

The determination of the energetic and/or structural heterogeneity of real solid surfaces plays a key role in the prediction and optimization of various chromatographic and adsorption processes that are carried out in their presence. One of the most informative characteristics of a heterogeneous surface is the distribution function Γ that appears in the fundamental integral equation [42,43]:

$$q(C) = q^* \int_{\Omega} Q_L(C,E,T) \Gamma(E,T) \, dE \qquad (1)$$

where C denotes the concentration of the solute in the mobile phase, $q(C)$ the adsorbate concentration in equilibrium with the solvent concentration C (overall adsorption isotherm at temperature T), q^* is the saturation capacity of the adsorbent, E is the adsorption energy in the interval Ω, and Q_L is the local model of adsorption isotherm.

The distribution function can be extracted by means of Eq. (1) by solving the inverse problem. In this case, having the experimental adsorption isotherm $q(C)$ and assuming a particular local adsorption isotherm, Q_L, one calculates Γ as a function of the adsorption energy or binding constant, k. The major potential advantage of such an approach is the fact that Γ can, at least in principle, be found for any functional relationship between q and C. It was demonstrated that $\Gamma(E)$ takes a closed form only when $q(C)$ is accounted for by one of the well-known and simple adsorption isotherm models, such as the Freundlich model used in this study. The Freundlich isotherm is an empirical equation of the form

$$q = AC^{\nu} = q^* K_F C^{\nu} \qquad (2)$$

where K_F is the adsorption (binding) equilibrium constant, and ν the heterogeneity parameter, between 0 and 1. However, in most cases and especially when the relationship between q and C is unknown, one has to use special computational techniques to find Γ [43].

Several numerical algorithms have been developed

to solve the integral Eq. (1) [43]. Among them, the affinity spectrum (AS) [44,45] method seems the simplest and the least computationally demanding. It has become the most popular method for the determination of the binding site heterogeneity of materials like MIPs [36–39,46]. Although this method is easy and straightforward, there are some questions regarding its accuracy that demand a discussion. In this section we comment on some practical problems originating from the application of the AS method combined with the Freundlich model.

Let us summarize briefly the basic features of the AS method. The only assumption made is that the local adsorption isotherm Q_L is a Langmuir-type isotherm. Then, by introducing the binding constant

$$k = e^{E/RT} \tag{3}$$

which relates the binding constant on a given site and its adsorption energy, Eq. (1) can be rewritten as follows:

$$q(C) = \int_{-\infty}^{+\infty} \frac{kC}{1+kC} \Phi(k)\, d(\ln k) \tag{4}$$

where

$$\Phi(k) = q^* RT\Gamma(E) \tag{5}$$

An approximate solution of Eq. (4) was derived by Ninomiya and Ferry [47,48] in terms of the theory of the viscoelastic behavior of polymers, by application of the second-order finite difference method. This approach was further adapted to adsorption systems by Hunston [44,45] who obtained the solution of Eq. (4) in the following form

$$\Phi^{AS}(k) = \left| \frac{F_1 - F_2}{2\ln a} - \frac{a[F_3 - F_4 - 2(F_1 - F_2)]}{2(a-1)^2 \ln a} \right| \tag{6}$$

where the parameters

$$F_1 = q\left(\frac{a}{k}\right); \quad F_2 = q\left(\frac{1}{ak}\right); \quad F_3 = q\left(\frac{a^2}{k}\right);$$

$$F_4 = q\left(\frac{1}{a^2 k}\right)$$

are derived from the experimental adsorption isotherm data, a being a constant larger than 1, and typically equal to $10^{0.2}$. The function $\Phi^{AS}(k)$ is called the affinity spectrum. The product of $\Phi^{AS}(k)$

and $d(\ln k)$ gives the number of sites having a binding constant in the interval ($\ln k$ to $\ln k + d(\ln k)$). Calculation of Φ^{AS} requires the interpolation of the experimental adsorption isotherm data over a sufficiently wide range of concentrations. In principle, the range of k over which the affinity spectrum can be determined is limited by $k_{min} = 1/c_{max}$ and $k_{max} = 1/c_{min}$ where c_{min} and c_{max} are, respectively, the smallest and the largest concentrations at which experimental data can be obtained. However, the AS method requires knowledge of q at c_{min}/a^2 and at $c_{max}a^2$, i.e. requires the extrapolation of the data beyond the concentration range within which they were acquired. These values are necessary to calculate F_3 and F_4 (see definitions above). In order to avoid the errors caused by these extrapolation procedures the original interval of concentrations should be reduced to the following one (c_{min}/a^2, $c_{max}a^2$). Fig. 2 illustrates this procedure. The shaded region in the figure represents the effective interval of concentrations that can be used in the calculation of Φ^{AS}. This reduction of the concentration interval, hence of the binding constant interval, makes the
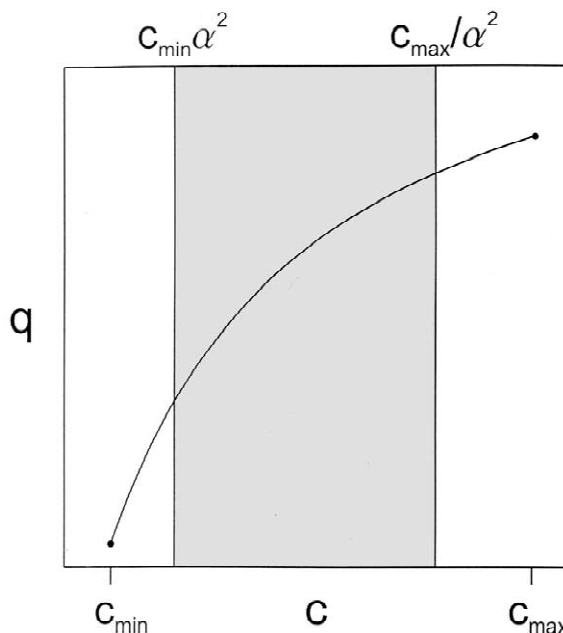

Fig. 2. The effective interval of concentrations (shaded region) that can be used in the reconstruction of the affinity spectrum. The minimal and the maximal concentrations available experimentally are denoted by c_{min} and c_{max}, respectively.

reconstruction of the affinity spectrum incomplete. This effect becomes particularly important when the experimental data are measured over a relatively narrow interval of concentrations. However, by assuming a specific binding model [37,38,46], instead of, for example, using a cubic spline to interpolate the experimental data, one can easily determine Φ^{AS} over the whole interval of k. In the case of the Freundlich model, it is sufficient to insert Eq. (2) into Eq. (6). This procedure results in a general function for the affinity distribution corresponding to the Freundlich model, given in the following form

$$\Phi_F^{AS}(k) = AS(\nu)e^{-\nu E/RT} = AS(\nu)k^{-\nu} \qquad (7)$$

$$S(\nu) = \frac{1}{2\ln a}\left|a^{\nu} - a^{-\nu} - \frac{a[(1-a^{\nu})^2 - (1-a^{-\nu})^2]}{(a-1)^2}\right| \qquad (8)$$

Eq. (7) is similar to the exact solution of Eq. (4) obtained for the Freundlich model [43,49], that is

$$\Phi_F(k) = A\frac{\sin(\pi\nu)}{\pi}k^{-\nu} \qquad (9)$$

Obviously, both ϕ_F^{AS} and ϕ_F are exponential functions with the same decay parameter. Therefore, the AS method predicts correctly the overall shape (inclination) of the energy distribution function. However, it does not predict correctly the amplitude (i.e. the pre-exponential factor). The reason can be explained by examining the basic properties of Eqs. (7) and (9).

The amplitudes of both the exact and the approximate distribution functions depend on the heterogeneity parameter. They differ merely by the substitution of $S(\nu)$, given by Eq. (8), in Eq. (7) for $f(\nu) = \sin(\pi\nu)/\pi$ in Eq. (9). We note that both functions have the same limit when ν tends toward 0. Fig. 3 shows a plot of $S(\nu)$ versus ν for different values of $x = \log a$ (solid lines) and the correct solution (dashed line denoted F). It shows that the AS method gives more accurate results for strongly heterogeneous surfaces. Also, $S(\nu)$ approaches $f(\nu)$ when ν tends toward 0 and is always very close when $\nu < 0.2$. This result confirms the fact that, from a mathematical point of view, the AS method becomes more accurate when a tends to 1. From the
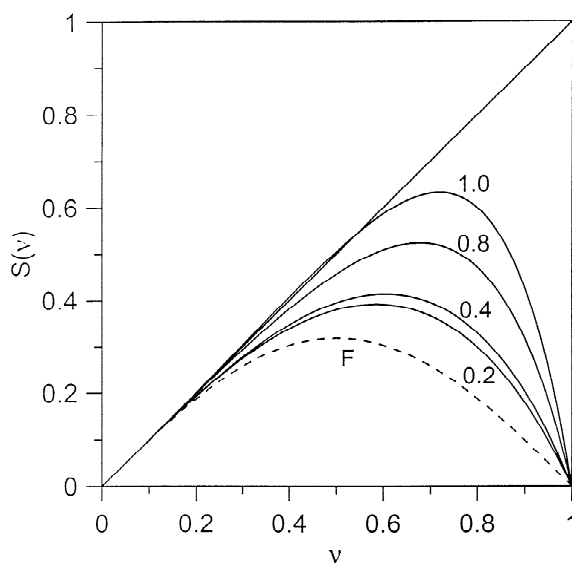

Fig. 3. The amplitude of the affinity spectrum as a function of the heterogeneity parameter, predicted by the AS method. The solid lines represent data calculated by means of Eq. (7) with $a = 10^x$, where $x = 0.2$, 0.4, 0.8 and 1. The dashed line marked by F denotes the exact dependence.

symmetry of the plot of $f(\nu)$ versus ν, we derive that its slope approaches -1 when ν tends toward 1. On the other hand, the slope of $S(\nu)$ becomes equal to $-(a+1)^2/2a$ in the vicinity of 1 and tends toward -2 when a tends toward 1. This means that the amplitude of the approximate distribution function decreases much faster with increasing ν in this region.

In conclusion, it seems that the AS method combined with the Freundlich model can be more useful for the estimation of the relative number, hence the proportion of adsorption sites characterized by different values of E than for the estimation of the absolute numbers of these adsorption sites, hence in the detection of possible changes in the number of high-energy sites.

The above discussion refers to the particular mathematical properties of $S(\nu)$ that lead to errors in the reconstruction of the distribution function. There is, however, one more drawback of the approach just described. It originates from the intrinsic properties of the Freundlich model. In order to estimate the total number of adsorption sites (i.e. the saturation

capacity of the isotherm), one has to calculate the following integral

$$q^* = \int_{\ln k_m}^{+\infty} \Phi_F(k) \, d(\ln k) \tag{10}$$

with

$$k_m = e^{\varepsilon/RT} \tag{11}$$

where ε is the minimum value of the adsorption energy. Unfortunately, the lower limit of this integral, i.e. the left-hand side cut-off of the distribution function, cannot be determined in the case of the Freundlich model since

$$K_F = \nu k_m^{\nu} / f(\nu) \tag{12}$$

hence k_m, is not directly available from the experimental data. The only parameter available is the lumped parameter A in Eq. (2). Therefore, the total number of adsorption sites cannot be calculated unless k_m is known. Another important question is how do the external factors like thermal annealing or the different other possible chemical post-treatments influence k_m? This problem is particularly important for the interpretation of the results presented here. It will be discussed in the following sections.

3. Experimental

The adsorption isotherms of LPA and DPA on an LPA-imprinted copolymer used as the stationary phase in HPLC were determined by conventional frontal analysis. A solution of an aqueous phosphate buffer (pH 5.8) in acetonitrile (3:7, v/v mixture) was used as the mobile phase. The experiments were carried out using laboratory-made columns packed with the native polymer and the one previously annealed at 120 °C.

Detailed description of the experimental set-up and of all the experimental procedures, including the thermal treatment of the stationary phase, were reported elsewhere [22,23].

4. Results and discussion

The single-component experimental adsorption-

isotherm data acquired for both enantiomers were fitted to several of the isotherm models that account for energetic heterogeneity of the stationary phase. These models included the Langmuir–Freundlich (LF), the Tóth (T), and the generalized LF (GLF) isotherm equations. Unfortunately, none of the above models was able to predict correctly the behavior of the experimental isotherms. The numerical algorithm used to fit the data was divergent in most cases and its application resulted in physically unrealistic values of the isotherm parameters. On the other hand, fitting of these data to the Freundlich equation (Eq. (2)) gave an excellent agreement, demonstrated by low residuals. The fits obtained were characterized by values of R^2 varying typically from 0.9985 to 0.9999. The isotherms calculated with this model are illustrated in Figs. 4 and 5. They correspond to the native and the annealed polymer, respectively. The
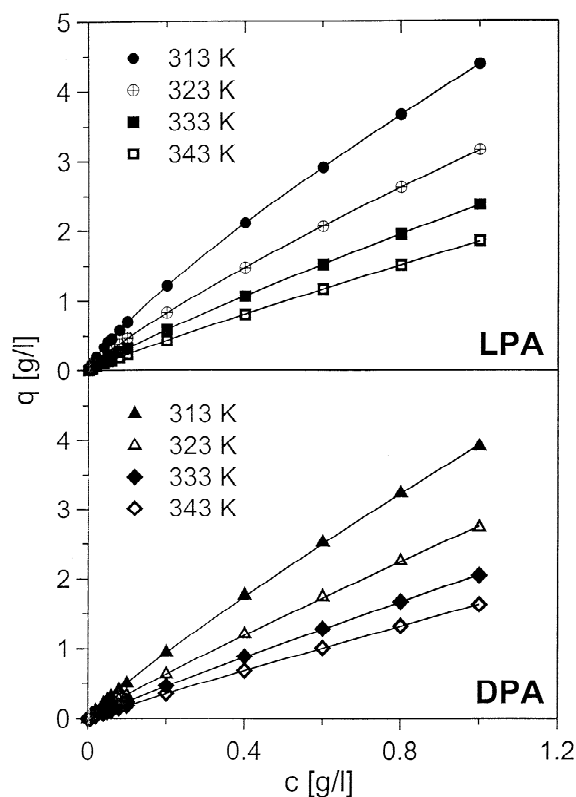

Fig. 4. Adsorption isotherms of LPA (top) and DPA (bottom) obtained for the native polymer. The symbols denote experimental data while the solid lines are their fits calculated using the Freundlich equation.
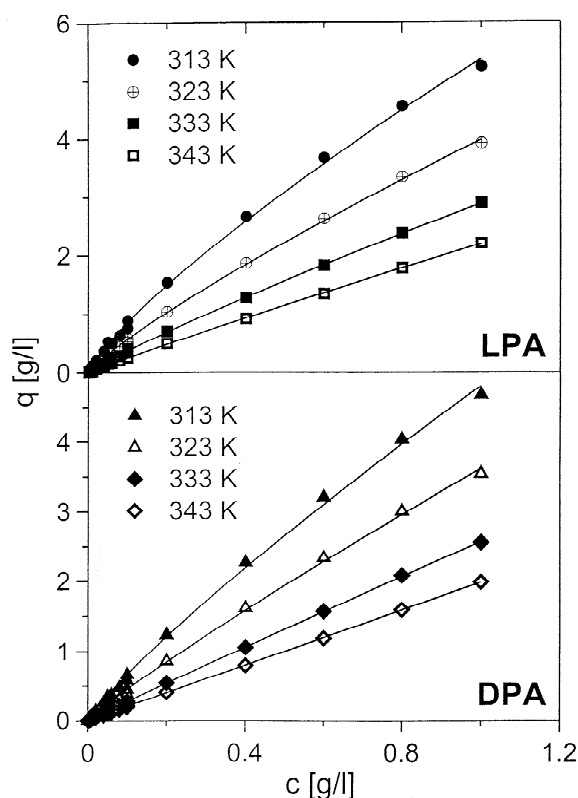
Fig. 5. Adsorption isotherms of LPA (top) and DPA (bottom) obtained for the annealed polymer. The symbols denote experimental data while the solid lines are their fits calculated using the Freundlich equation.

excellent accuracy of the Freundlich model observed in our case is, as mentioned earlier, a mere consequence of the experimental limitations and does not originate from some specific character of the adsorption system considered. In particular, this situation comes from the fact, because of the low solubility of the enantiomers in the mobile phase, their adsorption isotherms were measured over a narrow concentration range, spanning only the sub-saturation region. Such a situation is not unusual of the systems encountered in the study of the template/ MIPs described in the literature [37].

From a preliminary analysis of the curves shown in Figs. 4 and 5, it follows that thermal annealing does not influence strongly the shape of the adsorption isotherms. On the other hand we observe that q taken at constant C is, in general, larger for the annealed (A) than for the native (N) MIP. This is

true for LPA as well as for DPA. For example, for LPA at 40 °C, we have $q(C = 0.8 \text{ g/l})$ equals 3.67 for N and 4.56 for A while at 70 °C, q is equal to 1.52 for N and 1.79 for A. A similar increase in q upon thermal treatment takes place in the case of DPA. Here we observe that $q(C = 0.8 \text{ g/l})$ is 3.24 for N and 4.04 for A at 40 °C and 1.33 for N and 1.59 for A at 70 °C. This suggests that the thermal annealing produces an increase in the saturation capacity. However, as we will demonstrate later, this conclusion may be incorrect due to the particular properties of the Freundlich equation that were described earlier.

The influence of thermal annealing on the heterogeneity of the stationary phase was examined in more detail by comparing the isotherm parameters obtained for the native and the annealed MIPs. Fig. 6 shows the changes in the heterogeneity parameter, ν, caused by the thermal treatment of the polymer. The symbols represent the experimental data determined at four temperatures (40, 50, 60 and 70 °C) while the solid lines are their linear regressions. The results shown in Fig. 6 indicate that the adsorbing surfaces of both the native and the annealed polymer are characterized by a low to moderate degree of the energetic heterogeneity $(0.858 < \nu < 0.938)$. Of course, the annealed polymer exhibits, in general, a higher degree of homogeneity since ν becomes closer to 1 when annealing is applied. As is clearly seen from Fig. 6, the thermal treatment of the polymer causes ν to increase for both enantiomers. For example, ν increases from 0.897 to 0.938, i.e. by ~4.5%, for LPA at 70 °C. The only exceptions are the values of ν obtained for DPA at temperatures lower than 60 °C.

However, for both isomers we observe that ν increases linearly with the temperature of the adsorption process. This effect, also observed previously in different experimental systems [43] is consistent with theoretical predictions which indicate that ν should be proportional to T.

The influence of thermal annealing on the parameter A (see Eq. (2)) at different temperatures is illustrated in Fig. 7. In all cases studied, $\ln A$ is a linear function of the reciprocal temperature and A decreases exponentially with increasing temperature, following a classical Van't Hoff plot. In principle, this dependence could originate from the tem-
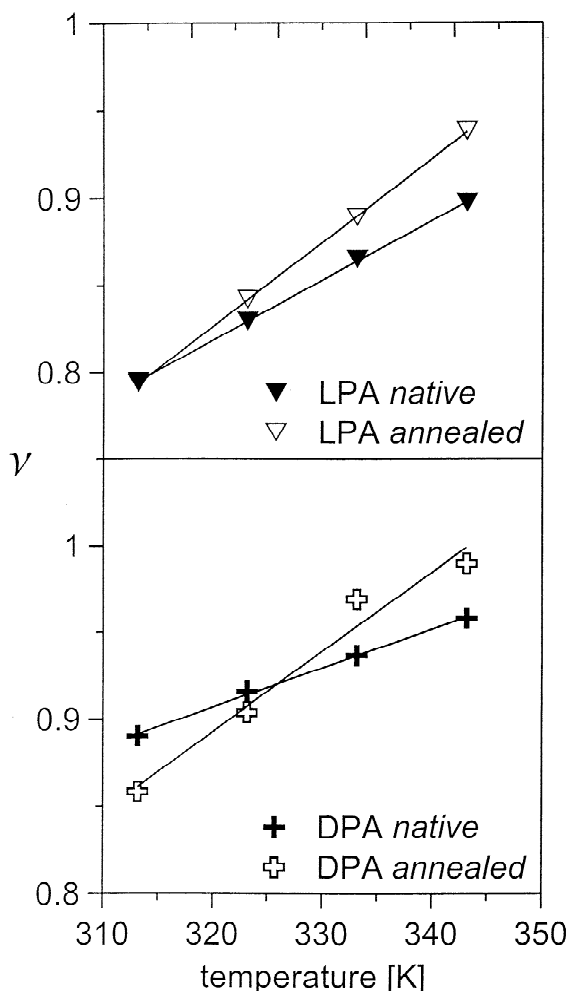
Fig. 6. Temperature dependence of the heterogeneity parameter. The symbols represent the experimental data while the solid lines are their linear regressions.

Fig. 7. Dependence of ln A on reciprocal temperature. The symbols represent the experimental data while the solid lines are their linear regressions.

parallel and vertically shifted. This shows that thermal annealing of the polymer produces an increase in ln A for both LPA and DPA, regardless of the temperature. As mentioned earlier, this effect suggests that the thermal annealing does produce an increase in the saturation capacity, q^*. However, taking the above observations into account, it is difficult to determine whether q^* alone increases upon thermal annealing. The reason is that A is the product of two parameters (cf. Eq. (2)) which can both be influenced by annealing. Unfortunately, with the Freundlich model, we cannot estimate q^* and K_F separately. Thus, changes in q^* induced by thermal annealing are difficult to acknowledge at this stage. In order to describe adsorption on MIPs more accurately, additional information is needed. This would involve the independent determination of the saturation capacity and the adsorption equilibrium constant. Measurements of the adsorption isotherms over a sufficiently wide range of C (up to the saturation region) and the subsequent fitting of the data to one of the isotherm models, e.g. the LF, T or GLF model, would provide this information. Unfortunately, in many phase systems such as the one studied here, the solubility of the solutes in the mobile phase is limited.

A last piece of evidence illustrating the reduction of the heterogeneity of the MIP surface caused by

perature-induced changes in q^* as well as from corresponding changes in K_F. However, the theoretical considerations reported earlier showed that ln A and ln K_F exhibit a similar dependence on T (see Eqs. (2), (11) and (12)). Therefore, it is more probable that, for a given enantiomer, the observed decrease in A with increasing T arises only from temperature dependence of K_F while it seems less probable that q^* varies considerably with T. The latter conclusion is also supported by the thermal stability analysis of the MIPs investigated in the experimental work [20,22,50].

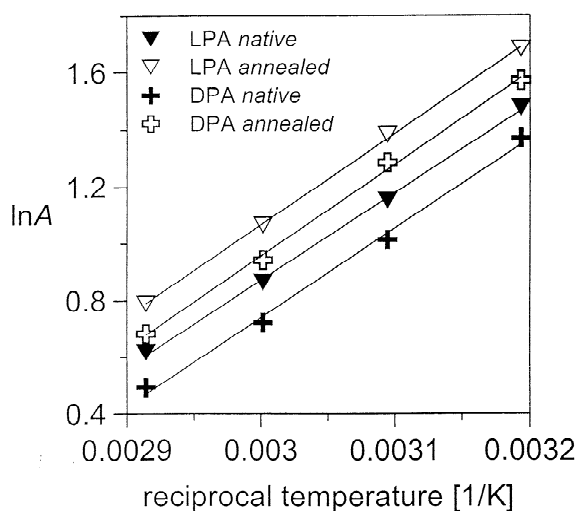The straight lines shown in Fig. 7 are almost

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the thermal treatment is given in Fig. 8. This figure shows plots of the isosteric heat of adsorption, Q_s, of LPA on the native (top part) and on the annealed MIP (bottom part) as a function of the surface concentration. The isosteric heat of adsorption was calculated according to the following equation

$$Q_s = RT^2 \left(\frac{\partial \ln C}{\partial T} \right)_q \qquad (13)$$

The results shown in Fig. 8 were obtained by interpolation of the experimental adsorption isotherms shown in Figs. 4 and 5. A detailed description
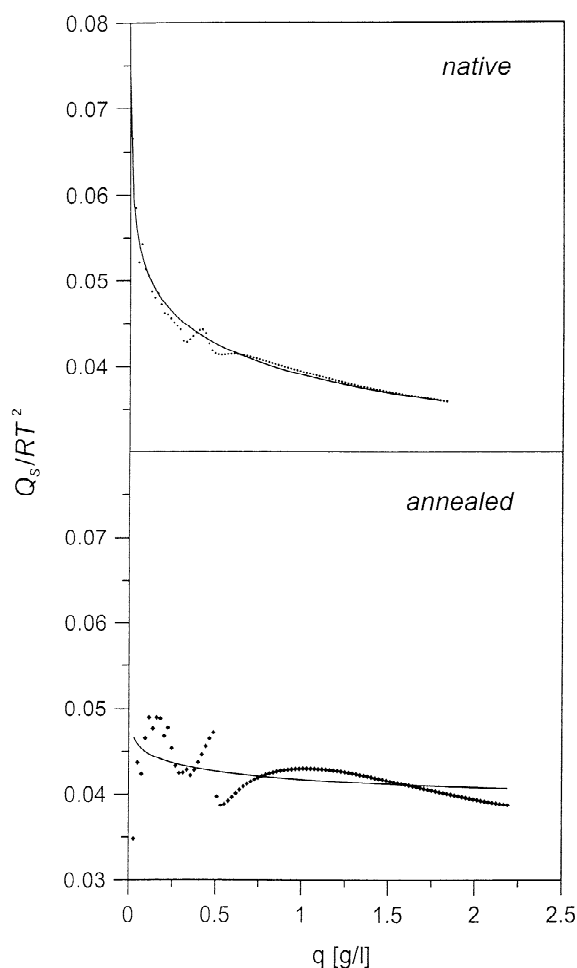

Fig. 8. Isosteric heat of adsorption of LPA on the native polymer (top) and on the annealed one (bottom) as a function of the amount adsorbed. The symbols represent the data calculated by means of Eq. (13) while the solid lines are their logarithmic regressions.

of this straightforward method of deriving the isosteric heat of adsorption can be found elsewhere [51]. Although the values of Q_s derived from Eq. (13) are far less accurate than those afforded by direct calorimetric measurements [52], the result gives basic qualitative information regarding the modifications made to the adsorption system. It is well known that the behavior of the experimental isosteric heats of adsorption is much more sensitive to the nature of the solute–adsorbent interactions than that of the corresponding isotherms [53]. Since Q_s measures the amount of energy released during adsorption, it can be easily used to derive the energetic properties of a given surface. The analysis of the shape of the curve Q_s versus q provides valuable information on the adsorption mechanism. In the top part of Fig. 8, it is seen that Q_s decreases sharply with increasing amount adsorbed. This effect is a typical manifestation of the energetic heterogeneity of the surface [42,43]. It is explained as follows. At very low solute concentrations, hence at low q, the adsorption sites having the highest affinity are occupied first. Thus, if there are a few high-energy sites, the released Q_s is relatively high. Then, with increasing q, the remaining sites are progressively filled.

Because these sites are characterized by decreasing affinity, Q_s decreases with increasing amount adsorbed. A similar situation takes place in the case of the annealed MIP. Here, however, the initial decrease in Q_s is much less intense. The curve shown in the bottom part of Fig. 8 is much flatter than the one above, for the native MIP. This suggests that the surface has become more homogeneous after the thermal treatment of the MIP. It is probable that the number of high energy sites has decreased and that they were converted into moderate to low energy sites. For this reason, the curve corresponding to the annealed MIP is above the tail of the curve obtained for the native polymer.

From Eqs. (2) and (13), it follows that

$$Q_s = \phi \log q + \gamma \qquad (14)$$

where ϕ and γ are numerical coefficients. In Fig. 8, we showed as a solid line the best fits of Q_s to Eq. (14). We observe (Fig. 8, top) that the curve determined from the experimental adsorption isotherms measured for the native polymer (symbols) follows

almost exactly Eq. (14). In the case of the annealed MIP (Fig. 8, bottom), the agreement between the experimental data and Eq. (14) is less accurate but still satisfactory.

To examine the influence of thermal annealing on the surface properties of the MIP we also calculated the corresponding affinity spectra (AS), by means of Eq. (10) with $a = 10^{0.2}$. We analyzed the systems at 70 °C because this is the temperature at which the largest changes in ν were observed. The top part of Fig. 9 compares the AS curves obtained for LPA adsorbed on the native and the annealed MIP at 70 °C. The bottom part compares the same data for DPA. The numbers by each curve are the associated values of the heterogeneity parameter ν. For both enantiomers, the distribution functions of the binding constant are significantly affected by thermal annealing. For LPA, however, this effect is smaller than for DPA. As seen in the figure, the changes in ν produced by thermal treatment are relatively small (~4.5%) for the system studied.

Therefore, shifts in the position of the distribution functions can be attributed mainly to changes in their amplitudes. From Fig. 3, it follows that $S(\nu)$ ($a = 10^{0.2}$) is a decreasing function of ν for ν greater than ~0.6. The decrease in $S(\nu)$ with increasing ν becomes faster when ν approaches 1. This explains why, for LPA, we observe a smaller shift in the distribution function upon annealing. Of course, the amplitude depends on A which, as seen in Fig. 7, increases upon thermal annealing. However, the increases in A are similar for both enantiomers, i.e. from 1.86 to 2.202 g/l and from 1.64 to 1.98 g/l for LPA and DPA, respectively. Furthermore, these increases cannot compensate the simultaneous decreases in $S(\nu)$. As a result, the overall effect is a decrease in the amplitude of both distribution functions.

The results described above suggest that the total number of sites, hence the adsorption capacity of the MIPs, does decrease upon thermal treatment. For example, one may conclude that for both LPA and DPA, the area under the corresponding curves shown in Fig. 9 decreases when annealing is applied. However, this conclusion is valid only if one assumes that the region of the Freundlich isotherm used (i.e. the left-hand side cut-off of Φ_F^{AS}) is the same for all the distribution functions considered.
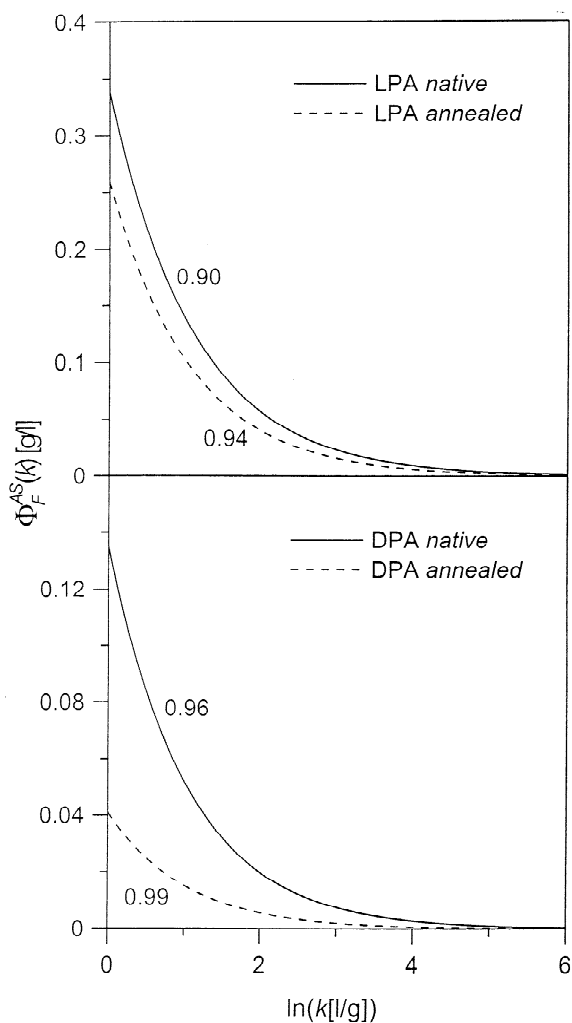

Fig. 9. The influence of thermal annealing on the affinity distributions. The curves shown in the figure correspond to the experimental data measured at 70 °C and they were calculated by means of the AS method (Eq. (7)). The numbers describing the curves are the values of the heterogeneity parameter corresponding to a given adsorption system.

Unfortunately, it seems that this approach has but a limited physical basis. Since the overall properties of the surface change upon thermal annealing, its energetic profile does so too and it is hard to accept that k_m remains constant. For example, if k_m was lower for the annealed than for the native polymer (which does not seem impossible), one would obtain a completely different conclusion. In this case, in

order to calculate q^* for the annealed polymer, it would be necessary to integrate Φ_F^{AS} with ln k from an interval wider than for the native polymer. Therefore, although, in general $\Phi_F^{AS}(N) > \Phi_F^{AS}(A)$, the area under the curve corresponding to the annealed polymer may be equal to or even greater than for the native one. As a result, the changes in q^* estimated as described above may suggest that the total number of adsorbing sites does not change or even increases when the stationary phase is subjected to thermal annealing.

In addition to the first drawback described above, which is inherent to the Freundlich model, there is a second one that is related to the behavior of $S(\nu)$. This was discussed in detail in the theoretical section. As an illustration, we compare in Fig. 10 the results obtained by means of the AS method with the exact solution of Eq. (1). The figure shows the affinity spectra calculated for LPA at 70 °C. The solid and the dashed lines with crosses correspond to the AS method while the analogous lines without crosses represent the data calculated by means of Eq. (9). Comparison of these curves demonstrates that the AS method overestimates both distribution functions. The observed effect originates from the differ-

ence between the shapes of $S(\nu)$ and $f(\nu)$ in the vicinity of 1 (Fig. 3).

5. Conclusion

The results obtained in this work indicate that thermal annealing causes a slight reduction of the binding site heterogeneity of an imprinted stationary phase. The temperature-induced alterations of the microscopic structure of the adsorbing surface predicted with the Freundlich model seem moderate and have only little influence on the relative proportion of selective to non-selective sites. In the case of both enantiomers the increase in the heterogeneity parameter observed upon thermal annealing is about 4.5%. On the other hand, annealing can strongly influence the saturation capacity of the polymer. This question cannot be answered with the help of the AS method combined with the Freundlich model. The saturation capacity and the minimal adsorption energy that determine the applicability region of the Freundlich equation cannot be determined as separate values. Thus, it is impossible to estimate q^*, either by fitting the experimental data to an adsorption isotherm or by integrating the calculated affinity spectra. Furthermore, the AS method introduces an error in the amplitude of the spectra when the Freundlich model is assumed. This error is smaller for strongly heterogeneous surfaces and tends toward 0 for heterogeneity parameters less than ~0.2. Fortunately, the inclination of the exponential distributions of the binding constant is predicted correctly by the AS method. For these reasons, the AS approach can be used only to estimate relative, not absolute changes in the population of adsorption sites.

As was demonstrated, the Freundlich isotherm equation applies not because it is ideally suited to account for the heterogeneity of MIPs but because the isotherms can be measured only in the low-concentration or sub-saturation region. This question is particularly important since a vast majority of the systems reported in the literature follow a similar behavior. We believe that other models, e.g. the LF, T, or GLF models could describe better the characteristics of adsorption on MIPs. This is because, with these models, the saturation capacity and the minimum or average value of the adsorption energy can
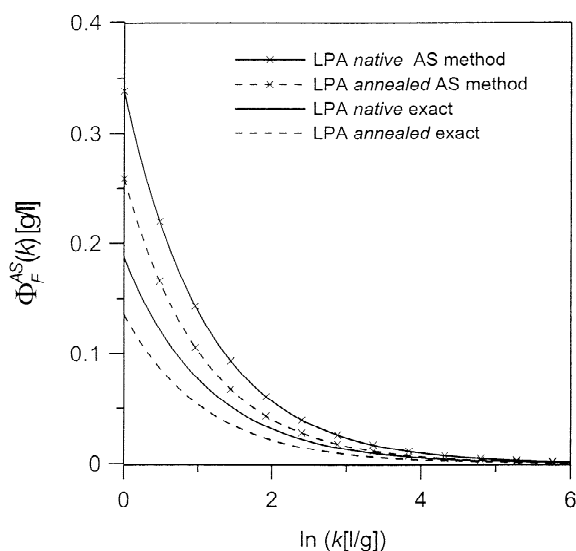

Fig. 10. Comparison between the affinity spectra calculated for LPA at 70 °C by means of the AS method (lines with crosses) and by means of Eq. (9) being the exact solution (lines without symbols).

be estimated more easily from fitting the experimental isotherms to a model. The only condition that must be fulfilled in this case is that measurements are made in a sufficiently wide concentration range (close to the isotherm plateau). Therefore, it seems that studies of adsorption on MIPs should be extended to higher concentrations, in order to understand better the influence of thermal or chemical post-treatments on the adsorptive properties of these materials.

Acknowledgements

References

[1] B. Sellergren (Ed.), Molecularly Imprinted Polymers. Man Made Mimics of Antibodies and Their Applications in Analytical Chemistry, Elsevier, Amsterdam, 2001.

[2] R.A. Bartsch, M. Maeda, Molecular and Ionic Recognition with Imprinted Polymers, Oxford University Press, Washington, DC, 1998.

[3] G. Wulff, Angew. Chem. Int. Ed. Engl. 34 (1995) 1812.

[4] K. Mosbach, O. Ramström, Biotechnology 14 (1996) 163.

[5] B.R. Hart, K.J. Shea, J. Am. Chem. Soc. 123 (2001) 2072.

[6] A. Rachkov, N. Minoura, Biochim. Biophys. Acta 1544 (2001) 255.

[7] S. Striegler, Tetrahedron 757 (2001) 2349.

[8] A.L. Jenkins, R. Yin, J.L. Jensen, Analyst 126 (2001) 798.

[9] A.C. Shrama, A.S. Borovik, J. Am. Chem. Soc. 122 (2000) 8946.

[10] K. Sreenivasan, J. Appl. Polym. Sci. 68 (1998) 1863.

[11] B. Sellergren, Anal. Chem. 66 (1994) 1578.

[12] M.T. Mouldon, L.H. Stanker, Anal. Chem. 69 (1997) 803.

[13] J. Matsui, M. Okada, T. Takeuchi, Anal. Commun. 34 (1997) 85.

[14] K. Haupt, K. Mosbach, Chem. Rev. 100 (2000) 2495.

[15] Ch. Luo, M. Liu, Y. Mo, J. Qu, Y. Feng, Anal. Chim. Acta 428 (2001) 143.

[16] F.W. Scheller, U. Wollenberger, A. Warsinke, F. Lisdat, Curr. Opin. Biotechnol. 12 (2001) 35.

[17] G. Vlataki, L.I. Anderson, R. Muller, K. Mosbach, Nature 361 (1993) 645.

[18] L.I. Anderson, Anal. Chem. 68 (1996) 111.

[19] G. Wulff, Chem. Rev. 102 (2002) 1.

[20] B. Sellergren, K.J. Shea, J. Chromatogr. 635 (1993) 31.

[21] B. Sellergren, K.J. Shea, J. Chromatogr. A 690 (1995) 29.

[22] Y.-B. Chen, M. Kele, P. Sajonz, B. Sellergren, G. Guiochon, Anal. Chem. 71 (1999) 928.

[23] P. Sajonz, M. Kele, G. Zhong, B. Sellergren, G. Guiochon, J. Chromatogr. A 810 (1998) 1.

[24] N.M. Maier, P. Franco, W. Lindner, J. Chromatogr. A 906 (2001) 3.

[25] G. Masci, G. Casati, V. Crescenzi, J. Pharm. Biomed. Anal. 25 (2001) 211.

[26] A.G. Mayes, in: B. Sellergren (Ed.), Molecularly Imprinted Polymers. Man Made Mimics of Antibodies and Their Applications in Analytical Chemistry, Elsevier, Amsterdam, 2001, p. 305.

[27] C. Sulitzky, B. Rückert, A.J. Hall, F. Lanza, K. Unger, B. Sellergren, Macromolecules 35 (2002) 79.

[28] L. Schweitz, P. Spègel, S. Nilsson, Analyst 125 (2000) 1899.

[29] M. Quaglia, E. De Lorenzi, C. Sulitzky, G. Massolini, B. Sellergren, Analyst 126 (2001) 1495.

[30] J. Matsui, T. Kato, T. Takeuchi, M. Suzuki, K. Yokoyama, E. Tamiya, I. Karube, Anal. Chem. 65 (1993) 2223.

[31] B. Sellergren, J. Chromatogr. A 673 (1994) 133.

[32] L. Schweitz, L.I. Anderson, S. Nilsson, Anal. Chem. 69 (1997) 1179.

[33] L. Schweitz, L.I. Anderson, S. Nilsson, J. Chromatogr. A 792 (1997) 401.

[34] Z.J. Tan, V.T. Remcho, Electrophoresis 19 (1998) 2055.

[35] O. Brüggemann, R. Freitag, M.J. Whitcombe, E.N. Vulfson, J. Chromatogr. A 781 (1997) 43.

[36] R.J. Umpleby II, M. Bode, K.D. Shimizu, Analyst 125 (2000) 1261.

[37] R.J. Umpleby II, S.C. Baxter, Y. Chen, R.N. Shah, K.D. Shimizu, Anal. Chem. 73 (2001) 4584.

[38] R.J. Umpleby II, G.T. Rushton, R.N. Shah, A.M. Rampey, J.C. Bradshaw, J.K. Berch Jr., K. Shimizu, Macromolecules 34 (2001) 8446.

[39] R.J. Umpleby II, S.C. Baxter, M. Bode, J.K. Berch Jr., R.N. Shah, K.D. Shimizu, Anal. Chim. Acta 435 (2000) 35.

[40] A. Katz, M.E. Davis, Macromolecules 32 (1999) 4113.

[41] M.A. Khasawneh, P.T. Vallano, V.T. Remcho, J. Chromatogr. A 992 (2001) 87.

[42] W. Rudziński, D.H. Everett, Adsorption of Gases on Heterogeneous Surfaces, Academic Press, New York, 1992.

[43] M. Jaroniec, R. Madey, Physical Adsorption on Heterogeneous Solids, Elsevier, Amsterdam, 1988.

[44] D.L. Hunston, Anal. Biochem. 63 (1975) 99.

[45] A.T. Thakur, P.J. Munson, D.L. Hunston, D. Rodbard, Anal. Biochem. 103 (1980) 240.

[46] T. Takeuchi, T. Mukawa, J. Matsui, M. Higashi, K.D. Shimizu, Anal. Chem. 73 (2001) 3869.

[47] K. Ninomiya, J.D. Ferry, J. Colloid Sci. 14 (1959) 36.

[48] J.D. Ferry, Viscoelastic Properties of Polymers, Wiley, New York, 1980.

[49] M.S. Da Rocha, K. Iha, A.C. Falfiros, E.J. Corat, M.E.V. Surez-Iha, J. Colloid Interface Sci. 185 (1997) 493.

[50] J. Svenson, I.A. Nicholls, Anal. Chim. Acta 435 (2001) 19.

[51] J. Ościk, Adsorption, PWN, Warsaw, 1982.

[52] W.-Y. Chen, C.-S. Chen, F.-Y. Lin, J. Chromatogr. A 923 (2001) 1.

[53] J. Narkiewicz-Michałek, P. Szabelski, W. Rudziski, Langmuir 15 (1999) 6091.