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SILANOPHILIC INTERACTIONS IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Basic compounds often are strongly adsorbed on chemically bonded silica-based reversed phases. Addition of amines to the mobile phases decreases the k' values. This effect is attributed to especially active centres on the silica surface, as it is not influenced by different modification procedures. Certain types of modified silica do not show this effect. In these instances, the k' values are only influenced by hydrophobic interactions between the solute and the stationary phase.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) has brought about a dramatic improvement in the fast and efficient separation of polar solutes. The separation mechanism, discussed in the literature[1–4], is attributed to hydrophobic interactions between the solutes and the alkyl chains of the chemically modified silica gel[5]. The "hydrophobic theory" of reversed-phase chromatography neglects the role of the silica in the chromatographic behaviour of stationary phases. However, practical experience indicates that the silanol groups do indeed affect the chromatographic behaviour of polar analytes, particularly basic compounds.

Owing to steric considerations, a maximum of *ca.* 50% of the silanol groups can react with the alkylsilane[6]. Therefore, at least 50% of the silanol groups remain on the surface of the stationary phase, but their influence on the chromatographic behaviour is almost neglected in theoretical treatments of the separation mechanism. On the other hand, these polar groups are often the cause of difficulties, and their influence can be easily monitored by chromatography of basic solutes. Tailing and/or irreversible adsorption on the stationary phase are observed. As a result of the different modification procedures, different functionalization of the modifying silane and different properties of the original silica gel used for the modification, commercially available reversed phases show enormous differences in their behaviour toward basic solutes. However, it has not been possible to quantify the influence of silanol groups on chromatographic separations with different stationary phases.

Amines, such as triethylamine (TEA), added in small amounts to the mobile phase have been proposed as tailing-reducing agents[7,8]. In this work, the influence of various amounts of TEA added to the mobile phase on the chromatographic behaviour of basic solutes with six different reversed-phase silicas was examined.

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EXPERIMENTAL

The stationary phases used were Spherisorb (Phase Separations, Queensferry, U.K.), ODS-2, Hypersil ODS (Shandon, Astmoor, U.K.), Nucleosil 100 C_{18} (Macherey, Nagel & Co., Düren, F.R.G.), Zorbax ODS (DuPont, Wilmington, MA, U.S.A.), LiChrospher CVH-18 (Merck, Darmstadt, F.R.G.) and laboratory-prepared LAB V. The phases were packed into 125 × 4.6 mm I.D. stainless-steel columns. All materials had a particle size of 5 μm. The modification procedure for LAB V is described elsewhere[9]. The unmodified silica was Nucleosil R (Macherey, Nagel & Co.).

Chromatograms were recorded with a Hewlett-Packard (Waldbronn, F.R.G.) Model 1084 liquid chromatograph, equipped with a variable-wavelength detector set at 254 nm. The nominal flow-rate was 1 ± 0.01 ml/min in all instances. Each chromatographic run was repeated at least three times; the retention times in replicate runs did not differ by more than 1.5%. Void volumes were determined by the method of Knox and Kaliszan[10]. The average of the peaks of ^2H_2O and C^2H_3CN measured with a refractive index detector was taken as t_0.

The mobile phase was 50% (v/v) aqueous acetonitrile, to which 1% acetic acid or various amounts of TEA were added. Basic solutes were examined with similar results. In this paper, we report the investigation of the antiarrhythmic drug disopyramid, purchased from Roussel Pharma (Wiesbaden, F.R.G.).

RESULTS AND DISCUSSION

Fig. 1 and Table I show the influence of the addition of TEA to the mobile phase on the k' value of disopyramid. If no TEA is present in the mobile phase, and with Spherisorb as the stationary phase, disopyramid is strongly adsorbed. Hypersil ODS, LiChrospher CH-18 and Zorbax show similar behaviour. Chromatographic elution is not possible. If relatively large amounts of TEA (2500 ppm) are added to

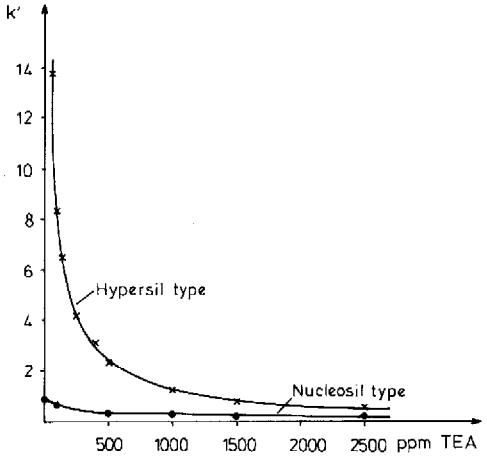

Fig. 1. Dependence of k' on TEA concentrations in the mobile phase for the two kinds of silica-based reversed phases.

TABLE I

RETENTION k' OF DISOPYRAMID AS A FUNCTION OF TEA CONCENTRATION IN THE MOBILE PHASE ON DIFFERENT STATIONARY PHASES

TEA added (ppm)	*Stationary phase*					
	Hypersil ODS	*Spherisorb ODS-2*	*LiChrospher CH-18*	*Zorbax ODS*	*Nucleosil C_{18}*	*LAB V*
2500	0.57	.56	0.30	0.35	0.21	0.24
1500	0.86	0.77	0.43	0.50	0.21	0.25
1000	1.28	1.11	0.52	0.65	0.29	0.22
500	2.31	1.69	0.99	0.89	0.31	0.22
400	3.10	2.02	1.13	1.12	0.36	0.26
250	4.12	2.90	2.00	1.87	0.42	0.21
150	6.54	5.00	3.12	2.53	0.57	0.25
100	8.40	6.79	4.26	3.49	0.60	0.21
50	13.73	14.63	8.47	7.53	0.84	0.23
0	*	*	*	*	0.97	0.26

* $k' > 20$.

the mobile phase, the basic solute has a small k' value and the peak symmetry is nearly 1. As the concentration of TEA decreases, the k' values increase with a corresponding increase in peak tailing. The dependence of the k' value of disopyramid on the amount of TEA in the mobile phase can be described by hyperbolic functions when using the stationary phases Spherisorb ODS-2, Hypersil ODS, Zorbax ODS and LiChrospher CH-18. Especially in the range 500–50 ppm of TEA in the mobile phase a tremendous increase in k' can be observed; small changes in TEA concentration cause a large variation in k', and this can be employed in chromatographic separations.

With Nucleosil C_{18} and LAB V as stationary phases, addition of TEA to the mobile phase has little effect on the k' value of disopyramid. Even if no TEA is present in the mobile phase, disopyramid is eluted with $k' = 0.97$ (Table I), and with 2500 ppm of TEA, $k' = 0.21$. For LAB V, there is almost no change in k' with variation in TEA concentration. Similar differences for these stationary phases are also observed with other basic solutes, such as aniline, pyridine, lutidine or collidine.

LAB V and Nucleosil C_{18} are prepared from the same basic silica gel, but by using different modifying procedures. Nucleosil C_{18} is modified with a trifunctional silane and subsequent end-capping, whereas LAB V is made with a monofunctional silane without end-capping. Despite these different procedures, both phases show the same characteristics. The behaviour of basic solutes as a function of TEA concentration allows a classification of stationary phases. The "Nucleosil-type" stationary phases show only a slight dependence of k' on TEA concentration in the mobile phase. Elution of basic solutes is possible even if no TEA is present in the mobile phase (Fig. 1). In all instances the effect of TEA is reversible. After washing with TEA-free solvent for 1 h the chromatographic behaviour of the columns is the same as the initial behaviour. The "Hypersil-type" stationary phases show a very strong dependence of k' on TEA concentration (Fig. 1). This difference between the two

TABLE II

CHARACTERIZATION OF THE DIFFERENT STATIONARY PHASES (DATA FROM PRODUCER)

Nume	*Silica*				*ODS-silica*			
	Diameter (μm)	*BET surface area (m^2/g)*	*Pore diameter (nm)*	*Pore volume (ml/g)*	*Functionality*	*End-capping*	*Packing density*	*C (%)*
LiChrospher	5	250	10	1.4	Di-	No	1.2	20.46
LiChrosorb	5	300	10		Di-	No	1.2	15.99
Zorbax	8	350	8		Mono-	No	1.3	14.30
Nucleosil	7	350	10	1.0	Tri-	Yes	1.2	12.99
Partisil	5	350	8.5	0.85	Tri-	Yes	1.2	10.67
Hypersil	5	170	12	0.7	Tri-	Yes	1.6	10.58
Spherisorb	5	220	8		Tri-	Yes	1.8	10.27

types of stationary phases cannot be attributed to the modification procedure, but must be caused by different properties of the basic silica[11].

The reasons for the increase in k' with decreasing TEA concentration in the mobile phase can be attributed to silanophilic interactions. Especially active centres of the silica can be masked by basic TEA molecules. In this masked form, the active centres are unable to interact with basic solutes, leading to a reduction in k'. The k' values at higher TEA concentration reflect the hydrophobic interaction of the solute with the stationary phase. It is interesting that the dramatic differences in the chromatography of basic solutes cannot be correlated with the physical data usually used to characterize stationary phases, as Table II shows. However, NMR characterization seems to be a more sensitive method, as will be shown in a subsequent publication. Further investigations will reveal whether the groups responsible for this phenomenon are especially active silanol groups or other constituents of the silica.

REFERENCES

1 Cs. Horvath, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
2 B. L. Karger, J. R. Gant, A. Hartkopf and P. H. Weiner, *J. Chromatogr.*, 128 (1976) 65.
3 D. C. Loche, *J. Chromatogr. Sci.*, 12 (1974) 433.
4 D. E. Martire and R. E. Boehm, *J. Phys. Chem.*, 87 (1983) 1045.
5 K. K. Unger, *Porous Silica (Journal of Chromatography Library*, Vol. 16), Elsevier, Amsterdam, 1979.
6 K. K. Unger, *Porous SIlica (Journal of Chromatography, Library*, Vol. 16), Elsevier, Amsterdam, 1979, p. 102.
7 Y. Tapuhi, N. Miller and B. L. Karger, *J. Chromatogr.*, 205 (1981) 325.
8 H. Engelhardt, in A. Henschen, K. P. Hupe, F. Lottspeich and W. Voelter (Editors), *High Performance Liquid Chromatography in Biochemistry*, Verlag Chemie, Weinheim, 1985, p. 15.
9 E. Bayer, K. Albert, A. Paulus, B. Peters, G. Laupp and J. Reiners, *J. Chromatogr.*, 364 (1986) 25.
10 J. H. Knox and R. Kaliszan, *J. Chromatogr.*, 349 (1985) 211.
11 A. Paulus, *PhD Thesis*, University of Tübingen, 1986.