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Evaluation of the application of liquid-phase titration to the examination of the adsorption activity of silica-based high-performance liquid chromatographic packings

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ABSTRACT

The theoretical basis of the chromatographic titration method was investigated and the correctness of the determination of the strong silanol fraction was confirmed. Application of liquid-phase titration to the comparison of reversed-phase high-performance liquid chromatographic packings is described and the possibility of sorbent evaluation is discussed.

INTRODUCTION

The variety of silica surface chemistry, caused by the structure of amorphous silica, *e.g.*, by variation in the distances of hydrogen-bonded silanols [1,2], is reflected in the broad range of pK_a and pH values of commercially available silicas and can influence the properties and quality of alkyl-bonded phases for high-performance liquid chromatography (HPLC). Because the strength of hydrogen bonds depends on the acidity of the silanols [3], the concept of the existence of acidic reactive silanols on the silica surface has been formulated and confirmed experimentally [2,4–7], supporting the previous concept of reactive silanols by Snyder and Ward [8]. Blockage of the acidic reactive silanols appeared unexpectedly to have a large effect on the packing efficiency [5], undesirable adsorption of basic compounds and hydrolytic stability of alkyl-bonded phases formed on silicas containing such groups [9,10]. Attention has also been paid to the influence of metal oxide impurities in chromatographic-grade silica gels on the chromatographic process [11]. Sadek *et al.* [12] and Nawrocki [13] have shown that the metal atoms can create "structural Lewis sites" and influence the silica surface atoms and their hydroxyl groups. This was confirmed by acid washing results [14–16]. A review by Nawrocki and Buszewski [17] summarizes work on reactive silanols on silica gel surfaces.

In a series of papers by Nawrocki and co-workers [18–24], the application of the gas-phase titration method to the examination of the adsorption activity of chromatographic packings was proposed. It was suggested that at least two types of adsorption centres differing significantly in adsorption energy exist on the surface of

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silica-based chromatographic packings. The method proposed by Nawrocki and co-workers should permit the determination of the number of strong adsorption centres on the sorbent (so-called acidic silanols), usually interfering with the chromatographic determination, causing peak tailing and even irreversible adsorption of sample substances. This method can be potentially useful for the evaluation of chromatographic packings and for their standardization.

This paper considers the evaluation of the effectiveness and correctness of the method of chromatographic titration on the basis of a simple theoretical description of the processes occurring on chromatographic columns. The assumed model permits the description of systems including either a gas or non-polar liquid as the mobile phase.

THEORETICAL

Chromatographic titration consists in the injection of a strongly adsorbed substance (so-called "blocker") followed by injections of small portions of a relatively weakly adsorbed substance ("marker"). The retention time of the "marker" changes during the elution of the "blocker", finally attaining the minimum value at the moment the "blocker" peak appears at the end of the column. A graphical dependence of the minimum retention time of the "marker" on the amount of "blocker" injected should be in the form of a broken line and its vertex should correspond to the number of strong adsorption centres.

The main assumptions of the chromatographic titration method can be defined as follows: (1) at the moment of "marker" elution (for the injection giving the minimum value of the retention time), the "blocker" molecules are uniformly distributed along the column, and (2) the "blocker" molecules adsorb only on the centres of high adsorption energy and "marker" molecules adsorb on the remaining centres.

Let us consider the latter assumption. According to Riedo and Kováts [25], we accept the following definition of the capacity factor k':

$$k' = (V_R - V_0)/V_0 = \frac{A}{V_0} \cdot \frac{d\Gamma}{dc} \tag{1}$$

where V_R = retention volume (dm^3), V_0 = total mobile phase volume in the column (dm^3), A = total adsorbent surface area in the column (m^2), Γ = excess Gibbs isotherm (the dividing plane is placed at the solid–liquid interface) (mol/m^2) and c = concentration of the chromatographed substance (mol/dm^3). An isotherm that can be utilized for the description of the considered system should possess one characteristic feature, *viz.*, it must be limited. We shall utilize the Langmuir isotherm (Li) and the isotherm which can be called the "limited Henry's isotherm" (LHi):

$$\Theta = \frac{Kc}{1 + Kc} \qquad \text{(Li)}$$

$$\Theta = \begin{cases} Kc & c \leqslant 1/K \\ 1 & c > 1/K \end{cases} \qquad \text{(LHi)} \tag{2}$$

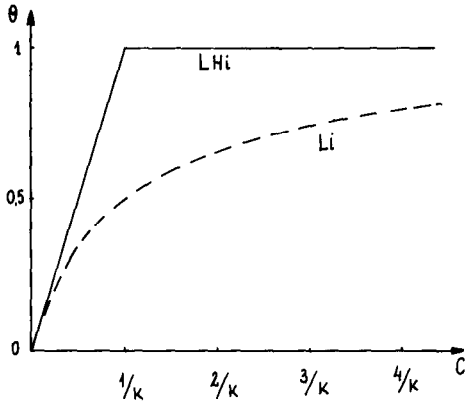

Fig. 1. The Langmuir isotherm (dashed line) and limited Henry's isotherm (solid line).

where Θ = the degree of surface coverage and K = Henry's constant (dm^3/mol).

The shapes of both isotherms are presented in Fig. 1. In order to simplify our model we shall consider only two types of adsorption centres, *viz.*, strong and weak, neglecting also the competitive adsorption of the mobile phase molecules. Owing to such a simplification, the results obtained can be related directly to gas chromatography. According to Langmuir [26], we use the above isotherms as local ones whereas a global isotherm is calculated from the following equation:

$$\Theta = \frac{\Theta_1 N_1 + \Theta_2 N_2}{N_1 + N_2} \tag{3}$$

where N_1 and N_2 are the number of strong and weak adsorption centres, respectively. From eqns. 3 and 2 we obtain the particular global isotherms:

$$\Theta = \frac{c}{N_1 + N_2}\left(\frac{K_1 N_1}{1 + K_1 c} + \frac{K_2 N_2}{1 + K_2 c}\right) \qquad \text{(Li)}$$

$$\Theta = \begin{cases} c \cdot \dfrac{K_1 N_1 + K_2 N_2}{N_1 + N_2} & c \leqslant 1/K_1 \\ \dfrac{N_1 + K_2 N_2 c}{N_1 + N_2} & 1/K_1 < c \leqslant 1/K_2 \quad \text{(LHi)} \\ 1 & c > 1/K_2 \end{cases} \tag{4}$$

where the subscripts 1 and 2 relate to strong and weak adsorption centres, respectively. Isotherms 4 describe the adsorption of the "blocker". Because the "marker" concentration is significantly lower than that of the "blocker" (theoretically infinitely low), it can be assumed that its molecules adsorb only on the free centres and do not

compete with the "blocker" molecules. Therefore, the global isotherm of the "marker" can be presented in the following form:

$$\Theta_M = \frac{(1 - \Theta_{B1})N_1\Theta_{M1} + (1 - \Theta_{B2})N_2\Theta_{M2}}{N_1 + N_2} \tag{5}$$

where the subscripts M and B relate to the "marker" and "blocker", respectively.

$$\Theta_M = \frac{c_M}{N_1 + N_2}\left(\frac{N_1K_{M1}}{1 + K_{B1}c_B} + \frac{N_2K_{M2}}{1 + K_{B2}c_B}\right) \qquad \text{(Li)}$$

$$\Theta_M = \begin{cases} K_{M1}c_M \cdot \dfrac{N_1(1 - K_{B1}c_B)}{N_1 + N_2} + K_{M2}c_M \cdot \dfrac{N_2(1 - K_{B2}c_B)}{N_1 + N_2} & c_B \leqslant 1/K_{B1} \\ K_{M2}c_M \cdot \dfrac{N_2(1 - K_{B2}c_B)}{N_1 + N_2} & 1/K_{B1} < c_B \leqslant 1/K_{B2} \quad \text{(LHi)} \\ 0 & c_B > 1/K_{B2} \end{cases} \tag{6}$$

During derivation of the above equations the assumption of infinite dilution of the "marker" was made and thus the Henry's isotherm was used as the local isotherm of the "marker".

According to the definition mentioned above, the surface excess of the "marker" can be expressed as follows:

$$\Gamma_M = \frac{(N_1 + N_2)\Theta_M - V_{mono}c_M}{A} \tag{7}$$

where V_{mono} denotes the monolayer volume (dm^3).

From eqns. 1, 7 and 6 we can obtain finally the dependence of the "marker" capacity coefficient on the "blocker" concentration:

$$k'_M(c_B) = \frac{1}{V_0}\left(\frac{N_1K_{M1}}{1 + K_{B1}c_B} + \frac{N_2K_{M2}}{1 + K_{B2}c_B}\right) \qquad \text{(Li)}$$

$$k'_M(c_B) = \begin{cases} K_{M1} \cdot \dfrac{N_1(1 - K_{B1}c_B)}{V_0} + K_{M2} \cdot \dfrac{N_2(1 - K_{B2}c_B)}{V_0} & c_B \leqslant 1/K_{B1} \\ K_{M2} \cdot \dfrac{N_2(1 - K_{B2}c_B)}{V_0} & 1/K_{B1} < c_B \leqslant 1/K_{B2} \quad \text{(LHi)} \\ 0 & c_B > 1/K_{B2} \end{cases} \tag{8}$$

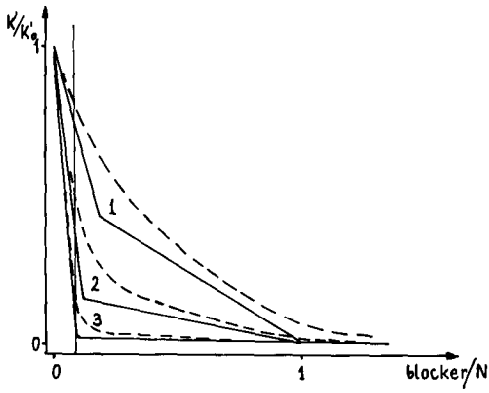

Fig. 2. Theoretical dependence of the capacity coefficient of a "marker" on the amount of "blocker" in the column. A constant "blocker" concentration in the mobile phase was assumed. Lines as in Fig. 1. The vertical line indicates the strong silanol fraction ($f = 0.1$). Parameter s values: (1) 10; (2) 50; (3) 500. Other parameters: $K_{B2} = 100$; $r = 1$.

In order to simplify matters, we have neglected in the above equations the term related to monolayer volume. In the case of strong positive adsorption taking place in the range of low concentrations, this term does not play a significant role. One should remember, however, that only the presence of this term permits negative values of the capacity coefficient, *e.g.*, for a substance whose molecules are excluded from the surface layer. It is worth noting that for typical adsorbents characterized by a specific surface area of *ca.* 100 m^2/g and porosity of the order of 1 ml/g monolayer volume ranges from several tens to hundreds of microlitres per gram of the adsorbent, depending on the molecular size of the adsorbate. In further considerations this term will be neglected.

Fig. 2 shows the dependence of the "marker" k' value on the amount of the "blocker" contained in the column (calculated from eqns. 4 and 6–8). A capacity coefficient is presented in the form of the fraction of the "marker" k' value on the "blocker"-free column and the unit of the amount of "blocker" is monolayer capacity ($N_1 + N_2$). Solid lines correspond to LHi and dashed lines to Li assuming the following parameter values:

$$f = N_1/(N_1 + N_2) = 0.1; \qquad r = V_0/(N_1 + N_2) = 1; \qquad K_{B2} = 100;$$

$$s = K_{B1}/K_{B2} = \begin{cases} 10 \text{ for } 1 \\ 50 \text{ for } 2 \\ 500 \text{ for } 3 \end{cases}$$

We have also assumed that $K_{M1}/K_{M2} = K_{B1}/K_{B2}$. The vertical line in Fig. 2 shows the fraction of the strong adsorption centres.

As one would expect, the line corresponding to the Langmuir isotherm is smooth. The higher the ratio of adsorption constants s, the closer this line is to the broken line obtained for the limited Henry's isotherm. The position of the vertex is obtained from the expression $f + r/K_{B1} + (1 - f)/s$. The vertex is always shifted to

the right so the strong centre fraction established on the basis of its position must be overestimated.

The ratio of the slopes of the two broken line sections can be calculated in a simple manner. We obtained the following expression:

$$\frac{1-f+r/K_{B2}}{1-f} \cdot \frac{s^2 f+1-f}{sf+1-f+r/K_{B2}} \approx s$$

Such an approximation is the better the greater is the product sf. The slope ratio values for broken lines presented in Fig. 2 are 5.8, 42.9 and 496.5 for $s = 10$, 50 and 500, respectively. The practical determination of the s value (*i.e.*, the difference in adsorption energy for centres 1 and 2 equal to $RT \ln s$) is limited by the too low accuracy of the slope determination for the second section of the broken line, especially for high s values. On the other hand, the evaluation of s at low levels is significantly underestimated.

Let us now consider the first assumption of chromatographic titration. It is well known that the "blocker" does not distribute uniformly along the column but forms a band in which the "blocker" concentration depends on the elution time and the distance from the column inlet. For the Langmuir isotherm the shape of such a band cannot be predicted in a simple manner, but it is possible for the limited Henry's

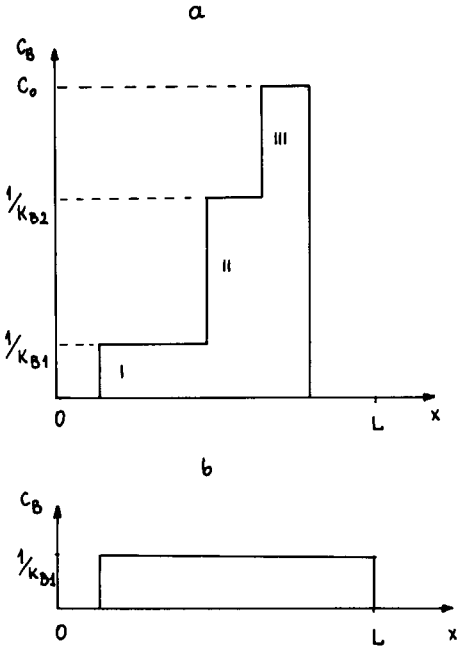

Fig. 3. Theoretical "blocker" band shape in a column of lengt L, (a) some time after injection and (b) at the moment of maximum strong centre blockage. c_0 denotes the "blocker" concentration of the injected solution.

isotherm. Neglecting the "diffusion" band broadening, we obtain a step band as presented in Fig. 3a, where c_0 denotes the "blocker" concentration of the injected solution.

According to Giddings [27], the migration rate of the band back edges can be expressed in the following form:

$$\begin{aligned} v_{\mathrm{I}} &= v_0 \Big/ \left\{ 1 + \frac{N}{V_0} \left[K_{\mathrm{B1}} f + K_{\mathrm{B2}}(1 - f) \right] \right\} \\ v_{\mathrm{II}} &= v_0 \Big/ \left[1 + \frac{N}{V_0} \cdot K_{\mathrm{B2}}(1 - f) \right] \\ v_{\mathrm{III}} &= v_0 \end{aligned} \tag{9}$$

where v_0 denotes the linear velocity of an unretained substance band.

Let us consider the moment at which the "blocker" distribution in the column is the most uniform. This takes place when step II (Fig. 3a) vanishes at the moment of the appearance of the "blocker" band front at the column outlet. Such a situation is presented in Fig. 3b. The retention time of the "blocker" band front t_{R} is equal to L/v_{II}, and the length of this part of the column where the "blocker" concentration is constant is equal to $L - v_{\mathrm{I}} t_{\mathrm{R}}$. We can now calculate the amount of "blocker" corresponding to the inflection point in Fig. 2 covering the correction for the band shape. Taking monolayer capacity as a unit, we obtain:

$$[f + r/K_{\mathrm{B1}} + (1 - f)/s][1 - (v_{\mathrm{I}}/v_{\mathrm{II}})] = [f + r/K_{\mathrm{B1}} + (1 - f)/s] \left(\frac{sf}{r/K_{\mathrm{B2}} + sf + 1 - f} \right) = f$$

Thus, the fact of taking into account the "blocker" band shape causes the inflection point abscissa to be equal to the actual strong adsorption centre fraction (within the developed model). We can also calculate the ratio of the slopes of the two sections of the broken line:

$$[0.5s + (1 - f)/2sf] \Big/ \left\{ 1 + (1 - 2f)/2sf - [0.5 + (1 - f)/2sf](1 - 1/s) \frac{K_{\mathrm{M2}}}{K_{\mathrm{B2}}} \right\}$$

For typical parameters the value of this ratio is close to $0.5s$, which is surprising and suggests a strong dependence of this ratio on the "blocker" band shape. The above expression contains a new parameter, $K_{\mathrm{M2}}/K_{\mathrm{B2}}$, which introduces the "marker" properties. Fixing its value at 0.01 we obtain slope ratios equal to 3.9, 23.4 and 249.3 for $s = 10$, 50 and 500, respectively. A characteristic feature of the above expression is a lack of parameter r connected with chromatographic column properties.

The most striking feature of chromatographic titration curves is the decrease in subsequent section slopes. Slope values for the first and second sections can be calculated from the following expressions:

$$\frac{K_{M1}}{r} \cdot \frac{1 + (1-f)/fs^2}{1 + (1-f)/sf[1 - (1 - 1/s)K_{M2}/K_{B2}]}$$

$$\frac{K_{M2}}{r} \cdot \frac{2 + (1-2f)/sf - [sf + (1-f)/sf](1 - 1/s)K_{M2}/K_{B2}}{1 + (1-f)/sf[1 - (1 - 1/s)K_{M2}/K_{B2}]}$$

We obtained 5.8, 42.6 and 491.3 for the first section and 1.47, 1.82 and 1.97 for the second (s = 10, 50 and 500, respectively). For the chosen set of parameters $K_{M1} = s$ and $K_{M2} = 1$. In practice, the accurate determination of the acidic silanol adsorption energy (on the basis of K_{M1}) is not possible because we cannot determine the total number of accessible silanols. Hence we cannot determine the parameter r and cannot express the injected "blocker" amount as the monolayer capacity fraction. On the other hand, it seems reasonable that if we can assume similar r values and similar silanol access for different sorbents, we can compare the slopes of corresponding sections and draw a conclusion concerning silanol adsorption energies of different packings.

In liquid-phase titration, the problem of the presence of water and other polar contaminants in the liquid mobile phase arises. Water can influence the "marker" retention in a very complicated manner, especially in the case of injection of a large amount of amine, which is believed to be able to displace all other adsorbates. In front of the amine band the water content on the column increases and after the band a region of decreased water content may be expected. Water evidently partially deactivates the packing surface and decrease the "marker" retention time, but it is not clear if it affects the slope of the LPT curve. Moreover, because an amine is much more strongly adsorbed than water, the position of the inflection point should not be affected by its presence.

In gas chromatography the experiment is performed at much higher temperatures and the low water content in the mobile phase may be easily maintained, so under proper experimental conditions the influence of water can be neglected.

From the above theoretical considerations we can draw the following conclusions:

(1) The position of the inflection point for the chromatographic titration curve should determine the actual strong adsorption centre fraction.

(2) The slope ratio of subsequent sections is a measure of the adsorption energy difference for two types of centres. In practice, we can say that this difference is high (above *ca.* 11 kJ/mol, which corresponds to s = 100 at room temperature) when the second section is horizontal (the slope is of order of 0.01 μmol^{-1}) or low when the second section slope can be determined with reasonable accuracy (it is of order of 0.1 μmol^{-1}).

(3) The slope of the section corresponding to strong silanols is a measure of their adsorption energy, but in practice it can only be used to compare sorbents.

EXPERIMENTAL

An attempt to apply the LPT method to a system with a liquid mobile phase (LiChrosorb C_{18} + hexane) was also made.

Apparatus and materials

Chromatographic measurements were performed using a chromatograph consisting of an HPP-4001 syringe pump, LC-2563 UV detector (Laboratorní Přístroje, Prague, Czechoslovakia) and Rheodyne Model 7120 injection valve. LiChrosorb Si-100 C_{18} packings[a] were synthesized in our laboratory using monochlorodimethyloctadecylsilane in the presence of the activator, morpholine dissolved in dry toluene (1:1) [28,29], and a high coverage density, α_{RP} = 3.5–3.55 μmol/m^2, was obtained. The specific surface area of the bare silica (measured by a sorptomatic method) was *ca.* 300 m^2/g. In order to reveal potential differences in the adsorption activities of the tested packings, two types of LiChrosorb Si-100 (with mean particle diameter 10 μm), *i.e.*, untreated and hydrochloric acid washed, were used. The hydrochloric acid (1:1) washing was performed at its boiling temperature in Soxhlet apparatus (a thin layer of adsorbent) for 1 week. Then the packing samples were washed with distilled water (two portions of fresh water per day) until the pH of the water did not change. This procedure required a long time of operation (*ca.* 3 weeks).

Columns of 100 × 4 mm I.D. contained the same amount of packing.

Liquid-phase titration procedure

The investigated columns were flushed with acetone and hexane (*ca.* 50-ml portions) at 320 K, then thermostated (water-jacket) at 306 K. Hexane containing 20 ppm of water was used as the mobile phase at a flow-rate of 1 ml/min. The UV detector was operated at 254 nm and its sensitivity was 0.01 a.u.f.s.

When a stable baseline was obtained, pure butylamine was injected. During the elution of the amine from the column, 10-μl portions of 0.5% ethyl methyl ketone in hexane were injected. For each amine injection the shortest ketone retention time was found. Dead volumes were determined by means of butylbenzene and values of 1.03 and 1.04 ml were obtained for packings based on untreated and acid-washed silica, respectively.

RESULTS AND DISCUSSION

The chromatographic titration curves for both packings are presented in Fig. 4. The inflection points correspond to 0.04 and 0.06 μmol/m^2 and the slope ratios obtained for packings based on untreated and washed silica are 10 and 70, respectively. The shape of the titration curve for untreated silica gel and the low slope ratio seem to indicate the existence of two types of strong adsorption centre differing slightly in adsorption energy. Another inflection point may be expected which is connected with blocking of all strong adsorption centres. We can estimate its position at *ca.* 0.15 μmol/m^2 of amine. This value cannot be obtained from the data presented here but on the basis of other results we can expect the value of k'/S_{BET} to be close to 0.025 m^{-2} starting from an amount of injected amine equivalent to all strong adsorption sites. In such a case acid washing of the silica gel would reduce the number of strong

[a] The commercial trade name of the RP packing material produced by Merck is LiChrosorb RP-18. We have synthesized our packing material on the basis of commercially available LiChrosorb Si-100 using monochlorodimethyloctadecyl silane, without endcapping. It differs from LiChrosorb RP-18 but we use a similar (not the same) name to express its origin and properties.

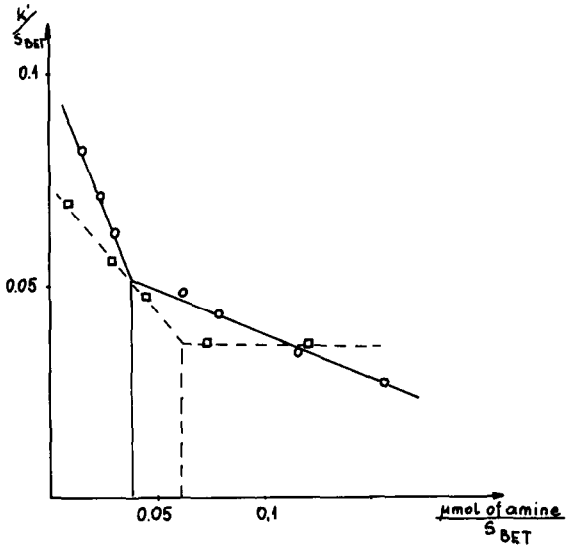

Fig. 4. Experimental LPT curves for (□) acid-washed and (○) untreated LiChrosorb C_{18}.

silanols by a factor of 2.5. This estimation is confirmed by the amine peak shapes presented in Fig. 5 and the way in which pyridine was eluted from the column. Additionally, 1 μl of pure pyridine was injected onto each column. For the acid-washed silica 110 ml of hexane were sufficient for its complete elution from the column. The peak descent to the baseline was clearly seen at a sensitivity of 0.01 a.u.f.s. With the untreated silica 200 ml of hexane could not elute pyridine completely and a clear descent of the peak to the baseline was not observed.

The density of coverage of the two packings and their surface areas per column are almost the same, so its is difficult to explain why the curves in Fig. 4 intersect. For

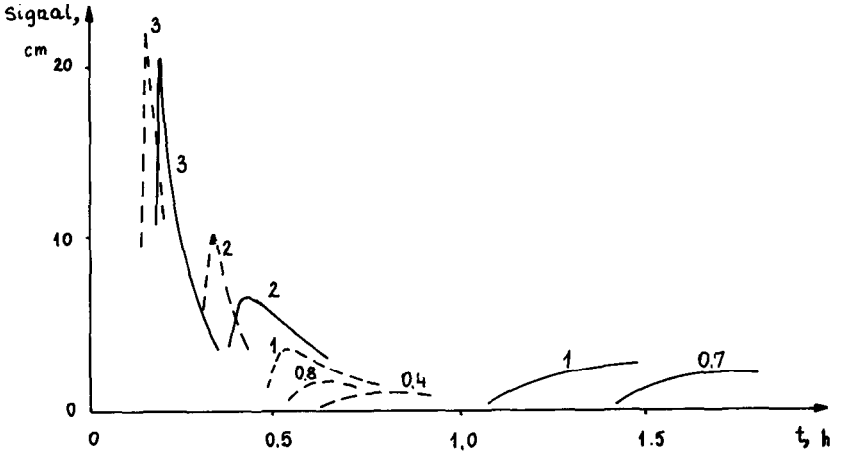

Fig. 5. *n*-Butylamine peak shapes for (dashed line) acid-washed and (solid line) untreated LiChrosorb C_{18}. Numbers indicate injected amine volumes (μl). 0.01 a.u. = 20 cm.

large amounts of injected amine the two curves should have horizontal parts at the same level. If there are no errors in the surface and coverage density determinations, this result seems to indicate an increase in the degree of surface hydroxylation after acid washing. This supposition, of course, needs further experimental evidence for confirmation.

CONCLUSIONS

The description of chromatographic titration presented here, in spite of its simplification, permits a quantitative evaluation of the main assumptions in the method. The position of the inflection point for the LPT curve should give precisely the strong adsorption centre fraction, but the slope of a particular section of the curve allows us only to distinguish between strong and weak centres.

Although LPT is time consuming and requires a large amount of hexane, it seems to be promising for the evaluation of HPLC packings. In comparison with gas-phase titration new problems appear: (1) the water adsorbed on the sorbent (we used hexane in equilibrium with atmospheric water vapour) partially deactivates the column and hinders the measurement of the effect of the injection of a small amount of a "blocker"; (2) the lower detection sensitivity and thus the necessity for the injection of a larger amount of "marker"; and (3) in some instances the "marker" tail is eluted too slowly and can deactivate the column.

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