Journal of Chromatography, 368 (1986) 39–47
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 886

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON DYNAMICALLY MODIFIED SILICA

VII*. INFLUENCE OF APPARENT SURFACE pH OF SILICA COMPARED WITH THE EFFECTS IN STRAIGHT-PHASE CHROMATOGRAPHY

STEEN HONORÉ HANSEN
Department of Organic Chemistry, Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen (Denmark)
and
PER HELBOE* and MOGENS THOMSEN
Drug Standardization Laboratory, National Board of Health, 378 Frederikssundsvej, DK-2700 Brønshøj (Denmark)
(First received May 5th, 1986; revised manuscript received June 20th, 1986)

SUMMARY

The protolytic characteristics of a series of silica packing materials have been determined by measuring the pH in 1% aqueous suspensions and by estimating the so-called "true pH" of the suspensions.

The effect of the protolytic characteristics on different modes of chromatography has been investigated by testing three columns in the liquid–liquid partition mode as well as in the dynamic modification mode. Large variations in selectivity towards mixtures containing acidic and basic solutes were seen in the straight-phase mode, this being contrary to the situation when chromatographing the solutes by reversed-phase partition in the dynamic modification mode.

The possible standardization of selectivity in the straight-phase mode by adjusting the apparent surface pH *in situ* is discussed.

INTRODUCTION

It has previously been shown that the apparent surface pH of different brands of silica may exhibit great variations[1]. These variations have been reported to affect the chromatographic properties in adsorption chromatography towards acidic and basic compounds to a degree that made it necessary to adjust the properties of the silica, *e.g.* by impregnation with a suitable buffer[1–4].

The use of bare silica as the column material for reversed-phase high-performance liquid chromatographic (HPLC) separations by the addition of quaternary am-

* For Part VI, see ref. 8.

0021-9673/86/$03.50

monium compounds to the eluent has been discussed in recent publications (*e.g.* refs. 5–9). One of the major advantages of this technique was demonstrated to be that the selectivity towards a given test mixture is independent of the brand of silica material[10,11].

The aim of the present work was to investigate the possibility of standardizing straight-phase separations by an *in situ* adjustment of the silica surface pH. Furthermore, it was confirmed that the apparent surface pH was without influence on selectivity during chromatography on dynamically modified silica.

EXPERIMENTAL

Apparatus

Testing of the individual chromatographic systems was performed on liquid chromatographs consisting of a Kontron Model 410 LC pump, a Pye-Unicam LC-UV detector or a Cecil Model 212 detector (both operated at 254 nm) and a Rheodyne Model 7125 injection valve; alternatively, a Waters liquid chromatograph was used consisting of a 6000 A pump, a 710 B WISP autoinjector, a 440 UV absorbance detector (operated at 254 nm), a 730 data module and a 720 system controller. Chromatograms were recorded on a Kipp & Zonen Model BD-8 recorder. Retention data were collected on a Hewlett-Packard Model 3353 A laboratory data system or on a Waters 730 data module.

Procedures

Determination of the amounts of cetyltrimethylammonium (CTMA) ions adsorbed onto the column material was performed by the elution method previously described[12].

The apparent surface pH values of the individual silica materials were determined by suspending 100 mg in 10 ml of distilled water; the suspension was stirred for 5 min and the pH was measured electrometrically using a Metrohm glass–calomel combination electrode.

Adjustment of the surface pH of silica packed into columns was performed by eluting the column with 160 ml of a 0.01 *M* potassium phosphate buffer followed by 60 ml of 50% methanol in water and finally by methanol.

Columns in the acid-washed condition were prepared by eluting with 50 ml of a mixture of 0.1 *M* nitric acid and methanol (1:1) followed by a similar volume of 50% methanol and finally by methanol.

Titration curves were constructed from the results of pH measurements of a suspension of 0.100 g of silica in 10 ml of distilled water following the addition of 50 μl portions of 0.01 *N* hydrochloric acid or 0.01 *N* sodium hydroxide (*cf.* ref. 13).

Chromatography

All experiments were performed on 120 × 4.6 mm I.D. columns from Knauer (Berlin, F.R.G.), packed by the dilute slurry technique with LiChrosorb Si 60 (5 μm) (E. Merck, Darmstadt, F.R.G.), Partisil 5 (Whatman, Clifton, NJ, U.S.A.), or Spherisorb S5 W (Phase Sep., Queensferry, U.K.). Activation of the columns before testing was performed by successive eluting with 60 ml of each of the following solvents: acetone, ethyl acetate, 1,2-dichloroethane and heptane. The number of theoretical

plates was calculated from a peak corresponding to naphthalene injected in heptane solution.

In liquid–liquid partition chromatography the eluent used was the upper phase from a mixture of cyclohexane–2-propanol–water (90:8:2) that had been stirred vigorously for 8 h, followed by standing overnight. Chromatography on dynamically modified silica was performed with methanol–water–0.2 *M* phosphate buffer (pH 7.5) (50:45:5) with the addition of 2.5 m*M* CTMA bromide as the eluent and prepared as previously described[7].

The chromatographic system, including columns, eluent, pump, and injection device, was thermostatted at 28.5°C.

During chromatography in the dynamic modification mode the column was guarded by a silica saturation column (120 × 8 mm I.D. dry-packed with LiChroprep Si 60 15–25 μm, Merck) situated between the pump and the injection device. After chromatography the column was rinsed by eluting with methanol–0.1 *M* nitric acid (1:1) and finally with methanol.

Chemicals

All chemicals were of analytical grade from Merck and were used as received from the manufacturer.

RESULTS AND DISCUSSION

Apparent surface pH

The surface pH values of a series of silica materials were determined using the technique previously described by Engelhardt and Müller[1]. The results are presented in Table I together with the results of Engelhardt and Müller. For several brands of silica the pH was measured on two or three different batches. The variations between different brands are quite large, the pH values ranging between 3.8 and 9.5. Variations between different batches of the same brand of material are not pronounced. Even between the results of the present measurements and those performed by Engelhardt and Müller only minor differences are seen.

It has been reported previously[14,15] that the pK_a of silica is 7.1 ± 0.5. In theory, all silica materials would be expected to exhibit uniform surface pH values, and the variations are most probably due to protolytic impurities originating from the manufacturing processes. To investigate this probability, titration curves were constructed for each of six materials and their true pH values were estimated according to the principles outlined by Ilver and Jackerott[13] in their investigations on protolytic impurities in drug substances. Fig. 1 shows titration curves for two silica materials, Nucleosil and Spherisorb. From the figure, and from Table I as well, it appears that the true pH is close to 6 for all the materials investigated. These results support the assumption mentioned above that the variations in surface pH are due to residual base or acid from the production processes.

Straight-phase chromatography

Three of the silica packing materials listed in Table I were investigated further, an acidic (Partisil), a neutral (LiChrosorb Si 60), and a basic material (Spherisorb). The columns were chromatographically tested using a liquid–liquid partition system

TABLE I

pH VALUES IN 1% AQUEOUS SUSPENSION OF SILICA

True pH values are calculated according to Ilver and Jackerott[13].

Trade name	*Batch*				*True pH*
	*A**	*B*	*C***	*D*	
LiChrosorb Si 40		6.1			
LiChrosorb Si 60	7.8	7.4	7.4	7.4	5.7
LiChrosorb Si 100	7.0	7.5	6.8	7.4	
LiChrospher Si 60		5.1			
LiChrospher Si 100	5.3	5.8	5.7		
LiChrospher Si 300		5.9			
LiChrospher Si 500	9.9	8.3			
LiChrospher Si 1000	9.2	8.6			
Hypersil	9.0	8.5	8.4		6.3
Nucleosil 50		8.1	6.7		
Nucleosil 100	5.7	6.0	6.0		5.9
Nucleosil 100V		8.1			
Polygosil 60	8.0	7.1			
Partisil	7.5	5.6	5.3	6.3	5.9
Sperisorb S5 W	9.5	9.0	9.5		6.2
Spherosil XOA 600		3.8			
Spherosil XOA 800		5.7			
Zorbax SIL	3.9	4.1	5.6		
Sepralyte SI		8.2			
Vydac 90		6.1			
Serva Si 60		7.1			5.9
Serva Si 100		7.1			
Serva Si 200		6.7			
Chromosorb LC 6		7.1			

* Batch series A are values measured by Engelhardt and Müller[1].

** Column materials used for further investigations, and for titration curve experiments.

based on a two-phase ternary mixture of cyclohexane, 2-propanol and water. The selectivity of the three silica packing materials towards a mixture of seven test solutes is shown in Table II. It appears that variations occur with the acidic compound ketoprofen and the basic compound lidocaine; their retention is controlled by the surface pH. A further problem is that the increased retention of acidic or basic compounds is accompanied by a deterioration of the peak shape (*cf.* Fig. 2). It has also been found that the retention and peak shape for the amines are clearly influenced by the amount of solute injected and by the number of injections previously performed of the same solute.

Straight-phase chromatography on pH-adjusted columns

It has been reported previously that more efficient separations of acidic or basic solutes on silica may be achieved by an adjustment of the surface pH of the column material prior to packing into a column[1–4]. An *in situ* buffer adjustment has also been reported[3]: this procedure includes a subsequent drying by purging the

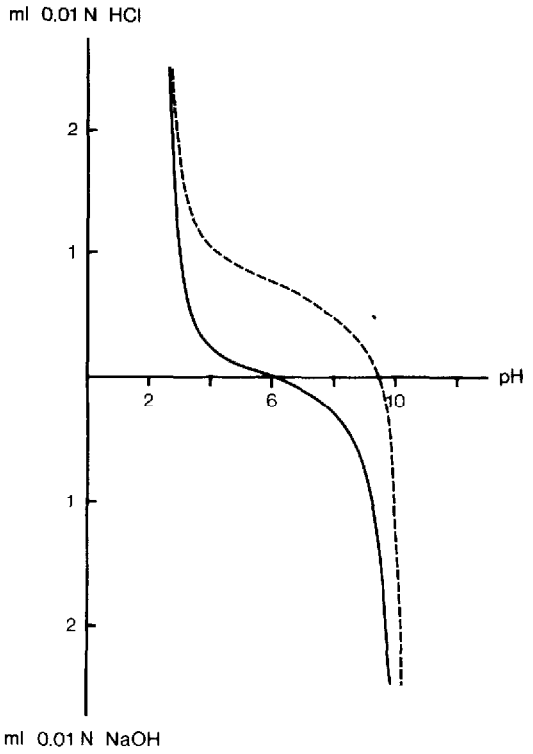

Fig. 1. Titration curves for Nucleosil 100-5 (———) and Spherisorb S5 W (– – –). 0.100 g of silica suspended in 10 ml of distilled water were titrated with 0.01 *N* hydrochloric acid. Another portion of the suspension was titrated with 0.01 *N* sodium hydroxide. The turning point of the curve corresponds to the true pH of the suspension (*cf.* ref. 13).

TABLE II

SEPARATION FACTORS BETWEEN PHENOL AND SEVEN DIFFERENT SOLUTES WHEN CHROMATOGRAPHED IN THE STRAIGHT-PHASE MODE ON THREE DIFFERENT COLUMN MATERIALS "AS IS"

Eluent: cyclohexane–2-propanol–water (90:8:2), upper phase.

Solute	*Column material*		
	Partisil	*LiChrosorb Si 60*	*Spherisorb*
Aniline	1.16	1.16	1.09
Ketoprofen	1.43	7.51	9.87
4-Nitrophenol	2.06	2.01	2.20
Testosterone	2.51	2.52	2.73
Benzocaine	2.94	2.73	3.25
Lidocaine	4.79	2.22	5.99
Cortisone acetate	7.43	7.54	8.34
Apparent surface pH	5.3	7.4	9.5

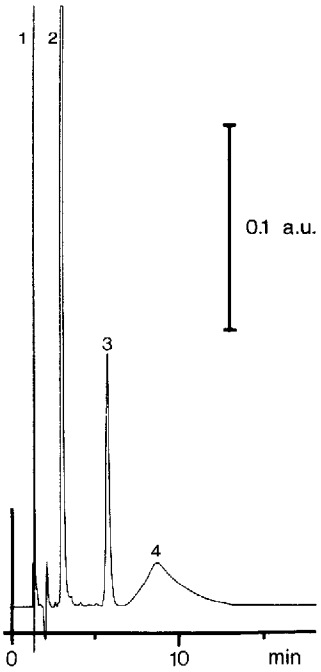

Fig. 2. Straight-phase separation of bases on Partisil "as is". Column, 120 × 4.6 mm I.D.; eluent, cyclohexane–2-propanol–water (90:8:2), upper phase; flow-rate, 1 ml/min; detection, UV 254 nm. Peaks: 1 = benzene; 2 = aniline; 3 = benzocaine; 4 = lidocaine.

column with nitrogen first at room temperature and then at 80°C. These procedures are time-consuming, especially treatment of the silica in bulk if a series of different pH adjustments are needed.

An attempt has therefore been made to perform pH adjustments of the silica materials *in situ* excluding the drying procedure. The adjustments performed were either an acid treatment or a treatment with buffer solutions of various pH values. The treatment was followed by elution with methanol–water and with methanol and subsequent activation. Table III shows the selectivity of the liquid–liquid partition chromatographic system towards the same seven test solutes as shown in Table II when using the three columns in an acid-washed condition and after treatment with pH 7.25 buffer, respectively (*cf.* Experimental). It appears from the table that the variations in selectivity have been considerably reduced, although some variations still occur for the basic solutes, and especially for aniline.

For one of the three columns investigated (Partisil) an attempt was made to return its surface pH to the original value by impregnation with a phosphate buffer of the same pH value as the one measured in an aqueous suspension of Partisil (*cf.* Table I). The results are shown in Table IV. It appears that the impregnation with a pH 5.3 buffer leads to a column that gives too high a retention for both an acidic solute (ketoprofen) and a basic solute (lidocaine), *i.e.* the column seems too basic for the acidic compound and too acidic for the basic compound relative to the untreated column. By impregnation with a buffer solution of pH *ca.* one unit lower and higher,

TABLE III

SEPARATION FACTORS BETWEEN PHENOL AND SEVEN DIFFERENT SOLUTES WHEN CHROMATOGRAPHED IN THE STRAIGHT-PHASE MODE ON THREE DIFFERENT COLUMN MATERIALS WHICH HAVE BEEN ACID WASHED OR TREATED WITH pH 7.25 BUFFER

Chromatographic conditions as in Table II.

Solute	*Acid treated*			*Buffer treated*		
	Partisil	*LiChrosorb*	*Spherisorb*	*Partisil*	*LiChrosorb*	*Spherisorb*
Aniline	2.97	2.45	24.20	1.13	1.07	1.06
Ketoprofen	1.07	1.07	1.03	17.04	12.56	14.96
4-Nitrophenol	2.00	1.95	2.03	2.27	2.05	2.14
Testosterone	2.78	2.60	2.72	2.47	2.27	2.39
Benzocaine	2.94	2.86	2.89	2.81	2.58	2.58
Lidocaine	>25	>25	>25	1.80	2.52	4.22
Cortisone acetate	8.25	7.83	7.50	7.74	7.11	7.30

respectively, the retention of the acidic and basic solute fits those originally obtained. These results are probably due to the fact that the surface pH resulting from the productions process is determined by other ionic species or relative concentrations than those of the potassium phosphate buffer used in this investigation.

The various treatments of the columns during the present investigation, including straight-phase and reversed-phase separations, buffer adjustments and acid treatment, were found in no way to influence their efficiency. The columns were tested before each series of experiments by activation to heptane through a series of solvents (*cf.* Experimental) and injecting naphthalene to calculate the number of theoretical plates. Throughout the whole series of experiments *ca.* 8000 theoretical plates were found for the Partisil column.

TABLE IV

SEPARATION FACTORS BETWEEN PHENOL AND SEVEN DIFFERENT SOLUTES WHEN CHROMATOGRAPHED IN THE STRAIGHT-PHASE MODE ON PARTISIL "AS IS" OR TREATED WITH BUFFERS OF VARIOUS pH VALUES

Chromatographic conditions as in Table II.

Solute	*Column treatment*				
	"As is"	*Acid*	*pH 5.3*	*pH 4.6*	*pH 6.3*
Aniline	1.16	2.97	1.13	1.17	1.15
Ketoprofen	1.43	1.07	1.57	1.40	2.95
4-Nitrophenol	2.06	2.00	2.09	2.07	1.99
Testosterone	2.51	2.78	2.53	2.58	2.50
Benzocaine	2.94	2.94	2.78	2.77	2.81
Lidocaine	4.79	>25	10.30	30.46	4.60
Cortisone acetate	7.43	7.83	7.93	7.92	7.46

TABLE V

SEPARATION FACTORS BETWEEN BENZENE AND TEN DIFFERENT SOLUTES WHEN CHROMATOGRAPHED IN THE DYNAMIC MODIFICATION MODE ON THREE DIFFERENT COLUMNS WITH AND WITHOUT A PRECEDING TREATMENT WITH ACID OR BUFFER OF pH 7.25

Eluent: methanol–water–0.2 *M* phosphate buffer (pH 7.5) (50:45:5) containing 2.5 m*M* CTMA bromide.

Solute	*Partisil*			*LiChrosorb*			*Spherisorb*		
	"As is"	*Acid*	*pH 7.25*	*"As is"*	*Acid*	*pH 7.25*	*"As is"*	*Acid*	*pH 7.25*
Aniline	0.42	0.42	0.42	0.43	0.43	0.43	0.43	0.43	0.42
Lidocaine	1.10	1.09	1.11	1.10	1.14	1.09	1.26	1.25	1.25
N,N-Dimethyl-aniline	1.26	1.25	1.26	1.26	1.26	1.25	1.24	1.27	1.26
Ketoprofen	6.74	6.65	6.81	6.85	6.96	6.59	7.05	7.21	7.25
Phenol	1.33	1.34	1.33	1.33	1.29	1.34	1.26	1.28	1.26
4-Nitrophenol	6.14	6.04	6.13	6.21	6.12	5.99	5.95	6.14	6.19
4-Hydroxy-benzoic acid	1.35	1.25	1.34	1.36	1.31	1.32	1.37	1.40	1.40
Methyl *p*-hydroxybenzoate	3.85	3.71	3.85	3.80	3.60	3.78	3.32	3.53	3.46
Testosterone	2.44	2.39	2.43	2.44	2.53	2.29	2.73	2.76	2.75
Cortisone	0.64	0.63	0.65	0.66	0.68	0.65	0.76	0.75	0.76
Prednisolone	1.10	1.06	1.10	1.13	1.12	1.10	1.19	1.21	1.21

Chromatography on dynamically modified silica

In previous investigations it has been found[10,11] that selectivity in reversed-phase chromatography on dynamically modified silica is independent of the brand of silica material used. The brands of silica investigated previously represent a wide range of apparent surface pH values. However, a treatment with acid was often used during the development of separation methods as a termination of the individual experiments, and hence the protolytic properties of the columns were determined by the acid treatment. Table V shows the selectivity towards a test mixture for the three columns investigated, both "as is" and following treatment with acid or with pH 7.25 buffer. It appears that the selectivity is independent of the surface pH as expressed by the results for each of the individual columns in the different conditions and by comparing the three columns. This means that the buffer and the CTMA in the eluent are able to control the characteristics of the surface. Some difference is seen, however, between the Spherisorb column and the two other columns in the retention of the steroids relative to phenol, but these differences are only minor compared with those exhibited by the straight-phase systems mentioned above. The differences observed may be a result of differences in the pore-size distribution, as shown previously[8,11].

CONCLUSION

It has been demonstrated that the apparent surface pH of silica column materials greatly influences the selectivity of straight-phase chromatographic systems, especially in the separation of acidic or basic compounds. By an *in situ* adjustment

of the surface pH it has been shown possible largely to standardize the selectivity of various brands of silica.

The selectivity of chromatographic systems based on dynamically modified silica was found not to be influenced by the surface pH.

The use of silica columns for reversed-phase chromatography using the dynamic modification approach does not prevent a subsequent use in the straight-phase mode on condition that proper rinsing for adsorbed CTMA by acid washing and subsequent activation of the column is performed (*cf.* Experimental).

ACKNOWLEDGEMENT

This work was supported by the Danish Medical Research Council, grant no. 12-0649.

REFERENCES

1 H. Engelhardt and H. Müller, *J. Chromatogr.*, 218 (1981) 395.
2 R. Schwarzenbach, *J. Liq. Chromatogr.*, 2 (1979) 205.
3 R. Schwarzenbach, *J. Chromatogr.*, 202 (1980) 397.
4 R. Schwarzenbach, *J. Chromatogr.*, 334 (1985) 35.
5 S. H. Hansen, P. Helboe and U. Lund, *J. Chromatogr.*, 270 (1983) 77.
6 S. H. Hansen and P. Helboe, *J. Chromatogr.*, 285 (1984) 53.
7 S. H. Hansen, P. Helboe and M. Thomsen, *J. Pharm. Biomed. Anal.*, 2 (1984) 165.
8 S. H. Hansen, P. Helboe and M. Thomsen, *J. Chromatogr.*, 360 (1986) 53.
9 M. Gazdag, G. Szepesi and M. Hernyes, *J. Chromatogr.*, 316 (1984) 267.
10 S. H. Hansen, P. Helboe, M. Thomsen and U. Lund, *J. Chromatogr.*, 210 (1981) 453.
11 P. Helboe, *J. Chromatogr.*, 245 (1982) 229.
12 P. Helboe, *J. Chromatogr.*, 261 (1983) 117.
13 K. Ilver and Aa. Jackerott, *Dan. Tidsskr. Farm.*, 37 (1963) 225.
14 M. L. Hair and W. Hertl, *J. Phys. Chem.*, 74 (1970) 91.
15 D. N. Strazhesko, V. B. Strelko, V. N. Belyakov and S. C. Rubanik, *J. Chromatogr.*, 102 (1974) 191.