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Review

Bare silica, dynamically modified with long-chain quaternary ammonium ions – the technique of choice for more reproducible selectivity in reversed-phase high-performance liquid chromatography

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ABSTRACT

Reversed-phase high-performance liquid chromatography (RP-HPLC) on chemically bonded phases has become the most popular technique in HPLC for use in pharmaceutical and biological chemistry during the last decade. However, it is unfortunate that standardization of RP-HPLC systems developed on column packing materials from different manufacturers with theoretically identical chemically bonded phases (*e.g.*, octadecylsilyl phases) is not possible. When using bare silica from different manufacturers and generating the reversed-phase systems by dynamic modification of the silica surfaces with long-chain quaternary ammonium ions, high reproducibility of selectivity is obtained. Further, the peak symmetries obtained for amine compounds chromatographed in these systems are very good.

CONTENTS

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1. INTRODUCTION

For half a century silica has been the support material of choice in chromatography, used either "as is" or modified in various ways. With the introduction of reversed-phase chromatography on chemically modified silica 20 years ago [1,2] the development in the analysis of biological samples was reinforced. This development has led high-performance liquid chromatography (HPLC) to become the most popular chromatographic technique in use today.

However, bare silica may also be used for HPLC separations similar to those obtained by reversed-phase chromatography on chemically bonded phases. This can be performed either in a cation-exchange mode [3,4] or by using dynamically modified silica [5,6], but only the latter technique is generally applicable to cationic, anionic and non-ionic solutes.

This review surveys the dynamically modified silica technique and its unsurpassed performance in offering reproducible selectivity, even when using column packing materials from different manufacturers. The high reproducibility of selectivity is unsurpassed even when cationic solutes are chromatographed.

2. CHEMICALLY BONDED PHASES

Reversed-phase (RP) HPLC on bonded-phase column packing materials offers several advantages over other modes of HPLC (*e.g.*, for analysing aqueous samples in biochemistry, clinical and environmental chemistry), and this is the main reason for the continued success of the technique [7,8].

The method is fairly simple to handle. When the type and the brand of column packing material have been chosen, the eluent composed of water as the basic solvent and modified with one of a few organic solvents, most often methanol, acetonitrile or tetrahydrofuran or a mixture thereof [9, p. 285] is pumped through the column system.

When ionic solutes are to be separated, secondary equilibria must also be taken into consideration [10]. The pH of the eluent must be properly adjusted and, occasionally, a counter ion has to be added to control the retention, changing the chromatographic mode into reversed-phase ion-pair partition [11,12]. This method has the further advantage that the selectivity between ionic and non-ionic compounds may easily be changed, as the retention of the non-ionic solutes is largely unaffected by ion-pairing additives [13,14].

When various additives, such as long-chain alkane sulphonates or quaternary ammonium ions, are used in the eluent to perform ion-pair chromatography, equilibrium for the system is reached more slowly. The column packing material will adsorb a certain amount of the additives depending on the equilibrium. One to two

hours or more are normally required. When the concentration of organic solvent is below the wettability limit [9, p. 298], long equilibrium times are also needed.

It is obvious that bonded-phase column packing materials do not behave in a uniform manner. During the late 1970s chromatographers [15,16] gradually became aware that materials that were labelled as being of the same type, *e.g.*, bonded ODS-silica, could perform differently. The first description of this behaviour was in 1980 by Ogan and Katz [17]. Such variations in selectivity are due to differences in the manufacturing processes of the bonded phases and in the basic silica material used, as described later.

The brand-to-brand variations in selectivity of bonded-phase materials may seem advantageous when, for example, the separation of a complex mixture of polynuclear aromatic hydrocarbons (PAHs) has to be achieved, as reported by Ogan and Katz [17]. Another example is shown in Fig 1, where the separation of five components of an analgesic tablet is shown [18]. The separation on LiChrosorb RP-8 has been optimized with respect to concentration and type of organic modifier (acetonitrile, methanol) and of the buffer pH in the eluent. Accordingly, the chromatogram represents the best possible separation of the five solutes on the column packing material used. However, the separation is not completely satisfactory as the peaks for ethallobarbital and phenacetin are not well resolved. A change in the column packing material to Nucleosil C_8 using the same eluent solves the problem and an optimum separation is shown in Fig. 1B.

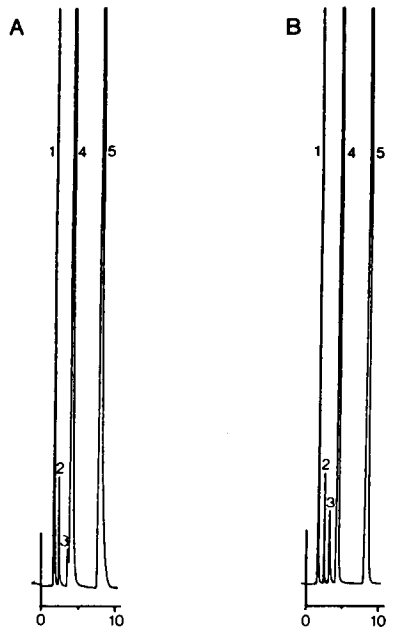

Fig. 1. Separation of analgesics. Columns: A, LiChrosorb RP-8, 5 μm (150 × 4.6 mm I.D.); B, Nucleosil 5 C_8 (150 × 4.6 mm I.D.). Eluent, acetonitrile–0.01 *M* sodium phosphate buffer (pH 7.0) (30:70, v/v); flow-rate, 1 ml/min; detection, UV (220 nm). Peaks: 1 = caffeine; 2 = codeine; 3 = ethallobarbital; 4 = phenacetin; 5 = propyphenazone. Reprinted from ref. 18 with permission.

Modifying the selectivity of a chromatographic system towards a given test mixture by changing the brand of column packing material may often prove useful in the development of separation methods, but is also time consuming and expensive. However, the brand to brand variation in selectivity most often implies several disadvantages. The variation will often make it impossible to reproduce a given separation when using other brands of column packing material than that used in the development of the method. Problems of this type have been reported in pharmaceutical analysis [19–21], clinical chemistry [22,23] and environmental analysis [16]. It was reported by Goldberg [24] that the separation factor between caffeine and theophylline when chromatographed on fifteen different ODS-silica materials ranged between 1.8 and 3.4. However, it is not possible to nominate a single brand of a given type of column packing material as the best choice for a certain separation.

In pharmaceutical analysis, the possible variations in selectivity may be especially critical in a pharmacopoeial context. HPLC has already been introduced in several monographs in international pharmacopoeias [25–27]. In some pharmacopoeias the column packing material is described in general terms as, for example, a chemically bonded C_{18} material, but adding information about the suitability of a special brand. An early example in the British Pharmacopoeia was the monograph on the antiviral drug substance idoxuridine. The monograph includes an assay and a test for related substances using HPLC on an ODS-silica material, μBondapak C_{18}, was mentioned as suitable. The elaboration of the method was published [28] and it appeared the μBondapak C_{18} column packing material was the only type tried. Using a series of different ODS-silica materials demonstrated that several types may be used. However, new problems concerning selectivity, retention and efficiency arose [19]. On certain columns it appeared that one of the impurities was only partly resolved from the internal standard whereas on others the retention was far too low. Finally, the efficiency and peak shape for certain solutes were unsatisfactory on some column packing materials.

The above problem should be taken into consideration whenever national or international methods of standardization are elaborated in, for example, clinical, drug, environmental or food analysis.

An even more unfortunate situation than the brand-to-brand variations is seen with changes from batch to batch of the same brand of column packing material. Such variations have been demonstrated, for example, by Atwood and Goldstein [29]. They tested over 20 batches of Vydac RP-18 column packing material with several test mixtures in an attempt to establish criteria for acceptance or rejection of batches for various types of separations. Three out of 24 batches were rejected because of large deviations from the mean characteristics.

When separating basic compounds on bonded-phase column packing materials, a further problem may arise. In addition to the variation in selectivity, considerable variations in peak tailing may occur, which makes it even more difficult to standardize methods using bonded-phase materials [30]. Such problems can to some extent be remedied by adding long-chain amines or quaternary ammonium compounds to the eluent as anti-tailing agents [30–33].

3. SILICA

The reason for the poorly reproducible selectivity when chromatographing mixtures of solutes on theoretically identical column packing materials with chemically bonded phases from different manufacturers lies partly in the structure of the bonded phase and partly in the physical and chemical properties of the silica matrix used.

The structure of the bonded stationary phase is dependent on the manufacturing process. In general, reversed-phase materials based on octadecylsilane (ODS) bonded phases, being those most often used, may be divided into two groups, monomeric and the polymeric phases. The monomeric phases are characterized by having a monomolecular layer of octadecyl chains on the silica surface whereas polymeric stationary phases are more like a sponge containing octadecyl groups [34].

The physical and chemical properties of the silica matrix play an important role in the chromatographic processes of solutes and thus on the selectivities obtained. The pore size and pore-size distribution are of importance especially if the former become less than 3–4 nm [35].

In recent years, attention has been drawn to the chemical properties of the silica matrix. It has been shown [36] that the surface pH of silica measured in an aqueous suspension may vary considerably, owing to contamination of the silica with protolytic impurities adsorbed to the surface [32]. The silica itself has also been shown to exhibit varying acidity toward solutes [37]. Such variations in acidity are ascribed either to contamination with metal impurities (adsorbed on the surface or incorporated in the silica matrix) or to the distribution of the silanol groups on the silica surface. In practice, problems with highly acidic phases are most often expressed as severe tailing of basic solutes. Hence a highly pure silica, totally hydroxylated [38] with a homogenous distribution of silanol groups on the surface, is to be preferred.

If chromatographic separation methods are used in stability testing of, *e.g.*, drugs, a high degree of reproducibility of selectivity is required because modifications of the chromatographic method during a stability study are undesirable. Manufacturers therefore make great efforts to improve the reproducibility of the individual ODS column packing materials that they produce, but one can hardly expect the different manufacturers to standardize their ODS column packing materials towards the same standard.

A possible means of achieving standardization in RP-HPLC is to use dynamically modified silica.

4. DYNAMICALLY MODIFIED SILICA

4.1. Equilibration of columns

The basis for obtaining a reversed-phase system using bare silica as the column packing material and an eluent containing a lipophilic cation is the anionic properties of silica.

The column system consists of the analytical column and a saturation column. The saturation column is packed with a silica having a large surface area and a particle diameter of 15–25 μm. This is to ensure saturation of the eluent with silica before it reaches the analytical column, and at the same time it should not contribute to the

back-pressure of the system. The saturation column is installed between the pump and the injection system. By use of the saturation column, dissolution of silica in the analytical column is prevented.

The process for the dynamic modification of the silica surface is easy to perform. The column system is flushed with methanol–water (50:50, v/v) then mobile phase is pumped through the system. The time of equilibration of the system depends partly of the surface area of the column packing material and partly of the pH and the concentration of the quaternary ammonium ion in the eluent. Using a new column system, the amount of eluent required is typically about 300–400 column volumes. The equilibration is most conveniently performed by eluting overnight. Once the equilibration has been established, after use the columns may be stored filled with the eluent and firmly closed. It is necessary, of course, to have a saturation and an analytical column at hand for each chromatographic system that is required to be immediately available.

4.2. Choice of quaternary ammonium compound and organic modifier

The adsorption of various quaternary ammonium compounds on the silica surface has been studied. As expected from ion-exchange theory, the amount adsorbed on the silica surface increases with increasing alkyl chain length of the alkyltrimethylammonium ion (Fig. 2).

On increasing the amount of quaternary ammonium ions in the eluent, a certain plateau of amount adsorbed on the silica surface is reached. The concentration where this plateau is attained coincides with the critical micellar concentration (CMC) of the ammonium ions in the eluent used. Above the CMC the concentration of free quaternary ammonium ions in the eluent is constant and in equilibrium with the adsorbed ammonium ions and with the ammonium ions in the micelles (Fig. 3). The micelles in the eluent will form a "secondary mobile phase" (the cores of the micelles)

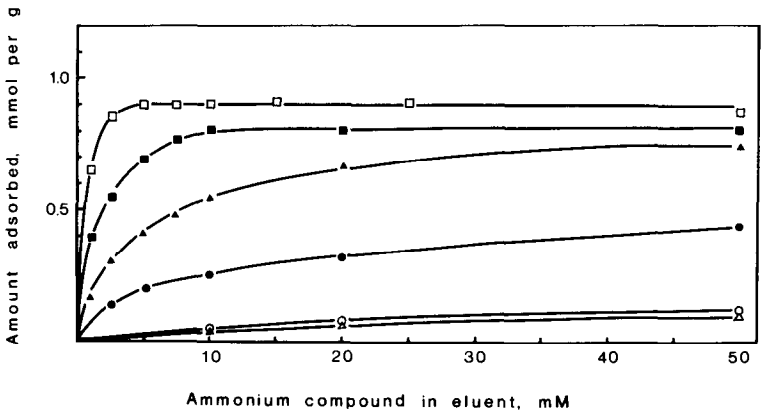

Fig. 2. Amounts of quaternary ammonium ions adsorbed per gram of silica *vs.* concentration in the eluent. Column, LiChrosorb Si 60, 5 μm (120 × 4.6 mm I.D.); eluent, methanol–water–0.2 *M* potassium phosphate buffer (pH 7.5) (50:45:5, v/v/v) with the addition of various kinds and concentrations of quaternary ammonium bromides. △ = Tetrabutylammonium; ○ = tetrapentylammonium; ● = dodecyltrimethylammonium; ▲ = tetradecyltrimethylammonium; ■ = CTMA; □ = stearyltrimethylammonium. Reprinted from ref. 46 with permission.

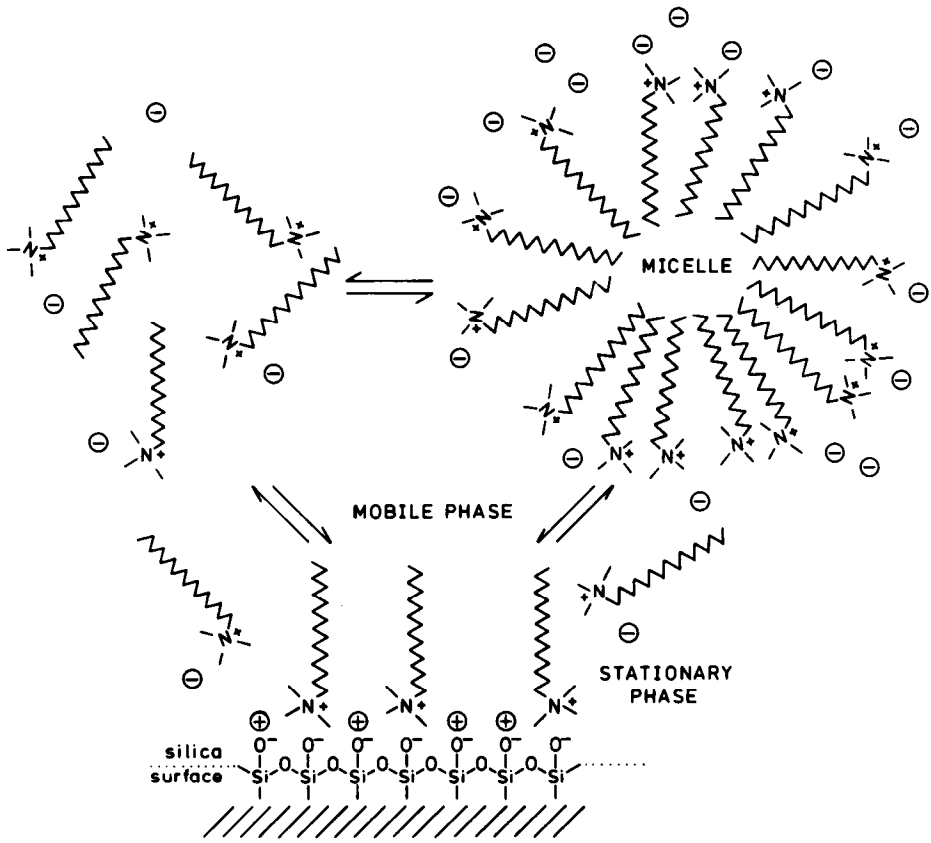

Fig. 3. Schematic presentation of the "three phases" in equilibrium when micelles are present in the eluent. A typical eluent is methanol–water–0.2 *M* potassium phosphate buffer (pH 7.5) (50:45:5, v/v/v) with the addition of 2.5 m*M* CTMA bromide.

into which the solutes may distribute. The cores of the micelles are apolar relative to the surrounding eluent and, as the micelles migrate with the velocity of the eluent, the retention of the solutes will decrease with increasing number and size of the micelles.

Symmetrical quaternary ammonium ions with a total number of carbon atoms similar to that of the long-chain alkyltrimethyl compounds are, under the same conditions, adsorbed on the silica surface to a much smaller extent (Fig. 2) and no reversed-phase effect is obtained (Fig. 4). This is due to the fact that the positive charge is evenly distributed over the large bulky symmetrical ammonium ions. Thus, the coulombic forces are weakened and the binding to the silanol group becomes much weaker, as is also seen from the lower ability to suppress the retention of 2-phenylethylamine by an ion-exchange mechanism. Using a buffer without an organic modifier but with symmetrical quaternary ammonium ions added, only a weak reversed-phase effect is seen [39].

Cetyltrimethyl ammonium (CTMA) bromide, being inexpensive and readily available as analytical-reagent grade material, is recommended as an additive to the

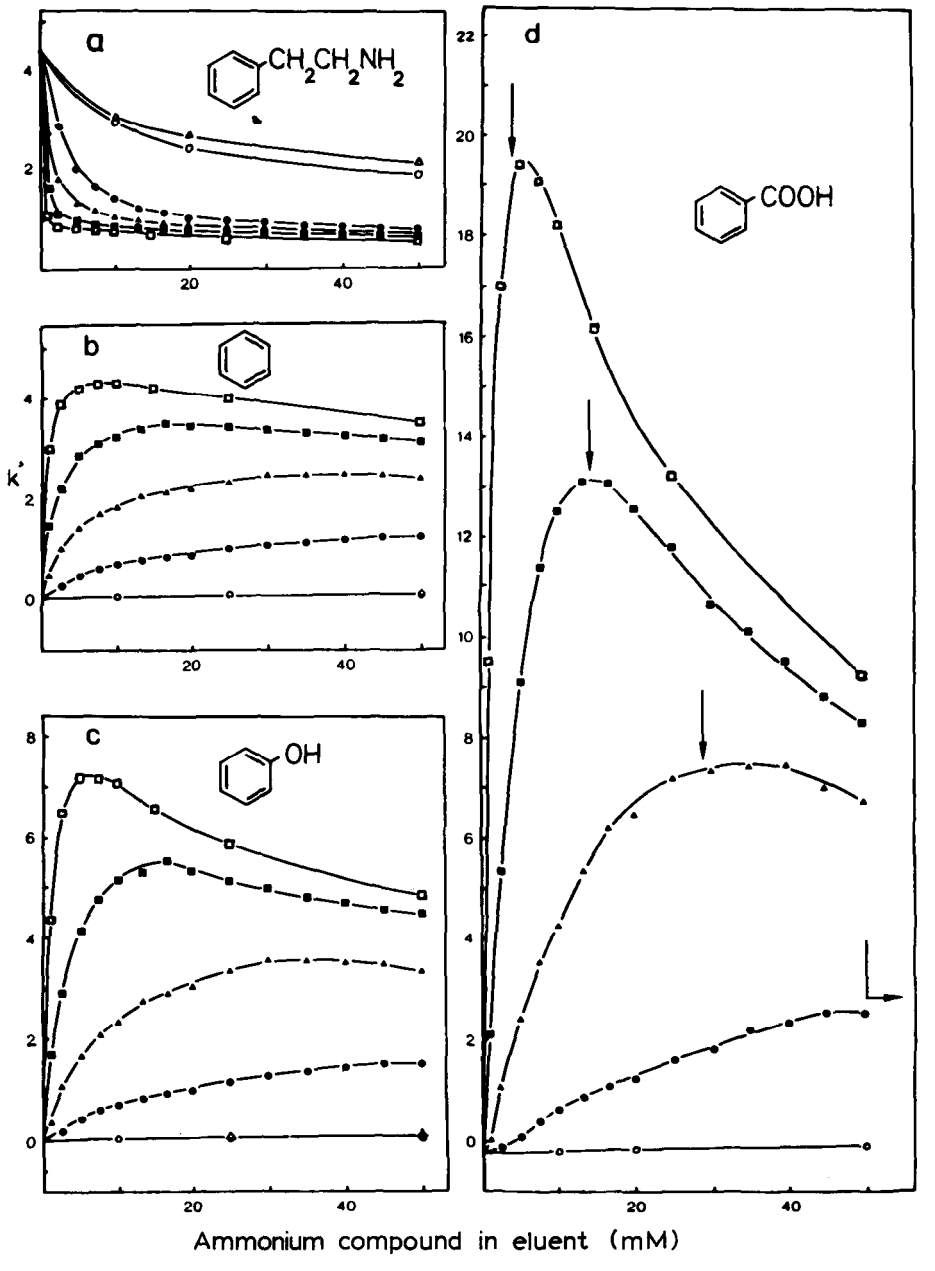

Fig. 4. Capacity factors (k') for four test solutes *vs.* the concentration of quaternary ammonium ions in the eluent. (a) 2-Phenylethylamine; (b) benzene; (c) phenol; (d) benzoic acid. Symbols and conditions as in Fig. 2. The arrows in (d) indicate the estimated critical micellar concentrations (CMC). Reprinted from ref. 46 with permission.

eluent when using dynamically modified silica. Methanol, acetonitrile and tetrahydrofuran, commonly used in reversed-phase chromatography on chemically bonded phases, are also the organic modifiers of choice with dynamically modified silica. In addition to controlling the eluting strength of the eluent, the modifier will also affect the amount of quaternary ammonium ions adsorbed on the silica surface [40]. If the eluting strength of the eluent is increased by increasing the amount of organic modifier in the eluent, fewer quaternary ammonium ions are adsorbed on the silica surface and the net result is a larger decrease in retention of solutes than expected from reversed-phase chromatography on chemically bonded phases.

The maximum adsorbable amount of CTMA ions found at low modifier concentrations (*e.g.*, 10% methanol) is 2.5–3 μmol/m^2. Only a monomolecular layer is adsorbed, corresponding to a coverage of one third of the silanol groups. In comparison, the coverage of the silanol groups in the chemically bonded phases is 50–60% at best [41]. If no organic modifier is added to the eluent the quaternary ammonium ions will build up a bi- or multi-molecular layer [42]. However, for most applications of HPLC on dynamically modified silica, the concentration of the organic modifier in the eluent is in the range 30–80%.

A change from one modifier to another (*e.g.*, from methanol to acetonitrile) will cause changes in selectivity similar to those known from reversed-phase chromatography on chemically bonded phases, and the separations may be optimized using the same principles [40], *e.g.*, by the method proposed by Schoenmakers *et al.* [43].

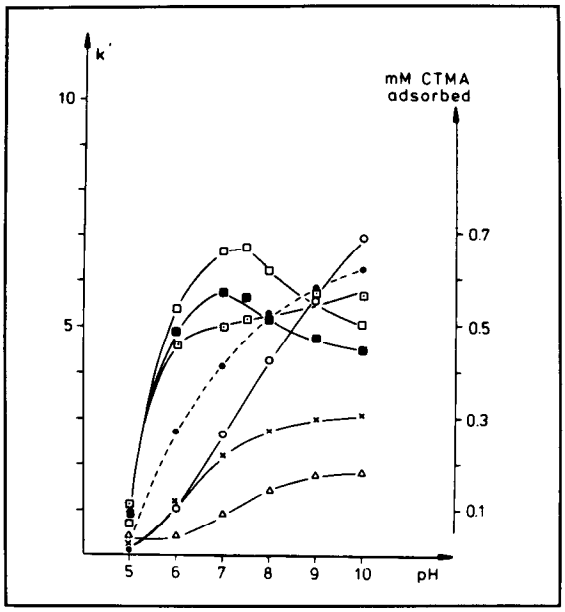

Fig. 5. Relationship between pH of the buffer in the eluent and the amount of CTMA adsorbed or retention (k') of various test solutes. Column, LiChrosorb Si 60, 5 μm (120 × 4.6 mm I.D.); eluent, methanol–water–0.2 *M* potassium phosphate buffer (50:45:5, v/v/v) with the addition of 2.5 m*M* CTMA bromide. ● = CTMA; △ = 2-phenylethylamine; × = benzene; ○ = phenol; □ = benzoic acid; ■ = sorbic acid; ⊡ = fumaric acid. Reprinted from ref. 44 with permission.

4.3. Influence of pH and ionic strength

The amount of quaternary ammonium ions adsorbed onto the silica surface increases with increasing degree of ionization of the silanol groups. As shown in Fig. 5, it is possible to use the system at high pH (above 9), but in order to prolong the lifetime of the saturation and analytical columns it is recommended that the pH of the buffers used should not exceed 7.5–8 owing to the solubility of silica at higher pH. However, in order to achieve special selectivity effects, the use of higher pH values may sometimes be worthwhile, as many aliphatic amines and phenols have pK_a values in the range 9–10.

The silica itself has a pK_a of about 6.5–7, and an S-shaped curve of the adsorption of CTMA *versus* pH in the range 5–9 is therefore obtained, as expected (Fig. 5). As a consequence of the increasing amount of stationary phase, a corresponding increase in the retention of neutral solutes is seen.

TABLE 1

EFFECTS OF ADDITION OF VARIOUS ANIONS TO THE ELUENT ON THE RETENTION (k') OF VARIOUS TEST SOLUTES IN DYNAMICALLY MODIFIED SILICA

Column, LiChrosorb Si 60, 5 μm (120 × 4.6 mm I.D.); eluent: methanol–water–0.2 *M* potassium phosphate buffer (pH 7.5) (500:487.5:12.5, v/v/v) containing 2.5 m*M* CTMA bromide; column temperature, 39.6°C. t_M = Void volume in minutes. Reprinted from ref. 44 with permission.

Solute	Initial k'	Inorganic salts added					
		Phosphate (pH 7.5)		Cl^-		Br^-	
		0.01 *M*	0.02 *M*	0.02 *M*	0.04 *M*	0.0215 *M*	0.043 *M*
Benzene	1.94	2.18	2.19	1.89	1.96	1.88	2.01
Toluene	3.84	4.15	4.08	3.57	3.47	3.68	3.57
β-Methylnaphthalene	13.7	15.0	15.2	13.6	13.3	13.9	13.7
Phenanthrene	28.9	31.4	31.4	28.8	27.9	30.0	29.2
Phenol	2.19	2.52	2.42	1.97	1.98	1.99	2.05
Phenethylamine	1.13	1.08	1.00	0.95	0.85	1.00	0.88
Tyramine	0.93	0.75	0.67	0.64	0.55	0.67	0.55
Tryptamine	2.90	2.62	2.33	2.20	1.84	2.27	1.90
Imipramine (IP)	22.1	22.0	20.5	20.9	19.6	21.2	19.8
Desmethyl-IP	13.4	12.4	11.1	10.8	9.2	11.1	9.4
Didesmethyl-IP	7.86	7.55	7.03	6.04	5.5	6.4	5.7
N-Methyl-IP	3.00	2.49	2.53	2.38	2.28	2.46	2.35
IP N-oxide	4.00	3.80	3.90	4.22	4.45	4.31	4.54
Benzenesulphonic acid	3.16	2.52	2.13	–	2.17	2.36	2.15
Benzoic acid	4.26	3.54	3.12	3.51	3.67	3.44	3.36
Phthalic acid	13.3	5.81	3.72	6.39	4.37	5.92	3.71
Isophthalic acid	13.0	6.64	4.65	7.45	5.61	7.03	4.84
Terephthalic acid	6.21	3.31	2.24	3.81	2.70	3.56	2.31
1,3,5-Benzenetricarboxylic acid	109.5	22.9	10.5	27.9	12.9	24.9	9.73
Sorbic acid	3.91	3.25	2.73	3.20	3.15	3.10	2.99
Maleic acid	4.50	2.21	1.57	2.38	1.57	2.22	1.34
Fumaric acid	4.83	2.49	1.60	2.85	1.92	2.62	163
t_w (water)	1.04	1.02	1.02	1.04	1.04	1.04	1.03

Using buffers with other cations and other anions [44] than potassium phosphate may contribute to changes in retention and selectivity between solutes. However, the addition of different anions to increase the ionic strength or as ion-pairing agents is of special interest, as shown in Table 1. To give a better survey of the selectivity changes induced, the relative changes for some additives are given in Table 2.

The addition of increasing amounts of buffer or other inorganic potassium salts to the eluent will result in a decrease in retention of anionic and cationic solutes, whereas the retention of neutral solutes generally remains unchanged. It has been shown [44] that the amount of CTMA ions adsorbed on the silica surface under these circumstances also remains at a constant level. Hence the causes of the change in the retention must be ascribed to interactions taking place in the eluent. As the CTMA

				n-Alkane sulphonates added			
NO_3^-		SO_4^{2-}		Hexane		Octane 0.02 *M*	Decane 0.02 *M*
0.02 *M*	0.04 *M*	0.02 *M*	0.04 *M*	0.02 *M*	0.04 *M*		
1.75	1.87	2.05	2.18	2.23	2.38	2.45	3.21
–	–	3.87	3.46	4.34	4.67	5.84	5.74
–	–	14.9	15.0	16.7	17.7	23.0	21.9
–	–	31.8	32.8	35.2	36.8	50.8	47.2
1.90	1.93	2.21	2.38	2.30	2.34	2.39	2.83
1.05	0.89	0.93	0.91	1.06	1.10	2.60	3.78
0.71	0.59	0.61	0.57	0.67	0.65	1.49	2.54
2.33	1.98	2.13	2.04	2.36	2.69	5.66	10.8
20.7	20.1	21.2	21.1	21.5	21.1	27.1	41.7
11.1	9.7	10.5	10.7	11.6	11.9	21.8	51.2
6.5	5.8	6.4	6.5	7.13	7.58	13.5	29.5
2.61	2.42	2.41	2.42	2.75	2.79	7.74	22.0
4.23	4.49	4.27	4.31	4.20	3.89	4.54	5.05
2.29	2.01	2.33	2.09	1.83	1.19	0.41	0.22
3.30	–	3.33	2.97	2.40	1.86	0.65	0.49
5.72	3.70	5.52	3.93	4.20	2.08	0.47	0.23
6.74	4.78	6.38	4.55	4.40	2.30	0.58	0.00
3.44	2.30	2.85	1.95	2.14	1.05	0.22	0.00
23.8	10.5	16.4	7.90	9.5	3.55	2.48	0.00
3.01	2.80	3.14	2.72	2.25	1.61	0.58	0.43
2.18	1.33	1.83	1.28	1.49	0.66	0.07	0.00
2.55	1.63	2.01	1.34	1.56	0.69	0.07	0.00
1.05	1.04	1.03	1.02	1.00	1.00	0.92	0.82

TABLE 2

RELATIVE CHANGE IN RETENTION FOR THE TEST SOLUTES IN TABLE 1 ON CHANGING FROM THE INITIAL CONDITIONS TO CONDITIONS WHERE 0.02 *M* SODIUM CHLORIDE OR 0.02 *M* *n*-ALKANE SULPHONATES ARE ADDED

Values relative to initial k' taken as 1.00.

Solute	0.02 *M* NaCl	0.02 *M* *n*-alkane sulphonates		
		Hexane	Octane	Decane
Benzene	0.97	1.15	1.26	1.65
Toluene	0.93	1.13	1.52	1.49
β-Methylnaphthalene	1.00	1.22	1.68	1.60
Phenanthrene	1.00	1.22	1.76	1.63
Phenol	0.90	1.05	1.09	1.29
2-Phenylethylamine	0.84	0.94	2.30	3.35
Tyramine	0.69	0.72	1.60	2.73
Tryptamine	0.76	0.81	1.95	3.72
Imipramine (IP)	0.95	0.97	1.23	1.89
Desmethyl-IP	0.81	0.87	1.63	3.82
Didesmethyl-IP	0.77	0.91	1.72	3.75
N-Methyl-IP	0.79	0.92	2.58	7.33
IP N-oxide	1.06	1.05	1.14	1.26
Benzenesulphonic acid	—	0.58	0.13	0.07
Benzoic acid	0.82	0.56	0.15	0.12
Phthalic acid	0.48	0.32	0.04	0.02
Isophthalic acid	0.57	0.34	0.04	0.00
Terephthalic acid	0.61	0.34	0.04	0.00
1,3,5-Benzenetricarboxylic acid	0.25	0.09	0.02	0.00
Sorbic acid	0.82	0.58	0.15	0.11
Maleic acid	0.52	0.33	0.02	0.00
Fumaric acid	0.59	0.32	0.01	0.00
t_M (water)	1.00	0.96	0.88	0.79

ions mask the ion-exchange sites on the silica surface, the cationic solutes are mainly retarded by a reversed-phase mechanism.

When *n*-alkane sulphonates, being more lipophilic anions, are added to the eluent, the effects described above are enhanced. Further, following such an addition, the retention of aromatic hydrocarbons increases owing to an increase in the amount of stationary phase, as ion pairs between CTMA and the alkane sulphonates are deposited on the surface. The increase in the amount of stationary phase is also reflected in a decrease in the hold-up volume, t_M, of the system.

Considering anionic solutes, a more dramatic decrease in retention is observed. This effect also increases with increasing valency of the carboxylic acid (number of anionic sites on the molecule). When using increasing amounts of inorganic salts this is mainly due to the increasing concentration of potassium ions in the eluent giving an ion-pair interaction that competes with the ion pairing between the anionic solutes and the CTMA ions. With increasing chain length of the *n*-alkane sulphonate added, the ion-pairing effect of these anions with CTMA ions becomes the determining factor for

the retention of anionic solutes, and thus a pronounced decrease in retention of these is seen.

The increase in retention of the cationic solutes is partly caused by the increase in the amount of stationary phase and partly by the formation of lipophilic ion pairs between the ammonium compounds and the *n*-alkane sulphonates. Decane sulphonate ions form the most lipophilic ion pairs with the quaternary N-methylimiprammonium ions, and at the same time the retention of the tricarboxylic acid decreases to zero.

The strong influence of ionic strength on the retention of anionic solutes may be applied for selective gradient elution of such compounds [45], as shown in Fig. 6.

4.4. Column packing material

The adsorption of quaternary ammonium ions on the surface of silica is strictly related to the presence of accessible silanol groups. Investigations have shown [40,46] that only up to one third of the silanol groups are covered. However, even if large differences in surface areas of different silicas are encountered, an excellent correlation between the surface area of the silica and the amount of quaternary ammonium ions

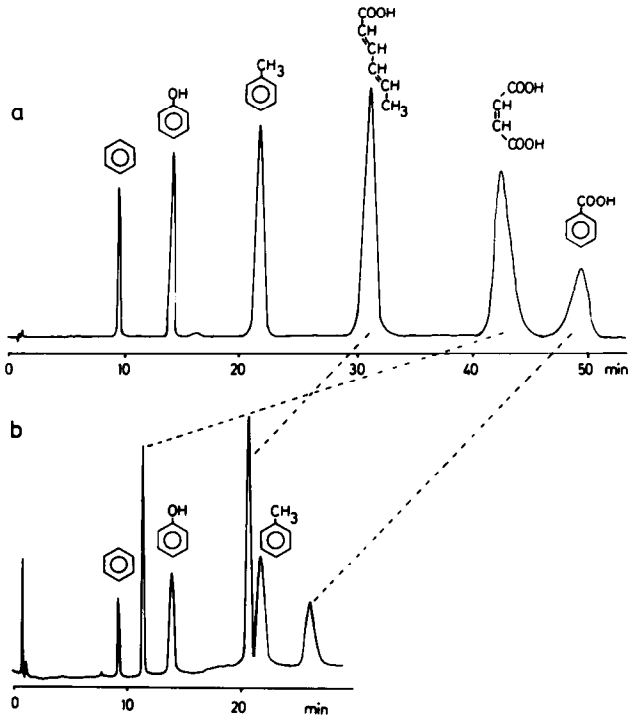

Fig. 6. (a) Isocratic and (b) ionic strength gradient elution of test solutes. Column, LiChrosorb Si 60, 5 μm (120 × 4.6 mm I.D.); eluent A, methanol–water–0.2 *M* potassium phosphate buffer (pH 7.5) (35:63.75:1.25, v/v/v) with 2.5 m*M* CTMA bromide added; eluent B, methanol–water–0.2 *M* potassium phosphate buffer (pH 7.5) (35:40:25, v/v/v) with 2.5 m*M* CTMA bromide added. (a) Isocratic elution with eluent A (1 ml/min); (b) gradient elution from 100% eluent A to 100% eluent B in 10 min at a flow-rate of 1 ml/min. Reprinted from ref. 45 with permission.

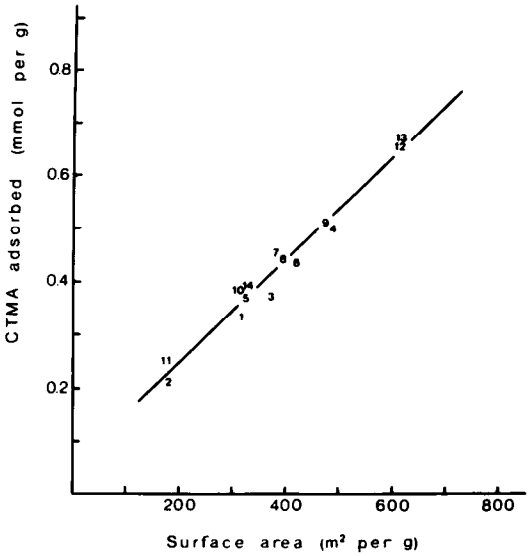

Fig. 7. Relationship between the surface areas of fourteen different silica column packing materials and the amounts of CTMA adsorbed. Other conditions as in Fig. 3. Reprinted from ref. 61 with permission.

adsorbed is found (Fig. 7). The amount of CTMA ions adsorbed may further be correlated with the retention of the non-ionic solute benzene (Fig. 8).

Despite the dissimilarity in the retention of solutes in the individual systems owing to differences in the surface areas of silica column packing materials from

TABLE 3

SEPARATION FACTORS BETWEEN BENZENE AND OTHER TEST SUBSTANCES MEASURED ON 14 DIFFERENT SILICA COLUMNS

Eluent as in Fig. 3. Reprinted from ref. 47 with permission.

Silica packing	Separation factor			
	Pyridine	Phenethylamine	Phenol	Benzoic acid
Chromosorb LC-6	0.13	0.48	1.27	2.64
Hypersil	0.12	0.46	1.33	2.64
LiChrospher Si 100	0.14	0.51	1.32	2.64
LiChrosorb Si 60	0.12	0.45	1.38	1.67
LiChrosorb Si 100	0.15	0.52	1.29	2.55
Nucleosil 50	0.11	0.52	1.38	2.63
Nucleosil 100	0.15	0.55	1.30	2.50
Nucleosil 100 V	0.13	0.54	1.30	2.59
Partisil	0.12	0.49	1.38	2.70
Polygosil 60	0.12	0.46	1.34	2.63
Spherisorb S W	0.12	0.46	1.38	2.52
Spherosil XOA 600	0.12	0.50	1.41	2.65
Spherosil XOA 800	0.18	1.17	1.55	2.26
Zorbax SIL	0.10	0.43	1.51	2.58

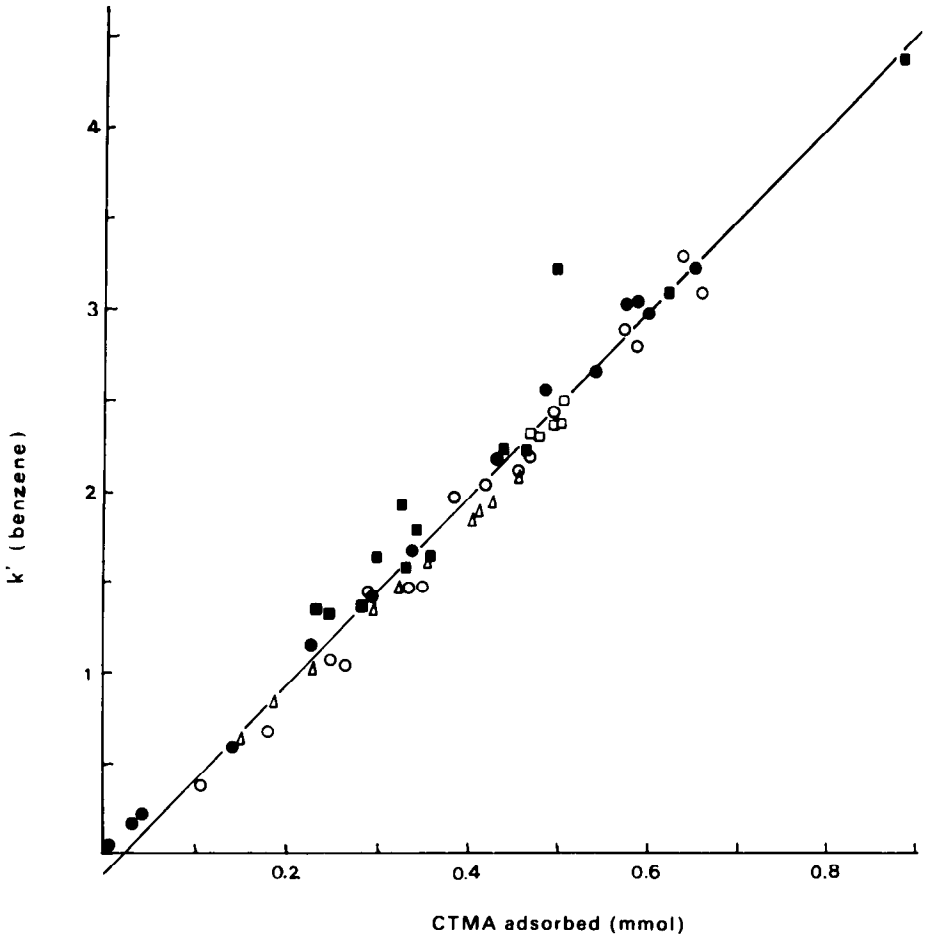

Fig. 8. Relationship between the capacity factor (k') of benzene and the amount of CTMA adsorbed to the silica for (■) different column materials, (○) CTMA concentrations, (△) buffer solutions, (●) pH values and (□) ionic strengths. Reprinted from ref. 61 with permission.

TABLE 4

SELECTIVITY EXPRESSED AS α-VALUES FOR SEVEN METABOLITES OR CONJUGATES OF SASP RELATIVE TO THE METABOLITE Ac-5-ASA

Eluent, methanol–water–0.2 *M* potassium phosphate buffer (pH 6.5) (45:10:45, v/v/v) with the addition of 3.75 m*M* CTMA bromide. Abbreviations: SASP = salazosulphapyridine; Ac-5-ASA = N-acetyl-5-aminosalicylic acid; 5-ASA = 5-aminosalicylic acid; SP = sulphapyridine; Ac-SP = acetylsulphapyridine; SP-OH = 4-hydroxysulphapyridine; Ac-SP-OH = 4-hydroxyacetylsulphapyridine; SP-O-GlcU = glucuronic acid conjugate of SP-OH; Ac-SP-O-GlcU = glucuronic acid conjugate of Ac-SP-OH. Reprinted from ref. 48 with permission.

Silica packing	SP	Ac-SP	SP-OH	SP-O-GlcU	Ac-SP-OH	5-ASA	Ac-SP-O-GlcU
Polygosil 60	5.70	3.47	1.98	2.13	1.51	1.24	1.10
Hypersil	5.37	3.30	2.20	2.07	1.62	1.36	1.08
LiChrosorb Si 60	5.37	3.63	2.02	2.21	1.55	1.32	1.11
Spherosil XOA 600	6.19	3.66	2.13	2.24	1.60	1.38	1.11
Chromosorb LC 6	5.43	3.34	2.22	2.00	1.73	1.34	1.04

different manufacturers, the chromatographic systems are equivalent and the selectivity of such systems towards a mixture of test solutes has been shown to be highly reproducible [47]. This is further commented upon and exemplified in the next section.

As mentioned above, the lack of reproducibility in selectivity between different chemically bonded reversed-phase column packing materials can be partly ascribed to differences in the apparent pH of the silica and the acidity of the non-derivatized silanol groups. These problems are not encountered when using dynamically modified silica as the buffer used in the eluent eliminates differences in apparent pH [36]. The quaternary ammonium ions eliminate differences in acidity as the more acidic silanol groups will be masked by the interaction with ammonium ions.

4.5. Standardization of RP-HPLC

The poorly reproducible selectivity encountered when using chemically bonded phases for RP-HPLC is most clearly seen when mixtures of solutes with different chemical functional groups are chromatographed. Several investigations have confirmed that the reproducibility of selectivity may be improved to a satisfactory level when using dynamically modified silica [47–50] (Tables 3–6).

TABLE 5

SEPARATION FACTORS BETWEEN HYDROCORTISONE AND ELEVEN OTHER CORTICOSTEROIDS MEASURED ON EIGHT DIFFERENT SILICA COLUMNS AND SIX DIFFERENT ODS-SILICA COLUMNS

Eluents: for silica, methanol–tetrahydrofuran–water–0.2 *M* phosphate buffer (pH 7.0) (25:13:57:5, v/v/v/v) containing 2.5 m*M* CTMA; for ODS-silica, acetonitrile–water (37:63, v/v). Identification: 1 = cortisone acetate; 2 = hydrocortisone; 3 = hydrocortisone acetate; 4 = prednisolone; 5 = prednisolone acetate; 6 = corticosterone; 7 = triamcinolone acetonide; 8 = methylprednisolone; 9 = betamethasone; 10 = fluocinolone acetonide; 11 = dexamethasone; 12 = desoxycortone acetate. Reprinted from ref. 50 with permission.

Packing	Separation factor from hydrocortisone										
	1	3	4	5	6	7	8	9	10	11	12
Silica											
LiChrosorb Si 60	1.21	1.29	0.97	1.24	1.69	1.49	1.65	1.71	2.39	2.15	3.07
Nucleosil 50-5	1.20	1.28	0.96	1.22	1.69	1.46	1.61	1.67	2.33	2.06	3.06
Zorbax SIL	1.30	1.39	0.96	1.31	1.67	1.55	1.59	1.70	2.46	2.14	3.30
Partisil 5	1.17	1.24	0.94	1.18	1.71	1.44	1.58	1.65	2.34	2.10	3.00
Spherisorb S5 W	1.33	1.42	0.97	1.37	1.63	1.50	1.59	1.63	2.42	2.05	3.30
LiChrosorb Si 100	1.25	1.35	0.97	1.27	1.60	1.43	1.62	1.66	2.29	1.98	3.06
Nucleosil 100-5	1.22	1.31	0.96	1.27	1.61	1.40	1.57	1.57	2.21	1.87	3.00
Hypersil	1.26	1.35	0.96	1.30	1.60	1.41	1.58	1.61	2.28	1.94	3.06
ODS-silica											
LiChrosorb RP-18	5.07	3.90	0.91	3.52	2.40	2.62	1.57	1.85	3.21	1.95	27.12
Nucleosil 5 C_{18}	5.07	3.93	0.91	3.58	2.41	2.68	1.54	1.80	3.25	1.89	27.85
Hypersil ODS	4.61	3.59	0.93	3.33	2.35	2.50	1.57	1.74	2.94	1.86	24.45
Zorbax ODS	3.88	3.24	0.95	2.87	3.04	2.10	1.60	1.45	2.07	1.49	25.27
Partisil ODS-3	5.02	3.88	0.97	3.71	2.35	2.83	1.55	1.81	3.25	1.89	26.34
Spherisorb S5 ODS	3.85	3.06	0.92	2.88	3.54	2.33	1.49	1.82	2.59	1.66	23.69

TABLE 6

SEPARATION FACTORS BETWEEN PROPRANOLOL AND THREE OF ITS POSSIBLE IMPURITIES, MEASURED ON EIGHT DIFFERENT SILICA COLUMNS AND EIGHT DIFFERENT ODS-SILICA COLUMNS

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Packing	Pore size (nm)	Separation factor[a]		
		II[c]	III	IV
Silica				
LiChrosorb Si 60	6	0.26	1.86	2.22
Nucleosil 50-5	5	0.26	1.76	2.13
Zorbax SIL	7	0.24	1.76	2.08
Partisil 5	7–8	0.25	1.66	1.98
Spherisorb S 5W	8	0.28	1.74	2.01
LiChrosorb Si 100	12	0.31	1.39	1.60
Nucleosil 100-5	10	0.26	1.43	1.66
Hypersil	10	0.26	1.40	1.66
ODS-silica				
LiChrosorb RP-18		0.40	2.43	3.81
Nucleosil 5 C_{18}		0.61[b]	2.40	4.69
Hypersil ODS		0.38	2.62	3.34
Zorbax ODS		0.38	2.97	4.38
Partisil 10 ODS		1.19[b]	2.05	4.48
Partisil 10 ODS 2		0.61	3.21	6.53
Partisil 10 ODS 3		0.56	2.42	3.71
Spherisorb S5 ODS		0.60	1.84	2.68

[a] Separation factor is defined as relative retention (k') with respect to propranolol.
[b] The broad propranolol peak overlaps the peak in the chromatogram.
[c] Compounds (R = 1-naphthyl): II = R–CH_2–CHOH–CH_2OH;
III = R–CH_2–CHOH–CH_2–N($CH(CH_3)_2$)–CH_2–CHOH–CH_2–O–R; IV = R–CH_2–CHOH–CH_2–O–R.

TABLE 7

COLUMN PACKING MATERIALS INVESTIGATED

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No.	Bonded-phase materials	No.	Silica materials
1	LiChrosorb RP-18	11	LiChrosorb Si 60
2	Nucleosil 5 C_{18}	12	LiChrosorb Si 100
3	Hypersil ODS	13	LiChrospher Si 100
4	Zorbax ODS	14	Nucleosil 50-5
5	Partisil ODS-3	15	Nucleosil 100-5
6	Spherisorb S5 ODS	16	Hypersil
7	Sepralyte C_{18}	17	Zorbax SIL
8	Spherosil XOA 600 C_{18}	18	Partisil 5
9	Supelcosil LC-18 DB[a]	19	Spherisorb S5 W
10	LiChrosorb RP-select B[a]	20	Sepralyte Si
		21	Spherosil XOA 600

[a] Deactivated ODS material.

TABLE 8

TAILING CHARACTERISTICS (ASYMMETRY FACTOR, A_S) AND SELECTIVITY (SEPARATION FACTOR, α, RELATIVE TO IMIPRAMINE) OF ELEVEN TEST SUBSTANCES CHROMATOGRAPHED ON TEN DIFFERENT BONDED-PHASE MATERIALS (*CF.*, TABLE 7) WITH NO ANTI-TAILING ADDITIVES IN THE ELUENT [METHANOL–WATER–0.2 *M* POTASSIUM PHOSPHATE (pH 4.0) (65:35:5, v/v/v)]

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Test substance	Parameter	Bonded phase										s_{rel}
		1	2	3	4	5	6	7	8	9	10	
Imipramine	k'	6.1	4.5	12.6	47.0	2.5	25.8	5.2	23.7	5.0	1.9	
	A_s	4.6	5.6	9.0	2.8	4.1	2.9	8.3	3.4	6.9	1.3	
Desipramine	α	0.94	0.84	0.89	0.45	0.94	0.52	0.70	0.54	0.88	1.00	26.7
Imipramine N-oxide	α	1.08	1.31	0.54	0.78	1.90	0.75	1.85	1.86	1.01	2.27	44.8
cis-Clopenthixol	α	2.64	2.61	1.68	—[a]	2.84	1.61	2.31	—	2.59	2.97	21.1
	A_s	3.7	3.1	4.7	—	2.9	3.1	4.6		3.1	1.7	
trans-Clopenthixol	α	2.69	2.79	1.85		3.04	1.74	2.50	—	2.77	3.19	20.3
Prochlorperazine	α	6.18	5.05	—	—	3.76	—	5.99	—	3.93	3.59	21.0
	A_s	4.6	6.6	—	—	6.1	—	7.1	—	5.5	2.9	
Chlorpromazine	α	2.60	2.06	1.88	—	1.76	1.84	2.09	2.10	1.85	1.78	13.1
	A_s	5.8	6.1	5.0	—	4.1	2.9	8.2	3.1	6.6	1.3	
Perphenazine	α	2.19	2.16	1.39	—	2.29	1.52	1.89	1.96	2.59	2.39	19.4
Propranolol	α	0.27	0.31	0.19	0.12	0.36	0.17	0.22	0.16	0.26	0.40	37.9
	A_s	5.6	4.0	6.8	5.3	2.4	3.7	4.9	3.2	2.6	1.9	
Nortriptyline	α	1.11	1.00	0.86	0.52	1.09	0.60	0.82	0.64	1.12	1.22	27.5
Amitriptyline	α	1.61	1.16	1.20	1.06	1.09	1.13	1.14	1.15	1.03	1.22	13.8
	A_s	3.9	4.5	4.8	4.8	4.6	3.8	6.1	3.1	4.5	1.4	

[a] Not measured as $k' > 50$.

TABLE 9

TAILING CHARACTERISTICS AS IN TABLE 8 BUT USING 2.5 m*M* DODECYLTRIMETHYLAMMONIUM BROMIDE AND 5 m*M* DECANE SULPHONATE AS ANTI-TAILING AND RETENTION-CONTROLLING ADDITIVES IN THE ELUENT (GIVEN IN TABLE 8)

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Test substance	Parameter	Bonded phase										
		1	2	3	4	5	6	7	8	9	10	s_{rel}
Imipramine	k'	6.0	6.9	5.5	12.1	7.2	7.1	8.7	10.3	6.2	5.0	
	A_s	1.5	1.4	1.8	1.2	1.3	1.6	1.4	2.2	1.5	0.8	
Desipramine	α	1.23	1.17	1.24	0.95	1.22	0.90	1.18	0.89	1.29	1.17	13.0
Imipramine N-oxide	α	0.75	0.80	0.65	1.34	0.79	1.42	0.93	2.41	0.69	1.06	49.6
cis-Clopenthixol	α	2.79	2.71	2.69	2.21	2.55	2.24	2.62	2.45	2.86	2.48	8.5
	A_s	1.2	1.1	1.2	1.2	1.5	1.2	1.0	2.6	1.1	0.8	
trans-Clopenthixol	α	3.30	3.15	3.14	2.62	2.89	2.63	3.15	2.94	3.40	2.82	8.9
Prochlorperazine	α	3.03	3.30	2.91	3.90	2.72	3.84	3.60	5.11	2.96	2.85	21.2
	A_s	1.9	2.4	2.5	2.2	1.5	1.7	3.4	2.2	2.0	1.4	
Chlorpromazine	α	2.03	2.04	1.98	2.11	1.89	1.91	2.17	2.25	2.06	1.83	6.4
	A_s	1.7	1.5	0.9	1.6	0.8	1.9	1.4	1.9	1.4	0.9	
Perphenazine	α	2.44	2.33	2.32	1.97	2.19	2.04	2.32	2.17	2.53	2.13	7.8
Propranolol	α	0.55	0.52	0.54	0.34	0.55	0.42	0.48	0.34	0.54	0.58	18.1
	A_s	0.7	0.7	0.8	0.8	1.0	0.4	0.7	2.9	0.8	0.2	
Nortriptyline	α	1.52	1.44	1.54	1.17	1.50	1.07	1.47	1.11	1.61	1.43	14.0
Amitriptyline	α	1.25	1.24	1.26	1.17	1.25	1.17	1.25	1.28	1.27	1.24	3.1
	A_s	1.1	1.2	1.3	1.6	1.3	1.2	1.1	2.1	1.5	0.8	

TABLE 10

TAILING CHARACTERISTICS AS IN TABLE 8 BUT CHROMATOGRAPHED ON ELEVEN DIFFERENT SILICA MATERIALS DYNAMICALLY MODIFIED WITH 2.5 m*M* CTMA IN THE ELUENT [METHANOL–WATER–0.2 *M* POTASSIUM PHOSPHATE BUFFER (pH 7.0) (55:40:5, v/v/v)]

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Test substance	Parameter	Dynamically modified silica											s_{rel}
		11	12	13	14	15	16	17	18	19	20	21	
Imipramine	k'	12.7	6.7	9.4	15.9	9.1	6.5	19.0	13.1	9.3	6.5	17.5	
	A_s	0.9	0.8	0.9	1.1	0.9	0.9	1.9	1.0	1.7	1.2	0.9	
Desipramine	α	0.55	0.55	0.54	0.56	0.54	0.58	0.57	0.55	0.56	0.58	0.54	2.7
Imipramine N-oxide	α	0.27	0.28	0.26	0.27	0.27	0.26	0.23	0.26	0.26	0.27	0.25	5.1
cis-Clopenthixol	α	0.91	0.88	0.85	0.89	0.87	0.86	0.82	0.91	0.88	0.89	0.92	3.3
	A_s	1.0	0.8	1.0	1.1	0.8	0.9	1.1	1.0	1.1	0.8	1.0	
trans-Clopenthixol	α	1.04	0.99	0.97	1.01	0.98	0.96	0.92	1.03	0.98	0.99	1.06	4.0
Prochlorperazine	α	2.43	2.23	2.13	2.50	2.25	2.07	1.84	2.17	2.04	2.11	2.49	9.2
	A_s	1.3	1.2	1.2	1.5	1.3	1.4	1.4	1.2	1.7	1.7	1.5	
Chlorpromazine	α	1.92	1.79	1.63	1.96	1.85	1.76	1.74	1.70	1.74	1.80	1.92	5.7
	A_s	1.0	0.9	0.8	0.8	0.7	1.0	1.0	0.9	1.0	0.9	0.9	
Perphenazine	α	0.93	0.88	0.78	0.93	0.88	0.85	0.82	0.85	0.92	0.89	0.93	5.6
Propranolol	α	0.37	0.37	0.38	0.38	0.38	0.35	0.29	0.35	0.32	0.34	0.38	8.2
	A_s	2.4	1.7	1.9	2.9	2.2	2.0	2.9	2.0	2.5	1.6	2.1	
Nortriptyline	α	0.54	0.56	0.52	0.57	0.54	0.57	0.52	0.54	0.54	0.57	0.53	3.5
Amitriptyline	α	0.95	0.97	0.94	0.98	0.97	0.95	0.90	0.94	0.94	0.97	0.97	2.4
	A_s	1.0	1.0	0.8	0.9	0.7	1.0	1.3	1.0	1.3	1.0	2.1	

TABLE 11

DRUG SUBSTANCES CHROMATOGRAPHED BY REVERSED-PHASE CHROMATOGRAPHY ON DYNAMICALLY MODIFIED SILICA

Drug substance	Typical column	Typical eluent	Detection	Ref.
Catecholamines	LiChrospher Si 100	Methanol–water–0.2 *M* phosphate buffer (pH 7.0) (80:15:5) + 2.5 m*M* CTMA	Electrochem., +400 mV	52
Opium alkaloids	LiChrosorb Si 60	Methanol–water–0.2 *M* phosphate buffer (pH 7.0) (35:60:5) + 2.5 m*M* CTMA	UV, 254 nm; fluorescence, 285/325 nm; electrochem., +550 mV	5, 53
Tricyclic antidepressants	LiChrosorb Si 60	Methanol–water–0.2 *M* phosphate buffer (pH 7.0) (55:40:5) + 2.5 m*M* CTMA	UV, 254 nm	5, 32, 44
Steroids	LiChrosorb Si 60	Methanol–water–0.2 *M* phosphate buffer (pH 7.5) (50:45:5) + 2.5 m*M* CTMA	UV, 254 nm	5, 36, 50 54
Penicillins	LiChrosorb Si 60	Acetonitrile–water–0.2 *M* phosphate buffer (pH 7.0) (30:65:5) + 2.5 m*M* CTMA	UV, 254 nm	55
β-Blocking agents	Zorbax SIL	Methanol–water–0.2 *M* phosphate buffer (pH 8.0) (70:25:5) + 2.5 m*M* CTMA	UV, 292 nm	32, 49
Salicylates	LiChrosorb Si 60	Methanol–water–0.2 *M* phosphate buffer (pH 8.0) (60:35:5) + 0.80 g/l CTMA	UV, 254 nm	5, 56
Lidocaine	Partisil	Methanol–water–0.2 *M* phosphate buffer (pH 7.5) (50:45:5) + 2.5 m*M* CTMA	UV, 254 nm	36
Salbutamol and terbutaline	LiChrosorb Si 60	Tetrahydrofuran–water–0.2 *M* phosphate buffer (pH 7.5) (28:67:5) + 2.5 m*M* CTMA	UV, 254 nm	40
Ketoprofen	Partisil	Methanol–water–0.2 *M* phosphate buffer (pH 7.5) (50:45:5) + 2.5 m*M* CTMA	UV, 254 nm	36
5-Aminosalicylic acid and metabolites	LiChrosorb Si 60	Acetonitrile–water–0.2 *M* phosphate buffer (pH 7.5) (30:65:5) + 2.5 m*M* CTMA	UV, 254, 315 nm; fluorescence, 315/430 nm; 315/500 nm; electrochem., +600, + 1000 mV	48,57
p-Aminobenzoic acid	LiChrosorb Si 60	Methanol–water–0.2 *M* phosphate buffer (pH 8.0) (60:35:5) + 0.80 g/l CTMA	UV, 254 nm	5

(Continued on p. 74)

TABLE 11 *(continued)*

Drug substance	Typical column	Typical eluent	Detection	Ref.
Barbiturates	LiChrosorb Si 60	Methanol–water–0.2 *M* phosphate buffer (pH 8.0) (50:45:5) + 0.80 g/l CTMA	UV, 254 nm	5
Preservatives	LiChrosorb Si 60	Methanol–water–0.2 *M* phosphate buffer (pH 7.5) (50:45:5) + 2.5 m*M* CTMA	UV, 254 nm	5, 40, 44, 46, 47, 54, 58
Idoxuridine	LiChrospher Si 100	Methanol–water–0.2 *M* phosphate buffer (pH 7.5) (4:91:5) + 2.5 m*M* CTMA	UV, 254 nm	59
Biogeneous amines	LiChrosorb Si 60	Methanol–water–0.2 *M* phosphate buffer (pH 7.5) (50:45:5) + 2.5 m*M* CTMA	UV, 254 nm	44
Tolfenamic acid	Polygosil 60	Acetonitrile–methanol–water–0.2 *M* phosphate buffer (pH 7.5) (25:20:50:5) + 1.25 m*M* CTMA	UV, 280 nm	60
Sulphonamides and metabolites	LiChrosorb Si 60	Methanol–water–0.2 *M* phosphate buffer (pH 6.5) (45:45:10) + 3.75 m*M* CTMA	UV, 254, 315 nm; fluorescence, 315/430 nm, 315/500 nm; electrochem., +600, +1000 mV	40, 48

When chromatographing basic analytes on chemically bonded phases, severe tailing problems are often seen in connection with the poor reproducibility. These problems are also solved by using dynamically modified silica [32] (Tables 7–10) owing to the deactivation of the active silanol groups.

5. APPLICATIONS

Dynamically modified silica has been used in pharmaceutical and biomedical analysis and in Table 11 an overview of the drugs analysed is given. The technique has also been used in occupational hygiene projects [51].

6. CONCLUSION

RP-HPLC with dynamically modified silica is a technique that has been shown to be superior to reversed-phase chromatography on chemically bonded phases with regard to reproducibility of selectivity and the technique should therefore be taken into consideration when such reproducibility is needed, *e.g.*, in stability testing of drugs during longer periods of time and in the international standardization of methods.

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