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Review

The silanol group and its role in liquid chromatography

Jacek Nawrocki

Faculty of Chemistry, A. Mickiewicz University, Drzymaly 24, 60-613 Poznań, Poland

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Abstract

Silica is the most widely used material in chromatography. Silica supports are still superior to other supports. There are, however, several problems with silica-based materials: severe peak tailing in the chromatography of basic compounds, limited pH stability, and irreproducibility for the same chemistry columns. The silanol group plays a key role in the chromatographic properties of silica. Therefore, this review discusses the current state of knowledge on silica surface chemistry and the impact of the chemistry on chromatography of basic solutes. The influence of the silica surface on the stability of bonded phases is also described. We discuss recent developments in IR and NMR spectroscopy of the silica surface, modern understanding of silica surface chemistry, and recent achievements in chromatography of basic solutes. HPLC of organic bases is troublesome due to poor understanding of the mechanisms responsible for difficult chromatography of the solutes. A significant part of the review concerns HPLC of organic bases and it emphasizes the importance of the ion-exchange mechanism for the retention of the bases. The paper discusses how to avoid and how to use ion-exchange for chromatography of organic bases. Factors controlling ion-exchange mechanisms on siliceous supports are discussed in detail. © 1997 Elsevier Science B.V.

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1. Introduction

SiO_2, silica gel or silica, is the most abundant material in the earth's crust. Silica is also the most widely used material in chromatography. Silica supports are still superior to other supports in terms of efficiency, rigidity, and performance. However, there are several problems with silica-based materials: severe peak tailing in the chromatography of basic compounds, limited pH stability, and irreproducibility for the same chemistry columns. The aim of this review is to discuss the current state of knowledge on silica surface chemistry and the impact of the chemistry on the chromatography of basic solutes. We will discuss recent developments in spectroscopy of the silica surface, modern understanding of silica surface chemistry, and recent achievements in chromatography of basic solutes. HPLC of organic bases is troublesome due to poor understanding of the mechanisms responsible for difficult chromatography of the solutes.

Many recent reviews and well-recognized books are complementary to the material which is reviewed below.

Chromatographic applications of silica-based materials are extensively described in the literature [1–3]. The chromatographic properties of silica are described in a fundamental book by Unger [1], while the chemistry of silica is a topic of a comprehensive book by Iler [4]. Silica was also the subject of many recent reviews: Sander and Wise [5] reviewed advances in bonded phases for HPLC, Nawrocki and Buszewski [6] the influence of silica surface chemistry on the properties of bonded phases for HPLC. Henry [7] concentrated on the design requirements of silica-based matrices for biopolymer chromatography, Engelhardt et al. [8] on the methods of characterization of silica-based reversed phases, while Albert and Bayer [9] focussed on the application of solid-state NMR spectroscopy for characterization of HPLC packings. Chromatography on dynamically modified bare silica has been the subject of an extensive article by Hansen et al. [10], while Freizer and Gooding [11] concentrated on silica-based supports for HPLC of biopolymers. reversed-phase HPLC of basic samples has been reviewed by Stadalius et al. [12]. In a comprehensive review Cox [13] described the influence of silica structure on reversed-phase retention while a two-part article by Nawrocki [14,15] describes the controversies in the silica surface.

Modified silica-based packing materials for size exclusion chromatography (SEC) were reviewed by Eksteen and Pardue [16]. A review by Berthod is exclusively devoted to silica as a backbone material for LC columns [17]. Properties of silica as a fundamental material for HPLC column packing are also reviewed by Andersen [18]. A modern understanding of retention mechanism in bonded-phase liquid chromatography has been described by Dorsey and Cooper [19]. Silylation of the silica surface has recently been reviewed by Van Der Voort and Vansant [20]. Despite these numerous papers there is a need for a paper summarizing the contemporary

developments in silica surface chemistry and the impact of silica chemistry on chromatography of basic solutes.

2. The silica surface and its structure

Silica gels used in chromatography are amorphous, non-crystalline materials which do not produce any X-ray diffraction pattern [17]. This is particularly true for high-surface area silicas [21]. Besides diatomites, all other silicas used in chromatography are synthesized [17]. Silica can be obtained by:

- Hydrolysis of inorganic silicates – this method leads to irregular particles, which are contaminated with trace metals such as Fe and Al.
- Hydrolysis of alkoxysilanes – this leads to much less contaminated product with irregular or spherical particles.
- To obtain spherical particles two main routes are used [1,22,23]. (a) The oil emulsion method (see, e.g. [237]) – sol–gel microsphere synthesis – the colloid of silica is emulsified in a water immiscible liquid, stabilized with surfactants and gelled by addition of a base. The diameter of the particles is controlled by stirring velocity (e.g. [24]). The resulting particles are subjected to a hardening process. (b) The coacervation method (also called microencapsulation or polymer-induced colloid aggregation (PICA)) [25,26] (see also [27]) – this consists of the generation of stable sol of uniformly sized colloid particles, addition of an organic polymerizable and water-soluble material, initiation of the polymerization-induced colloid aggregation and, finally, burning off the organic material. This method produces extremely uniform silica particles [26].
- Pyrogenic silica (fumed silica) is obtained by burning silica tetrachloride in a flame or by high-temperature hydrolysis of silicon tetrachloride [2,4]. Aerosils and Cab-O-Sils are the pyrogenic silicas. Aerosil is a low-density powder composed of loosely connected nonporous colloidal silica particles of spherical shape. Pyrogenic silicas are often used for spectroscopic studies of the surface properties [2]. The reader can find more details on the production of silica in [1,2,4].

Since chromatographic processes (i.e. adsorption) proceed on the support surface, chromatographers ought to understand the silica surface chemistry. Amorphous silica with a porous structure bears three kinds of silanols on the surface: isolated, geminal, and vicinal. The surface also contains exposed siloxane bonds (Si–O–Si). The silanols are considered strong adsorption sites [28], while siloxane sites are usually considered hydrophobic [1]. The δ bonds in the Si–O–Si moiety are strengthened by d_π–p_π interaction. Both lone pairs of electrons on oxygen are involved in π interaction and this is responsible for the inability of the siloxane moiety for donor–acceptor interactions, e.g. siloxane sites on a silica surface cannot form H-bonds with H-donor adsorbates [29,59]. The much lower electron-donor properties of siloxane than the C–O–C bond was confirmed by the study of West et al. [241]. Scott and Kucera [30] proved chromatographically (NPLC) that siloxane groups present in silica gel contribute little or nothing to solute retention.

However, the hydrophobic character of the siloxane bond [1,4] makes it possible to observe some retention of non-polar molecules on this site. Bij et al. [31] showed that retention of propylbenzene is significant on naked silica only at very high water concentrations. According to them, hydrophobic interactions between the solute and siloxane are responsible for the phenomenon. Similarly, Cox and Stout [32] observed that, for silica as a packing, the plot of log capacity factor for toluene against methanol concentration was linear. The retention on the siloxane was small compared with that found for an alkyl-bonded phase. Bidlingmeyer et al. also observed reversed-phase phenomena on bare silica for lipophilic amines in aqueous phases. The k' values were high, well above 10 for some compounds [33]. The hydrophobic siloxane site can be a center of reversed-phase interactions on bare silica when predominantly aqueous phases are used [13,32,33]. Silica is an extremely weak reversed-phase packing; similar retention occurs for a given neutral solute if the percent of acetonitrile is reduced by half when compared to the mobile phase for a C_{18} column [244].

Silanols are much more interesting to chromatographers than are siloxanes. Silanols can exist on the surface in single, geminal or vicinal forms. A

pair of vicinal silanols can form the so-called bonded pair. Recently it was postulated that also more than two silanols can form bonded species [34]. Dehydration of three or more bonded silanols leads to the increase of isolated silanol number upon increase of temperature, which has actually been observed (see Fig. 2). It was also postulated that geminal silanols can also form bonded pairs [35]. However, non-empirical methods used by Sauer and Schroeder [36] proved the hypothesis of intramolecular H-bond highly improbable. Also the geminal, intramolecular H-bond was not found in several model compounds [37,38] and, finally, the lack of the intramolecular H-bond in a geminal pair was confirmed by ^{1}H CRAMPS NMR spectra [39]. Internal silanols (i.e. those buried in silica matrix and inaccessible for external solutes) are also postulated to exist in silica. All the silanol species present in silica structure are schematically illustrated in Fig. 1.

· Our contemporary knowledge indicates that the silica surface is composed of siloxanes and silanols. Silanols exist as single (isolated), geminal or bonded. Bonded silanols can be formed of two or more species. Silanols are hydrophilic while siloxanes are hydrophobic. The hydrophobicity of siloxanes can lead to a retention of hydrophobic solutes in HPLC provided that the mobile phase is water rich.

2.1. Silanols: methods of determination

A variety of physical and chemical methods can be used to determine the surface concentration of silanols [1]. All the methods require the use of silica

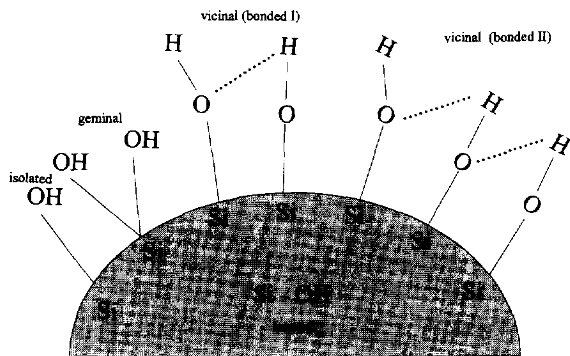


Fig. 1. The silanol species present in the silica structure.

with no physically adsorbed water on the surface, which is quite important and has been extensively discussed in the literature. In general, the bulk of water is easy to remove thermally but it is particularly important to avoid a monolayer of water which is strongly held by H-bonds to surface silanols. The presence of water would lead to incorrect results as water reacts with all reagents used for silanol determination. It is now well established that heating of the silica at 200°C in vacuum ensures obtaining a dry surface [40]. Among the physical methods, IR spectroscopy (including FT-IR) and, more recently, ^{29}Si CP-MAS NMR spectrometry are the most frequently used. The physical methods are particularly useful when relative measurements are carried out. The advantage of these methods is their ability to distinguish between different kinds of silanols. For example, IR can distinguish free and bonded silanols but cannot (practically) distinguish between free and geminal sites. On the other hand ^{29}Si CP MAS NMR can distinguish between single and geminal silanols but cannot differentiate between free and bonded sites. However, another NMR method (^{1}H MAS NMR) can see isolated and H-bonded silanols. There are attempts to quantify single and bonded silanols by IR [41]. Also, another physical method, i.e. temperature-programmed desorption (TPD) was used to determine quantities of bonded and free silanols on the silica surface [42].

The following chemical methods are used for the determination of total silanols: (1) isotopic exchange with D_2O [43–45], HTO [1] or ND_3 [34]; (2) titration with NaOH in the presence of salt [46,47]; (3) reaction with methyl (or other alkyl) lithium [1,48,49]; (4) reaction with Grignard reagents [1]; (5) reaction with dimethylzinc [50,51]; (6) reaction with diborane and organoboron reagents [1,48,59]; (7) reaction with alkali metal naphtenides [52].

Weight loss upon ignition of the silica at 1200°C can also be used for a determination of silanol surface concentration [53,54], however such a high temperature leads also to the removal of internal silanols [240].

Holik and Matějková [55] developed a NMR method for determination of silica hydroxyls based on an exchange of the silanol protons with deuterium atoms of CF_3COO^2H and a measurement of resulting CF_3COOH signal. The results presented for

some silicas are slightly higher than usually observed.

The reactions with metalloorganic reagents are hindered in micropores since the reagents always exist in the form of complexes with solvent molecules. This results in considerably lower values of determined surface silanol concentration as the complexes cannot penetrate the smallest pores. Satisfactory results can be achieved with the reagents when the silica under question does not contain pores with diameters lower than 5 nm [1]. Takeuchi et al. [62] have recently published a GC method for estimation of a number of silanols on silica and a modified silica surface. However, the method neglects the heterogeneity of silanols which has been previously shown in numerous papers [6,14,15,53,54,63,64].

Isotopic exchange with D_2O [43], HTO [1] or ND_3 [34] are considered to give the most reliable results. The most reliable methods of determination of the SiOH surface concentration gave the result 8.0 ± 1.0 $\mu mol/m^2$ and this value is generally accepted for the SiOH surface concentration [1,43] and, moreover, it is sometimes considered as a physicochemical constant [40,59].

There are, however, some reports that the surface concentration of silanols on chromatographic grade silica is somewhat lower, i.e. equal to 5.5 ± 1.0 $\mu mol/m^2$ [54]. Morrow and McFarlan [34] also noticed lower silanol content on non-rehydroxylated silicas (i.e. 4.1–5.8 $\mu mol/m^2$). The possible reason is that manufacture of silica involves some kind of thermal treatment. If such a silica is not rehydroxylated later by the manufacturer, then its surface cannot be considered as fully hydroxylated. This, in turn, will impact on the silica's chromatographic properties. Similar values were obtained by the dimethylzinc method [65,66] and with organoboron reagents [48], which gave the surface concentration of silanols on Nucleosil-5-100 of 6.73–6.97 $\mu mol/m^2$. Low silanol values for Zorbax-type silicas were reported by Köhler et al. [53,54]. The result for Zorbax-Sil was confirmed by Goworek et al. [67] who reported 6.2 $\mu mol/m^2$ by means of isotopic exchange in HPLC system. LiChrosorb Si-60 measured by the same method [67] showed 8.23 μmol $-OH/m^2$. Fóti and Kováts [44,45] have recently found, by isotopic exchange with D_2O, a total concentration of SiOH on LiChrosorb Si-100 as 8.44 ± 0.1 $\mu mol/m^2$. According to Köhler et al. [53,54] fully a hydroxylated surface contains more H-bonded silanols and it is more favorable for chromatographic purposes. Silicas with lower concentrations of silanols contain relatively higher surface concentrations of isolated, free silanols.

Hexamethyldisilazane (HMDS) may be used for determination of silanol surface concentration, provided that silica is partially dehydroxylated and does not contain more hydroxyls than the densest possible surface coverage of trimethylsilyl (TMS) groups. The number is reported as 4.07 [56], 4.48 [57,58], or 4.65 $\mu mol/m^2$ [59]. See also a discussion of that value in [6,60]. The same limitations are valid for the method presented by Yoshinaga et al. [61] which involves a reaction of silica with chlorodimethylsilane and FT-IR spectroscopy. The use of chlorosilanes or HMDS for the determination of surface silanols is obviously less useful for chromatographic purposes. It is also true that the size of the reactant affects the result. Thus we can sometimes talk about determination of 'accessible silanols', i.e. only those which can be determined with a reactant of a given size. The number of 'accessible silanols' decreases with increasing reactant size [34].

In general:

- isotopic exchange seems to be the most reliable method to determine surface hydroxyl concentration;
- the results of ignition of silica at 1200°C will inform about the sum of surface and internal silanols;
- organic complexes can be used for determination of surface silanol concentration only on wide-pore silicas;
- silylorganic reactants are useful for silanol determination only on partially dehydroxylated silicas (remember that the size of the reactant affects the results);
- 'as received' silicas may contain fewer silanols than the usually accepted value of 8 $\mu mol/m^2$.

2.2. Activity of silanols

Different adsorption or reaction activity of various kinds of silanols were thoroughly discussed in previous reviews [6,14,15]. It has been known for

years that different silanols have different adsorption activity, e.g. different behavior of isolated and bonded silanols was noted for adsorption of basic solutes very early [68]. It is obvious that an estimation of a relative ratio of more and less active sites on a silica surface is of primary importance for chromatography. The first attempt to quantify more and less reactive silanols on the silica surface was done by Snyder and Ward [240] in 1966. They concluded from their experiments that H-bonded silanols form pairs, and the pairs are more reactive than isolated silanols due to an enhanced acidity of the proton not engaged in the H-bond. Our contemporary knowledge indicates isolated silanols as the more reactive ones (see e.g. [243]). The hypothesis of Snyder and Ward seemed logically well justified, and yet it was proved wrong. In my opinion, H-bonded silanols do not form pairs; probably H-bonding engages more than two silanol groups. Such H-bonded vicinal silanols would form rather linear or two-dimensional structures in which there is no more reactive hydrogen, as all hydrogens would be involved in H-bonding in such structures. This is schematically shown in Fig. 2.

Such structures can only be sensitive to other H-donors. And, indeed, Mauss and Engelhardt [243] showed that retention of hydroxyl-containing molecules in chromatography depends on the quantity of bonded silanols on the silica surface. Free silanols are considered more active (adsorption, reactivity) as well as undesirable adsorption sites on siliceous HPLC-bonded phases [53,54]. In 1990, Van Der Voort et al. [41] estimated a relative ratio of free and bonded silanols by using integration IR absorption

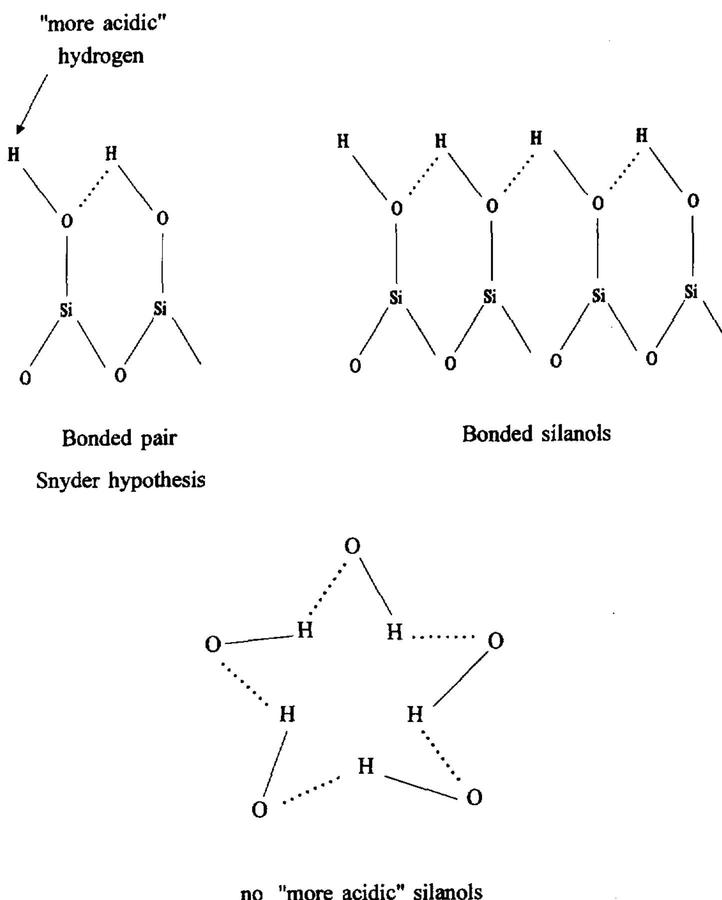


Fig. 2. H-bonded silanols on a silica surface.

bands of free and total hydroxyls on the silica surface. They have shown that isolated, free silanols comprise only 6% of total hydroxyls. This would strongly support the hypothesis of Kirkland et al. [53,54] concerning undesirable adsorption sites. (Our previous reviews [6,14,15] as well as other studies [12,13,69] postulated the existence of a small population of silanols which are able to enter into strong interactions with polar solutes. The population was said to constitute less than 1% of silanols. According to Kirkland, isolated silanols are responsible for strong, undesired interactions with basic solutes in liquid chromatography.) A similar number of strongly interacting sites was also mentioned by Marshall et al. [70,71]. However, in the next paper by Gillis D'Hammers et al. [42], on a temperature-programmed desorption of pyridine from the silica surface, a relative ratio of free to bonded silanols was found to be approximately one. The combined results of ^{29}Si CP-MAS NMR and FT-IR on a quantitative distribution of all types of silanols are presented in Fig. 3 [20]. The number of isolated silanols depicted in Fig. 3 is much higher than 6% determined in [41].

According to the survey of results collected by Vansant et al. [59] the surface concentration of the free silanol group ranges from 1.5 to 2.4 $-OH/nm^2$ (i.e. 3.15±0.8 $\mu mol/m^2$) for silicas treated at 473 K. The value 1.1 $-OH/nm^2$ is very close to the surface concentration of isolated silanols found by Morrow and McFarlan [34]. A combined analysis of IR and

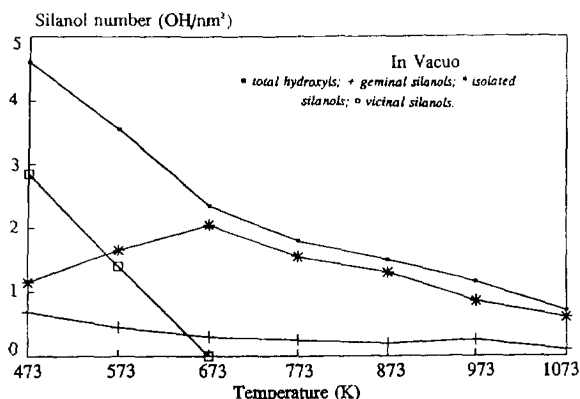


Fig. 3. Silanol type distribution on the silica surface as a function of pretreatment temperature [20].

gravimetric results allowed Morrow and McFarlan to conclude that roughly 19% of total silanols are isolated [34].

Such a large discrepancy between the results obtained by these methods leaves the question of a relative ratio of free to bonded silanols still open. In general: the methods used for the determination of bonded and isolated silanols can provide various results as the phenomena measured on these two kinds of sites (reactivity, IR absorption, adsorption) always overlap due to a chemical similarity of the sites, also the methods of determination of relative concentration of the sites are not precise enough. Moreover, various silicas can contain various relative amounts of the sites. Kirkland et al. [53,54] showed, however, that the ratio of isolated to bonded silanols can be controlled to some extent; the rehydroxylation process can substantially increase the number of bonded silanols and simultaneously decrease the adsorptivity for which isolated silanols are blamed.

The temperature-programmed desorption studies of pyridine allowed also to measure an energetic heterogeneity of the silica surface. Bonded silanols were considered as low-energy adsorption sites (with desorption energy E_d=50–65 kcal/mol) while isolated silanols (with E_d=~90 kcal/mol) were found to be much stronger adsorption sites [42].

The existence of a small, strongly interacting population of silanols on the silica surface was again supported by the difference in fluorescence decays when chlorosilane versus hydroxysilane reagent was used to bind pyrene to the surface [72]. Hydroxysilane was found to react predominantly with the active silanols [72].

Wang and Harris also stated that the active surface sites were clustered [72]. Reactivities of various functional groups at silane modifiers were examined by Lork et al. [73]; chlorosilanes were among the most reactive. However, there is growing evidence in the literature that chloro- and alkoxysilanes do not react with a dry silica surface below 200°C [20,72,74–77]. That resembles earlier findings of Snyder and Ward [240] on slow and selective reactivity of trimethylchlorosilane with the silica surface. According to Nawrocki's studies on deactivation of the silica surface, the adsorption of chlorosilane on the silica surface was fast and nonselective, while the adsorption of silazane was reversible [242].

Chlorosilanes can react with a dry silica surface (silanols) only at temperatures >200°C [75]. The importance of amine catalyst in this reaction is emphasized [20,74,78,79]. Blitz et al. [79] have shown that amines with exchangeable protons are better catalysts than tertiary amines. The base-promoted silylation of the silica surface is described in detail by Tripp et al. [80]. To summarize:

- fully hydroxylated silica contains more bonded than isolated silanols;
- different silanol types have different reactivity and different adsorption activity;
- isolated silanols are particularly active in adsorption of amines;
- rehydroxylation can increase the number of bonded silanols and decrease the number of isolated silanols;
- chlorosilanes and alkoxysilanes need a catalyst to react with silanols (at T<200°C).

2.3. Silica surface de- and rehydroxylation

Silanols present on the the silica surface can be thermally removed with formation of the siloxane bond and release of water molecules. As this can be used as a method for determination of the silanol surface concentration, it is necessary to know the behaviour of physically adsorbed water. It is commonly believed that a 'dry' silica surface is also essential for successful synthesis of bonded phases (e.g. [17]). There is an increasing number of reports on the important role of adsorbed water in the synthesis of bonded silylalkyl phases.

In an important paper, Zhuravlev [40] showed that at 190±10°C essentially all physically adsorbed water is removed. Dehydration and dehydroxylation of silica were shown to be very heterogeneous processes. According to Zhuravlev, the majority of physically adsorbed water can be easily removed from the surface at 25°C in vacuum. The same was observed by Bronniman et al. [81] by means of a ^{1}H CRAMPS NMR study of silica dehydration. But, a monolayer (or less) of water is retained on the surface up to ca. 200°C [40]. According to Kinney et al. [39], the first monolayer of strongly hydrogen-bonded water plays a significant role in bridging any gaps between adjacent silanols on the silica surface. Ellipsometric measurements of water film thickness on quartz surface also confirm the particular importance of the first adsorbed monolayer. According to Gee et al. [82] the adsorption of water on a partially dehydroxylated surface proceeds through clusters, and remaining isolated silanols are considered as the main adsorption sites. As the hydration water seems to be necessary for the successful surface modification, the clusters can lead to patches of modified surface which had been frequently previously reported [72,83]. Further heating results in a rapid decrease in the surface concentration of vicinal silanols. At a temperature of 400–500°C dehydroxylation slows down due to an increase in the average distance between the neighboring free hydroxyl groups. This was nicely confirmed by Van Der Voort et al. [42,59,84] who proved by IR that the population of H-bonded silanols sharply decreases with temperature increasing above 200°C, while the amount of free silanols increases with the heat treatment (see Fig. 3). The increasing number of isolated silanols indicates the existence of 'clusters' of bonded silanols containing uneven number of the species (e.g. dehydroxylation of three bonded silanols leads to siloxane+isolated silanol). Temperature-programmed desorption studies showed, however, that isolated silanols are much more numerous (than detected by IR) and their amount also decreases with temperature. The relative content of isolated sites increases with heat treatment [42,59,84]. At about 450°C the bridged hydroxyl concentration is almost reduced to zero [40]. Similar observations were made by Bronniman et al. [81] by ^{1}H NMR. Also, the pore structure affects the dehydroxylation process; for small-pore silicas a decrease in the number of bridged silanols proceeds at lower temperatures than that for silica gels with wider pores [59,234]. This means that any excessive heat treatment can influence silica surface chemistry. It is therefore safer to use fully hydroxylated silicas for chromatographic or spectroscopic experiments. As has been shown in Section 2.1, there are silicas which have less silanols than normal (8 μmol/m^{2}).

Rehydroxylation of the silica surface is often applied before synthesis of chemically bonded phases [15,53,54]. Various methods of rehydroxylation and their impact on the quality of bonded phases were thoroughly evaluated by Köhler and Kirkland [53,54] and also discussed in a review [15]. Later,

Unger et al. [85] studied acidic/hydrothermal treatment of a series of commercially available silicas. It was shown that acid treatment does not change the specific surface area, but the silanol concentration may increase up to 10% after treatment. The authors noticed that acid treatment removes some trace metals from silicas. (Relevant discussion concerning the removal of metals reader can be found in [14].) According to Unger et al. [85], acidic treatment of native silica improved the column performance in terms of plate numbers and peak symmetry. For chromatographers, it meant that silica which had been subjected to any heat treatment would contain more isolated silanols which are stronger adsorption sites and are, therefore, according to Kirkland et al. [53,54], considered undesirable. The dehydration and dehydroxylation processes are shown schematically in Fig. 4.

To summarize:

- removal of strongly held monolayer of water on the silica surface requires a treatment at 200°C;
- further heating (200–400°C) results in dehydroxylation of bonded silanols (the number of isolated silanols may slightly increase);
- above 400°C the number of isolated silanols decreases;
- rehydroxylation increases the number of bonded silanols on the silica surface.

2.4. *IR of the silica surface*

IR is probably the most important tool which has been used for investigation of silica surface chemistry. Contemporary research is based mainly on Fourier transform technique (FT-IR) (e.g. [243]), diffuse reflectance infrared Fourier transform (DRIFT) (e.g. [53,54,153,154]) or FT-IR with photoacoustic detection (FT-IR PAS) (e.g. [84]). Most

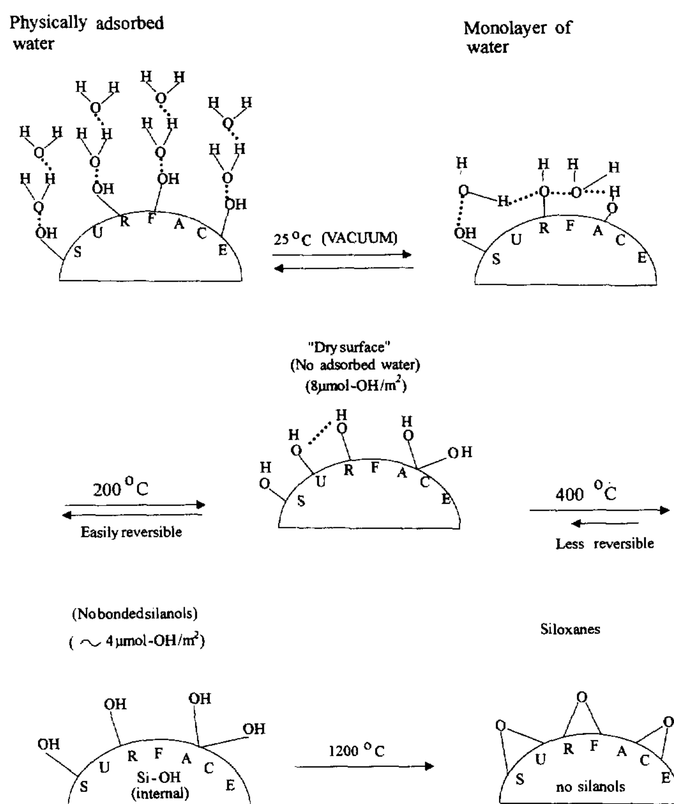


Fig. 4. Dehydration and dehydroxylation of a silica surface.

often the range of 3900–3000 cm^{-1} is explored. This requires removal of all adsorbed water as it absorbs IR light in the same region. To overcome this difficulty, near infrared spectroscopy (NIR) is sometimes used (e.g. [101,102]). As has already been mentioned, IR can distinguish between isolated, internal and bonded silanols. However, the bands often overlap and it is difficult to use IR for quantitative determination of particular silanol types. Geminal silanols absorb at almost the same wavenumber as isolated species.

Silanols absorb IR radiation in the broad region of 3800–3000 cm^{-1}. Usually a sharp band at about 3730–3750 cm^{-1} is accompanied by a broad absorption in the range 3600–3000 cm^{-1}. The band at 3730–3750 cm^{-1} is assigned to isolated silanols [1]. Hair and Hertl [86] first noted the splitting of the band into three. This was later confirmed by Van Cauwelaert [87] and Van Roosmalen and Mol [88,89]. According to Sauer and Schroeder [36], the band also contains absorption of geminal silanols. A confirmation can be found in a paper by McFarlan and Morrow [90]. The experimental –OH stretching frequencies of trimethylsilanol and dimethylsilanediol differ by only 2 cm^{-1} [37]. This is, however, a matter of controversy as, for example, very detailed DRIFT spectra of silica gels in the expanded range 3660–3780 cm^{-1} failed to show any fine structure [53]. Köhler and Kirkland noted, however, that silicas with absorption maximum slightly above 3740 cm^{-1} strongly retained amines, while silicas with the maximum below 3740 cm^{-1} have more favorable chromatographic properties. Morrow and McFarland [91] have shown that the IR absorption band at 3747 cm^{-1} (for aerosil) or 3743 cm^{-1} (for precipitated silica) is a result of overlapping of absorption of truly isolated silanol (at 3750 cm^{-1}) and of slightly perturbed vicinal pairs of silanols (at 3740 cm^{-1}). The Morrow and McFarlan assignment is (to some extent) in agreement with the Kirkland [53,54] hypothesis which was the basis for the classification of chromatographic silicas into types A and B. Low hydroxylated silicas with a maximum of absorption of SiOH above 3740 cm^{-1} were assigned type A, while fully hydroxylated silicas with a SiOH band below 3740 cm^{-1} were classified as type B [54]. The bonded phases based on type B silicas are believed to be better (less adsorptive toward amines) than those synthesized from type A silicas [12]. It is shown by Vansant et al. [59] that the wavenumber of absorption maximum (at approx. ~3740 cm^{-1}) moves to higher values when silica is subjected to thermal treatment. The importance of the 3740 cm^{-1} band limit between 'good' and 'bad' silicas was not confirmed by the studies of Sagliano et al. [92]. They showed that both 'good' and 'bad' silicas gave a single silanol band at 3735 cm^{-1}. However, the importance of free (unbonded) silanols for the stability of the chemically bonded phase was confirmed. Sagliano et al. [92] showed that hydrolysis of the less-stable phase led to the reappearance of an isolated silanol band. Conversely, the hydrolysis of the stabler phases produced only a minor amount of isolated silanols [92]. The isolated silanol absorption band at ~3750 cm^{-1} varies from one to another silica in a range of about 3760–3735 cm^{-1}. Thus, it seems impossible to use IR spectra as a single criterion for classification of the silica to less or more acidic type (B or A) as postulated by Kirkland et al. [53,54]. More generally we can say that the methods which provide information on bulk properties of silica (such as IR absorption, NMR resonance, apparent surface pH, etc.) cannot distinguish subtle differences in silica surface chemistry which can significantly influence the chromatographic performance. This fact has also been suggested by Unger et al. [85] and Cox [13]. It is worth adding here that Cab-O-Sil (fumed silica) does not adsorb amines [93,94]. The absorption maximum for fumed silicas is at ~3750 cm^{-1}, and for precipitated silicas is at ~3740 cm^{-1} [86,87,93,94]. According to Kováts [95], fully hydroxylated fumed and precipitated silicas are very similar.

The 3580–3540-cm^{-1} band is assigned to vicinal silanols [1] and it disappears upon heating. Another absorption band at 3680 cm^{-1}, usually overlapped by a sharp absorption band of isolated silanols and a broad band of vicinal silanols is assigned to internal hydroxyls [1,96]. The absorption by internal hydroxyls has been assigned to 3660- or 3650-cm^{-1} bands. This is discussed and well referenced in the paper of I-Ssuer Chuang et al. [97]. Kondo et al. [96] noticed that the shape of the –OH stretching vibration depends not only on a heat treatment but also on the method of synthesis of the silica. The same

group showed later [98] that silica obtained by hydrothermal treatment of silica hydrogels, at pH> 11 adjusted with base, has a new type of –OH group with an IR band at ca. 3500 cm^{-1}. This group can exchange as easily as free silanols to –OD group by adsorption of D_2O. Those –OH groups were, however, unreactive toward methanol and trimethylchlorosilane in contrast to free silanols [98]. The 3500-cm^{-1} band was, however, observed by Hockey [99] and the assignment of the band had been disputable even earlier.

A very broad absorption band, 3900–3000 cm^{-1}, of wet silica sample was resolved into its components by means of a curve resolver [98]. It appeared that the band is composed of six basic bands with maxima at 3870, 3750, 3630, 3470, 3260 and 3030 cm^{-1}. The band at 3030 cm^{-1} was assigned to physically adsorbed water and 180°C treatment easily removed it. The H-bonded –OH with various OH . . . H distances were responsible for 3260-, 3470- and 3630-cm^{-1} bands. Two former bands were removable by heat treatment at 680°C. The band at 3750 cm^{-1} was assigned to free silanols while the origin of the 3870 cm^{-1} remains unknown. The maxima of the low frequency bands changed upon increasing the temperature, while the bands at 3870 and 3750 cm^{-1} were virtually stable. This indicated, according to [100], that each component band might be composed of a few secondary components.

It is worth mentioning here that transmission near infrared (NIR) technique was successfully applied for quantitative surface silanol measurements [101]. An advantage of NIR over the fundamental IR region is a clear separation of silanol and water absorption bands [102]. Also, the suitability of photoacoustic near-IR spectrophotometry for the observation of silanols was shown [102]. The most interesting, for chromatographers, is the relation between IR-observed silica surface features and its chromatographic properties. Mauss and Engelhardt [243] showed by FT-IR that chromatographic retention of amines proceeds mainly on isolated silanols, while bonded silanols are responsible for the retention of –OH-containing compounds. Sudo [153,154] showed a relation between chromatographic properties of high-temperature endcapped supports and their DRIFT spectra. He found that the separation factor pyridine:phenol was positively correlated with the band area absorption of silanols.

IR spectroscopy is very often used for a characterization of modification reactions proceeding on the silica surface. The observations of silanol absorption bands which change upon modification permit better understanding of the reactions and the properties of the modified silicas. This will be referred to in the following sections.

To summarize:

- the 3760–3735-cm^{-1} band (more or less sharp) indicates the presence of isolated and geminal silanols (sometimes splitting of the band is observed);
- the 3680-cm^{-1} band is assigned to internal silanols;
- the 3620–3200-cm^{-1} broad absorption (centered at 3500 cm^{-1}) is due to bonded silanols (the absorptions often overlap the isolated and internal silanol bands);
- on the fully hydroxylated silica the isolated silanol IR band can be totally overlapped by the bonded silanol band.

2.5. NMR investigations of silica structure

NMR has proven to be an invaluable tool for investigation of silica surface chemistry. which has been reviewed by Albert and Bayer [9]. The ^{29}Si CP-MAS NMR is used to distinguish between geminal and isolated silanols. Higher reactivity of geminal vs. isolated (single) silanols was detected by means of ^{29}Si CP-MAS NMR [9,35]. The ^{13}C CP-MAS NMR is used mainly to investigate a mobility of the alkyl chains in chemically modified silicas [9]. In recent years further insights into the chemistry of silica was possible due to this technique. Scholten [103] has shown by a deuterium exchange and ^{29}Si CP-MAS NMR that some silanols (apparently seen by IR) are in fact internal structures, i.e. inaccessible for chemical modification. Their concentration was calculated as an equivalent of 0.82 $\mu mol/m^2$. This has been confirmed by I-Ssuer Chuang et al. [97] who found that about 9% of silanols on low surface silica are internal hydroxyls. The same method was used to show a shielding effect, i.e. inability of some silanols to interact with solutes due to the shielding by methyl groups of dimethyloctadecylsilane modi-

fier. According to the results [103], approximately half of the residual silanols on a typical octadecyl phase is inaccessible for interactions with solutes. However, in the same thesis contradictory results are presented. By inverse gas chromatography, Scholten [103,104] showed that the isolated silanol left after modification of the geminal site is more active in adsorption than the parent geminal site. This contradicts the earlier shielding hypothesis, as it is difficult to imagine silanol more susceptible for shielding than that left after modification of the geminal site. The ^{1}H CRAMPS NMR was used by Bronniman et al. [81] to show the silica dehydration process. The bulk of the adsorbed water was shown to be easily removable (vacuum at ambient temperature) while a monolayer of water is strongly held on the surface by H-bonds. Such a monolayer can be used for the synthesis of the so-called 'horizontally polymerized phase' (see Section 4.2).

3. Stability of bonded phases

Stability of bonded phases has been thoroughly investigated at low as well as at high pHs. Low pH stability of chemically bonded phases are of particular interest due to the fact that most of peptide and protein separations is carried out at pH 2 to overcome problems with the ion-exchange mechanism. Low pH stability was exhaustively examined by Kirkland's group [53,54,105]. They have found and thoroughly discussed the dependence of the stability of the bonded phase on the quality of the initial silica. The silica used for a synthesis can determine the stability of the resultant bonded phase [106]. According to Refs. [53,54], a fully hydroxylated silica is necessary to produce a stable chemically bonded phase. Such a silica exhibits: (1) a larger number of associated silanols, (2) higher apparent surface pH value, (3) lower adsorptivity for basic compounds, (4) better hydrolytic stability of bonded-phase ligands, and (5) increased mechanical stability.

The use of a bulkier substituent on the silicon of the stationary-phase modifier molecule should increase the hydrolytic stability of the phase. Since most RPLC separations of peptides and proteins are carried out at pH 2, it was proved by Kirkland's group [53,54,105] that the dimethyloctadecylsilane phases are susceptible to rapid destruction at such conditions. To overcome this disadvantage of conventional chemically bonded octadecyl phases, new, sterically protected phases were designed [111]. Instead of two methyl groups at the silicon atom, silanes with two isopropyl or isobutyl groups were proposed. Such a moiety is less vulnerable to destruction at low pH, and better shields the underlying silanols [103,107]. Coverage density is, however, much lower than for dimethyl ODS phases. Sterically protected phases are commercially available under the brand name Zorbax SB $C_{18/8}$ with a ligand density of 2 $\mu mol/m^2$ when compared to the related classical dimethyloctadecylsilane Zorbax Rx-C_{18} with a ligand density of 3.37 $\mu mol/m^2$ [108,109]. A higher stability of a bulky substituted phase was shown earlier by Ackerman [110] for triethylphenoxysilane compared to trimethylphenoxysilane. Sagliano et al. [106] demonstrated the higher stability of a triethylsilyl phase in comparison to a butyldimethylsilyl phase and attributed this to a shielding effect.

High-coverage stationary phases have the highest stability under acidic conditions [92,106]. Long-chain-bonded phases are stabler under acidic conditions than shorter alkyl phases [54,106]. The opposite is stated in a paper of Freiser et al. [112], however, the authors did not explicitly state which alkyl-chain modifier was used for the synthesis of the bonded phase. Trifunctional silanes lead to more stable polymeric phases (e.g. [106]).

The separation of certain organic bases at high pH would be very attractive. However, it is commonly believed that silica dissolves at pH>7.5. Recent developments in high pH application of bonded phases show that it is not necessarily true. In fact, in organic-rich mobile phases the dissolution of silica is very slow and acceptable for chromatographic purposes [113]. Miller and DiBussollo [114] observed that, despite the apparent destruction of the bonded phase at high pH (pH 9), *N*-acetylprocainamide retention was not affected. They could not identify the point of hydrolytic cleavage on the packing. The results of Kirkland et al. [109] suggest that the base attacks the silica matrix. A gradual dissolution of silica matrix in an alkaline mobile phase causes an increase of the relative (%C) carbon coverage (in

fact the carbon content in the column stays at approximately the same amount). It should be mentioned that saturation of an aggressive mobile phase (e.g. 0.1 *M* NaOH/80% MeOH as in [114]) with silicate considerably extends the column lifetime [114]. After reports of Wehrli et al. [115], Atwood et al. [116], Wheals [117] and Law and Chen [113], Kirkland's group started to investigate the possibility of using chemically bonded phases at pH as high as 9–12. The use of high pH for the separation of organic bases had been shown by Law in numerous papers [113,118,119]. Systematic studies [109,120–122] of commercially available octadecyl phases at high pH have shown that:

- densely bonded monomeric dimethyl-C_{18} ligands better protect silica from dissolution than bulky substituents;
- an aggressive mobile phase (high pH) dissolves rather the bulk of the support, while the hydrolysis of a bonded phase is low (this is also supported by [123]);
- the nature of a bonded phase (monomeric or polymeric) does not significantly influence column stability;
- the nature of porous silica determines the rate of dissolution (column packings made with silicas prepared by aggregating silica sols (sol–gel silicas) show better resistance to dissolution than silicas made by gelling soluble silicates [247]); pure silicas dissolve more rapidly, silicas contaminated with metals dissolve more slowly;
- acetonitrile prolongs column life in contrast to methanol [109];
- buffers dissolve silica (carbonate and phosphate buffers are much more aggressive than organic buffers);
- more concentrated buffers dissolve silica faster than less concentrated;
- high temperature stimulates silica degradation;
- buffer cations also influence silica dissolution: $NH_4^+ > K^+ > Na^+ > Li^+$ [120,121].

Experiments [109,120,121] indicate that silica-based chemically bonded packings are surprisingly resistant to high pH. Careful selection of material and conditions should allow working with such packings at pH 10–12 [121]. The resistance of silica-based columns to highly alkaline conditions is well illustrated in Fig. 5, which shows that ca. 30 000 column volumes of a pH 11 mobile phase do not change the column performance.

Those observations indicate that for high pH separations the A type silica is better suited than the B type. The experiments proved a quite high stability of silica columns at pH 9, no substantial degradation was noted up to 25 000–30 000 bed volumes [109]. The observation that metal-contaminated silica is more resistant to alkaline conditions has already been confirmed by studies of Falkenhagen and Dietrich [123,124]. They found that silica doped with Al^{3+} can be successfully used at pH 10 for about 10 000 bed volumes. Earlier, similar effects were observed for silicas modified with zirconia [125]. Magnesia–silica supports have been shown to generate symmetrical peaks of organic bases. High-temperature calcination of mixed oxides ($MgO-SiO_2$) led to a considerable increase in surface acidity [126]. Studies of Kareko et al. [127] confirm that metal oxides generate considerable acidity in mixed impure silica. Even such an alkaline metal as Mg^{2+} generates highly acidic sites on a silica surface. Results in [127] fully confirm the conclusions of a discussion concerning acidity in our previous review [14], as well as our earlier results [128,129]. However, the increased acidity of certain silanols does not substantially influence chromatography of bases when they are in the nonprotonated state.

4. HPLC of organic bases

There are many controversial opinions in the literature concerning the HPLC of amine bases. Some authors advocate low pH, others high pH, for the chromatography of amines, while commercial companies advocate the use of their 'base-deactivated' packings[1]. In some papers metal-free, pure silica is recommended, while in others silica containing trace metals is said to be better due to its high pH stability. The average reader is no doubt confused by these opinions. In this paper we will

[1]The terms: 'base' or 'basic solute' used throughout this paper refer to the uncharged forms of the organic bases. The author realizes that under chromatographic conditions those basic solutes often exist in forms of charged species which are not bases from the point of view of Lewis theory.

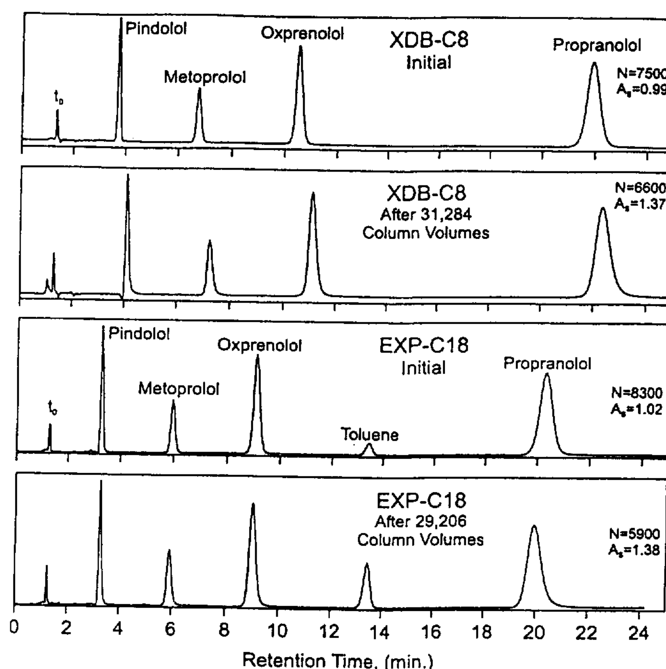


Fig. 5. Separation of highly basic β-blocker drugs at high pH. Conditions: columns, 15×0.46 cm, 5 μm, experimental, double end-capped, dimethyl-C_{18} (EXP-C_{18}) and dimethyl-C_8 (XDB-C_8) phase; mobile phase, 55% methanol/45% 0.05 *M* 1-methyl-piperidine–HCl; 24°C; flow-rate, 1 ml/min; detector, UV 215 nm; sample, pindolol, metoprolol, oxprenolol, propanolol, pK_a=9.5–9.7 (0.165, 0.413, 0.413, and 0.083 mg/ml, respectively) and toluene (~3 mg/ml), 5 μl injection [247].

summarize the knowledge concerning the chromatography of bases to show the complexity of the problem. The chromatography is so complex due to several retention mechanisms which can possibly govern the chromatographic behavior of the bases. The following mechanisms are usually considered in RPLC of organic bases: (1) hydrophobic interactions (with both hydrocarbon and siloxane moieties); (2) ion-exchange; (3) salting-out effect; (4) hydrogen-bond interactions.

It is definitely easier to predict chromatographic properties of an organic base when one of the mechanisms dominates. However, when several retention mechanisms contribute to the retention, then the problem becomes much more difficult. It has been shown in numerous papers that ion-exchange is one of the important retention mechanisms in chromatography of bases, and this will be described in detail.

The following phenomena are often observed when chromatography of basic compounds is performed: (1) unsymmetrical peaks (tailing); (2) no elution (in extreme cases); (3) low efficiency of chromatographic column; (4) strong dependence of retention on sample size.

The literature specifies three reasons for such behavior of the bases: (1) interactions with silanols; (2) interactions with metals; (3) interactions with a small group of particularly acidic silanols.

Popular belief blames residual silanols for the undesired interactions with organic bases (see, e.g. [130]). However, all chemically bonded phases contain residual silanols and it has been shown in numerous papers that some phases are much better than others. Bidlingmeyer et al. [33] showed that, even when silanol concentration increases (from C_{18} silica through partially covered 50% C_{18} to bare silica), tailing of bases actually decreases. A similar example of lower efficiency of the C_8 phase over a bare silica is shown by Cox and Stout [32]. This contradicts the concept of per se 'bad' silanols. Interactions with metals were described widely in

previous reviews [14,15]. We can say that on high-purity silicas which are widely used the interactions with metals are negligible. However, the metal traces buried in silica matrix can be responsible for considerable enhancement of some silanol acidity. This was also discussed in the previous review [14,15].

To get satisfactory results in the chromatography of bases the user has the possibility to manipulate: (1) the stationary phase; (2) the mobile phase; (3) the sample size.

Stationary phases available on the market show very different chromatographic affinities toward organic bases. The user has to determine by himself the silanophilic properties of the column or packing he uses, and the way to do it is described in Section 4.1.

4.1. Detection of silanophilic interactions: methods of characterization of bonded phases

Since there is a great variety of silica-based materials there is a need for some simple characterization tests. As we have emphasized in our previous review [14], commercially available bonded phases vary in their properties and it is risky to replace a column from one manufacturer with a column from another. Reproducibility has improved over the years and, according to Dorsey and Cooper [19], modern columns typically show variations of capacity factor of <5% from lot to lot. However, there are some reports in the literature that the problem has not been solved satisfactorily to HPLC column users. This is particularly true for chromatography of proteins [131]. Even highly sophisticated phases based on type B silica were shown to vary from batch to batch [132]. The silanophilic tests should be divided into two groups:

(1) The first group tests are based on detection of H-bonding ability of silanols toward polar compounds. Those tests are usually performed in nonpolar (or nonaqueous) solvents. Not only amines are able to detect silanol H-bonding activity – also other compounds capable of H-bonding (e.g. ketones) can give some idea on the activity. Gas-phase (GC) testing of the siliceous materials also belongs to the same groups. In the gas-phase, H-bonding occurs between amine molecules and silanols (e.g. [133]).

(2) The second group of tests is designed to detect ion-exchange ability of some silanols toward protonated amines. Those tests are carried out in a polar (aqueous) environment.

The degree of consistence of the two kinds of tests has not been clearly established yet. However, when conditions of the second type of tests are not properly chosen (e.g. amine used as a solute is not protonated – e.g., pyridine at pH 7 is not protonated) then the ion-exchange ability of the packing is not tested while a hydrogen bonding between the amine and the silica is highly improbable (in the aqueous mobile phase the amine molecule will be hydrated with water molecules), the same is true for silanols – thus a hydrogen bond between them is hardly possible. The situation changes when silanols are ionized and amine is protonated. McCalley discusses the case where strong bases (pK_a=8–10) improve symmetry of their peaks upon lowering pH from 7 to 3 [134,135]. In the same case, peaks of pyridine worsen on the same set of packings. The explanation can be that: strong bases are protonated at pH 7, the silanols are also ionized and there are favorable conditions for ion-exchange interactions. At the same pH pyridine is not protonated – it is not a sensitive probe for ion-exchange sites. When pH changes to 3 there are far fewer ion-exchange sites on the surface (only the most acidic sites are ionized) – thus there is much less ion-exchange interactions with strong bases. Pyridine is protonated at pH 3 and it is now sensitive to ion-exchange sites.

Strong silanophilic interactions with basic solutes are manifested as a severe tailing and low mass recovery for proteins, particularly when the amount injected is less than 5 μg [136]. In routine practice there is a need for a rapid estimation of a new column or packing ability to interact with organic bases. The literature proposes a number of the so-called 'silanophilic tests' for the purpose. According to Bogusz et al. [137], the test mixture should be made taking into consideration the following points: (1) efficiency of the column for neutral, acidic, and basic solutes; (2) resolution of selected close-eluting compounds; (3) elution of all compounds in a reasonable time.

The methyl red method which was briefly described in an earlier review [14,15] (see also [138]) was proposed for estimation of accessible silanols, but the application of the method appeared to be too

complicated and dependent on too many variables [139]. Sadek and Carr [140] proposed to use a cyclic tetraaza compound as a silanophilic additive to the methanolic mobile phase. The test is said to be extremely sensitive for silanol activity. A simple test to characterize bonded phases was developed by Daldrup and Kardel [130] for pharmaceutical use. Three compounds were used for the test: diphenhydramine, MPPH (5-(*p*-methylphenyl)-5-(phenylhydantoin)) and diazepam. Therefore the test is often called the 'DMD test'. Diphenhydramine, which is a basic drug, is extremely sensitive to strongly interacting sites (in the paper authors have stated that the compound is 'sensitive to residual polarity, especially that arising from free silanol groups', however, all chemically bonded phases contain unreacted silanols, but only some of them has shown strong interactions with diphenhydramine). MPPH served as a reference compound in the test, while diazepam was used to indicate the resolution of the column. Another simple test to characterize HPLC columns was developed by Engelhardt and Jungheim [141]. According to them a 'good' column should give symmetrical peaks for basic solutes (symmetry <1.3) and retention time independent of sample size, even when the latter is increased up to 20 times. Engelhardt and Jungheim propose to use a mixture of aniline and phenol as the test solutes. For a 'good' column one should expect that: aniline will elute before phenol, a ratio of the asymmetry of the aniline peak to that of phenol should not exceed 1.3. In additional tests they advise to use *N,N*-dimethylaniline, *o*-, *m*-, *p*-toluidines and toluene. For the 'good' column *N,N*-dimethylaniline should elute before toluene, while three isomeric toluidines should hardly separate as they have similar hydrophobicity and different basicity. The test is carried out with a methanol/water (55/45) mobile phase.

Nondek et al. [142] proposed to use retention of pyridine and 2,6-dimethylpyridine as a test for residual silanols. Quinine and quinidine were proposed by McCalley [143] as sensitive probes for a detection of silanophilic interactions. Of several columns investigated, LiChrosorb C_8 Select B yielded the best results. The test has also shown the lack of compatibility with the earlier proposed method for a detection of underivatized silanols on the bonded phase surface [143]. The old method developed by Majors [144] consisted of measuring k' of nitrobenzene with dry *n*-hexane as a mobile phase.

4.2. Stationary phase requirements

Most HPLC users do not synthesize their own packings, they have to rely on commercial columns; however, we want to show what are the important characteristics of a stationary phase.

High purity silica: it has been previously shown (see [14,15] and references therein) that metals buried in silica matrix can have a detrimental effect on silica's chromatographic properties by generating strongly acidic silanol sites on its surface. The mechanism of the generation of acid sites has been described in detail [14] on the basis of catalytic literature [145–147]. Presently, many commercially available silicas are advertized as 'high-purity' or 'metal-free' materials [122,148,149]. However, when siliceous packing is intended to be used at high (>8) pH, higher stability is expected for silicas containing metal contaminants [109,120,121,123–125]. (See the above discussion concerning the stability of phases.)

Rehydroxylation of the silica surface: silica which is excessively heated first looses physically adsorbed water and then silanols. Dehydroxylation proceeds first on H-bonded silanol groups, i.e. the relative coverage of isolated silanols increases. Since those isolated silanols are blamed for the undesirable adsorption of organic bases, it is reasonable to search for methods for their removal or minimalization. Köhler and Kirkland [53] have shown that effective rehydroxylation of the silica surface can be achieved by treatment with acids or bases. In general, bases lead to well-rehydroxylated silica but also to a heterogeneous surface of SiOH groups. Satisfactory rehydroxylation was obtained with quaternary ammonium bases and some amines. The best acceptable results were achieved with HF. It was also found [53] that the procedure of rehydroxylation proposed by Gobet and Kováts [150] cannot give suitable results. A similar procedure of hydrothermal hydrofluoride acid treatment of silica prior to modification with octadecylsilane was proposed by Hetem [3]. According to him, such treatment increases the number of geminal silanols on the surface.

It is necessary to add here that many of the applied

methods used for 'cleaning' a silica surface (mainly removal of trace metals), such as the earlier described (see [15] and references therein) acid treatments can also give a more homogeneous, fully hydroxylated surface. Conversely, rehydroxylation procedures also remove trace metals from the silica matrix [3].

What is the aim of rehydroxylation? The procedure is usually applied mainly to restrict the amount of free, isolated silanols. Those silanols are believed to be responsible for the ion-exchange interactions. Cox and Stout [32] have proved that the major difference between de- and rehydroxylated materials is their ability to ion-exchange (see Fig. 19). The differences observed can be caused by a lower acidity of rehydroxylated material or lower amounts of ion-exchange sites on the rehydroxylated silica. Now, two questions arise: whether H-bonded silanols are capable of ion-exchange? and whether rehydroxylation change the acidity of ion-exchanging silanols? The former implies that H-bonded silanols are less acidic and they do not take part in ion-exchange at the pH of the experiment (not stated unfortunately in [32]). Single, isolated silanols retain their ability to dissociate, i.e. they must be more acidic. Probably, the rehydroxylation changes (decreases) the number of isolated silanols and this is why we observe a lower ability of rehydroxylated surface to ion-exchange. This would also mean that bonded silanols are not participating in ion-exchange.

End-capping: very often used to improve the quality of the original chemically bonded phase by blocking access to some residual silanols on the silica's surface. The process is well known and when the initial coverage in a C_{18}/C_8 phase is not too high the end-capping can substantially improve retention of organic bases (see, e.g. [149]). End-capping is usually carried out with compounds able to generate trimethylsilyl groups ($(CH_3)_3Si$–), the most popular being trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS). McMurtrey [151] has shown that of a variety of such compounds trimethylsilylimidazole is the most reactive. On the other hand, Buszewski [152] has proved that the application of HMDS can lead to the partial destruction of the C_{18}/C_8 chemically bonded phase. NH_3, the side product of the reaction, has been blamed for the destruction. A high-temperature (350°C) HMDS end-capping procedure also led to a decrease of carbon coverage. The decrease was the highest for monofunctional C_{18} phases while trifunctional phases gained some carbon after this procedure [153].

Recently [153,154] it has been shown that cyclic organosilanes can be used for end-capping of polymeric chemically bonded phases originated from trichlorosilanes. Phases end-capped at high temperature with cyclosiloxanes were shown to perform excellently on the Engelhardt test. It was explained by the formation of dimethylsilyl loop structures on the silica surface leading to elimination of a vast amount of silanols. The elimination was confirmed by DRIFT spectra of the modified silicas. A linear relationship was found between relative retention of phenol/pyridine and IR absorption in the OH region. It has also been spectroscopically confirmed that isolated silanols interact more strongly with basic solutes than H-bonded silanols do [154]. Zhang et al. [155] have shown that the residual activity of RP columns used in supercritical fluid chromatography can be significantly reduced by a dynamic in situ silanization with diphenyltetramethyldisilazane.

Bulky substituents: see discussion in Section 3.

Horizontally polymerized phases: They can be understood as an extension or transfer of the concept of 'self-assembled monolayers' (SAM) [156] to chromatographic supports. SAM are densely packed, ordered films attached to the surface via chemical bonds. Regular SAM should have a density of 8 $\mu mol/m^2$. However, such a dense, closely packed phase would be hardly penetrable by solute molecules. Wirth at al. [157–160] in a series of papers characterized synthesis and chromatographic performance of mixed, horizontally polymerized monolayers on silica gels. The applicability of such phases to chromatography of bases has not been evaluated yet. It was proved that the aniline peak on the SAM phase is more symmetrical than on the conventional C_{18} phase [159]. The surface is fully covered irrespective of the number of surface silanols. Not all silane molecules are covalently bonded to the surface. According to the estimation of LeGrange et al. [83], on a flat silica surface only 10–20% of chains in a monolayer are bonded to the surface. In the view of Tripp and Hair [75,76], bonding of the layer to the

silica surface is doubtful. According to them chlorosilanes do not form covalent bonds with silanols. A polymerized layer is simply adsorbed on the surface. (Similar results were presented by Silberzan et al. [77].) The main feature of this technique is deposition of the coating molecules from an organic solvent on a cleaned hydrated surface [59]. Water intrinsically adsorbed to the surface (a monolayer – see Bronniman et al. [81]) is used to promote the polymerization of trifunctional silane on the silica surface. Strictly controlled amounts of water provide a reproducible stationary phase [161]. When the silica surface is highly dehydrated, submonolayer coverages are obtained, and islands of molecules with ordered chains are observed [83]. It has to be emphasized that SAM can only be obtained with trifunctional silanes. Dimethyloctadecylsilane cannot form SAM due to steric hindrance [162]. Horizontal polymerization of silanes provides an excellent sterical barrier which reduces exchange of protons between silanols and the mobile phase. Wirth and Fairbank synthesized mixed C_{18}/C_1 and C_{18}/C_3 phases. The phases were shown to be stable at low and high pH [161]. The total coverage obtained with this method is comparable to the theoretical value: 3.1 μmol C_{18}/m^2+4.9 μmol C_3/m^2 reported in [158]. The concept of horizontally polymerized phases is similar to another approach of stationary-phase manipulations, i.e. to encapsulation of rigid silica particles into polymer cover.

Polymer encapsulation: this is another method which was intended to overcome the problem of strongly interacting sites on a silica surface as well as low stability of silica at higher pHs. Porous polymers are used in HPLC and they can be applied over a much wider range of pH than siliceous packings. However, they exhibit a number of drawbacks: lower column efficiency, weaker pressure resistance, swelling or shrinking upon change of solvent. Thus, the intention of the research was to develop porous silica particles covered with a thin layer of polymer. Such a packing should be characterized by the mechanical stability of the silica core, a wider pH range of application, it should not swell or shrink, and the strong adsorption sites should be covered with a polymer layer. The ideal polymer layer should be thin, to assure a rapid mass transfer, to totally cover the silica surface and not impede access to the pores. Immobilization of organic polymers may be achieved by: adsorption, insolubility of the polymer in the used eluent, attachment of the polymer to the surface by a chemical bonding, and crosslinking of immobilized polymer chains [163]. There are several approaches to do that: Schomburg et al. [164,165] immobilized polymer molecules on a porous silica surface; Ohtsu et al. [166] coated silica with organosilicon prepolymer, then polymerized it and finally modified it with C_{18} alkyl moieties. A similar approach was presented by Engelhardt et al. [167] who had initially modified silica with vinyltrichlorosilane and then polymerized vinyl groups with acrylic derivatives. The polymer-coated packings were shown to have better than initial silica chromatographic properties in respect to pH stability [166] (however, somewhat disappointing in [167]) and comparable to conventional ODS phases efficiency [166]. Polymer-encapsulated packings were also shown to produce symmetrical peaks of simple amines [167]. These papers and [168] have also shown that polymerization mainly proceeds in micro- and mesopores, considerably reducing pore volume and surface area (see, e.g. [166]). According to Hanson et al. [168], the polymer coatings do not result in a homogeneous polymer film but in an inhomogeneous loading where the bulk polymer is mainly located in the pores of the silica. The variety of silica stationary phases with immobilized polymers has been comprehensively reviewed by Petro and Berek [163].

Electrostatic shielding: The idea of the shielding is shown in Fig. 6. Bonded silane contains amino or peptide [169] groups which can be charged under acidic or neutral conditions (similarly to solute organic base). When a positive charge is located close to the surface it will repel a positively charged solute. The repulsion force protects the solute molecule against ion-exchange with dissociated silanols (negatively charged SiO^- sites). This is employed in Suplex pK_b-100 packing by Supelco Inc. [170], however, the detailed chemical structure of the packing has not been revealed. Suplex pK_b-100 packing can easily separate with symmetrical peaks a mixture of dimethylpyridine derivatives at pH 7, or paraquat and diquat (quaternary ammonium compounds) at pH 3 [170]. The Suplex pK_b-100 packing was followed by another modified silica from the

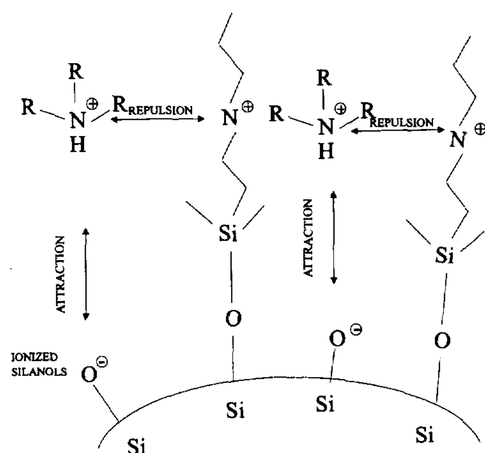


Fig. 6. The idea of electrostatic shielding.

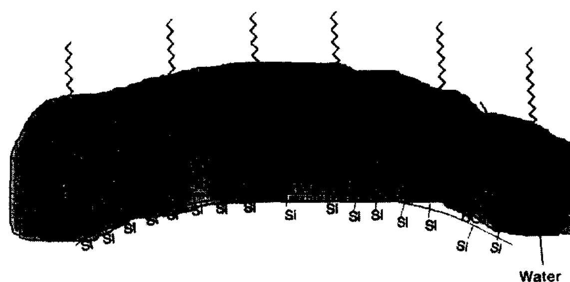


Fig. 7. The structure of Supelcosil ABZ^{+}-Plus [172].

same company, i.e. Supelcosil LC-ABZ designed for separation of compounds of zwitterionic nature. The chromatographic properties of the column were compared to that of the ODS phase [171]. ODS was found to be more hydrophobic than LC-ABZ. Later, the LC-ABZ phase evolved to the ABZ^{+}-Plus phase and more structural features have been revealed [172]. According to Ascah et al. [172] the support is synthesized in a one-step procedure contrary to the earlier version of the phase. The more hydrophobic nature of the ODS phase was attributed to a higher degree of hydration of the ABZ phases. The higher affinity of amide-functionalized supports for water adsorption was observed by Buszewski et al. [173,174], and was explained by the presence of polar groups in alkylamide phases. Significant excess of water in the shorter-chain alkylamide phases decreased the retention time for some polar compounds such as amines. (However the effect of short chains has also been reported for alkyl-bonded phases [112].) Buszewski et al. [175,176] have also noted that a mild thermal treatment of the alkylamide phase considerably worsens its selectivity toward hydrophobic solutes. Another feature of the alkylamide phases is engagement of the amide group in H-bond with unreacted silanols [172,177]. Altogether: the higher hydration layer and H-bonded silanols means the better shielding of the residual silanols, and thus the better chromatography of polar solutes including organic bases [172]. The structure of Supelcosil ABZ^{+}-Plus is shown in Fig. 7.

Alkylamide phases are usually synthesized in two-step synthesis; first by bonding of aminopropylsilane and then reacting the aminogroup with a suitable alkanoyl chloride. Such phases were synthesized by Buszewski et al. and are described in numerous papers [169,173–177]. A similar concept of the bonded phase is presented by Czajkowska and Jaroniec [178]. A disadvantage of the two-step procedure is the proportion of unreacted amino groups on the modified surface, which makes the surface less defined. One-step modification procedures have also been recently developed and they are reported for LC-ABZ^{+}-Plus packing [172]. Another possibility of one-step modification of silica to get 3(chlorodimethylsilyl)propyl-*N*-octylcarbamate is presented by O'Gara et al. [179]. Much lower retention of amitriptyline on the carbamate phase when compared to the similar C_8 phase was noted [179].

A packing with a peptide group chemically bonded to the silica surface [169] was also able to separate the Daldrup and Kardel [130] mixture with symmetrical peaks. Another concept of silanol shielding has recently been presented by Feibush [180]. In his approach, basic/polar ligands are chemically bonded to the silica matrix. Their role is to form hydrogen bonds and ion-pairs with free silanols. As the complete structures of the bonded moieties have not been revealed it is difficult to discuss the exact mechanism of chromatographic separation on such phases [180].

The troublesome chromatography of organic bases has forced workers to invent a variety of techniques which were designed to improve the chromatography

or to improve the results. Some of these measures are simply 'practical' (i.e. obtained by a 'trial and error' method), while some of them required intensive research. Of those methods we can mention: (1) addition of a simple amine or quaternary ammonium salt to the mobile phase – this approach is used very often, however, it does not guarantee positive results; (2) application of especially designed, deactivated HPLC stationary phases for chromatography of organic bases; (3) ion-exchange chromatography on plain silica (applicable only to ionic solutes); (4) application of a dynamically modified silica chromatography (applicable to ionic as well as to non-ionic solutes).

All of those methods will be briefly described below.

4.2.1. 'Base-deactivated' packings

Following numerous reports in the chromatographic literature, companies started to produce especially designed packings for analysis of organic bases. There is a variety of such packings on the market and the best known are listed in Table 1.

A comparison of the properties of all columns is impossible for two reasons: (1) there is no universal test applied by everyone. Of the variety of 'base-deactivated' columns usually only a few are compared by a single author with the same test; (2)

Table 1
'Base-deactivated' columns

Packing	Manufacturer	Ref.
LiChroCART Superspher RP-8e	Merck	[184]
LiChroCART Superspher RP-18e		[184]
LiChrosorb RP-Select B		[187]
Polyspher RP-18		[186,230]
Purospher RP-18		[191]
Lichrospher 60 RP Select B		[187,186]
LiChrospher RP-8 Select B		[135]
Ultrabase C_8	Shandon SFCC	[184]
Ultrabase C_{18}		[184]
Zorbax SB-C_{18}	Rockland Technologies	[122,191]
Zorbax RX C_8		[184,205,207]
Zorbax RX C_{18}		[122,184,190,191,205,207]
Spherisorb ODS-B	Phase Separations	[186]
Hypersil BDS-C_{18}	Shandon UK	[186,190,191]
Supelcosil LC-ABZ	Supelco	[186,191,230]
Supelcosil LC-ABZ+ Plus		[191]
Suplex pK_b-100		[185,187]
Nucleosil C_{18}-AB	Macherey-Nagel	[137,184,185,191]
μ-Bondapack C_{18}	Waters-Millipore	[187,190]
Novapack C_{18}		[187]
Symmetry C_{18}		[191]
Exsil 100 ODS B	Exmere	[187]
Kromasil KR-100-5-C_{18}	Eka Nobel	[187,190]
ACT-1 C_{18}	Interaction	[187]
Chromspher B	Chrompack	[186,191,230]
Bakerbond BDC	J.T.Baker	[186]
Asahipack ODP-50	Asachi Chem. Co.	[230]
Inertsil ODS	GL Sciences	[137,185,190,191]
Prodigy 5 ODS-2	Phenomenex	[191]
YMC-Basic	YMC Inc.	[190,191]
Techsphere ODS-BDS	HPLC Technology	[137]
Encapharm RP-18 TS	Dr. I.Molnar, Berlin,	[137]
Synchropack RP-SCD	Synchron Inc.	[137]

chromatographic effects are compound dependent, and a column superior in one application can appear worse in another.

We will try to compare the available material as far as possible.

At first let us compare bare silicas which can be used in normal-phase chromatography or as ion-exchanger for chromatography of organic bases. Surface and structural properties of several silica gels used in HPLC were compared by nitrogen adsorption–desorption isotherms and characterized by adsorption energy distribution [181]. The studies showed that HPLC silicas are similar. Basically two kinds of adsorption sites are found on the surfaces with maxima located at about 6 and 13 kJ/mol. Small fractions of high-energy sites were also noted for Hypersil, Partisphere and Vydac. The differences were also found for pore size distributions: Inertsil, Altima, Kromasil and Apex silicas have a relatively narrow pore-size distribution with a maximum at approx. 10 nm, while Hypersil, Vydac and Partisphere silicas have a much broader pore distribution range from 10 to 100 nm [181]. In the following studies on modified (C_8) silicas the authors have shown that the modification has removed virtually all high-energy sites from the silica surface. This indicates that such results are not necessarily relevant to chromatographic properties observed in reality [182]. Kirkland et al. [183] compared several bare silicas in normal-phase chromatography. The silicas were arbitrarily chosen as those belonging to type B, i.e. highly purified, fully hydroxylated with low activity toward amines. A test mixture contained: toluene, acetoacetanilide, catechol, and 3-pyridylacetonitrile. Catechol was missing in the chromatogram from Hypersil, while 3-pyridylacetonitrile did not elute from Zorbax SIL column. Let us remember that the apparent pH of Hypersil's surface is 8.5–9.0 [14], while that of Zorbax is 3.9–5.6. An analysis of the peak shapes and retentions allowed Kirkland et al. [183] to rank the silicas from best to worst: Zorbax RX>Nucleosil>Kromasil>Hypersil>Spherisorb> Zorbax SIL>Ultron>Novapack. Since it is the only ranking of bare silicas to our knowledge, we will compare base-deactivated phases to demonstrate that such a ranking is too compound dependent. We will compare the number of plates generated under the same conditions.

Verne-Mismer et al. [184] compared several deactivated commercial columns for the analysis of a quaternary ammonium analogue of α-tocopherol and some related compounds. According to them, Ultrabase packings were superior for the analysis. The results are presented in Table 2.

McCalley compared performances of several base-deactivated columns for some basic compounds in order to find the best column for determination of tobacco alkaloids. The results are shown for two mobile phases (buffered and unbuffered) in Table 3 and Table 4.

None of these columns seemed suitable for determination of alkaloids, but buffering of the mobile phase significantly improved the applicability and efficiency of the examined columns.

As can be seen from the results, benzylamine and nicotine are much more 'demanding' solutes than pyridine, which is not charged at neutral pH and thus is not sensitive to ion-exchange mechanism.

Another comparison of the performance of the base-deactivated column can be found in the paper of Paesen et al. [186], who evaluated the columns for determination of erythromycin. The results are shown in Table 5.

A qualitative comparison of several base-deactivated phases was provided by Vervoort et al. [187]. The columns were compared by chromatographing a set of organic bases using methanol–10 mM sodium phosphate buffer (pH 7.4) as the mobile phase, taking into account the peak shapes, number of plates generated and stability of retention on the volume of the mobile phase pumped over the columns. The comparison is shown in Table 6.

Table 2

Efficiency of several base-deactivated packings in analysis of the quaternary ammonium analogue of α-tocopherol [184]

Base-deactivated packing	N (plates per column)
Ultrabase C_8	3500
Ultrabase C_{18}	3400
Nucleosil C_{18}-AB	2450
Zorbax RX-C_8	800

Conditions: mobile phase, 0.15 M NaH_2PO_4–methanol (65/35 v/v); apparent pH 5.2; flow-rate, 1 ml/min; temperature, 40°C; detection, UV at 280 nm; sample, 9 μg of the compound in 50 μl of water.

Table 3
Performance of the test compounds on deactivated ODS columns

Base-deactivated column	N/m (plates per 1 m of column)		
	Pyridine	Benzylamine	Nicotine
Nucleosil C_8-AB	5320	<1000	<1000
Suplex pK_b-100	33 000	2700	2890
Inertsil	44 400	<1000	1300
LiChrospher RP8 Select B	26 500	retained	5760

Mobile phase: methanol–water (55:45) (unbuffered) [185].

Table 4
Performance of the test compounds on deactivated ODS columns

Base-deactivated column	N/m (plates per 1 m of column)		
	Pyridine	Benzylamine	Nicotine
Nucleosil C_8-AB	4920	1990	5100
Suplex pK_b-100	36 200	9130	22 300
Inertsil	40 700	31 800	42 300
LiChrospher RP8 Select B	24 300	10 600	8400

Mobile phase: methanol–0.05 *M* phosphate, pH 6.25 (55:45) [185].

According to Bogusz et al. [137], a quaternary ammonium compound in the mobile phase improved efficiency of all tested base-deactivated columns (except Inertsil ODS). This indicates that some active silanols are still available for interactions with basic compounds despite the 'deactivation' of the phases [137].

According to the results presented in Tables 2–6 all theses can be proved. There is no single best universal column. All the results are too compound dependent to decide which stationary phase should be used for particular separation.

Table 5
Performance of the base-deactivated columns in chromatography of erythromycin [186]

Base-deactivated column	N_{EA}	N_{EAEN}
Supelcosil LC-ABZ	890	25 000
Zorbax RX C_{18}	440	8600
Zorbax SB C_8	920	7900
Hypersil BDS C_{18}	1080	8200
LiChrospher 60 RP Select B	900	460
Spherisorb ODS-B	1420	8800
Chromspher B	820	8800
Bakerbond BDC	240	—

Mobile phase: acetonitrile–0.2 *M* phosphate buffer (pH 6.0)–water (x:5:(95−x)). The concentration of acetonitrile (x) in the mobile phase was adjusted for each stationary phase in order to obtain comparable retention of EA. Flow-rate, 1.5 ml/min.
N_{EA}, N_{EAEN}, number of plates for erythromycin and erythromycin A enol ether, respectively.

Mutton [188] examined several base-deactivated columns by recording a gradient chromatogram of a test mixture containing nine substances (including pyridine, benzylamine and *N*-acetyprocainamide hydrochloride). The results confirmed again a great diversity of the columns. Of the nine columns tested, Inertsil ODS 2 appeared to perform the best. Also, in another independent comparison of base-deactivated columns, McCalley indicated Inertsil ODS as superior to other packings [185].

Eight RP phases were thoroughly investigated by Claessens et al. [189]. The retentions of test mixtures in three isoeluotropic mobile phases based on methanol, acetonitrile and THF were examined. Of the columns, Zorbax RX performed best, however the selection of columns was relatively narrow. The observations made by Claessens et al. [189] confirmed again significant differences among nominally the same C_{18} columns.

Seventeen ODS packings for HPLC were examined with three test mixtures and principal component analysis by Olsen and Sullivan [190]. The analyses have shown great differences among columns designed for chromatography of basic com-

Table 6
A comparison of the stationary phases [187]

Column	Peak shape	Plate number	Stability	Remarks
μ-Bondapack C_{18}	0	0	0	
Nova-Pack C_{18}	–	+	0	
Kromasil KR100-5- C_{18}	0	+	0	
Zorbax Rx-C_8	0	+	0	
LiChrosorb RP-Select B	0	+	0	
Exil 100 5μm ODS-B	– –	–	0	can be used without buffer
Suplex pK_b-100	+	+	0	can be used without buffer

– –, very bad; –, bad; 0, acceptable; +, good.

pounds. Columns were categorized and the columns belonging to the same category were shown to behave similarly in several separations of pharmaceutical compounds. On the other hand, secondary grouping appeared to be a very rough approximation as the columns belonging to the same secondary class showed a totally different chromatographic behavior. Also Vervoort et al. [191] examined 14 base-deactivated commercially available phases. The influence of silanol blocking compounds was investigated at pH 3 and 7. Both papers show that the results depend very much on the analyte, e.g. in most experiments in [191] a Hypersil BDS column gave poorly asymmetric peaks. However, it appeared to be the column of choice for the analysis of dirithromycin and its degradation products [190].

4.3. Mobile-phase manipulations

4.3.1. Organic solvent modifier

The authors of three papers [134,189,192] examined organic solvent modifiers in the HPLC of organic bases. It has been proved that the organic part of the mobile phase is an important factor influencing the peak shape and the efficiency of the column. The authors agreed that of three organics (methanol, acetonitrile and THF) examined, acetonitrile gave the most asymmetrical peaks. This was explained by the inability of acetonitrile to form hydrogen bonds with residual silanols, in contrast to methanol and THF [189]. Also, according to Kazakevich and McNair [193], acetonitrile molecules interact mostly with alkyl groups of the bonded phase and they do not interact with silanols. A similar observation was made by Bliesner and Sentel [194]. However, for pH>9 separations, acetonitrile inhibits a dissolution of silica supports, and it is considered a safer organic modifier than methanol [109].

4.3.2. Dynamically modified silica

The method of chromatography on dynamically modified silica was introduced in 1981 by Hansen [195]. The method was systematically investigated in the 1980s, and thoroughly reviewed [10,196,197]. The authors proved that their method can be successfully applied for separation of basic solutes.

According to Hansen et al. [10], chromatography on dynamically modified silica is simple to handle and can be applied for the separations of cationic, anionic and nonionic solutes. The most important achievement of the method is its reproducibility on various brands of silica (modified and unmodified). The dynamic modification is easy to perform: a mobile phase containing quaternary ammonium salt is pumped through the column to reach equilibrium. Equilibration time depends on the ammonium compound, pH, and specific surface area of the silica. As the quaternary ammonium salts tend to dissolve silica, it is necessary to saturate the mobile column with silica by a special saturation column usually installed between the pump and the injection system. The amount of adsorbed ammonium compound depends also on the alkyl chain length [10] and can reach a maximum surface concentration of 2.5–3 $\mu mol/m^2$ (only a monolayer of the ammonium compound is adsorbed). The concentration of the compound in the mobile phase should be above its critical micelle concentration (CMC). Thus, at equilibrium in the chromatographic system three phases are present: the layer of adsorbed quaternary ammonium ions as the stationary phase; micelles;

and dissolved molecules in the mobile phase. Typically, for RP-HPLC, mobile phases are used. The system of three phases is presented in Fig. 8.

Hansen et al. [198] showed that the separation of some tricyclic antidepressants (imipramine, desipramine and imipramine *N*-oxide) was impossible even on an ODS base-deactivated column (Supelcosil LC-18 DB), while the application of the dynamic modification permitted an excellent separation on LiChrosorb Si 60, known rather for its acidic properties [12]. The separation is shown in Fig. 9.

It is also worth mentioning that, for 11 silica columns examined, capacity factor, k', for imipramine varied in the range of 6.5–19 while relative retention for desipramine (for those 11 silica columns) had a standard deviation of only 2.5% [198].

Despite the addition of the quaternary ammonium salt to the mobile phase, UV detection at 254 nm is still possible. Also, other detection systems, such as fluorescence and an electrochemical detector, were used [10].

The question is why this method is so successful in blocking active, strongly interacting sites on a surface of silica? A possible answer may be that this is because the mobile phase contain a large number of positively charged species (quaternary ammonium ions) which compete for the strongly acidic, dissociated sites on the surface of the stationary phase. Dynamically modified silica method does not allow gradient elution to be performed and hence its practical meaning is still low.

4.3.3. *The effect of amine modifiers on retention of organic bases*

Silanophilic interactions of basic solutes with siliceous supports can be suppressed by increasing the water content in the mobile phase as well as (in a much more pronounced way) by the addition of amine modifiers to the mobile phase. The role of

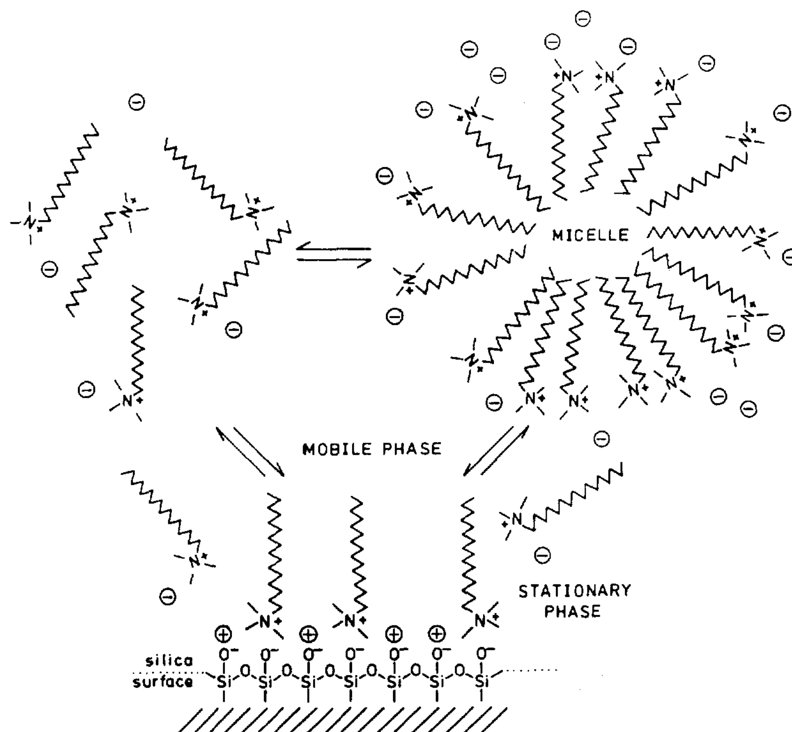


Fig. 8. Schematic presentation of the 'three phases' in equilibrium when micelles are present in the eluent [10]. Reprinted from S.H. Hansen, P. Helboe, M. Thomsen, J. Chromatography 544 (1991) 33; with kind permission of Elsevier Science NL, Sara Burgerharstaat 25, 1055 KV Amsterdam, The Netherlands.

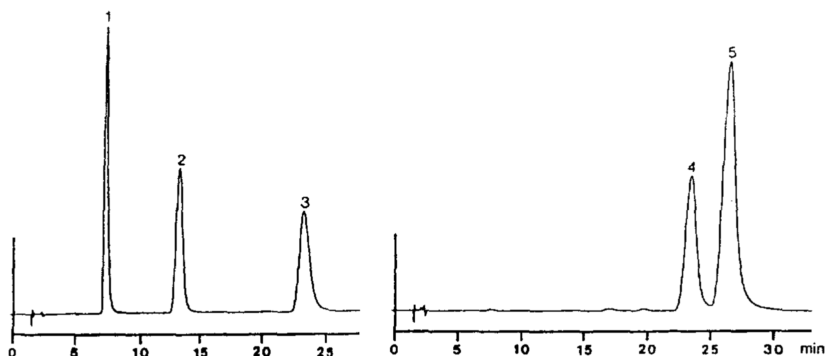


Fig. 9. Separation of imipramine and structurally related compounds of *cis*- and *trans*-clopenthixol on dynamically modified silica. Column: LiChrosorb Si 60 (120×4.6 mm I.D.). Eluent: methanol–water–0.2 *M* potassium phosphate buffer (pH 7.0) (55:40:5) with the addition of 2.5 m*M* CTMA bromide. Peaks: 1, imipramine *N*-oxide; 2, desipramine; 3, imipramine; 4, *cis*-clopenthixol; 5, *trans*-clopenthixol [198]. Reprinted from S.H. Hansen, P. Helbow, M. Thomsen, J. Chromatogr. 409 (1987) 71; with kind permission of Elsevier Science NL, Sara Burgerharstaat 25, 1055 KV Amsterdam, The Netherlands.

amine additive is to interact with silanol sites and, thus, by competing with solute molecule, reduce the interactions of the latter. According to Kiel et al. [204], basic solute can interact with the reversed-phase support through several mechanisms: hydrophobic interactions, ion-exchange and hydrogen bonding.

Also, a tertiary amine modifier can interact with the support through the same mechanisms. Therefore it will decrease the interactions of a solute with the stationary phase by blocking ion-exchange or hydrogen bond site, or by repelling a charged solute molecule as is depicted in Fig. 10.

However, in the view of Hill and Kind [207], results of hydrogen bonding seem to be at least disputable, as they showed that the amine additives decrease only the retention of ionized solute molecules. The use of amine blocker in the mobile phase ensures obtaining similar chromatographic results on both silica type A and type B [205].

Amine modifiers did not have any substantial effect on the retention of partly ionized solutes, and obviously no effect at all on the retention of non-ionized solutes [207]; this is shown in Fig. 11.

In practice, strong adsorption sites significantly contribute to the retention of basic solutes. This fact was observed in liquid [199–201] and gas chromatography [64,202], and is illustrated by the data in Table 7, which show that the retention of tricyclic antidepressants is greatly affected by amine additive to the mobile phase.

The amine additives were first treated as 'tail reducers' [203] and indeed in many cases they substantially improved the chromatograms. Retention

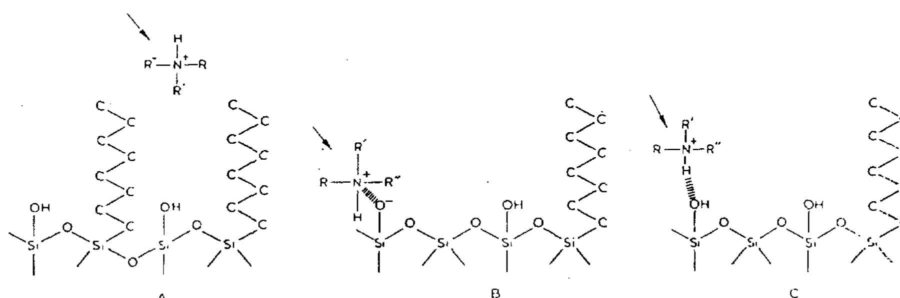


Fig. 10. Potential interactions of a tertiary amine molecule with the reversed-phase surface and silica support: (A) hydrophobic interactions; (B) ion-exchange; (C) hydrogen bonding [204]. Reprinted from J.S. Kiel, S.L. Morgan, R.K. Abramson, J. Chromatography 320 (1985) 313; with kind permission of Elsevier Science NL, Sara Burgerharstaat 25, 1055 KV Amsterdam, The Netherlands.

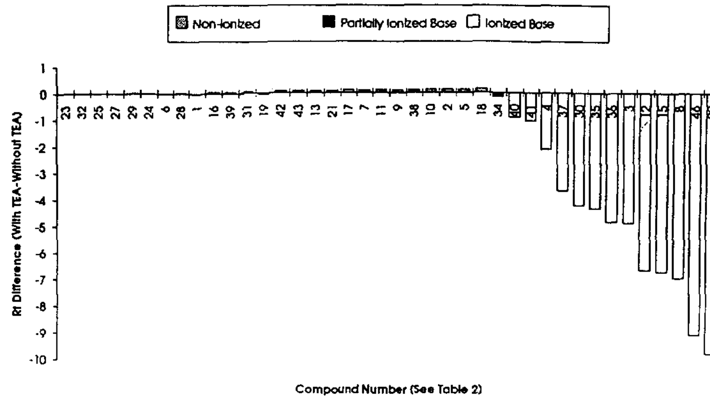


Fig. 11. Retention time difference of compounds analyzed on a Zorbax C_8 column with and without TEA in the mobile phase (Compounds 23, 28 and 34 are partially ionized bases) [207].

Table 7
The influence of amine additive on the retention (k') of basic solutes on regular reversed-phase packings

	Lichrosorb RP-8		ODS Hypersil		Lichrosorb RP-18	
	No amine	DMOA	No amine	DMOA	No amine	DMOA
Desipramine	8.24	2.04	12.5	2.53	17.34	2.54
Imipramine	8.60	1.76	13.6	2.17	20.8	2.14
Trimipramine	10.3	2.28	17.3	2.82	29.5	2.78

Eluent: 1:1 methanol–phosphate buffer (pH 3) and 0.05 *M* dimethyloctylamine (DMOA) in 1:1 methanol–phosphate buffer (pH 3) [199].

on base-deactivated columns is much less affected by an amine additive to the mobile phase than retention on a 'normal' stationary bonded phase; evidence for this is shown in Table 8.

A systematic study of amine modifiers was done by Kiel et al. [204]. They investigated primary, secondary, tertiary and quaternary amine additives. Longer-chain amines appeared more effective in decreasing retention of basic solutes. Also, tertiary amines appeared to be more effective than primary or secondary amines. Tertiary amines also appeared to be the most effective in improving the peak shape. Primary and quaternary amines decreased peak asymmetry only to a limited extent. Better efficiency of tertiary over primary amines was explained by their greater ability for hydrogen bond formation [204]. Quaternary amine modifiers with longer chains (C_{10}–C_{16}) were examined by Bij et al. [31],

Table 8
The influence of amine additive on the retention (k') of basic solutes on base-deactivated column [137]

	Imipramine		Amitriptyline	
	No amine[a]	With amine[a]	No amine[a]	With amine[a]
Superspher RP-18	10.70	8.71	13.27	10.77
Nucleosil C_{18}-AB	6.08	5.27	7.40	6.44
Encapharm RP-18	8.47	6.86	10.41	9.13
LiChrosorb RP Select B	7.86	4.55	9.94	5.57
Synchropack RP SCD	5.97	4.71	6.86	7.75
Inertsil ODS-2	6.46	5.45	7.92	6.71

[a] Mobile phase: ACN–phosphate buffer (30:70), pH 3.
[b] Mobile phase: ACN–triethylammoniumphosphate buffer (30:70), pH 3.

and hexadecyltrimethylammonium ion was found to have the highest formation constants in reaction with surface silanols, and hence the highest efficiency in decreasing solute silanophilic interactions. Also, Stadalius et al. [12] suggested that the best results for the suppression of undesirable ion-exchange interactions can be achieved with tertiary amines (possibly dimethylalkylamines) as well as with trimethylalkyl ammonium salts, as these two types of compound are known for their strong interactions with silanols. According to Hill [205], triethylamine (TEA) and dimethyl *n*-octylamine (DMOA) are the most efficient suppressors. The effect of amine additives was shown to be important for solutes with high pK_a values. For substances with $pK_a<7$ there was no improvement in peak shape noticed [187]. Also, triethylamine appeared to be a much better silanol suppressor than tetramethylammonium ions [187]. The amount of amine additive to the mobile phase is also an important issue. In general, the amount of amine depends on the quality of the silica base used for the synthesis of a bonded phase. More acidic silica (more heterogeneous) requires more amine suppressor in the mobile phase. Such differences were shown by Bayer and Paulus [200]. For a relatively 'good' bonded phase a 0.02–0.05 m*M* amine concentration in the mobile phase should be sufficient. Below the optimum amine concentration, the chromatographic behavior of a basic solute will strongly depend on the suppressor concentration. Thus, it is rather better to use a slight excess of amine than necessary. Dolan [206] recommends working with amine concentration of at least 20–25 m*M*.

Also, hydrogen-bonding interactions were investigated in non-polar, non-aqueous mobile phases. Stuurman and Wahlund [208] showed the contribution of hydrogen bonding to the retention of phenol and ketones in an *n*-hexane mobile phase. Trioctylphosphine oxide (TOPO) was used to modify the mobile phase. The adsorption of TOPO on LiChrosorb RP-8 was studied and a two-site Langmuir model was applied. They found two kinds of proton-donating sites on the surface. The total monolayer capacity of those sites was less than 10% of the available unreacted silanols. The capacity of the strongest sites was less than 2% of the total remaining silanols [208].

Conclusions:

- there is no sufficient confirmation in the literature that amine molecules can interact with silanols via H-bonding in an aqueous/organic environment;
- if the interactions between amine and the stationary phase are of electrostatic nature then quaternary ammonium salts and tertiary amines seem to be the best silanol blockers;
- quaternary ammonium salts of the type $R(CH_3)_3N^+X^-$ where R is a long alkyl chain, are particularly good silanol blockers;
- H-bonding can contribute to the retention of polar compounds (including amines) in non-polar, non-aqueous mobile phases.

4.4. The solute

4.4.1. The nature of the solute and its interactions with silanols

In general, the same rules for interactions of basic solute molecules with silanols are valid as discussed above. McCalley [192,209] has proved that steric hindrance around the basic nitrogen atom of the solute reduces the interactions with silanols. This effect was shown for a series of substituted pyridines, for which a substituent in the 2-position improved peak symmetry. Another factor affecting the chromatographic behavior of a basic analyte is its protonation. The pH* of organic solvent/aqueous buffer mobile phases may be significantly different from the pH of their aqueous component, measured before organic solvent addition; e.g. McCalley [209] shows that the pH* value for methanol–phosphate buffer, pH 7 (55:45 v/v), is 8.25.

On the other hand, pK_a values for pyridine and 2,4-dimethylpyridine in the mobile phase were lower than those determined in aqueous solutions [209]. These two facts imply that some of the bases may be only partially protonated or even not protonated in the actual HPLC conditions. This would explain why the retention of pyridines was not dependent on their pK_a values. Sŷkora et al. [210] have estimated that, to achieve the same dissociation of the basic compound in the approx. 60% methanol mobile phase, as in aqueous solution, an aqueous buffer (used for the preparation of the mobile phase) should be 2.5 pH units lower. This is a more general issue; pK_a in

aqueous/organic solvent is different from that measured in aqueous solution. Lewis et al. [245] shows an average change in pK_a values for aniline derivatives of −0.7 for 25% MeOH. Wan et al. [217] shows an average reduction in pK_a of −1.2 units for anilines and 35% MeOH. The study of Bosch et al. [246] shows a net change of −0.3 units per 10% addition of organic, when combined effects of phosphate buffer and aniline pK_a are taken into account. All these results [210,217,245,246] summarize a consistent pattern of a change of about −0.3 pK_a units per 10% addition of organic. Cox and Stout [32] show that an organic part of the mobile phase also affects the dissociation of silanols.

The retention is expected to depend on the pK_a of the analyte if ion-exchange contributes to the retention mechanism. When the ion-exchange dominates then the dependence is very clear, as shown by Law [118] (see Fig. 15). As pointed out by Stadalius et al. [12], the higher the pK_a the stronger will be the interactions with the ion-exchange site. Similar observation was made by Sýkora et al. [210]. Vervoort et al. [187] examined a dependence of asymmetry of the solute peaks as a function of the solute's pK_a values. In general, substances with high pK_a values gave more asymmetrical peaks than compounds with lower ones. It was observed for the whole pH range investigated (3.5–10) and is shown in Fig. 12.

McCalley [134] also noticed higher asymmetry for substances with high pK_a values, however, he pointed out the importance of the steric factors (substituents around the basic nitrogen atom) and their effect on the peak shape. Substituents at the basic nitrogen significantly influence the ion-exchange process [119]. According to Sýkora et al. [210] the best indicators of unwanted ion-exchange sites are compounds with high basicity (high pK_a) and low hydrophobicity (low $\log P_{o/w}$ ($P_{o/w}$, octanol/water partition coefficient)). However, this seems to be in conflict with the above-cited abilities of long-chain amines to block ion-exchange sites. At sufficiently high pH the bases should exist in the non-protonated form. Then, peak shapes should again be good as the bases cannot interact electrostatically with the stationary phase surface.

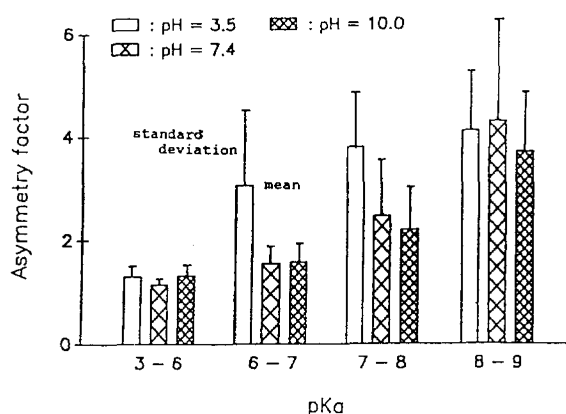


Fig. 12. Influence of the buffer pH on the peak asymmetry [187]. Reprinted from R.J.M. Vervoort, F.A. Maris, H. Hindrics, J. Chromatography 623 (1992) 207; with kind permission of Elsevier Science NL, Sara Burgerharstaat 25, 1055 KV Amsterdam, The Netherlands.

To summarize:

- electrostatic interactions between amine and silanols depend on the amine pK_a;
- steric hindrance at basic nitrogen can significantly decrease the interactions;
- pK_a of amine in an aqueous/organic mobile phase differs from that measured in aqueous solution;
- pH* of the buffered mobile phase differs from pH of buffer used to prepare the mobile phase.

4.4.2. Sample size effect

Sample size dependence of the retention for basic analytes was observed early. Bij et al. [31] have shown that the retention of smaller basic samples is much higher that of larger samples. The effect was partially suppressed by amine additives to the mobile phase. They have also shown that the effect was dependent on pH: at higher pH the effect was stronger while lowering pH substantially decreased the sample size effect. Ionization of silanols and thus availability of ion-exchange sites was an explanation of the observed phenomena [31]. McCalley [209] investigated an asymmetry dependence on sample size. Larger sample size of pyridine resulted in higher asymmetry of the solute's peak. Simultaneously the column efficiency was shown to be relatively independent of the sample size. The studies were performed on a relatively well-base-deactivated packing (Inertsil ODS) [209]. Vervoort et al. [187] confirmed the asymmetry dependence on the sample size. The effect was particularly evident for solutes with high pK_a values. For compounds

with pK_a<8 almost no difference in peak shape was noticed. The results were observed for μ-Bondapack column which seems to be relatively active when compared to the base-deactivated packings. Stadalius et al. [12] have shown that a decrease of sample size may result in a higher efficiency of the chromatographic column. This is illustrated in Table 9.

According to Eble et al. [211] the effect is a result of overloading conditions for active sites which are available only in a very limited number. This explanation was found by adsorption studies of angiotensin on RP (Zorbax ODS) silica. The studies suggested adsorbent heterogeneity with two kinds of adsorption sites available. The adsorption capacity for the more active site was found to be much less than that for weaker adsorption sites. According to Stadalius et al. [12] samples greater than 0.5 μg for a 25-cm column may cause overloading of strongly interacting sites. The ratio of adsorption capacity on strong to weak sites for angiotensin was found to be 1:60 on the Zorbax ODS column. (The ratio of unreacted silanols to grafted $C_{8/18}$ chains is roughly equal to 1. Which means that only a very limited proportion of unreacted silanols can be considered as a strongly interacting site.)

Table 9 also shows the increase in efficiency with increasing ionic strength as well as decreasing pH of the mobile phase. The latter restricts the number of available sites for ion-exchange (at lower pH fewer sites are dissociated), thus the effect is similar to that observed in gas chromatography when strongly adsorbing sites are blocked with an amine blocker [202]. According to Vervoort et al. [187] changing the pH from 3.5 to 2.5 does not provide much improvement in peak shape. This means that sites which are dissociated at pH 3.5 are also dissociated at pH 2.5, i.e. their pK_a<2.5. However, an addition of amine at pH 2.5 substantially improves the peak shapes. The amines are protonated at pH 2.5 and, hence, are able to block those residual ion-exchange sites. Such an effect was observed on Zorbax Rx-C_{18}, μ-Bondapack C_{18}, and to a lesser extent on Suplex pK_b-100. This effect is a function of silica support used, although it shows that even on deactivated surfaces some highly acidic sites exist. Also, a similar efficiency increase was found by Pfleiderer and Bayer [201] when they blocked geminal sites with Fe^{3+} ions in chromatography of diphenylhydramine hydrochloride. There again a blockage of a small number of strongly interacting sites resulted in an over three-fold increase of the plate number. This can probably be explained by the transition from the overloading conditions (with two adsorption sites available) to the system with one retention mechanism of greater capacity, as was shown in the paper by Eble et al. [211]. To summarize:

- when few acidic, ion-exchange sites exist on the silica surface then it is easy to overload the sites with the sample;
- thus the decrease in sample size should improve the efficiency of the column.

4.5. *Ion-exchange mechanism*

Chromatography of basic solutes still poses difficulty to many practitioners. This is because a very complicated system of mechanisms is responsible for the retention of the solutes. As it was noted above, the following phenomena are considered as the possible mechanisms: ion-exchange on silanols, salt-

Table 9
Column efficiency as a function of chromatographic conditions

Conditions	Column plate number		
	Morphine	Codeine	Oxymorphone
1. pH 6, no TEA, 2 m*M* acetate	2860	730	624
2. pH 6, no TEA, 25 m*M* phosphate	2620	1160	2950
3. pH 3.5, 25 m*M* phosphate	7260	3950	5100
4. pH 3.5, 0.1% TEA, 25 m*M* phosphate	7000	5370	6630
5. As above, sample weights reduced 10-fold	7060	7770	8580

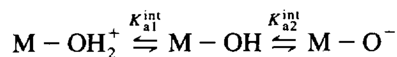
Column: Zorbax Rx (sterically protected bonded phase composed of diisopropyl C_8 groups). Mobile phase: acetonitrile (ACN)–buffer (40/60 in 1, 2 and 4; 7/93 in 3; 6/94 in 5); flow-rate, 1.5 ml/min [12].

ing out effects, formation of ion-pairs and partition, as in chromatography of non-polar compounds. When mixed mechanisms are responsible for a retention of the analytes then it is more difficult to control the chromatographic process. When the more important retention (e.g. ion-exchange) has limited column capacity and even small samples can overload the sites then the efficiency of the HPLC column is low (see, e.g. [32,211]).

First, we have to understand that silica can behave as an ion-exchanger, secondly we have to understand the mechanism of ion-exchange and the conditions that would allow control of the process. This is necessary for two reasons: (1) we can take advantage of ion-exchange mechanism and we can apply it under controllable conditions; (2) we can learn how to eliminate ion-exchange when the process is undesired.

Two parameters are usually used to characterize the ability of an oxide to act as an ion-exchanger: the pK_a value of the ion-exchange site, i.e. silanol and point of zero charge (pzc, i.e. the pH at which the surface is neutral); above the pzc the oxide should show cation-exchange properties, while below the pzc anion-exchange is also possible (ZrO_2, Al_2O_3). Ion-exchange properties are common for metal oxides (SiO_2, ZrO_2, TiO_2, Al_2O_3, Fe_2O_3). Silica's pzc has a low value of 4 [27] or 3.5 [212] or below 2 [213], and it means that practically only cation exchange is possible. As will be discussed below, anion-exchange properties were observed for some silicas, thus we can suppose that for such silicas pzc values can be higher.

In general, silica behaves according to the site binding model proposed for metal oxides by Yates et al. [214]. The model assumes a simplified picture of porous oxide surfaces:



$$M-OH_2^+ \overset{K_{a1}^{int}}{\rightleftharpoons} M-OH \overset{K_{a2}^{int}}{\rightleftharpoons} M-O^-$$

where K_{a1}^{int} and K_{a2}^{int} are the intrinsic ionization constants. This is illustrated in Fig. 13.

Potential of the surface is determined mainly by OH^- and H^+ ions. The resulting surface charge depends on an excess of one type of charged site over the other and is a function of the solution pH. At the pzc the number of positively charged sites

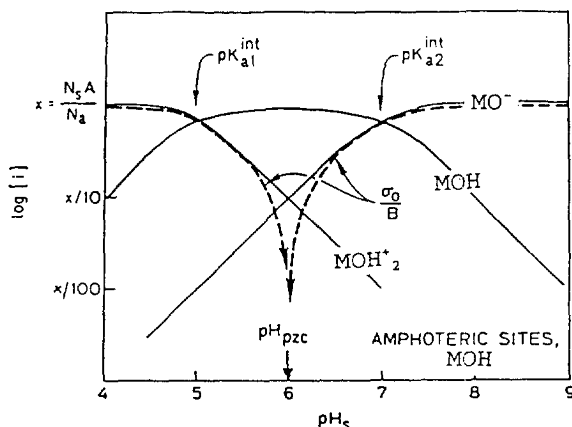


Fig. 13. Variation of charged and uncharged species concentration with the surface pH $(pH)_s$ on an amphoteric metal oxide [27]. Reprinted from J. Nawrocki, R.P. Rigney, A. McCormick, P.W. Carr, J. Chromatography A, 657 (1993) 229; with kind permission of Elsevier Science NL, Sara Burgerharstaat 25, 1055 KV Amsterdam, The Netherlands.

($M-OH_2^+$) is equal to the number of negatively charged sites ($M-O^-$) on the surface. It is easily seen that pH_{pzc} depends on the ionization reactions and is related to the ionization constants by the equation:

$$pH_{pzc} = 0.5(pK_{a1}^{int} + pK_{a2}^{int})$$

As one can see in Fig. 13, pH_{pzc} is always lower than pH pK_{a1}. Various values reported for pzc for silica indicate that the acidity of the silica surface can also vary – probably due to the differences in chemical composition of the silicas (metal traces).

At normal pH~7 half of silanols exist in ionized form and they will attract any positively charged species (protonated organic bases) by strong, electrostatic forces. The pK_a of silanols should theoretically be 7.1±0.5 [218] and, in fact, pK_a of silica determined by IR spectroscopy was found to be 7.2±0.2 [219]. According to Iler [4], the dissociation constant is 6.5. In the literature the whole variety of pK_a values for silica can be found from as low as 1.5 to as high as 10 [14].

There are many reports on the cation-exchange properties of silica below the above-mentioned values of silica's pzc. This implies the existence of very acidic sites on the silica surface which can dissociate below pH 2 [4,187,212,213,215,217,232,233] (see

also Fig. 14, after [216]). Usually it is assumed that pH 4 would fully protonate all SiO^- sites. According to Hill [205], pH 2.1 suppresses the ionization of silanols with an exception of the most acidic. The acidity of neighboring silanols can be considerably increased by trace metals [14,69,126,127,146, 147,220]. Such strongly acidic sites are detrimental for chromatography of charged species, such as organic bases at low pH. This would also explain why lowering the pH to 2 is sometimes not fully successful. As such sites usually exist at a very low surface concentration, they will be easily overloaded, as shown by Eble et al. [211]. It has to be emphasized here that the pK_a of the silanol group is another 'bulk' value which is measured as an average for all silanols present on the silica surface. It means that pK_a does not necessarily reflect the existence of strongly acidic sites on the surface, as the sites can be chromatographically active at very low surface concentrations.

Anion-exchange properties of several C_8 silica have also been observed [132]. Interestingly, the properties were observed on Zorbax Rx C_8, i.e. packing derived from type B silica. Zorbax C_8 (type A) has not been able to anion exchange which confirms the higher acidity of type A silica. This can also explain the good performance of type B-derived silicas in chromatography of amines at low pH [132].

Let us consider ion-exchange relationships: theoretical dependencies governing the ion-exchange were given by Stout et al. [221] and will be cited in extenso below. Retention factor (k') in chromatography depends on phase ratio and a distribution constant:

$$k' = \phi K \quad (1)$$

where ϕ = phase ratio, K = distribution constant.

The distribution constant is the ratio of concentration of solute in the stationary to the mobile phase:

$$K = \frac{[SiO^- NR_4^+]}{[NR_4^+]} \quad (2)$$

where NR_4^+ is the protonated form of basic solute.

Retention in chromatography is given by:

$$k' = \frac{V_r - V_m}{V_m} \quad (3)$$

for a cation-exchange separations the following equilibria are important:

$$SiOH \rightleftarrows SiO^- + H^+ \quad (4)$$

and

$$SiO^- M^+ + NR_4^+ \rightleftarrows SiO^- NR_4^+ + M^+ \quad (5)$$

where M^+ = buffer cation.

Ionization and ion-exchange equilibria constants are given as:

$$K_{SiOH} = \frac{[SiO^-][H^+]}{[SiOH]} \quad (6)$$

$$K_{ix} = \frac{[SiO^- NR_4^+][M^+]}{[SiO^- M^+][NR_4^+]} \quad (7)$$

Since under normal conditions the concentration of M^+ is well in excess over both the concentration of H^+ and of solute, it is reasonable to assume:

$$[SiO^-] = [SiO^- M^+] \quad (8)$$

and

$$K_{ix} = \frac{[SiO^- NR_4^+][M^+][H^+]}{[NR_4^+] K_{SiOH} [SiOH]} \quad (9)$$

let us combine Eq. (2) and Eq. (9):

$$K = K_{ix} K_{SiOH} \frac{[SiOH]}{[M^+][H^+]} \quad (10)$$

and finally from Eq. (1) and Eq. (10):

$$k'_{ix} = \phi K_{ix} K_{SiOH} \frac{[SiOH]}{[M^+][H^+]} \quad (11)$$

This means that k' at a constant pH should be a function of $1/[M^+]$. If the ionic strength is varied, a plot of k' versus the reciprocal of concentration of the competing ion should yield a straight line which passes through the origin of the coordinate system.

Another important feature of the ion-exchange chromatography on silica is the independence of the retention on solute lipophilicity.

Now, when the dependencies governing the ion-exchange are known why not take advantage of them for the separation of organic bases? There are several ways to do that: (1) the ion-exchange chromatography of organic bases can be realized on bare silica

with nonaqueous solvents; (2) the ion-exchange chromatography can be realized on bare silicas with aqueous/organic solvent; (3) the ion-exchange can be realized on chemically bonded phases with aqueous/organic solvent.

Ion-exchange chromatography requires ion-exchanging sites, i.e. this implies a relatively high pH in order to dissociate virtually all silanols. High pH can be detrimental for silica [1,4], however recent research shows that siliceous packings are surprisingly resistant to high pH, and reasonable conditions (mobile phase, buffer) allow to use pH 10 for an acceptable time [109,120–122]. But at higher pH organic bases exist in a non-protonated state. Thus, there should be some trade off – silanols should be reasonably dissociated while the separated bases should be protonated.

On the other hand, if we want to avoid ion-exchange we should keep the pH as low as possible. Another way to avoid the ion-exchange mechanism is to carry out the chromatography at high pH – i.e., at which the organic bases are deprotonated. High pH causes a rapid degradation of polymerized bonded phases [12,205]. This also requires a silica gel resistant to high pH. Recently, intensive research has been focused on this line of research [109,120–124,247].

4.5.1. Ion-exchange on bare silica with non-aqueous solvents

Flanagan and Jane [216] confirmed that ion-exchange is an important process in chromatography of basic solutes on plain silica with the use of non-aqueous, ionic mobile phases. They noticed that an increase in retention of organic bases was similar to the ionization profile of silica silanols; the increase in retention being greatest in the region pH 7–9. There is also a noticeable increase of retention in the pH range of 0–2. This indicates that silica contains also acidic sites with $pK_a \approx 1$, i.e. silanols more acidic than normal. The increase of retention with increasing pH for emepronium is shown in Fig. 14 [216].

The maximum retention is usually observed for $pH = pK_a$ of the basic solute. Flanagan and Jane also showed that pH can influence the peak shape at almost constant retention [216]. As earlier predicted by Stout et al. [221], the retention at constant pH is easily controllable by ionic strength. Flanagan et al.

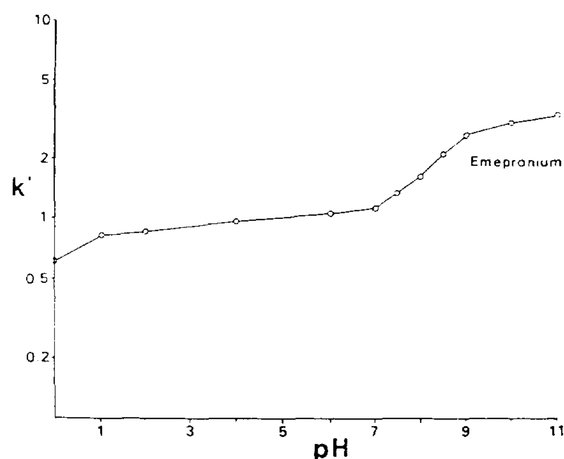


Fig. 14. Variation of retention with eluent pH at constant ionic strength for emepronium, a quaternary ammonium compound. Column: 125 mm Spherisorb S5W silica; eluent, sodium perchlorate (0.1 *M*) in methanol adjusted to an appropriate pH; detection UV, 240 nm; injection, 10 μl of methanolic solution containing emepronium (10 mg/l). The eluent pH was adjusted by the addition of either perchloric acid or methanolic sodium hydroxide (0.1 *M*) [216]. Reprinted from R.J. Flanagan, I. Jane, J. Chromatography 323 (1985) 173; with kind permission of Elsevier Science NL, Sara Burgerharstaat 25, 1055 KV Amsterdam, The Netherlands.

[222] have separated amiodarone (tertiary amine, anti-arrhythmic drug) and its analogues on bare silica (Spherisorb S5W) with a non-aqueous mobile phase, methanol–diethyl ether (85:15) containing 0.02% perchloric acid. Under the conditions employed the system was characterized by the following features: (1) base compounds were retained only when they were appreciably ionized; increase in ionic strength of the eluent decreased the retention; (2) retention could be predicted to a large extent from the pK_a of the analyte; (3) a mobile phase composition also could be used to control the selectivity of the system. This indicated ion-exchange as a main mechanism for controlling the retention [222].

Another approach to solve the problem of contribution of various mechanisms to chromatographic retention was presented by Schwarzenbach in a series of papers [223–227]. The method consisted of 'buffering' of a silica surface (i.e. a simple procedure of coating) with the substance (1.5–2.5%, w/w) of desired pH. Then, the resulting adsorbent was used in chromatography with water-immiscible mobile

phases. According to the author [227] the stability of 'buffered' silica was comparable to that of untreated material, however, for more polar mobile phases the lifetime of the column was considerably shortened. In this approach an acidic or basic character of the surface will suppress the dissociation of ionogenic solute, allowing its chromatography as a non-dissociated molecule. A variety of separations of ionogenic substances is presented on silica adsorbents prepared in that way. It is clear that such a procedure can be successful only when the chromatographed sample contains substances with the same ionic character.

4.5.2. Ion-exchange on bare silica with aqueous/organic solvents

An interesting example of an application of ion-exchange properties of bare silica for a separation of basic solutes was presented by Bidlingmeyer et al. [33]. They showed that retention of some basic amines on silica under reversed-phase conditions depends on ion-exchange and adsorption mechanisms. The chromatographic process was adjustable with various concentrations of inorganic salts as well as with organic amine additives. Bidlingmeyer et al. measured the influence of ionic strength, pH, ion-exchange capacity, as well as the solvent strength, on chromatographic retention of bases. They have proved that the chromatographic process of a separation of basic anesthetics or tricyclic antidepressants is governed by the ion-exchange, as the retention was strongly dependent on the concentration of inorganic salt in the mobile phase. Moreover, they showed the dependence of the retention volumes on the pH of the mobile phase. The dependence is shown in Fig. 15.

At low pH, the retention is low due to the undissociated silanols; the dissociation increases with increasing pH, thus the retention increases too. At a pH value close to the pK_a value of the organic base the retention reaches the maximum, then it drops due to deprotonation of the base. Retention of maleic acid, which does not form positively charged species, is not affected by the changing pH. Bidlingmeyer et al. have also shown that a contribution of adsorption to the retention of organic amines is not negligible.

They observed a linear relationship of a plot of

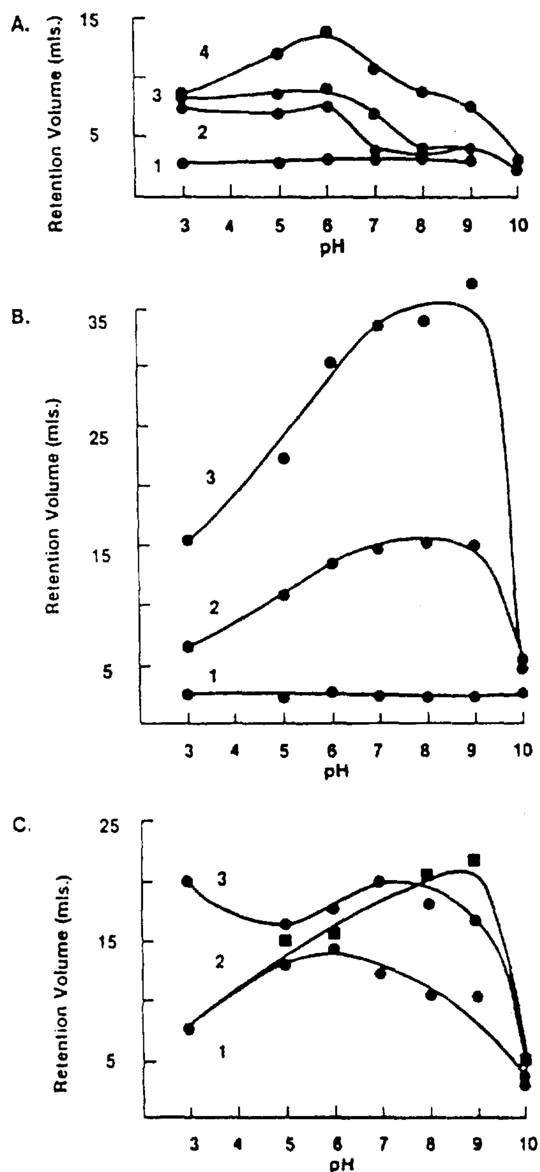


Fig. 15. Effect of pH in solute retention with a silica column. (A) Compounds are: (1) benzocaine (pK_a 2.8); (2) lidocaine (pK_a 7.9); (3) etidocaine (pK_a 7.9); (4) tetracaine (pK_a 8.5). (B) Compounds are: (1) maleic acid; (2) chlorpheniramine (pK_a 8.9); (3) propranolol (pK_a 9.4). (C) Compounds are: (1) promethezine (pK_a 9.1); (2) phenylpropanolamine (pK_a 9.0); (3) quinidine (pK_a 8.3). Mobile phase is acetonitrile/water (60:40) with 4 m*M* sodium chloride. The pH is the value of the aqueous stock solution containing sodium chloride before the mobile phase is prepared. Column is Radial-Pack silica [33]. Reprinted from Analytical Chemistry with kind permission of the American Chemical Society.

log k' vs. solvent composition, which is typical for reversed-phase systems. This is shown in Fig. 16.

According to Law [118], such a reversed-phase-type eluent used with naked silica is a versatile tool for separation of basic compounds. The system is efficient (N=50 000 plates/m, h_{red}=4 [118] or N=70 000 plates/m, h_{red}=2–3 [119]), and analytes are eluted with highly symmetrical peaks. Another feature of retention in such systems is its independence of the solute's lipophilicity. Numerous examples of separations of pharmaceutically important compounds are shown [118]. A wealth of chromatographic data for monofunctional arylalkylamines and five different groups of drugs is presented by Law [119] for the organic/aqueous (pH 9.1) mobile phase and bare silica system.

As postulated by the theory of ion-exchange chromatography the k' values should be a function of solute pK_a values. This was demonstrated [119] for two sets of simple arylalkylamines and is illustrated in Fig. 17. The data were obtained for methanol–ammonium acetate buffer (9:1), pH 9.2.

General dependence of log k vs. pK_a observed for ion-exchange systems can be influenced by substituents at or near the basic center. This is well documented for a series of 69 arylalkylamines [119]. In the earlier paper, Law proved that such a high-pH phase can be used for months with the same efficiency [113]. However, other studies have proved that the system with an aqueous/organic mobile phase at apparent pH 9.2–9.5 is very sensitive for minor differences in silica (e.g. differences between various batches of the same brand), and for subtle differences in the mobile-phase composition [228,229]. Probably due to these drawbacks, ion-exchange on bare silica has not turned out to be a routinely used method in practice.

4.5.3. *Ion-exchange on chemically bonded phases with aqueous/organic solvents*

Chemically bonded phases can also be used in an ion-exchange mode. Retention is then governed by two main mechanisms: ion-exchange and hydrophobic forces. A typical example of the ion-exchange mechanism is shown in Fig. 18.

An increase of retention with decreasing ionic strength was shown by Cox and Stout [32] for thiamine and morphine on a partially covered C_8

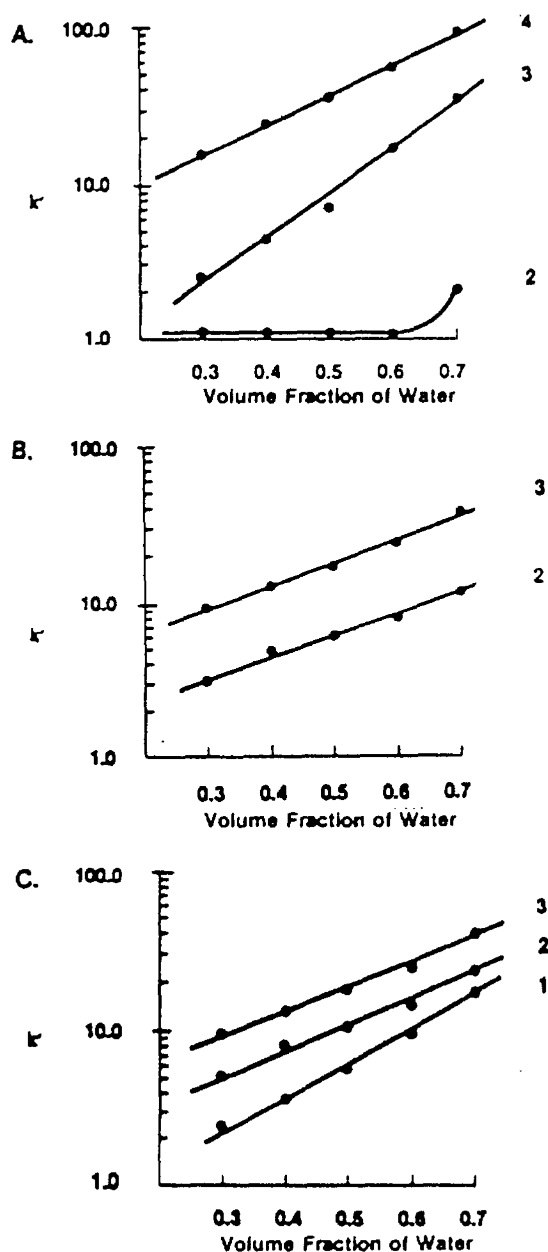


Fig. 16. Effect of solvent strength upon retention with constant pH and ionic strength. (A) Compounds are lidocaine (2), etidocaine (3), tetracaine (4), and benzocaine (1), always eluted near V_o and is not shown. (B) Compounds are: chlorpheniramine (2), propranolol (3), and maleic acid (1) always eluted near V_o and is not shown. (C) Compounds are: promethazine, chlorpheniramine (2), and quinidine (3). Mobile phase is acetonitrile/water with 4 mM dibasic ammonium phosphate at pH 7.8. Column is RadialPAK Silica [33]. Reprinted from Analytical Chemistry with kind permission of the American Chemical Society.

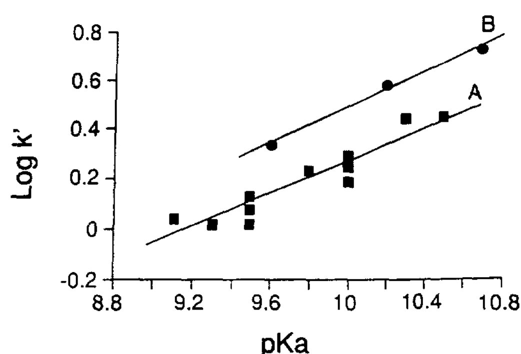


Fig. 17. Graph showing the relationship between logarithm of capacity factor (log k') and solute pK_a for two sets of amines. Unbranched phenylalkylamines (A), and N-methylphenylalkylamines (B) [118]. Reprinted from B. Law, Trends. Anal. Chem. 9(1) (1990) 31; with kind permission of Elsevier Science NL, Sara Burgerharstaat 25, 1055 KV Amsterdam, The Netherlands.

phase. In the same figure the opposite, i.e. decrease of the capacity factor with increasing value of 1/[Na^+] is shown. This is the evidence for the salting out effect. The slope of the line is a function of the concentration of silanol groups. It also depends on the acidity of silanols – thus it can used to measure differences between silicas of different origin or thermal history. This was confirmed by Cox and Stout [32] who measured the slopes of k' vs. 1/[Na^+] for two silicas: one heat treated at excessive temperature before rehydroxylation, and the other prepared (rehydroxylated) according to the Köhler and Kirkland procedure [53]. Indeed, the slopes of the plots were different, confirming the differences between the silicas. The major difference between the materials was the ion-exchange character. This also confirmed the hypothesis of Köhler and Kirkland of a decrease of the number of highly acidic silanols due to the treatment. The plots for both silicas for thiamine and morphine are shown in Fig. 19. When another, not influenced by the ion-exchange mechanisms are present, there will be an intercept. Moreover the slope is also a function of the acidity of silanols and can be used to compare silicas of various acidity [12,32,221].

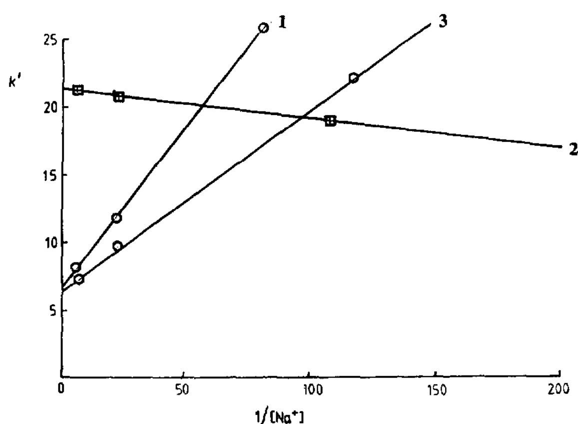


Fig. 18. Plot of k' against 1/buffer concentration on a partially bonded C_8 packing. Mobile phase: 15% methanol in phosphate buffer (pH 4.6). (1) thiamine, (2) caffeine, (3) morphine [32]. Reprinted from G.B. Cox, R.W. Stout, J. Chromatogr. 384 (1987) 315; with kind permission of Elsevier Science NL, Sara Burgerharstaat 25, 1055 KV Amsterdam, The Netherlands.

Cox and Stout concluded that silicas with higher slopes of k' vs. 1/[M^+] give rise to bonded phases with poor elution properties. Ionic character is important when mobile phases of low ionic strength are used [32].

The retention of toluene (the non-ionic solute) was shown to be dependent on a quantity of the available siloxane bonds which were said to be capable of hydrophobic interactions [32]. The retention of

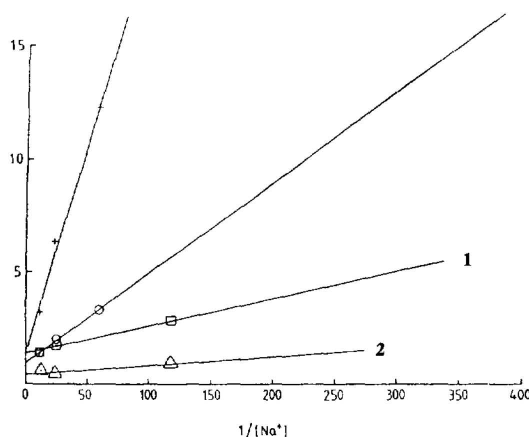


Fig. 19. Plot of k' against 1/buffer concentration on a partially bonded C_8 packing. +, thiamine, packing a; o, morphine, packing a; 1, thiamine, packing b; 2, morphine, packing b. Packing a, silica 170 m^2/g, 120 Å pore diameter; packing b, the same silica but rehydroxylated according to the procedure of Köhler and Kirkland [53,54]) [32]. Reprinted from G.B. Cox, R.W. Stout, J. Chromatogr. 384 (1987) 315; with kind permission of Elsevier Science NL, Sara Burgerharstaat 25, 1055 KV Amsterdam, The Netherlands.

toluene on rehydroxylated silica was markedly lower, which was considered as the evidence of hydrophobic interactions with siloxane since the number of the siloxane bonds should decrease as a result of rehydroxylation.

A wealth of data on the chromatographic behavior of organic bases on silica and ODS silica can be found in the paper of Sugden et al. [231]. According to them, the retention mechanisms are very complex and involve ion-exchange with surface silanols, partition of ion-pairs and salting out effects. Also according to Kiel et al. [204], retention and peak shape of basic solutes depends primarily on ion-exchange and hydrogen bond mechanisms. Flanagan et al. [222] have also shown the possibility of using chemically bonded phases for the analysis of amiodarone and its analogues. The dominant mechanism of separation was said to be ion-exchange, however, the ODS system offered substantially different selectivity. This is shown in Fig. 20, which is drawn for the case when, as a mobile phase, a methanolic solution of perchloric acid (0.03%, 2.78 m*M*) was used.

4.5.4. Competing ions

The process of an ion-exchange retention can also be controlled by the size of the competing ion. Larger competing ions are more easily displaced by solute ions than smaller competing ions, i.e. a retention of the solute will be higher when the competing ion is larger. This was shown by Papp and Vigh [232,233] for hydrated sodium and potassium ions, and by Lingemannet et al. [212] for several tetraalkylammonium ions. Papp and Vigh have found that the retention of amines is very sensitive to the changes of the type of buffer cation [232]. The retention-decreasing power of the cations increased in the order: $Na^+ < K^+ < TMA^+$ (TMA, tetramethylammonium) [233]. This indicates a possible way to control the ion-exchange mechanism when chromatographing organic bases. Papp and Vigh also found that the behavior of organic bases depends on the relative ratio of the pK_a values of silica silanols and the organic base. According to them, the contribution of ion-exchange to the retention is large over the entire range of methanol concentration [233]. According to Hill [205], buffers

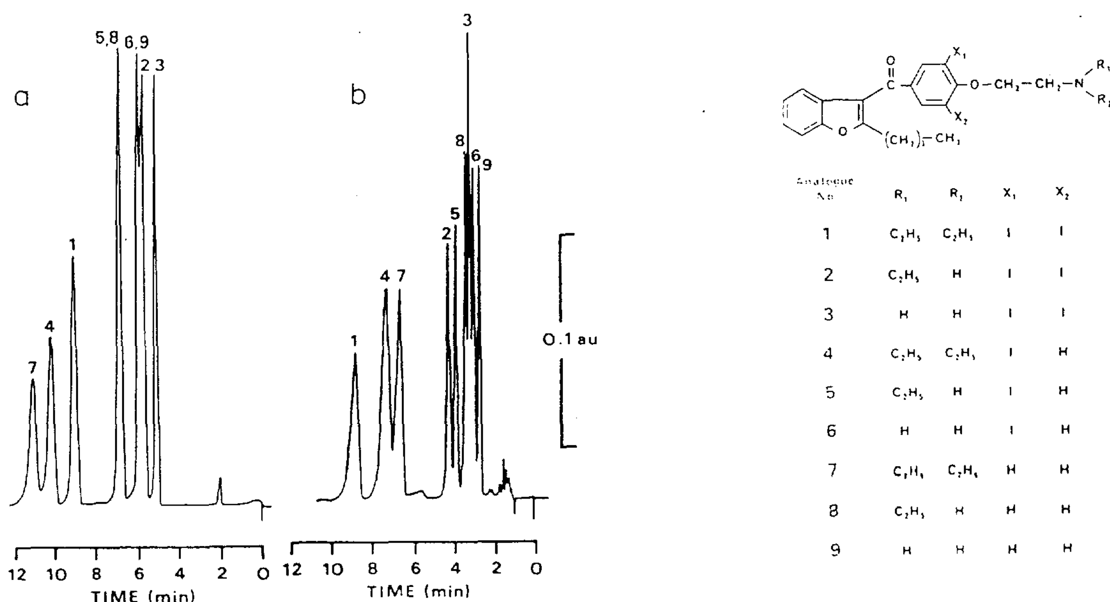


Fig. 20. Chromatography of amiodarone, desethylamiodarone and seven amiodarone analogues on a 250-mm column. Eluent: methanol-containing perchloric acid (0.03% v/v, 2.78 m*M*). Flow-rate, 2.0 ml/min; detection, UV, 240 nm; injection, 100 μl of methanolic solution (20 mg/l) of each compound. (a) Column packed with Spherisorb 5 silica. (b) Column packed with Spherisorb 5 ODS1 [222]. Reprinted from R.J. Flanagan, G.C.A. Storey, R.K. Bhamra, I. Jane, J. Chromatogr. 247 (1982) 15; with kind permission of Elsevier Science NL, Sara Burgerharstaat 25, 1055 KV Amsterdam, The Netherlands.

containing Na^+ and K^+ tended to precipitate in acetonitrile-rich mobile phases.

4.6. The two-site model

When two kinds of sites are present on the stationary phase then tailing of the solutes can have a dual origin:

(1) A thermodynamic origin is when one type of site (strongly interacting) is sparsely populated and sample size is comparable to the concentration of the sites. Then the site will work in overloaded conditions (nonlinear mode), while the weaker site would work in a linear mode. This type of tailing will be sensitive to the amount of sample injected.

Eble et al. [211] have shown experimentally that the two-site model of adsorption leads to a bimodal Langmuir isotherm. They have shown that on a modified silica surface a small subset of stronger adsorption sites exists together with a more numerous subset of hydrophobic sites. The smaller set was shown to have much lower (ca. 1/60) sample capacity when compared to the sites of lower strength. It is also important to say that only some solutes were able to detect chromatographically the existence of the strong sites. The less-populated, stronger adsorption sites were said to be silanols, while hydrophobic sites were identified as the other sites. The distribution constant for angiotensin II was estimated to be 200 times larger on stronger sites than that on hydrophobic sites. Due to the lower sample capacity of less-populated sites, chromatography in overloaded conditions can be the reason for asymmetric peaks. The two-site model [211] gave a quantitative fit of the data over a >1000-fold range in sample size, which verifies the two-site model and connects this with the degree of tailing and band-broadening depending on sample size. Later, the same group showed that decreasing the sample size can improve peak symmetry and efficiency of the column [12]. The question which requires an explanation is why only 1/60 of silanols can strongly interact with angiotensin II? The ratio of residual silanols to hydrophobic C_8 chains on Zorbax C_8 should be approximately equal to 1. This would mean that only less than 2% of silanols are able to undergo strong interactions with basic solutes. According to Scholten's [103] hypothesis, only 20% of residual silanols are able to interact with solutes due to a shielding effect of the methyl groups at the silicon atom of the modifier molecule. Together with the results with angiotensin II, this would suggest that about 5% of remaining silanols are able to strongly interact with organic bases. This, in turn, is approximately the number of isolated (non-H-bonded) silanols on a silica surface determined by IR-PAS measurements [234].

(2) The tailing can be of kinetic origin when one adsorption site interacts much more strongly than the other one, i.e. when the desorption rate from the stronger site is much lower than that from the weaker site. Kinetic tailing will not be affected by sample size since it is not a non-linear effect.

The influence of overloading conditions on peak shape was examined by Fornsted et al. [235]. According to them, when mass transfer kinetics is fast on the second type of sites (strongly adsorbing) peak tailing has only a thermodynamic origin, i.e. is caused by overloading of the type II sites. This becomes negligible when $a_1/a_2>100$ (a_1/a_2=a ratio of the analyte adsorbed on type I and type II sites, respectively). For kinetic tailing the same is true for $a_1/a_2>1000$. Due to a much higher curvature of the isotherm on the type II sites, in practice the density of the type II sites should be decreased to about 1 ppm or less to avoid tailing of kinetic or thermodynamic origin [235]. For chromatography this means that even a negligible number of strong adsorption sites (less than 1 per 1 000 000) when compared to 'normal' sites can cause tailing. This also shows why it is so difficult to remove all the undesirable sites from the silica surface.

Giddings [236] has estimated the influence of the two-site model on the shape of the chromatographic peak. He has found that a very few active sites on the stationary phase may lead to tailing under linear conditions. For this, slow desorption kinetics from the stronger sites is necessary; i.e. a residence time on the stronger site should be comparable to the standard deviation of the undisturbed peak (in time units). Recent, detailed, theoretical studies of mass transfer kinetics in linear and nonlinear chromatography have been carried out by Guiochon's group [235,237]. They have shown that substantial tailing can be caused by sparsely populated sites with slow adsorption–desorption kinetics despite their minor

(or even negligible) contribution to the retention. When the contribution becomes very small, then the tailing of peak appears close to baseline. Fronstedt et al. [237] have shown that even when strong sites constitute only ca. 0.1% of total sites, a definite tailing can be observed. It is a common practice to use the asymmetry factor (asf) calculated at 10% peak height for a comparison of the efficiency of various columns. These authors [237] have shown that for sparsely populated sites asf becomes useless as the tailing appears close to the baseline.

When the retention is governed by mixed mechanisms, e.g. hydrophobic and ion-exchange, then the retention data can be fitted to a two-adsorption-site model originally proposed by Sokolowski and Wahlund [199] as well as by Horvath's group [31,238].

A solution to the two-site model presented by Sokolowski and Wahlund [199] resulted in the following expression:

$$\frac{1}{k'V_m - a} = \frac{1}{b} + \frac{K^*_{BZ}[Z^-]_m}{b}[B^+]_m \qquad (12)$$

where k' is the capacity ratio of a basic solute, V_m is the column hold-up volume, $[Z^-]_m$ is concentration of counter ion in the mobile phase, $[B^+]_m$ is the concentration of competing amine in the mobile phase, K^*_{BZ} is the equilibrium constant of masking amine B with counter ion Z on the active site,

$$a = W_s K_o K_{QZ} [Z^-]^m$$

$$b = W_s K^*_o K^*_{QZ} [Z^-]^m$$

W_s is weight of the stationary phase, K_o and K^*_o are adsorption capacities of sites 1 and 2, K_{QZ} and K^*_{QZ} are equilibrium constants on sites 1 and 2.

Site 1 is hydrophobic while site 2 is polar (ion-exchange).

According to Eq. (12) a plot of the left side against concentration of competing amine $[B^+]_m$ should yield a straight line. Such straight lines were obtained for several antidepressants with dimethylamine as a competing amine [199].

The Horvath model is simpler and can be described by the equation [31,232,238,248]:

$$\frac{[A]}{k'_o - k'} = \frac{1}{k'_2 K_A} + \frac{[A]}{k'_2} \qquad (13)$$

where k'_o is the measured k' in the absence of a competing masking agent, k' is the measured k' in the presence of a competing masking agent, k'_2 is the silanophilic (ion-exchange) contribution to the actual k', K_A is the binding constant and [A] is the concentration of masking agent in the mobile phase. This model takes into account an attenuation of silanophilic retention by the competing amine. The K_A values show the ability of the amine to block the silanol site. According to Horvath, the longer alkyl chain in the trimethylammonium ion the higher the K_A value.

In general, both models are very similar: the Sokolowski and Wahlund model takes into account that part of the competing amine (alkylammonium ion) can also be distributed to the stationary phase as an ion-pair with a counter ion present in the buffer [199].

5. Conclusions

The undesired adsorption ability of many silica-based packings toward basic solutes is well known to chromatographers. The authors agree that the unwanted interactions are caused by a subfraction of the surface silanols with enhanced acidity. However, there is no agreement as to what causes the acidity. Generally there are two main hypothesis explaining the source of difficulties:

(1) There have been some attempts at explaining the phenomenon with some bulk properties of silica, e.g. acidity of the isolated silanols. This has been presented by Köhler et al. [53,54], who blamed highly acidic, isolated silanols for the strong interactions. They believe that total rehydroxylation can significantly improve the adsorption properties of silica. However, as Cox [13] has emphasized, such a bulk parameter as an apparent surface pH does not correlate with 'good' or 'bad' classification of the silicas introduced by Köhler et al. [54]. For example, Hypersil which is considered 'more acidic' (i.e. belongs to type A [12]) has the apparent surface pH of 8.1–9.0 [14]; similarly, less acidic (type B [12]) Nucleosil was shown to have pH values in the range of 5.15–8.1; while Vydac (also type B [12]) appeared to have pH values in the range of 4.1–6.1. The

fumed silicas which have their SiOH absorption band at 3750 cm^{-1} do not adsorb amines [93,94], which does not fit to the observations Köhler et al. Moreover, Köhler et al. [53,54] have neglected all literature estimations of the surface concentration of the deleterious silanols. Most literature sources assume that the concentration of 'bad' sites is less than 1% of all silanols [12–15,201,239]. The concentration of isolated silanols, even in low-hydroxylated silicas (with ca. 5.5 $\mu mol/m^2$) should be equal to ca. 3.5 $\mu mol/m^2$ (in agreement with their ^{29}Si CP-MAS NMR calculations of relative geminal/isolated silanol content). In favor of the Köhler et al. [54] hypothesis, we must add that some isolated silanols can be engaged in H-bonded groups (vicinal silanols). The first attempt to estimate the proportion of isolated/vicinal silanols was made by Snyder and Ward [240]. The IR studies of Van Der Voort et al. [59,84] showed that only a very small portion of the silanols belongs to the isolated (non-engaged in H-bond) sites and this supports the above hypothesis. However, the subsequent studies of TPD of pyridine from the silica surface showed that the population of isolated sites comprises about half of the total silanols (and this is contrary to the hypothesis) [42]. The same silica (Kieselgel 60) was used in both studies [42,59]. Also, other results concerning surface concentration of isolated silanols collected by Vansant et al. in their book [59] do not support Kirkland hypothesis.

(2) It is believed that the observed phenomena cannot be explained by observation of bulk properties, since only a very small group of silanols is responsible for the strong interactions with basic solutes. Consequently, the methods of investigation and/or removal should take this into account. Such a point of view was presented in our earlier reviews [6,14,15], as well as by Cox [13]. We believe that the trace metals buried in the silica matrix are responsible for the generation of the more acidic group of silanols. This is well known in catalysis and it was earlier discussed in detail [14]. In addition, Cox's group has postulated and proved that the main mechanism of interactions between the acidic sites and basic solutes is ion-exchange [12].

If we agree that the enhancement of acidity of some silanols is due to metal impurities in the silica matrix, we can expect a rather wide range of silanol pK_a values due to various metals as well as various distances of metal atoms to silanols. According to Qian Hong Wan et al. [217] such a wide range of pK_a values is to be expected.

Chromatography of organic bases is difficult due to several mechanisms governing their retention. Among mechanisms responsible for retention of organic bases, the following phenomena are listed: ion-exchange, hydrogen bonding, salting-out effect and hydrophobic interactions. The existence of ion-exchange under HPLC conditions is well documented in the literature. The same cannot be said about hydrogen-bonding. There is no convincing example in the literature of an H-bonding mechanism being responsible for the retention of organic bases under usual HPLC conditions. In our opinion, H-bonding can be rather blamed for tailing of polar compounds in gas–solid chromatography and in liquid chromatography of polar solutes when a non-polar mobile phase is used [208]. The mechanisms depend on the sample, stationary-phase, mobile-phase, pH, ionic strength, etc. The most difficult are the separations which involve contributions of different mechanisms. In such a situation it is extremely difficult to control the process. So many variables make such chromatography troublesome. One of the most important phenomena in chromatography of bases is ion-exchange. Before we consider an analysis of organic base we should answer the question: do we want to use the ion-exchange mechanism in analysis of our sample or do we want to avoid it.

If we want to avoid ion-exchange:

Then we should try to suppress the ion-exchange by:

- lowering the pH of the mobile phase to suppress the ionization of silanols. However, this will also cause an increased protonation of our analytes. At higher pH the solute is deprotonated, but simultaneously more silanols will be in a dissociated form. The ion-exchange is proportional to the product of the fractional solute ionization and the fractional silanol ionization. When the solute is completely de-ionized (at high pH for bases) silanol ionization no longer matters and vice versa;
- the manipulation of pH requires a knowledge of the pK_a values of solutes;
- the actual pH* of the mobile phase may differ

from the pH of the buffer used to prepare the mobile phase;

- the pK_a of the analyte in the aqueous/organic mobile phase differs from that determined in water;
- we can use amine additives to the mobile phase which will compete with solute molecules to ion-exchange sites.
- increase in ionic strength can further suppress silanol retention;
- use of more retentive buffer cations (K better than Na) also suppresses the contribution of ion-exchange to retention;
- use a column with low silanol activity.

If we want to use ion-exchange for separation:

- We can consider the use of plain silica which contains more ion-exchange sites than modified silica;
- then we should choose a pH for the process to assurs an ionization of silanols as well as protonation of analytes;
- the ion-exchange process can be controlled by buffer concentration, pH, and cation of the buffer.

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References

[1] K.K. Unger, Porous Silica, Elsevier, Amsterdam, 1979.
[2] K.K. Unger (Ed.), Packings and Stationary Phases in Chromatographic Techniques, M. Dekker, New York, 1989.
[3] J.J. Hetem, Chemically Modified Silica Surfaces in Chromatography. A Fundamental Study, Hüthig Buch Verlag, Heidelberg, 1993.
[4] R. Iler, The Chemistry of Silica, Wiley, New York, 1979.
[5] L.C. Sander, S.A. Wise, CRC Crit. Rev. Anal. Chem. 18(6) (1987) 299.
[6] J. Nawrocki, B. Buszewski, J. Chromatogr. 449 (1989) 1.
[7] M.P. Henry, J. Chromatogr. 544 (1991) 413.
[8] H. Engelhardt, H. Löw, W. Götzinger, J. Chromatogr. 544 (1991) 371.
[9] K. Albert, E. Bayer, J. Chromatogr. 544 (1991) 345.
[10] S.H. Hansen, P. Helboe, M. Thomsen, J. Chromatogr. 544 (1991) 53.
[11] H.H. Freizer, K.M. Gooding, J. Chromatogr. 544 (1991) 125.
[12] M.A. Stadalius, J.S. Berus, L.R. Snyder, LC-GC 6(6) (1988) 494.
[13] G.B. Cox, J. Chromatogr. A 656 (1993) 353.
[14] J. Nawrocki, Chromatographia 31(3/4) (1991) 177.
[15] J. Nawrocki, Chromatographia 31(3/4) (1991) 193.
[16] R. Eksteen, K.J. Pardue, in: Chi-san Wu (Ed.), Handbook of Size Exclusion Chromatography, M. Dekker Inc., New York, 19.
[17] A. Berthod, J. Chromatogr. 549 (1991) 1.
[18] D.J. Anderson, Anal. Chem. 67(12) (1995) 475R.
[19] J.G. Dorsey, W.T. Cooper, Anal. Chem. 66(17) (1994) 857A.
[20] P. Van Der Voort, E.F. Vansant, J. Liq. Chromatogr. Rel. Technol. 19(17/18) (1996) 2723.
[21] C.L. Guillemion, M. Le Page, A.I. de Vries, J. Chromatogr. Sci. 9 (1971) 470.
[22] R. Arshady, J. Chromatogr. 586 (1991) 181.
[23] R. Arshady, J. Chromatogr. 586 (1991) 199.
[24] U. Trüdinger, G. Müller, K.K. Unger, J. Chromatogr. 535 (1990) 111.
[25] J.J. Kirkland, J. Chromatogr. Sci. 10 (1972) 593.
[26] N.D. Danielson, J.J. Kirkland, Anal. Chem. 59 (1987) 2501.
[27] J. Nawrocki, R.P. Rigney, A. McCormick, P.W. Carr, J. Chromatogr. A 657 (1993) 229.
[28] M.L. Hair, W. Hertl, J. Phys. Chem. 73 (1969) 4269.
[29] V.V. Strelko, S.K. Rubanik, Adsorbtsia i Adsorbenty (Naukova Dumka, Kiev 1974) 2 (1974) 82.
[30] R.P.W. Scott, P. Kucera, J. Chromatogr. Sci. 13 (1975) 337.
[31] K.E. Bij, C. Horváth, W.R. Melander, A. Nahum, J. Chromatogr. 203 (1981) 65.
[32] G.B. Cox, R.W. Stout, J. Chromatogr. 384 (1987) 315.
[33] B.A. Bidlingmeyer, J.K. Del Rios, J. Korpi, Anal. Chem. 54 (1982) 442.
[34] B.A. Morrow, A.J. McFarlan, Langmuir 7 (1991) 1695.
[35] M.L. Miller, R.W. Linton, G.E. Maciel, B.L. Hawkins, J. Chromatogr. 319 (1985) 9.
[36] J. Sauer, K.-P. Schroeder, Z. Phys. Chem. (Leipzig) 266 (1985) 379.
[37] G.I. Harris, J. Chem. Soc. (1963) 5978.
[38] A.M. Ferrari, P. Uglieno, E. Garrone, J. Phys. Chem. 97 (1993) 2671.
[39] D.R. Kinney, I-Ssuer Chuang, G.E. Maciel, J. Am. Chem. Soc. 115 (1993) 6786.
[40] L.T. Zhuravlev, Pure Appl. Chem. 61 (1989) 1969.
[41] P. Van Der Voort, I. Gillis-D'Hamers, E.F. Vansant, J. Chem. Soc. Faraday Trans. 86(22) (1990) 3751.
[42] I. Gillis-D'Hamers, I. Cornelissens, K.C. Vrancken, P. Van Der Voort, E.F. Vansant, J. Chem. Soc. Faraday Trans. 88(5) (1992) 723.
[43] L.T. Zhuravlev, A.V. Kiselev, V.P. Naidinja, A.L. Polyakov, Russ. J. Phys. Chem. 37(113) (1963) 1216.

[44] G. Fóti, C. Martinez, E.sz. Kováts, J. Chromatogr. 461 (1989) 243.
[45] G. Fóti, E.sz. Kováts, Langmuir 5 (1989) 232.
[46] G.W. Sears, Anal. Chem. 30 (1956) 1981.
[47] A.L. Khurama, C.-T. Ho, J. Liq. Chromatogr. 11 (1988) 3205.
[48] G. Schomburg, A. Deege, J. Köhler, U. Bien-Vogelsang, J. Chromatogr. 282 (1983) 27.
[49] T. Welsch, H. Frank, Z. Prakt. Chem. 325 (1983) 325.
[50] L. Nondek, V. Vyskočil, J. Chromatogr. 206 (1981) 581.
[51] L. Nondek, A. Reissova, J. High Resolut. Chromatogr. Chromatogr. Commun. 7 (1984) 153.
[52] J. Kijenski, R. Hombek, S. Malinowski, J. Catal. 50 (1977) 186.
[53] J. Köhler, J.J. Kirkland, J. Chromatogr. 385 (1987) 125.
[54] J. Köhler, D.B. Chase, R.D. Farlee, A.J. Vega, J.J. Kirkland, J. Chromatogr. 352 (1986) 275.
[55] M. Holik, B. Matějková, J. Chromatogr. 213 (1981) 33.
[56] A.C. Zettlemayer, H.H. Hsing, J. Colloid Interface Sci. 58 (1977) 263.
[57] K.K. Unger, N. Becker, P. Raumeliotis, J. Chromatogr. 125 (1976) 115.
[58] D.W. Sindorf, G.E. Maciel, J. Phys. Chem. 86 (1982) 5208.
[59] E.F. Vansant, P. Van Der Voort, K.C. Vranken, Characterization and Chemical Modification of the Silica Surface, Elsevier, Amsterdam, 1995.
[60] P. Van Der Voort, S. Vercauteren, K. Peeters, E.F. Vansant, J. Colloid Interface Sci. 157 (1993) 518.
[61] K. Yoshinaga, H. Yoshida, Y. Yamamoto, K. Takakura, M. Komatsu, J. Colloid Interface Sci. 153 (1992) 207.
[62] T. Takeuchi, T. Miwa, N. Nagae, Chromatographia 35(7/8) (1993) 375.
[63] D.B. Marshall, C.L. Cole, A.D. Norman, J. Chromatogr. 361 (1986) 71.
[64] J. Nawrocki, J. Chromatogr. 362 (1986) 117.
[65] B. Buszewski, A. Jurášek, J. Garaj, L. Nondek, I. Novák, D. Berek, J. Liq. Chromatogr. 10 (1987) 2325.
[66] B. Buszewski, K. Šebekova, P. Božek, D. Berek, J. Chromatogr. 367 (1986) 171.
[67] J. Goworek, F. Nooitgedacht, H. Rijkhof, H. Poppe, J. Chromatogr. 352 (1986) 399.
[68] M.R. Basila, J. Chem. Phys. 35 (1961) 1151.
[69] P.C. Sadek, C.J. Koester, L.D. Bowers, J. Chromatogr. Sci. 25 (1987) 489.
[70] D.B. Marshall, K.A. Stutter, C.H. Lochmüller, J. Chromatogr. Sci. 22 (1984) 217.
[71] D.B. Marshall, C.L. Cole, D.E. Connolly, J. Chromatogr. 361 (1986) 71.
[72] H. Wang, J.M. Harris, J. Phys. Chem. 99 (1995) 16999.
[73] K.D. Lork, K.K. Unger, J. Chromatogr. 352 (1986) 199.
[74] J.P. Blitz, R.S.S. Murthy, D.E. Leyden, J. Colloid Interface Sci. 121 (1988) 63.
[75] C.P. Tripp, M.L. Hair, Langmuir 8 (1992) 1961.
[76] C.P. Tripp, M.L. Hair, Langmuir 8 (1992) 1120.
[77] P. Silberzan, L. Léger, D. Ausserré, J.J. Benattar, Langmuir 7 (1991) 1647.
[78] J.P. Blitz, R.S.S. Murthy, D.E. Leyden, J. Am. Chem. Soc. 109 (1987) 7141.
[79] J.P. Blitz, R.S.S. Murthy, D.E. Leyden, J. Colloid Interface Sci. 126 (1988) 387.
[80] C.P. Tripp, R.P.N. Veregin, M.L. Hair, Langmuir 9 (1993) 3518.
[81] Ch.E. Bronniman, R.C. Zeigler, G.E. Maciel, J. Am. Chem. Soc. 110(7) (1988) 2023.
[82] M.L. Gee, T.W. Healy, L.R. White, J. Colloid Interface Sci. 140(2) (1990) 450.
[83] J.P. LeGrange, J.L. Markham, C.R. Kurkjian, Langmuir 9 (1993) 1749.
[84] I. Gillis-D'Hamers, K.C. Vrancken, E.F. Vansant, G. De Roy, J. Chem. Soc. Faraday Trans. 88(14) (1992) 2047.
[85] K.K. Unger, K.D. Lork, B. Pfleiderer, K. Albert, E. Bayer, J. Chromatogr. 556 (1991) 395.
[86] M.L. Hair, W. Hertl, J. Phys. Chem. 73 (1969) 2372.
[87] F.H. van Cauwelaert, P.A. Jacobs, J.B. Uytterhoeven, J. Phys. Chem. 76 (1972) 1434.
[88] A.J. Van Roosmalen, J.C. Mol, J. Phys. Chem. 82 (1978) 2748.
[89] A.J. Van Roosmalen, J.C. Mol, J. Phys. Chem. 83 (1979) 2485.
[90] A.J. McFarlan, B.A. Morrow, J. Phys. Chem. 95 (1991) 5388.
[91] B.A. Morrow, A.J. McFarlan, J. Phys. Chem. 96 (1992) 1395.
[92] N. Sagliano Jr., R.A. Hartwick, R.E. Patterson, B.A. Woods, J.L. Bass, N.T. Miller, J. Chromatogr. 458 (1988) 225.
[93] J.P. Hendra, J.R. Horder, E.J. Loader, J. Chem. Soc. A, (1971) 1766.
[94] J. Adams, C.S. Giam, J. Chromatogr. 285 (1984) 81.
[95] E.sz. Kováts, private communication, October 1989.
[96] S. Kondo, K. Tomasi, C. Pak, Bull. Chem. Soc. Jpn. 52 (1979) 2046.
[97] I-Ssuer Chuang, D.R. Kinney, G.E. Maciel, J. Am. Chem. Soc. 115 (1993) 8695.
[98] S. Kondo, H. Yamauchi, Y. Kajiyama, T. Ishikawa, J. Chem. Soc. Faraday Trans. I 80 (1984) 2033.
[99] J.A. Hockey, Chem. Ind. (1965) 57.
[100] S. Kondo, M. Muroya, K. Fuji, Bull. Chem. Soc. Jpn. 47 (1974) 553.
[101] S.G. Bush, J.W. Jorgenson, J. Chromatogr. 503 (1990) 69.
[102] S.G. Bush, J.W. Jorgenson, M.L. Miller, R.W. Linton, J. Chromatogr. 260 (1983) 1.
[103] A.B. Scholten, PhD Thesis, Technische Unversiteit Eindhoven, Eindhoven, 1996.
[104] A.B. Scholten, H.-G. Janssen, J.W. de Haan, C.A. Cramers, J. High Resol. Chromatogr. 17(2) (1994) 77.
[105] J.L. Glajch, J.J. Kirkland, J. Köhler, J. Chromatogr. 384 (1987) 81.
[106] N. Sagliano Jr., T.R. Floyd, R.A. Hartwick, J.M. DiBussolo, N.T. Miller, J. Chromatogr. 443 (1988) 155.
[107] A.B. Scholten, H.A. Claessens, J.W. de Haan, C.A. Cramers, J. Chromatogr. A 759 (1997) 37.
[108] A.B. Scholten, J.W. de Haan, H.A. Claessens, L.M. van de Ven, C.A. Cramers, J. Chromatogr. A 688 (1994) 25.
[109] J.J. Kirkland, M.A. Van Straten, H.A. Claessens, J. Chromatogr. A 691 (1995) 3.

[110] E. Ackerman, Acta Chem. Scand. 10 (1956) 298.
[111] J.J. Kirkland, J.L. Glajch, R.D. Farlee, Anal. Chem. 61 (1989) 2.
[112] H.H. Freiser, N.P. Nowlan, D.L. Gooding, J. Liq. Chromatogr. 12 (1989) 827.
[113] B. Law, P.F. Chan, J. Chromatogr. 467 (1989) 267.
[114] N.T. Miller, J.M. DiBussolo, J. Chromatogr. 499 (1990) 317.
[115] A. Wehrli, J.C. Hildenbrand, H.P. Keller, R. Stampfli, R.W. Frei, J. Chromatogr. 149 (1978) 199.
[116] J.G. Atwood, G.J. Schmidt, W. Slavin, J. Chromatogr. 171 (1979) 109.
[117] B.B. Wheals, J. Chromatogr. 187 (1980) 65.
[118] B. Law, Trends Anal. Chem. 9(1) (1990) 31.
[119] B. Law, J. Chromatogr. 407 (1987) 1.
[120] H.A. Claessens, M.A. van Straten, J.J. Kirkland, J. Chromatogr. A 728 (1996) 259.
[121] J.J. Kirkland, J. Chromatogr. Sci. 34 (1996) 309.
[122] J.J. Kirkland, J.W. Henderson, J. Chromatogr. Sci. 32 (1994) 473.
[123] J. Falkenhagen, P.G. Dietrich, Am. Lab. 26 (1994) 48.
[124] P.G. Dietrich, J. Falkenhagen, I. Molnar, Fachz Lab. GIT 37 (1993) 23.
[125] R.W. Stout, J.J. DeStefano, J. Chromatogr. 326 (1985) 63.
[126] K. Nobuhara, M. Kato, M. Nakamura, M. Tokami, S. Kaneko, J. Chromatogr. A 704 (1995) 45.
[127] S. Kaneko, T. Mitsuzawa, S. Ohmori, M. Nakamura, K. Nobuhara, M. Masatami, J. Chromatogr. A 669 (1994) 1.
[128] J. Nawrocki, D.L. Moir, W. Szczepaniak, Chromatographia 28 (1989) 143.
[129] J. Nawrocki, D.L. Moir, W. Szczepaniak, J. Chromatogr. 467 (1989) 31.
[130] T. Daldrup, B. Kardel, Chromatographia 18(2) (1984) 81.
[131] B.S. Welinder, T. Kornfelt, H.H. Sorensen, Anal. Chem. 67(1) (1995) 39A.
[132] S.L. Secreast, J. Chromatogr. 544 (1991) 99.
[133] C.P. Tripp, M.L. Hair, J. Phys. Chem. 97 (1993) 5693.
[134] D.V. McCalley, J. Chromatogr. A 738 (1996) 169.
[135] D.V. McCalley, J. Chromatogr. A 769 (1997) 169.
[136] P.C. Sadek, P.W. Carr, L.W. Bowers, J. Liq. Chromatogr. 8(13) (1985) 2369.
[137] M. Bogusz, M. Erkens, R.D. Mayer, I. Schröder, J. Liq. Chromatogr. 15(1) (1992) 127.
[138] M. Verzele, J. Lammens, M. van Roelenbosch, J. Chromatogr. 186 (1979) 435.
[139] I. Wouters, I. Quintens, E. Roets, J. Hoogmartens, J. Liq. Chromatogr. 5(1) (1982) 25.
[140] P.C. Sadek, P.W. Carr, J. Chromatogr. Sci. 21 (1983) 314.
[141] H. Engelhardt, M. Jungheim, Chromatographia 29(1/2) (1990) 59.
[142] L. Nondek, B. Buszewski, D. Berek, J. Chromatogr. 360 (1986) 241.
[143] D.V. McCalley, J. Chromatogr. 357 (1986) 221.
[144] R.E. Majors, J. Chromatogr. Sci. 18 (1980) 492.
[145] Ch.L. Thomas, Ind. Eng. Chem. 41 (1949) 2564.
[146] K. Tanabe, T. Sumiyoshi, K. Shibata, T. Kiyoura, J. Kitagawa, Bull. Chem. Soc. Jpn. 47 (1974) 1064.
[147] K. Tanabe, in: J.R. Anderson M. Boudart (Eds.), Catalysis, Science and Technology, Springer-Verlag, Berlin, 1981, p. 231.
[148] K. Larsson, W. Hermann, P. Möller, D. Sanchez, J. Chromatogr. 450 (1988) 71.
[149] Y. Ohtsu, Y. Shiojima, T. Okumura, J. Koyama, K. Nakamura, O. Nakata, K. Kimata, N. Tanaka, J. Chromatogr. 481 (1989) 147.
[150] J. Gobet, E. Kovàts, Adv. Sci. Technol. 1 (1984) 77.
[151] K.D. McMurtrey, J. Liq. Chromatogr. 11(16) (1988) 3375.
[152] B. Buszewski, Chromatographia 28(11/12) (1989) 574.
[153] Y. Sudo, J. Chromatogr. A 737 (1996) 139.
[154] Y. Sudo, J. Chromatogr. A 757 (1997) 21.
[155] S. Zhang, B. Schindler, G. Nicholson, E. Bayer, J. High Resolut. Chromatogr. 18 (1995) 579.
[156] J. Sagiv, J. Am. Chem. Soc. 102 (1980) 92.
[157] M.J. Wirth, H.O. Fatunmbi, Anal. Chem. 64 (1992) 2783.
[158] M.J. Wirth, H.O. Fatunmbi, Anal. Chem. 65 (1993) 822.
[159] R.W.P. Fairbank, Yang Xiang, M.J. Wirth, Anal. Chem. 67 (1995) 3879.
[160] H.O. Fatunmbi, M.D. Bruch, M.J. Wirth, Anal. Chem. 65 (1993) 2048.
[161] M.J. Wirth, R.W.P. Fairbank, J. Liq. Chromatogr. Rel. Technol. 19(17/18) (1996) 2799.
[162] D.J. Angst, G.W. Simmons, Langmuir 7 (1991) 2236.
[163] M. Petro, D. Berek, Chromatographia 37(9/10) (1993) 549.
[164] G. Schomburg, A. Deege, J. Köhler, U. Bien-Vogelsang, J. Chromatogr. 282 (1983) 27.
[165] G. Schomburg, J. Köhler, H. Figge, A. Deege, U. Bien-Vogelsang, Chromatographia 18 (1984) 265.
[166] Y. Ohtsu, Y. Shiojima, T. Okumura, J. Koyama, K. Nakamura, M. Nakano, O. Nakata, Y. Fujiyama, Chromatographia 24 (1987) 380.
[167] H. Engelhardt, H. Löw, W. Eberhardt, M. Mauss, Chromatographia 27 (1989) 535.
[168] M. Hanson, B. Eray, K. Unger, A.V. Neimark, J. Schmid, K. Albert, E. Bayer, Chromatographia 35(7/8) (1993) 403.
[169] B. Buszewski, J. Schmid, K. Albert, E. Bayer, J. Chromatogr. 552 (1991) 415.
[170] T.L. Ascah, B. Feibush, J. Chromatogr. 506 (1990) 357.
[171] D. Korakas, K. Valkó, J. Liq. Chromatogr. 17(16) (1994) 3571.
[172] T.L. Ascah, K.M.L. Kallury, C.A. Szafrański, S.D. Corman, F. Liu, J. Liq. Chromatogr. Rel. Technol. 19(17/18) (1996) 3049.
[173] B. Buszewski, M. Jaroniec, R.K. Gilpin, J. Chromatogr. A 668 (1994) 293.
[174] B. Buszewski, R.K. Gilpin, M. Jaroniec, Chemia Anal. (Warsaw) 39 (1994) 653.
[175] B. Buszewski, R.K. Gilpin, M. Jaroniec, Chemia Anal. (Warsaw) 39 (1994) 673.
[176] B. Buszewski, P. Kasturi, R.K. Gilpin, M.E. Gangoda, M. Jaroniec, Chromatographia 39(3/4) (1994) 155.
[177] B. Buszewski, M. Jaroniec, R.K. Gilpin, J. Chromatogr. A 673 (1994) 11.
[178] T. Czajkowska, M. Jaroniec, J. Liq. Chromatogr. Rel. Technol. 19(17/18) (1996) 2829.

[179] J.E. O'Gara, B.A. Alden, T.H. Walter, J.S. Petersen, C.L. Niederländer, U.D. Neue, Anal. Chem. 67 (1995) 3809.
[180] B. Feibush, J. Liq. Chromatogr. Rel. Technol. 19(14) (1996) 2315.
[181] Y. Bereznitski, M. Jaroniec, M. Kruk, J. Liq. Chromatogr. Rel. Technol. 19(10) (1996) 1523.
[182] Y. Bereznitski, M. Jaroniec, M. Kruk, B. Buszewski, J. Liq. Chromatogr. Rel. Technol. 19(17/18) (1996) 2767.
[183] J.J. Kirkland, C.H. Dilks Jr., J.J. DiStefano, J. Chromatogr. 635 (1993) 19.
[184] J. Verme-Mismer, M. Lamard, J. Wagner, J. Chromatogr. 645 (1993) 251.
[185] D.V. McCalley, J. Chromatogr. 636 (1993) 213.
[186] J. Paesen, P. Claeys, E. Roets, J. Hoogmartens, J. Chromatogr. 630 (1993) 117.
[187] R.J.M. Vervoort, F.A. Maris, H. Hindrics, J. Chromatogr. 623 (1992) 207.
[188] I.M. Mutton, J. Chromatogr. A 697 (1995) 191.
[189] H.A. Claessens, E.A. Vermeer, C.A. Cramers, LC·GC Int. 6(11) (1993) 692.
[190] B.A. Olsen, G.R. Sullivan, J. Chromatogr. A 692 (1995) 147.
[191] R.J.M. Vervoort, M.W.J. Derksen, A.J.J. Debets, J. Chromatogr. A 765 (1997) 157.
[192] D.V. McCalley, J. Chromatogr. A 708 (1995) 185.
[193] Yu.V. Kazakevich, H.M. McNair, J. Chromatogr. Sci. 33 (1995) 321.
[194] D.M. Bliesner, K. Sentel, Anal. Chem. 65 (1993) 1819.
[195] S.H. Hansen, J. Chromatogr. 209 (1981) 203.
[196] S.H. Hansen, P. Helbow, M. Thomsen, Trends Anal. Chem. 4(9) (1985) 233.
[197] S.H. Hansen, P. Helbow, M. Thomsen, Adv. Chromatogr. 28 (1989) 195.
[198] S.H. Hansen, P. Helbow, M. Thomsen, J. Chromatogr. 409 (1987) 71.
[199] A. Sokolowski, K.-G. Wahlund, J. Chromatogr. 189 (1980) 299.
[200] E. Bayer, A. Paulus, J. Chromatogr. 400 (1987) 1.
[201] B. Pfleiderer, E. Bayer, J. Chromatogr. 468 (1989) 67.
[202] J. Nawrocki, J. Chromatogr. 407 (1987) 171.
[203] K.-G. Wahlund, A. Sokolowski, J. Chromatogr. 151 (1978) 299.
[204] J.S. Kiel, S.L. Morgan, R.K. Abramson, J. Chromatogr. 320 (1985) 313.
[205] D.W. Hill, J. Liq. Chromatogr. 13(16) (1990) 3147.
[206] J.W. Dolan, LC·GC Int. 4 (1991) 16.
[207] D.W. Hill, A.J. Kind, J. Liq. Chromatogr. 16(18) (1993) 3941.
[208] H.W. Stuurman, K.-G. Wahlund, Chromatographia 16 (1982) 147.
[209] D.V. McCalley, J. Chromatogr. A 664 (1994) 139.
[210] D.S. Kora, E. Tesařová, M. Popl, J. Chromatogr. A 758 (1997) 37.
[211] J.E. Eble, R.L. Grob, P.E. Antle, L.R. Snyder, J. Chromatogr. 384 (1987) 45.
[212] H. Lingeman, H.A. van Munster, J.H. Beynen, W.J.M. Underberg, A. Hulshoff, J. Chromatogr. 352 (1986) 261.
[213] R.L. Smith, D.J. Pietrzyk, Anal. Chem. 56 (1984) 610.
[214] D.E. Yates, S. Levine, T.W. Healy, J. Chem. Soc. Faraday Trans. I 70 (1974) 1807.
[215] J. Coene, M. Ghijs, E. van den Eeckhout, W. van den Bossche, P. Sandra, J. Chromatogr. 553 (1991) 285.
[216] R.J. Flanagan, I. Jane, J. Chromatogr. 323 (1985) 173.
[217] Wan Qian-Hong, M.C. Davies, P.N. Shaw, D.A. Barrett, Anal. Chem. 68 (1996) 437.
[218] M.L. Hair, W. Hertl, J. Phys. Chem. 74 (1970) 91.
[219] K. Marshall, G.L. Ridgewell, C.H. Rochester, J. Simpson, Chem. Ind. (1974) 775.
[220] D. Heidrich, D. Volkmann, B. Żurawski, Chem. Phys. Lett. 80(1) (1981) 60.
[221] R.W. Stout, G.B. Cox, T.J. Odiorne, Chromatographia 24 (1987) 602.
[222] R.J. Flanagan, G.C.A. Storey, R.K. Bhamra, I. Jane, J. Chromatogr. 247 (1982) 15.
[223] R. Schwarzenbach, J. Chromatogr. 129 (1976) 31.
[224] R. Schwarzenbach, J. Chromatogr. 202 (1980) 397.
[225] R. Schwarzenbach, J. Liq. Chromatogr. 2 (1979) 205.
[226] R. Schwarzenbach, J. Chromatogr. 251 (1982) 339.
[227] R. Schwarzenbach, J. Chromatogr. 334 (1985) 35.
[228] R. Gill, M.D. Osselton, R.M. Smith, T.G. Hurdley, J. Chromatogr. 386 (1987) 65.
[229] R.M. Smith, T.G. Hurdley, J.P. Westlake, R. Gill, M.D. Osselton, J. Chromatogr. 455 (1988) 77.
[230] R.J.M. Vervoort, M.W.J. Derksen, F.A. Maris, J. Chromatogr. A 678 (1994) 1.
[231] K. Sugden, G.B. Cox, C.R. Loscombe, J. Chromatogr. 149 (1978) 377.
[232] E. Papp, G. Vigh, J. Chromatogr. 259 (1983) 49.
[233] E. Papp, G. Vigh, J. Chromatogr. 282 (1983) 59.
[234] P. Van Der Voort, I. Gillis-D'Hamers, K.C. Vrancken, E.F. Vansant, J. Chem. Soc. Faraday Trans. 87(24) (1991) 3899.
[235] T. Fornstedt, G. Zhong, G. Guiochon, J. Chromatogr. A 742 (1996) 55.
[236] C. Giddings, Anal. Chem. 35(13) (1963) 1999.
[237] T. Fornstedt, G. Zhong, G. Guiochon, J. Chromatogr. A 741 (1996) 1.
[238] A. Nahum, Cs. Horvath, J. Chromatogr. 203 (1981) 53.
[239] L.A. Ciolino, J.G. Dorsey, J. Chromatogr. A 678 (1994) 201.
[240] L.R. Snyder, J.W. Ward, J. Phys. Chem. 70 (1966) 3941.
[241] R. West, L.S. Whatley, K.J. Lake, J. Am. Chem. Soc. 83 (1961) 761.
[242] J. Nawrocki, Chemia Anal. (Warsaw) 41 (1996) 11.
[243] M. Mauss, H. Engelhardt, J. Chromatogr. 371 (1986) 235.
[244] P.E. Antle, A.P. Goldberg, L.R. Snyder, J. Chromatogr. 321 (1985) 1.
[245] J.A. Lewis, D.C. Lommen, W.D. Raddatz, J.W. Dolan, L.R. Snyder, I. Molnar, J. Chromatogr. 592 (1992) 183.
[246] E. Bosch, P. Bou, H. Allemann, M. Rosés, Anal. Chem. 68 (1996) 3651.
[247] J.J. Kirkland, J.J. DeStefano, GIT Special – Chromatography International 96, June 1996, p. 62.
[248] Z. Varga-Puchony, Gy. Vigh, J. Chromatogr. 257 (1983) 380.