Journal of Chromatography, 506 (1990) 253–264
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1821

Regulation of peak compression effects for substituted benzamides in reversed-phase liquid chromatography

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SUMMARY

The possibilities of regulating peak compression effects, *i.e.*, of making the analyte peak coelute with the system peak, were investigated for substituted benzamides. The changes in capacity factor for the system peak relative to the retention of the analyte were studied by varying the composition of the mobile phase. The parameters useful for altering the capacity factor ratio were found to be the ionic strength of the phosphate buffer and to some extent the pH, whereas the amount of acetonitrile and the concentration of the amine modifier gave negligible effects. Variation of the amount of silanol groups on the solid phase could also be used as an efficient means for regulation of the capacity factor ratio.

INTRODUCTION

Mobile phases in reversed-phase liquid chromatography often contain additives, *e.g.*, ion-pairing reagents and competing ions, in order to change the retention and/or the selectivity or to improve the peak symmetry. When using such mobile phases, the injection of a sample not identical with the mobile phase will disturb the column equilibria and generate two kinds of peaks. The first kind is the ordinary peaks corresponding to the injected compounds. The other kind originates from the equilibrium disturbance and is called system peaks, as they reflect the concentration changes of the mobile phase components[1–5]. A system peak is thus a zone with an excess or a deficit of a mobile phase component, eluting at a retention volume characteristic of that component. The theoretical treatment of system peaks has so far been limited to those developed by very small equilibrium disturbances (*e.g.*, ref. 6).

It has been shown that, under special circumstances, peak compression[7–9] or peak deformation[10] will occur for analytes coeluting with the system peak. The peak compression effect might give extremely narrow peaks and apparent efficiencies up to $5 \cdot 10^6$ plates/m have been observed[11], so this effect is of interest as a means of improving the sensitivity in liquid chromatography. One bioanalytical application has been published in which peak compression was applied to the determination of low levels of FLA 908, a minor remoxipride metabolite, in urine[8]. To be able to make

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further use of the peak compression effects and to learn how to avoid peak deformation, a better understanding of these phenomena is necessary.

In earlier studies with tertiary amines as model compounds, the amine modifiers used in the mobile phase were N,N-dimethylalkylamines, the alkyl group varying from hexyl to dodecyl[7–9]. The system peaks for these modifiers have different capacity factors, giving retention "windows" in between. For analytes eluting in these retention windows, it has not been possible to obtain useful peak compression effects. The aim of this work was to find parameters that can be used for the fine regulation of the ratio of the system peak capacity factor to the analyte capacity factor and thereby broaden the applicability of the peak compression effect.

EXPERIMENTAL

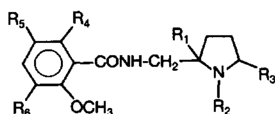
Chemicals

N,N-Dimethyloctylamine (DMOA) was obtained from ICN Pharmaceuticals (Plainview, NY, U.S.A.) and N,N-dimethylheptylamine (DMHA), N,N-dimethylnonylamine (DMNA) and N,N-dimethyldecylamine (DMDA) from Ames Labs. (Millford, CT, U.S.A.). Other chemicals, of HPLC or analytical-reagent grade, were obtained from the usual commercial sources and used as received.

The model compounds (Fig. 1) were substituted benzamides related to the neuroleptic compound remoxipride and synthesized at CNS Research and Development, Astra Research Centre (Södertälje, Sweden). Their syntheses have been described: compound II[12], compounds III–V[13], compound VI[14] and compound VII[15].

Chromatography

Six different columns were used: a factory-packed Spherisorb ODS-1 (3 μm; 100 × 4.6 mm I.D.) from Phase Separations (Queensferry, U.K.), a factory-packed Nucleosil 120-3 C_{18} (100 × 4.6 mm I.D.) from Macherey-Nagel (Düren, F.R.G.), and four columns (100 × 4.6 mm I.D.) with 5-μm Spherisorb ODS-1 and Spherisorb ODS-2 (Phase Separations) in different proportions (100:0, 75:25, 50:50 and 0:100). The mixed packing slurries were stirred for 1 h before the columns were packed. Methyl isobutyl ketone was used as a slurry medium and hexane as the eluent. The packing pressure was 400 bar.



	R_1	R_2	R_3	R_4	R_5	R_6
I (remoxipride)	H	C_2H_5	2H	OCH_3	H	Br
II	H	C_3H_7	2H	OCH_3	H	Br
III	H	C_2H_5	2H	OCH_3	Cl	Br
IV	H	C_2H_5	2H	OCH_3	Cl	Cl
V	H	C_2H_5	2H	OH	Cl	Cl
VI	H	C_2H_5	2H	OH	Br	OCH_3
VII	OH	C_2H_5	O	OCH_3	H	Br

Fig. 1. Structure of model compounds.

The mobile phases were different concentrations of acetonitrile in phosphate buffers of various pH and ionic strengths, with or without the addition of amine modifiers (DMHA to DMDA). The flow-rate was 1.0 ml/min. The analytes were dissolved in phosphate buffer with the same pH and ionic strength as the mobile phase. The injection volume was 100 μl.

The chromatographic system consisted of a Model 590 programmable pump (Waters Assoc., Milford, MA, U.S.A.), a Perkin-Elmer (Überlingen, F.R.G.) ISS-100 autosampler and a Perkin-Elmer LC-95 UV detector. The cell volume was 1.4 μl and the detector was operated at 208 nm. A refractive index (RI) detector (Waters Model 410) was coupled in series with the UV detector and used to study the retention of the system peak. The UV detector signal was monitored with a Model SP 4270 integrator (Spectra-Physics, San Jose, CA, U.S.A.) and the RI detector signal was recorded with a Kipp & Zonen (Delft, The Netherlands) BD 41 recorder. To prolong the lifetime of the analytical column, a guard column dry-packed with Corasil (Waters Assoc.) was placed between the pump and the autosampler.

Determination of capacity factors

The column mobile phase volume, V_m, was measured by the injection of potassium nitrate. The system peak is the result of fairly large changes in the concentration of the amine modifier in the mobile phase. Being on the non-linear part of the adsorption isotherm, the retention volume for the system peak depends strongly on how it is generated. In this work, the retention volume was measured for the small positive system peak generated when phosphate buffer was injected. Effects on the peak shape can be expected when the capacity factor of this system peak is the same as the isocratic capacity factor of the analyte, *i.e.*, the ratio of these capacity factors is 1.0.

PRINCIPLE OF THE PEAK COMPRESSION EFFECT

A mobile phase with a UV-transparent cationic modifier, *e.g.*, DMNA, is used. The UV-absorbing amine analyte is injected dissolved in a solution of an organic anion, *e.g.*, nonyl sulphate, with a larger retention than the cationic modifier. This anion generates a system peak with a DMNA deficit and a nonyl sulphate peak containing the corresponding excess of DMNA. These peaks can be observed with RI detection (Fig. 2, top trace). The system peak is thus a zone of mobile phase with a lower concentration of DMNA, giving an increased retention of the amine analytes within this zone. If the analytes are injected dissolved in phosphate buffer, all three compounds show normal peaks (Fig. 2, middle trace). When the analytes are injected in a solution of a counter ion, the peak shapes for the analytes eluting before or after the system peak are hardly influenced at all, whereas the analyte coeluting with the system peak will elute in a narrow band (Fig. 2, bottom trace). This analyte will be trapped by the system peak and, owing to its higher retention within this zone of DMNA deficit, it will move more slowly than the zone until it reaches the higher DMNA concentration of the surrounding mobile phase where the analyte retention is equal to, or even higher than, the system peak retention. The result is that the analyte is concentrated in a narrow band at the back of the system peak[7].

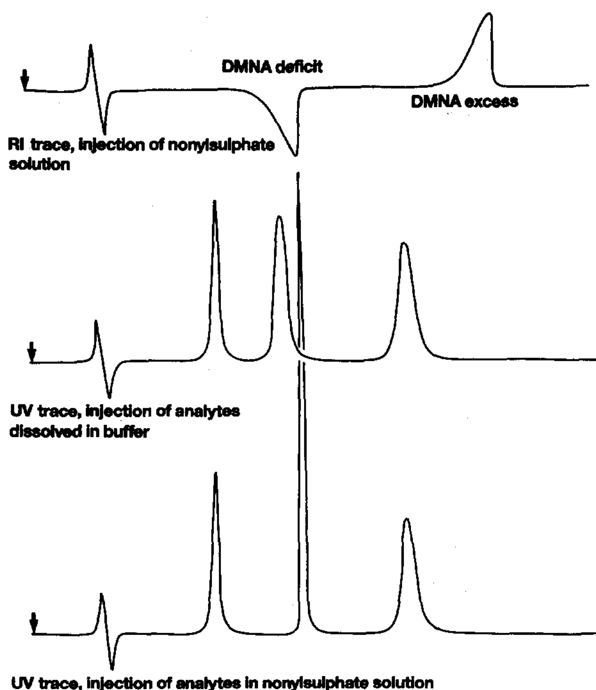


Fig. 2. Schematic representation of the peak compression effect exemplified with a mobile phase containing DMNA as the amine modifier and using nonyl sulphate in the solution injected to create the system peak.

RESULTS AND DISCUSSION

Choice of solid phases

The retention of basic compounds such as amines has been found to be correlated with the surface concentration of residual silanols[16]. Several simple chromatographic tests for residual silanols have been published[16–18]. In this paper, a test was performed by injecting a mixture consisting of two amines (compounds I and II, Fig. 1) with a high affinity to silanols, and a lactam (VII), a neutral compound expected not to be retained on silanols. Mobile phases with and without DMOA added to mask the residual silanols[19] were used, and the influence of the DMOA addition on the retention of these three analytes was studied.

Two octadecylsilica columns with different specifications were initially compared: Spherisorb ODS-1, a low-carbon-content (7%) support with a large number of residual silanol groups, and Nucleosil 120-3 C_{18}, with a higher carbon-content (11%). On the latter (Table I) the amines elute earlier than the lactam for both mobile phases and the addition of DMOA does not change either the retention or the peak efficiency and symmetry. Hence, this batch of the support can be regarded as fully end-capped.

The results for Spherisorb ODS-1 (5-μm) are completely different (Table I). The retention of the amines is high without DMOA, but decreases drastically when DMOA is added. Simultaneously, the peak efficiency and symmetry are improved. The retention, efficiency and symmetry of the lactam peak are not influenced by the

TABLE I

INFLUENCE OF AMINE MODIFIER ON CAPACITY FACTORS FOR TWO DIFFERENT OCTADECYLSILICA COLUMNS

Mobile phase: acetonitrile–phosphate buffer (pH 2.00, I = 0.05) (28:72, v/v) without or with 0.4 mM DMOA.

Compound	*Column*			
	Nucleosil 120-3 C_{18}		*Spherisorb ODS-1 (5 μm)*	
	Without DMOA	*With DMOA*	*Without DMOA*	*With DMOA*
I	0.78	0.72	9.26	2.01
II	1.37	1.25	18.9	3.95
VII	2.02	1.91	1.64	1.59

addition of DMOA. These results were as expected for the Spherisorb ODS-1 supports and indicate a high number of residual silanols. The results (not shown) for the 3-μm Spherisorb ODS-1 gave an almost identical pattern but slightly shorter retention and higher efficiency.

For the study of peak compression effects, it is essential to use a support where the addition of an amine modifier, responsible for the system peak, has a pronounced effect on the retention of the analyte amines. Hence there should be a large difference in retention for an amine analyte within the system peak compared with outside. A support with a high number of residual silanols is, therefore, expected to give the strongest compression effects and the Spherisorb support was chosen for the peak compression studies.

Variation of the lipophilicity of the amine modifier

In order to obtain peak compression effects for a specific analyte, an amine modifier giving a system peak with a retention close to that of the analyte must be chosen. The retention of the system peak and the retention of the analytes using mobile phases with amine modifiers of different lipophilicity were studied (Table II). With

TABLE II

CAPACITY FACTORS WITH AMINE MODIFIERS OF DIFFERENT LIPOPHILICITY

Mobile phase: acetonitrile–phosphate buffer (pH 2.00, I = 0.05) (26:74, v/v) with 0.5 mM amine modifier. Spherisorb ODS-1 (5 μm) was used.

Amine modifier	*Compound*				
	I	*VII*	*II*	*III*	*System peak*
DMHA	3.40	2.18	6.40	7.82	1.81
DMOA	2.21	2.21	4.19	5.31	2.50
DMNA	1.49	2.20	2.80	3.45	3.54
DMDA	0.87	2.14	1.69	2.26	5.15

increasing lipophilicity of the amine modifier, the system peak retention increases, whereas the amine analyte retention decreases. The lactam retention was constant, however. These results were as expected, but of more importance for the purpose of peak compression was the change in retention for the system peak relative to the analyte. This change is best illstrated by plotting the ratio of the capacity factor for the system peak to that of the analyte (Fig. 3). Peak compression effects, as mentioned above, can be expected when the ratio is about 1.0. Under these conditions, DMNA seemed to be a perfect choice if a peak compression effect was required for III, and DMOA might be useful for I. For II, DMNA gave too high a capacity factor ratio whereas the ratio using DMOA was too low. To be able to obtain peak compression effects also for II, a change of amine modifier is obviously not a suitable approach, and alternative means of regulating the capacity factor ratio must be found.

The compounds giving the system peak and the analytes are all tertiary amines, and changes in the mobile phase composition might be expected to have a similar influence on the retention of both kinds of peaks. However, amines might have different retentions as solutes and as mobile phase components, reflected in the different expressions for their capacity factors[4]. Further, in the present system an amine is added to the mobile phase to mask residual silanols and therefore the silanophilic part of the retention is probably much greater for the amine modifier, giving the system peak, than for the analytes. The influence of the mobile phase components on the retetion for both kinds of peaks was studied to see if these assumed differences in retention mechanism could be useful for changing the capacity factor ratio and, thus, regulating the peak compression effects.

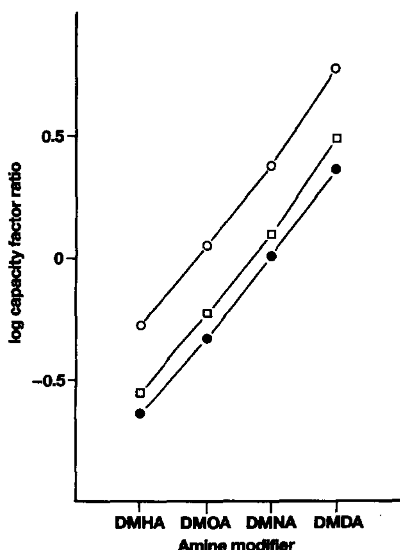


Fig. 3. Influence of the lipophilicity of the amine modifier on the capacity factor ratio. Spherisorb ODS-1 was used and the mobile phase was acetonitrile–phosphate buffer (pH 2, I = 0.05) (26:74, v/v) with 0.5 mM amine modifier. ○ = k' (system peak)/k' (I); □ = k' (system peak)/k' (II); ● = k' (system peak)/k' (III).

Variation of the pH and ionic strength of the phosphate buffer in the mobile phase

The ionic strength of the phosphate buffer was varied from 0.02 to 0.20 at pH 2.00, 2.50 and 3.50. Five amine model compounds and the lactam were injected. The capacity factor of the lactam was the same for all mobile phases. The plots of log k' against ionic strength at pH 2.00 show a small decrease in the capacity factor for the amine compounds when the ionic strength increases, but on the other hand the capacity factor for the system peak increases (Fig. 4). The plots for the analytes are parallel, except for a slight decrease in retention when the analyte elutes close to the system peak. A probable explanation is that the system peak resulting from an injection of buffer is a zone with an excess of DMNA, thus giving coeluting analytes a shorter retention. At pH 2.50 (not shown), the results were very similar to those at pH 2.00, whereas at pH 3.50 the decrease in retention for the analytes and the increase in retention for the system peak were more pronounced than at pH 2.00 (Fig. 5).

The capacity factor ratio plots (pH 2.00 in Fig. 6 and pH 3.50 in Fig. 7) illustrate the rapidly decreasing ratios at ionic strengths below 0.1. At pH 3.50, it is possible to obtain an almost 3-fold change in ratio. A probable explanation for the opposite behaviour of the system peak and the amine analytes is a more extensive distribution of the amine modifier to the solid phase with increasing ionic strength. The amine analytes will then experience an increasing competition for the sites and the retention will decrease. It shoud be noted that at pH 3.50, using the same amine modifier, it is possible to obtain peak compression effects for four of the five analytes by using buffers with different ionic strengths. For V, a more lipophilic amine modifier must be used.

The influence of pH on the capacity factor ratio at low ionic strength is shown in Table V. The ratios are the same at pH 2.00 and 2.50 but decreases at pH 3.50. At higher ionic strengths, the ratios are independent of pH.

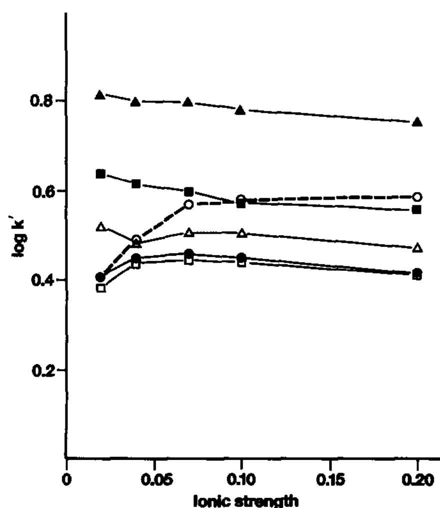


Fig. 4. Influence of ionic strength on the capacity factor at pH 2.00. The column was Spherisorb ODS-1 and the mobile phase was acetonitrile–phosphate buffer (pH 2, I = 0.02–0.20) (30:70, v/v) with 0.5 mM DMNA. ○ = System peak (broken line); □ = II; △ = III; ● = IV; ■ = VI; ▲ = V.

TABLE III

INFLUENCE OF THE ACETONITRILE CONCENTRATION IN THE MOBILE PHASE ON THE CAPACITY FACTOR RATIO

Mobile phase: acetonitrile–phosphate buffer (pH 2.00, I = 0.05) (28:72, v/v) with 0.6 mM DMOA. Spherisorb ODS-1 (5 μm) was used.

Acetonitrile concentration (%)	*Compound*		
	I	*II*	*III*
23	1.13	0.58	0.44
25.5	1.10	0.57	0.45
28	1.08	0.55	0.45
30.5	1.05	0.52	0.44
33	1.01	0.52	0.45

Variation of the acetonitrile and amine modifier concentrations

With DMOA as the amine modifier, the acetonitrile concentration of the mobile phase was varied from 23 to 33%, giving a 2-fold decrease in the capacity factors for both the system peak and the analytes. For I, with about the same capacity factor as the system peak, and also for II there was an insignificant decrease in capacity factor ratio with increasing acetonitrile concentration, whereas for III the ratio was constant (Table III).

Changing the amine modifier concentration from 0.4 to 1.2 mM gave a more than 2-fold decrease in all capacity factors, but the ratios were constant (Table IV). Variation of the acetonitrile or amine modifier concentration is therefore not useful for changing the capacity factor ratio. In a study by Fornstedt *et al.*[20,21], using protriptyline as the amine modifier, changes in the capacity factor ratios were observed when amine modifier concentrations of 0.0076, 0.20 and 0.95 mM were compared. These changes are probably a reflection of the change in coverage of the silanophilic site. The coverage was found to be 3, 41 and 78%, respectively. Peak compression effects were found only at the two highest concentrations[21].

TABLE IV

INFLUENCE OF THE MOBILE PHASE DMOA CONCENTRATION ON THE CAPACITY FACTOR RATIO

Mobile phase: acetonitrile–phosphate buffer (pH 2.00, I = 0.05) (28:72, v/v) with DMOA. Spherisorb ODS-1 (3 μm) was used.

DMOA concentration (mM)	*Compound*		
	I	*II*	*III*
0.4	1.26	0.71	0.57
0.6	1.22	0.70	0.54
0.8	1.21	0.70	0.54
1.0	1.18	0.68	0.51
1.2	1.20	0.70	0.52

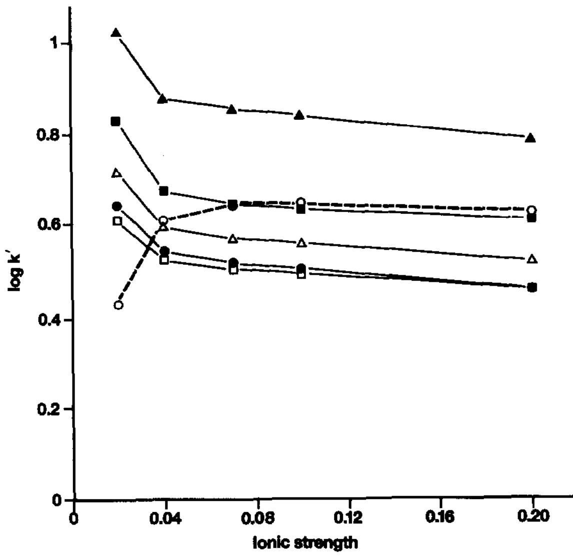
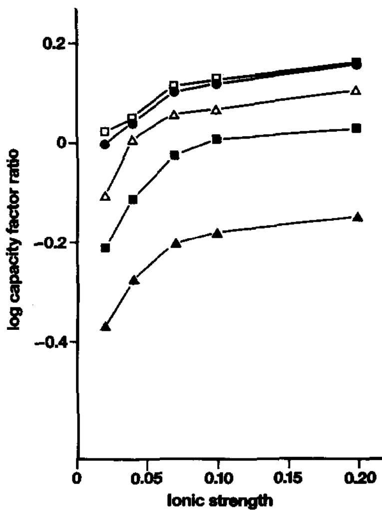


Fig. 5. Influence of ionic strength on the capacity factor at pH 3.50. Conditions and symbols as in Fig. 4.



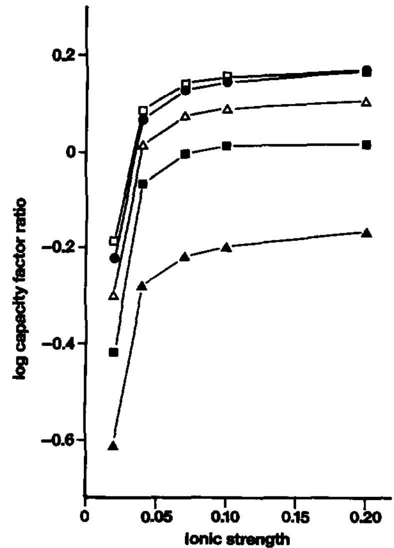


Fig. 6. Influence of ionic strength on the capacity factor ratio at pH 2.00. Conditions as in Fig. 4. □ = k' (system peak)/k' (II); △ = k' (system peak)/k' (III); ● = k' (system peak)/k' (IV); ■ = k' (system peak)/k' (VI); ▲ = k' (system peak)/k' (V).

Fig. 7. Influence of ionic strength on the capacity factor ratio at pH 3.50. Conditions as in Fig. 4 and symbols as in Fig. 6.

TABLE V

INFLUENCE OF pH ON THE CAPACITY FACTOR RATIO

Mobile phase: acetonitrile–phosphate buffer (pH 2.00–3.50, I = 0.02) (30:70, v/v) with 0.5 mM DMNA. Spherisorb ODS-1 (5 μm) was used.

pH	*Compound*				
	II	*IV*	*III*	*VI*	*V*
2.00	1.06	1.00	0.77	0.58	0.39
2.50	1.06	1.00	0.76	0.58	0.38
3.50	0.66	0.61	0.51	0.39	0.25

Varying the ionic strength, and at low ionic strengths also varying the pH, can thus be useful for the fine regulation of the peak compression effect. However, it is not the ideal tool as very small variations in the ionic strength can have a drastic effect on the capacity factor ratio. An extremely careful preparation of the buffer is necessary.

Influence of the support

As the amount of silanols is very important for the retention of amines, experiments were designed in which the amount of silanols in the column was varied. This was done by packing columns where Spherisorb ODS-1 was mixed with Spherisorb ODS-2. According to the manufacturer's specification, ODS-2 is "fully capped" with a bonded phase loading of 0.5 mmol/g (12%), whereas ODS-1, as mentioned above, is "partially capped" with a loading of 0.3 mmol/g (7%). Four columns were packed, with ODS-1 to ODS-2 ratios of 100:0, 75:25, 50:50 and 0:100. All four columns gave excellent efficiency (60 000–80 000 plates/m, calculated using the peak width at half-height) for the neutral compound VII, which is not sensitive to the presence of silanols. As expected for amines retained mainly on the residual silanols, the capacity factors decreased with increasing amounts of ODS-2 (see Table VI). The efficiency of the amine peaks decreased with an increasing amount of

TABLE VI

INFLUENCE OF THE SUPPORT ON THE CAPACITY FACTORS

Column 1, 100% ODS-1; column 2, ODS-1–ODS-2 (75:25); column 3, ODS-1–ODS-2 (50:50); column 4, 100% ODS-2. Mobile phase: acetonitrile–phosphate buffer (pH 2.00, I = 0.05) (28:72, v/v) without or with 0.5 mM DMNA.

Compound	*Column 1*		*Column 2*		*Column 3*		*Column 4*	
	Without DMOA	*With DMOA*	*Without DMOA*	*With DMOA*	*Without DMOA*	*With DMOA*	*Without DMOA*	*With DMOA*
VII	1.87	1.83	1.71	1.64	1.58	1.49	1.37	1.33
I	17.0	1.83	10.5	1.15	8.38	0.97	3.80	0.67
II	33.5	3.49	21.2	2.30	16.8	1.98	6.87	1.45
System peak	–	3.54	–	3.08	–	3.00	–	2.92

ODS-2, from about 30 000 plates/m for 100% ODS-1 to about 4000 plates/m for 100% ODS-2.

The addition of DMNA to the mobile phase gave drastically reduced capacity factors for the amines on all four columns (Table VI). The decrease in capacity factor with increasing amount of ODS-2 still occurred, but to a smaller extent. The efficiency for the lactam peak was not changed whereas an improvement was seen for the amines, giving about 80 000 plates/m for 100% ODS-1 and 50 000 plates/m for 100% ODS-2. The retention of the system peak decreased only slightly with an increasing percentage of ODS-2, resulting in an increase in capacity factor ratio (Fig. 8). The use of mixed supports is obviously an interesting means of regulating peak compression effects as a more than 2-fold change in capacity factor ratio occurred. The manipulation of the amount of silanols on the column gives the possibility of optimizing peak compression effects also at high ionic strength. As small changes in the ionic strength of the phosphate buffer then will not affect the capacity factor ratio, this should give more robust systems.

For the ODS-2 column, it can also be noted that the drastic decrease in retention for amines on adding DMNA to the mobile phase, and also the inferior peak shape and efficiency without DMNA, indicate a considerable amount of residual silanols also on this "fully capped" support.

CONCLUSION

There are several ways of regulating peak compression effects. First, the proper amine modifier is chosen. Fine regulation can then be achieved by selecting a column with a suitable amount of silanols and by adjusting the ionic strength and the pH of the

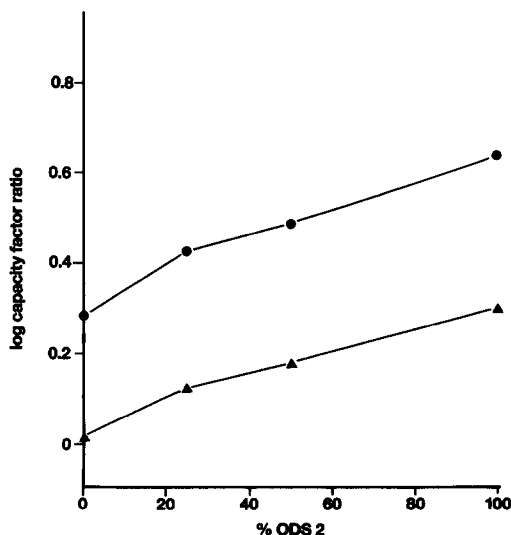


Fig. 8. Influence of the support on the capacity factor ratio. The columns were packed with mixed supports: column 1, 100% Spherisorb ODS-1; column 2, ODS-1–ODS-2 (75:25); column 3, ODS-1–ODS-2 (50:50); column 4, 100% ODS-2. The mobile phase was acetonitrile–phosphate buffer (pH 2, I = 0.05) (28:72, v/v) with 0.5 mM DMNA. ● = k' (system peak)/k' (I); ▲ = k' (system peak)/k' (II).

mobile phase. Using these methods, it seems possible to obtain peak compression effects for all amine analytes within the studied capacity factor range. Work is in progress to demonstrate how these findings can be used in practical work.

ACKNOWLEDGEMENTS

The author thanks Prof. Douglas Westerlund and Dr. Thomas Nordgren for valuable comments on the manuscript.

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