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Method for determination of the chromatographic surface area of reversed-phase surfaces

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Abstract

A method for determination of the chromatographically accessible area of a reversed-phase column is developed. The electrostatically modified linear adsorption isotherm, in combination with the solution of the linearized Poisson–Boltzmann equation, forms the theoretical basis of the developed method. The column's surface area is calculated by applying this theory to measured adsorption isotherms of charged amphiphiles. The consistency of the method is tested for different negatively charged amphiphiles using mobile phases of different ionic strengths, pH values and different methanol content. It is found that the chromatographically accessible column area is unchanged for different amphiphilic solutes and when buffer solutions of different compositions are used as the mobile phase (222–230 m^2/g). The surface area increases to approximately 250 m^2/g when the mobile phase contains 5 or 10% methanol. The developed method can be used by column manufacturers and by the practising chromatographer to characterize reversed-phase columns. The method is also of theoretical interest since it can be used to calculate the column's phase ratio, which is needed to obtain adsorption entropies.

Keywords: Surface area; Adsorption isotherms; Stationary phases, LC; Toluenesulfonate

1. Introduction

Reversed-phase liquid chromatography (RPLC) material usually consists of porous silica particles to which *n*-alkyl groups are bound to give a hydrophobic surface. The pore diameter of common commercial RPLC phases generally range from 60–300 Å and the porosity of the silica particles results in a high internal surface area. The area of the bare silica surface can be determined experimentally by the BET method in which the nitrogen adsorption isotherm is measured and the so-called nitrogen BET value is calculated. This value for the area is often specified by the manufacturer and is also often used by the user, e.g. [1–4]. However, when the silica has been modified with long *n*-alkyl chains, the area obtained by the BET method on pure silica may not be correct, since the changed surface properties probably change the surface area as well. Another question concerning the use ofthe BET method in RP chromatography is that N_2 molecules penetrate into micro-pores and cracks that are inaccessible for the larger solute molecules and these consequently experience a much smaller surface. This was recently discussed in a work by Farin and Avnir [5] where it was concluded that for proper calculation of the accessible area of porous silica for silylating reagents, either the effective area for the ligand or the

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fractal dimension of the surface should be used and not the nitrogen BET value.

In this paper, a method for determination of the accessible surface area of a RP-18 phase under typical RP conditions is presented and such a method has, to our knowledge, not been accomplished before. The presented method is of great interest for column characterization, using the measured area as the characteristic parameter. Column characterization is of interest, for example, for the control of long-term stabilities and tests of reproducibility from column to column. Techniques that were used previously for the characterization of stationary phases are spectroscopic (IR, NMR, ESCA, fluorescence), thermal, elemental analysis and chromatographic techniques, e.g. [3,6,7], including measurements of retention indices [8].

Furthermore, for the prediction of a specific capacity factor the stationary phase area determined under chromatographic conditions is of interest. For the prediction of a capacity factor using a particular column knowledge about the phase ratio, as well as the free energy of retention, facilitates the calculations [9,10]. The determination of the phase ratio is not straightforward in RP chromatography, especially with respect to the definition of the volume of the stationary phase. Under the conditions used in this method, the phase ratio is defined as the stationary phase area divided by the column dead volume and the phase ratio can be calculated subsequently from the determined area. It is also of theoretical interest to know the column's phase ratio. From retention measurements at various temperatures for a given solute, the enthalpy of adsorption can be calculated. When the phase ratio is known, the entropy of adsorption can be calculated from such measurements [11]. The phase ratio of the column is also of interest for calculations where the adsorption isotherm of the organic compounds forms the basis, e.g., for explaining deviations of isotherm data that have been obtained for different columns [12] or for calculations of peak profiles [7].

The presented method is based on measurement of the adsorption isotherm for a negatively charged amphiphile to the stationary phase surface. The electrostatic surface potential modified Langmuir isotherm [13–16] that was used previously in combination with ion pair chromatography has been used in this work for the description of the experimentally measured isotherms. In this isotherm, the electrostatic surface potential created by the adsorbed charged amphiphile is included. Combination of this isotherm and the solution of the linearized Poisson–Boltzmann equation in cylindrical coordinates have been used to derive an equation of a straight line, where the area is calculated from the slope. In this work, the adsorption isotherms using different chromatographic systems and different negatively charged amphiphiles have been measured experimentally by frontal analysis.

2. Theory

2.1. Method of frontal analysis

Isotherm frontal analysis was used for the determination of amphiphile adsorption. According to chromatographic theory, the velocity, u_f (m^3/s), with which a front moves through the column is given by Eq. (1):

$$u_f = \frac{u_0}{1 + \frac{A}{V_0} \cdot \frac{n_A}{c_A}} \quad (1)$$

where u_0 is the flow velocity of the mobile phase (m^3/s), A is the area of the stationary phase (m^2), V_0 is the dead volume of the column (m^3), n_A is the amount of solute adsorbed (mol/m^2) and c_A is the mobile phase concentration of the solute (mol/m^3).

A relationship between the total amount of solute adsorbed on the stationary phase and its concentration in the mobile phase can be derived from Eq. (1). Since n_A equals the adsorbed amount of solutes per m^2, it can be written as:

$$n_A = N_A/A \quad (2)$$

where N_A is the total amount adsorbed on the stationary phase and A is the area of the stationary phase. From Eqs. (1,2), combined with the relationships $u_0 = V_0/t_0$ and $u_f = V_0/t_r$, respectively, the following equation is obtained:

$$N_A = u_0 \times c_A(t_r - t_0) \quad (3)$$

t_r is the elution time for the front (s) and t_0 is the column dead time (s).

2.2. The adsorption isotherm

The relationhip between the concentration of species in the mobile and the stationary phase at equilibrium is described by the adsorption isotherm. For charged amphiphiles, the electrostatically modified Langmuir isotherm has been used previously for describing the adsorption isotherm for the ion pair reagent in ion pair chromatography. This adsorption isotherm includes a term for the electrostatic potential created at the surface by the charged amphiphile and is derived in the following way:

Consider the equilibrium:

$$A(l) + S \Leftrightarrow AS \tag{4}$$

where A(l) and AS represent the amphiphile in the mobile phase and on the stationary phase, respectively, and S is the part of the surface that is not occupied by an amphiphile. The condition for equilibrium is:

$$\mu_A + \mu_S = \mu_{AS} \tag{5}$$

where μ represents the electrochemical potential of the species. In the ideal case, the electrochemical potential for each species is:

$$\mu_A = \mu_A^0 + RT \ln c_A \tag{6}$$

$$\mu_S = \mu_S^0 + RT \ln X_S \tag{7}$$

$$\mu_{AS} = \mu_{AS}^0 + RT \ln X_{AS} - z_A F \psi_0 \tag{8}$$

where

$$X_S + X_{AS} = 1 \tag{9}$$

F is the Faraday constant, μ_0 is the electrochemical potential of the standard state, z_A is the charge of the amphiphile and Ψ_0 is the electrostatic potential. This electrostatic potential is generated by the amphiphile itself at the stationary phase surface. X_S and X_{AS} are the fractions of unoccupied and occupied surface, respectively.

The fraction of the stationary phase occupied by amphiphilic molecules is:

$$X_{AS} = n_A / n_0 \tag{10}$$

where n_0 is the monolayer capacity of the surface (mole/m^2) and n_A is the surface concentration of the amphiphile (mole/m^2). Combination of Eqs. (5–10) gives the adsorption isotherm:

$$n_A = \frac{n_0 K_{AS} \cdot c_A \cdot e^{-\frac{z_A F \Psi_0}{RT}}}{1 + K_{AS} \cdot c_A \cdot e^{-\frac{z_A F \Psi_0}{RT}}} \tag{11}$$

where

$$K_{AS} = \exp - \left(\frac{\mu_{AS}^0 - \mu_A^0 - \mu_S^0}{RT}\right) \tag{12}$$

When $n_A / n_0 < 0.2$, the denominator in Eq. (11) is close to one and, to a first approximation, the linear form of the adsorption isotherm is valid:

$$n_A = n_0 K_{AS} \cdot c_A \cdot e^{-\frac{z_A F \psi_0}{RT}} \tag{13}$$

This electrostatically modified linear isotherm has been used in this work for describing the adsorption of different types of charged amphiphiles.

By using Eq. (2), the adsorption isotherm can also be written in terms of the total amount of amphiphile adsorbed in, e.g., a column:

$$N_A = N_0 K_{AS} \cdot c_A \cdot e^{-\frac{z_A F \psi_0}{RT}} \tag{14}$$

where N_0 is the monolayer capacity of the column (mol).

A change in the electrostatic surface potential is obtained when the charged amphiphile adsorbs on the surface of the stationary phase. This change in electrostatic surface potential as a function of the surface concentration of adsorbed charged amphiphiles is obtained from a solution of the linearized Poisson–Boltzmann equation in cylindrical coordinates [17]:

$$\psi_0 = \frac{z_A n_A F}{\kappa \epsilon_0 \epsilon_r} \cdot \frac{I_0(\kappa r)}{I_1(\kappa r)} \tag{15}$$

where n_A is the surface concentration of charged solute (mole/m^2), κ is the reciprocal Debye length, ϵ_r is the dielectric constant of the mobile phase, ϵ_0 is the permittivity of the vacuum and $I_0(\kappa r)$ and $I_1(\kappa r)$ are the modified Bessel function of the first kind of order zero and one respectively and r is the pore radius of the stationary phase. A pore radius of 50 Å,

according to the manufacturer, is used in the calculations. The inverse Debye length is defined as:

$$\kappa = F \cdot \left(\frac{2I}{\epsilon_0 \epsilon_r RT}\right)^{1/2} \quad (16)$$

where I is the ionic strength of the mobile phase. Eq. (15) can only be used for $\psi_0 < 40$ mV; for higher surface potentials, a numerical solution of the Poisson–Boltzmann equation must be used. From Eq. (15) it is also seen that the electrostatic surface potential is related to the concentration of surface charges, i.e., the surface concentration of amphipiles in mol/m^2, n (mol/m^2).

2.3. The surface area

The equilibrium constant K_{AS}, Eq. (12), can also be defined as [7]:

$$K_{AS} = \frac{k_0' \cdot V_0}{N_0} \quad (17)$$

where V_0 is the dead volume of the column and k_0' is the capacity factor for the amphiphile at some mobile phase composition where the electrostatic potential that is caused by its own adsorption is zero. Inserting Eqs. (15,17,2) into Eq. (14) gives the relationship:

$$\ln\frac{N_A}{c_A} = \ln k_0 V_0 - N_A \frac{z_A^2 F^2}{A\kappa\epsilon_0\epsilon_r RT} \cdot \frac{I_0(\kappa r)}{I_1(\kappa r)} \quad (18)$$

In this equation, the parameters c_A, F, κ, ϵ_0, ϵ_r, T, I_0 and I_1 are known parameters for a specific chromatographic system and N_A is determined experimentally by frontal chromatography. From this equation, it follows that the surface area, A, can be calculated from the slope of a plot of ln N_A/c_A vs. N_A.

$$\ln\frac{N_A}{c_A} = K_1 - N_A \times \frac{K_2}{A} \quad (19)$$

where

$$K_1 = \ln k_0 V_0$$

and

$$K_2 = \frac{z_A^2 F^2}{\kappa\epsilon_0\epsilon_r RT} \cdot \frac{I_0(\kappa r)}{I_1(\kappa r)}$$

As for other determinations of the area of porous materials, the value of the area is dependent on the experimental method and the conditions used [18]. Furthermore, fractal geometry tells us that the measured area also depends on the size of the probe used. In this method, the area is obtained from the relation between the electrostatic surface potential and the surface concentration of charges. A relevant question is therefore "What is the size of the probe used in this system?" This is a complicated issue which is beyond the scope of this paper. The measured area is controlled by the electrostatic repulsion between the adsorbed amphiphiles, where the Debye length, $1/\kappa$, is a measure of the distance of interaction between the amphiphiles. We therefore suggest that the area is measured using a probe with an area proportional to $(1/\kappa)^2$.

3. Experimental

A LC system from Perkin-Elmer was used for the chromatography and it was composed of a LC-gradient pump (series 200), a LC sample processor (ISS 200), a LC oven (101), a diode array detector (235 C) and, for measuring the non-UV-absorbing compounds, a refractive index detector (series 200). The system was controlled by a data system, Turbochrom 4, also from Perkin-Elmer. The pH measurements were performed using a Radiometer PHM 64 Research pH meter. The column was a LiChrospher 100 from Merck, Darmstadt, Germany (RP-18, particle diameter=5 μm) with the dimensions 12 cm×4 mm I.D. and the same column was used in all experiments.

According to the manufacturer, the bulk density of LiChrospher Si 100 is approximately 0.40 g/ml. The amount of material in the column is calculated to be 0.60 g and this value is used when converting from column area to area per gram in the ensuing calculations.

Toluene-4-sulfonic acid (Merck), 2,5-dimethylbenzenesulfonic acid sodium salt (Eastman Kodak), 1-hexane sulfonic acid, sodium salt (Aldrich-Chemie, Steinheim, Germany) and octane-1-sulfonic acid sodium salt (Merck) were used as the solutes. For the buffer solutions, sodium dihydrogenphosphate monohydrate (p.a.; Merck) and orthophosphoric acid (85% p.a.; Merck) were used. Methanol (gradient-grade LiChrosolv) and acetoni-

trile (for chromatography LiChrosolv), both from Merck, were used as the mobile phase solvents. The water used was from a Milli-Q system and had a resistivity of 18.2 MΩ·cm. All buffer solutions were filtered through a 0.45-μm filter (Millipore) before use.

A phosphate buffer solution (pH 3), containing 0.1 mg $NaNO_3$/ml, was pumped into the column as a front. Since a gradient pump was used, the front was created by an instantaneous change of the mobile phase reservoir. The value of t_0 is the time by which the flow goes from the reservoir, containing the solute, to the detector. This time was experimentally found to be 235 s when the flow-rate was 1.0 ml/min. The column temperature was 298 K and the flow-rate was 1.0 ml/min in all experiments.The amount of amphiphile adsorbed at various concentrations of the mobile phase were calculated from the retention time of the breakthrough front, according to Eq. (3).

4. Results and discussion

In Fig. 1 the adsorbed amount of *p*-toluenesulfonate onto a LiChrospher 100 RP-18 phase is plotted as a function of its concentration in a mobile phase with ionic strengths of 0.025, 0.05 and 0.1 *M*, respectively. As expected, the electrostatic surface potential, created by the charged amphiphile itself, gives rise to a non-linear adsorption isotherm. From these experimentally determined adsorption isotherms, it is also clear that the amount of *p*-toluenesulfonate adsorbed at a given mobile phase concentration increases as the ionic strength increases. This dependence of the ionic strength on the amount adsorbed is due to a decrease in electrostatic surface potential with increasing ionic strength [see Eq. (15)].

Since the proposed method for determination of the area is based on Eq. (19), the linearity of a plot of $\ln N_A/c_A$ vs. N_A and its dependence on the ionic strength are both critical tests of its validity. The three data sets shown in Fig. 1 are used for constructing such plots (see Fig. 2) and it is seen that they all exhibit good linearity and that the slope changes with the ionic strength. By inserting values for the physical constants in Eq. (19), the surface

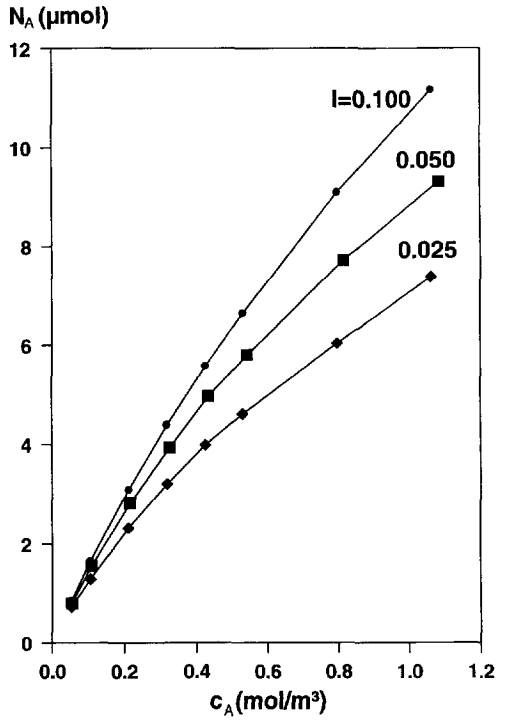

Fig. 1. Plots of the experimentally obtained adsorption isotherms of *p*-toluenesulfonate to a LiChrospher RP-18 stationary phase with ionic strength as a parameter. N_A = adsorbed amount of *p*-toluenesulfonate (μmol/column); c_A = concentration of *p*-toluenesulfonate in the mobile phase (mol/m^3). Mobile phase: phosphate buffer, pH 3, and ionic strengths of 0.025, 0.050 and 0.100 *M*, respectively.

area of the stationary phase in the column can be calculated from the slope of these plots. From these three sets of data, it is found that the determined surface areas in the column are approximately the same (133, 135 and 138 m^2/column, respectively). The good adherence between the three calculated areas, in combination with the good linearity and the theoretically expected dependence on the ionic strength for the adsorption, shows that the physical description of the theory used is correct. In the following discussion and in Table 1 these numbers are converted into m^2/g stationary phase by using 0.60 g as the estimated amount of material in the column.

According to the theory, the surface area measurements are based on the created electrostatic surface potential, which is expected to be independent of the

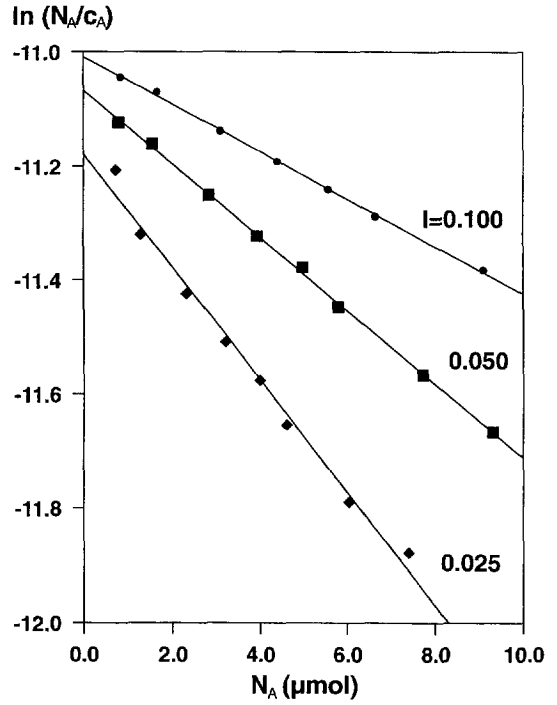

Fig. 2. Plots [according to Eq. (19)] of the experimental data from Fig. 1, i.e. ln (N_A/c_A) vs. N_A. Experimental conditions and symbols are as for Fig. 1.

amphiphile used. In order to test this, several types of amphiphiles were used and the surface area was calculated from the slope of the ln N_A/c_A vs. N_A plot. The results for two different negatively charged amphiphiles, dimethylbenzenesulfonate and *p*-toluenesulfonate, using a mobile phase of the same composition are shown in Fig. 3. It is seen from the plot that the slopes are equal (the found areas are 225 and 222 m^2/g, respectively), verifying the theory for this method. In conclusion, when different phosphate buffers are used as the mobile phase, the obtained surface area is entirely consistent with the proposed theory.

In Fig. 4 the plots are shown for dimethylbenzenesulfonate and hexylsulfonate as amphiphiles, using a mobile phase consisting of phosphate buffer containing 5% (v/v) methanol. The calculated areas are 247 and 255 m^2/g, respectively, showing that the accessible surface area increases when methanol is added to the mobile phase. Also, octanesulfonate was used as the amphiphile using the same mobile phase and the area found, in this case, was 247 m^2/g. In an experimental series with octanesulfonate as the am-

Table 1
Summary of the measured surface area for a LiChrospher RP-18 column under different experimental conditions

Modifier	pH	Ionic strength (*M*)	MeOH (%, v/v)	R^2	Area (m^2/g)
PTS	3	0.05	0	0.999	222
PTS	3	0.025	0	0.989	225
PTS	3	0.1	0	0.999	230
DMBS	3	0.05	0	0.995	222
DMBS	3	0.0475	5	0.999	247
HexS	3	0.0475	5	0.999	255
OctS[a]	3	0.0475	5	0.955	247
OctS	3	0.045	10	0.999	258

[a] The result was obtained from two separate series of experiments.
R^2 = the correlation coefficient for the plot of ln(N_A/c_A) vs. N_A.
PTS = *p*-Toluenesulfonate; DMBS = dimethylbenzenesulfonate; HexS = hexanesulfonate and OctS = octanesulfonate.

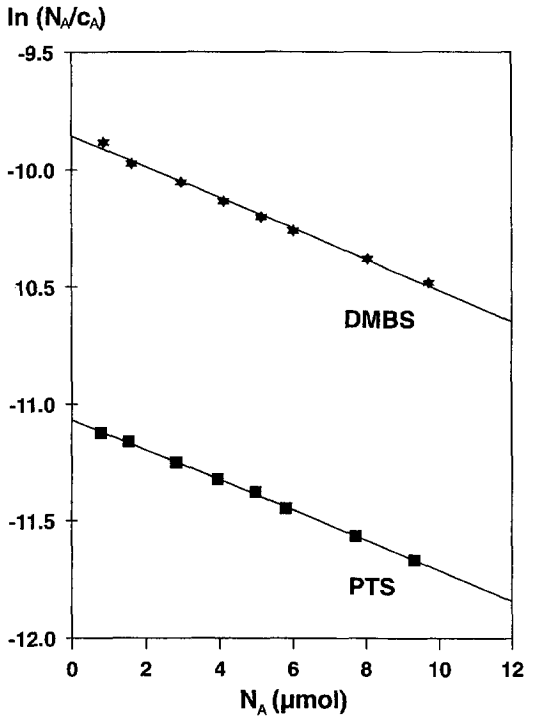

Fig. 3. Plots [according to Eq. (19)] of experimental adsorption data for *p*-toluenesulfonate and dimethylbenzenesulfonate to the LiChrospher RP-18 column. N_A = adsorbed amount (µmol/column); c_A = concentration in the mobile phase (mol/m^3). Mobile phase: phosphate buffer with a sodium ion concentration of 0.05 *M*, pH 3.

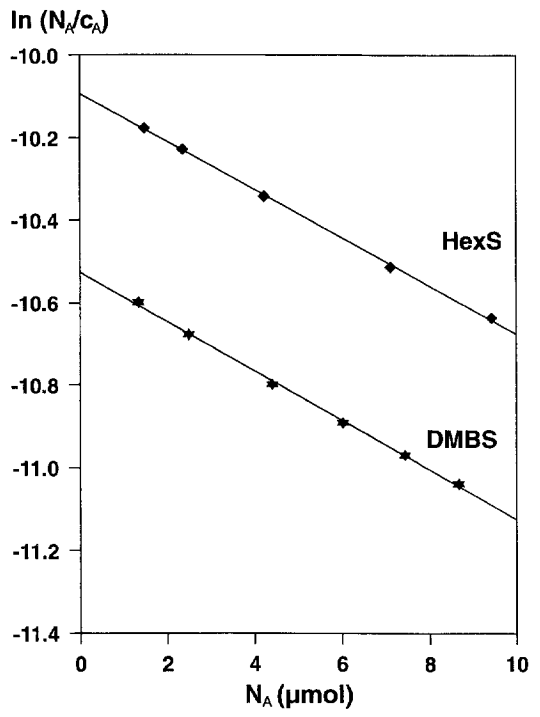

Fig. 4. Plots (according to Eq. (19)) of experimental adsorption data for hexanesulfonate (HexS) and dimethylbenzenesulfonate (DMBS) to the LiChrospher RP-18 column. N_A = adsorbed amount (µmol/column); c_A = concentration in the mobile phase (mol/m^3). Mobile phase: phosphate buffer, pH 3, with 5% (v/v) methanol and an ionic strength of 0.0475 *M*.

phiphile, a mobile phase containing 10% (v/v) methanol was used and the measured surface area was found to be 258 m^2/g. The increase in the accessible surface area on addition of methanol to the mobile phase (see Table 1) is in accordance with earlier observations regarding the phase ratio dependence on the content of organic modifier (see Ref. [19] and references therein). A possible explanation for the increase in surface area is a lowering of the surface tension between the liquid and stationary phases as the methanol content of the mobile phase increases, facilitating the penetration of the mobile phase into the pores. Another conclusion from the results is that the accessible area for this mobile phase (in agreement with the theory) is independent of the chemical structure of the amphiphile.

As discussed in the Section 2, the measured surface area depends on the size of the probe molecule. In this method, it is bigger than the N_2 molecule used in the BET method. Furthermore, the proposed method determines the surface area for the liquid–solid interface, in contrast to the gas–solid interface determined by the BET method. Accordingly, the found area is smaller than the area measured by the BET method, which, according to the manufacturer, is 350 m^2/g for the stationary phase used here. Therefore, it is reasonable to assume that the surface area determined by the presented method is more closely related to the surface area that is accessible under chromatographic conditions.

When Eq. (19) is applied to the experimental data, it is assumed that the denominator in Eq. (11) is approximately one. This assumption was tested using the experimentally obtained area in combination with an approximated amphiphile surface area of 50 Å^2 per molecule. When 10 µmol of amphiphile is adsorbed, i.e. the highest amount used, the denominator in Eq. (11) is calculated to be 1.07, confirming the correctness of the assumption. Another assumption is that when calculating the adsorbed amount of amphiphile, no considerations was given to the fact that the mobile phase concentration close to the surface is lower than that in the bulk solution. The real adsorbed amount is therefore slightly higher than the value reported. However, an approximate calculation shows that, for the worst case, the largest possible deviation is 3% for the adsorbed amount of amphiphile.

5. Conclusion

A method for determination of the chromatographically accessible surface area of RP column material has been developed. The electrostatically modified Langmuir adsorption isotherm is used to describe the amount of negatively charged amphiphiles adsorbed. The method has been thoroughly tested by systematically varying the mobile phase composition and it is found that the experimental results are consistent with the theory, verifying the usefulness of the developed method. Although we have only used this method on one particular column, the consistency of our results show that it can be applied to any RP column.

The surface area of the accessible stationary phase was found to be around 225 m^2/g, when the mobile

phase consisted of plain phosphate buffer and when it contained 10% methanol the accesssible area increased to 258 m^2/g. The obtained area is significantly lower than that obtained by the BET method (350 m^2/g), indicating that the surface area determined by the BET method is significantly larger than that accessible under chromatographic conditions. The proposed method to measure the accessible stationary phase's surface area is therefore believed to be better for characterization of RP stationary phases. Other areas where this method could be applied are in the calculation of a column's phase ratio, which allows the calculation of adsorption entropy, investigation of retention differences between different columns due to differences in hydrophobicity and/or surface area.

References

[1] M.G Batham and C.F. Simpson, Anal. Proc., 20 (1983) 618.
[2] L.C. Sander and S.A. Wise, Anal. Chem., 67 (1995) 3284.
[3] H. Lu and S.C. Rutan, Anal Chem., 68 (1996) 1387.
[4] J.J. Kirkland, M.A. van Straten and H.A. Claessens, J. Chromatogr. A, 691 (1995) 3.
[5] D. Farin and D. Avnir, J. Chromatogr., 406 (1987) 317.
[6] M.T. Aubel and G. Guiochon, J. Chromatogr., 498 (1990) 281.
[7] I. Hägglund and J. Stålberg, J. Chromatogr. A, 761 (1996) XXX.
[8] V. Pacáková and L. Feltl, Chromatographic Retention Indices, Ellis Horwood, Chichester, 1992.
[9] S.V. Galushkoo, J. Chromatogr., 552 (1991) 91.
[10] P. Jandera, H. Colin and G. Guiochon, Anal. Chem., 54 (1982) 435.
[11] J.H. Knox and G. Vasvari, J. Chromatogr., 83 (1973) 181.
[12] H. Guan and G. Guiochon, J. Chromatogr. A, 724 (1996) 39.
[13] J. Ståhlberg and I. Hägglund, Anal Chem., 60 (1988) 1958.
[14] J. Ståhllberg and Á. Bartha, J. Chromatogr., 456 (1988) 253.
[15] J.T. Davies and E.K. Rideal, Interfacial Phenomena, Academic Press, New York, 1963.
[16] R.S. Deelder and J.H.M. van den Berg, J. Chromatogr., 218 (1981) 327.
[17] R.E. Rice and F.H. Horne, J. Colloid Interface Sci., 105 (1985) 172.
[18] Technical Report, Pure and Appl. Chem., 66 (1994) 1739.
[19] P. Jandera, in R.M. Smith (Editor), Retention and Selectivity in Liquid Chromatography, Elsevier, Amsterdam, 1995, p. 248.