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CHARACTERIZATION OF STYRENE-DIVINYLBENZENE COLUMN PACKINGS FOR LIQUID CHROMATOGRAPHY

ELUTION OF SOME ACIDIC COMPOUNDS

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SUMMARY

The elution behaviour of nineteen substituted benzoic acids on a PRP-1 column packing was investigated. The relationships between capacity factors (k') and mobile phase composition (φ_0) were studied in methanol–water systems under different ionization suppression conditions. The effect of the pH of the eluent was evaluated. It was found that the relationships between $\log k'$ and φ_0 were linear and that the selectivity can be enhanced by varying the pH of the mobile phase. Further, from a comparison of the behaviour of the solutes on PRP-1, on a laboratory-prepared Chromosorb 101 and on a C₁₈ column packing, it can be concluded that the solute elution may be explained by solvophobic theory.

INTRODUCTION

Polystyrene–divinylbenzene resins have played an essential part in gas and liquid chromatography for many years^{1,2}. Polymeric column packings are becoming used more frequently in modern liquid chromatographic applications as improvements in polymeric packing materials are realized and earlier problems associated with their use have been overcome. In addition, such materials offer distinct advantages over silica packings for particular applications, where chemical stability under different pH conditions is required. The potential of polymeric adsorbents prompted us to extend our studies of these stationary phases.

In a previous paper³, we have described the preparation of polymeric column packings in the laboratory and their characterization. The polymer employed was Chromosorb 101, which has good chromatographic properties but a lower efficiency than that of a similar commercial column packing, PRP-1.

The aim of this study was to investigate the elution behaviour of some acidic compounds on a PRP-1 column packing. A review of liquid chromatographic determinations of carboxylic acids was recently presented by Schwarzenbach⁴. An important question is how increasing organic solvent concentrations in the eluent change the retention factor of a given solute. A number of workers^{5–7} have observed that plots of the logarithm of the retention factor *versus* volume fraction of methanol

in methanol-water mixtures are linear. However, other workers have observed marked deviations from linearity⁸⁻¹⁰. In this paper, the relationships between capacity factors (k') and mobile phase composition (ϕ_0) were studied, employing water-methanol eluents under different ionization suppression conditions. Further, the effect of the pH of the mobile phase on retention was evaluated. Some workers^{11,12} have shown that retention on some styrene-divinylbenzene gels resembles that on a C₁₈ bonded phase; therefore, a comparison of retention on PRP-1, laboratory-prepared Chromosorb 101 and C₁₈ column packings was performed with the purpose of acquiring some information about the retention mechanism.

EXPERIMENTAL

The chromatographic measurements were carried out on a Spectra-Physics (San Jose, CA, U.S.A.) Model 8700 liquid chromatograph, equipped with a Model 770 spectrophotometric detector, set at 254 nm. Chromatograms were recorded on a Spectra-Physics SP 4270 integrator. Sample introduction was made with a Rheodyne Model 7125 injector (supplied by Spectra-Physics), equipped with a 10- μ l sampling loop.

HPLC-quality methanol (Rudi Pont, Eurobase, Milan, Italy) and deionized, distilled water were filtered through a 0.45- μ m Millipore filter and degassed with a helium purge. The pH was controlled with 0.01 *M* phosphoric acid and 0.01 *M* phosphate salts. The reported pH values are the pH of the solution before the addition of methanol. Solvent mixtures are expressed as percent by volume. The solutes, of analytical-reagent grade from Alltech (Milan, Italy), were dissolved in methanol. The solute concentrations, ranging between 10 and 500 ppm, allowed suitable responses from the detector without overloading the column.

The PRP-1 (10- μ m particle diameter) column (150 \times 4.1 mm I.D.) was supplied by Hamilton (Bonaduz, Switzerland). The column of Chromosorb 101 was prepared as described previously³. Potassium nitrate was used as the unretained solute.

All experiments were run in triplicate, and all statistics were based on a 95% confidence level.

RESULTS AND DISCUSSION

Non-polar stationary phases are suitable for the separation of carboxylic acids with buffered aqueous organic solutions as the mobile phase. Among this class of compounds, we studied the benzoic acids listed in Table I together with their pK_a values¹³.

We studied the relationships between capacity factors and methanol volume fraction, ϕ_0 , ranging from 0 to 0.9, at two pH values of the mobile phase (2.2 and 11). Under the former conditions all acids are completely protonated, whereas under the latter they are completely dissociated.

Figs. 1 and 2 show plots of $\log k'$ vs. mobile phase composition, obtained at pH 2.2. The relationships are linear. In Table II the parameters of the regression

$$\log k' = \log k'_w - S\phi_0 \quad (1)$$

TABLE I
 pK_a VALUES OF BENZOIC ACIDS

Compound	pK_a	Compound	pK_a
Benzoic acid	4.20	2-Methylbenzoic acid	3.91
2-Hydroxybenzoic acid	2.98	3-Methylbenzoic acid	4.27
3-Hydroxybenzoic acid	4.08	4-Methylbenzoic acid	4.38
4-Hydroxybenzoic acid	4.58	2-Chlorobenzoic acid	2.92
2-Aminobenzoic acid	4.79	3-Chlorobenzoic acid	3.82
3-Aminobenzoic acid	4.72	4-Chlorobenzoic acid	3.99
4-Aminobenzoic acid	4.85	2-Methoxybenzoic acid	4.09
2-Nitrobenzoic acid	2.17	3-Methoxybenzoic acid	4.09
3-Nitrobenzoic acid	3.49	4-Methoxybenzoic acid	4.48
4-Nitrobenzoic acid	3.44		

according to Snyder *et al.*¹⁴, are reported. Schoenmakers *et al.*¹⁰ proposed the use of a quadratic expression, in accordance with their finding of a non-linear behaviour of a large number of solutes on a C_{18} column. We therefore also investigated whether eqn. 1 should be replaced with a quadratic relationship between $\log k'$ and ϕ_0 , but no marked improvement was found in the fit of retention factor–composition data.

A larger data dispersion was sometimes observed at large volume contents of methanol. It should be borne in mind that under such conditions the pH of the aqueous–organic solvents can differ significantly from that of neat aqueous solutions^{15–17}. Clearly, this difference gives a systematic error that may be relevant to the retention if the pH is near the pK_a value; a correction factor should be utilized when using ionization control for separation.

It can be seen from the data in Table II that the elution order of benzoic acids is not dependent on solute acidity; there is a weak “*ortho* effect” for amino and hydroxy acids, whereas for other solutes *meta* or *para* acids are more retained.

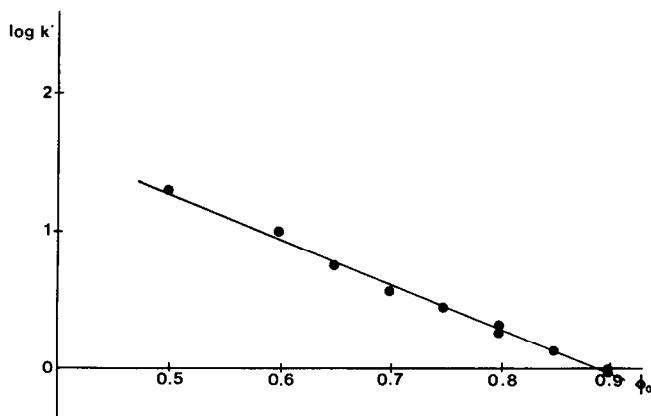


Fig. 1. Plot of $\log k'$ vs. ϕ_0 for benzoic acid on a PRP-1 column. Mobile phase, water–methanol (pH 2.2).

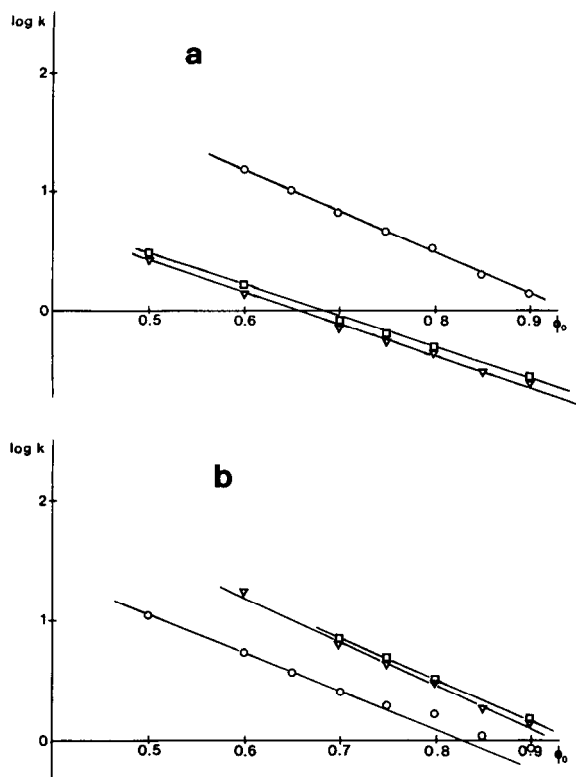


Fig. 2. Plot of $\log k'$ vs. ϕ_0 for (a) hydroxybenzoic acids and (b) methoxybenzoic acids on a PRP-1 column. Mobile phase, water-methanol (pH 2.2). \circ , *ortho*; \square , *meta*; ∇ , *para*.

In Fig. 3 plots of $\log k'$ vs. methanol content at pH 11 are shown for some acids. The retention decreases as all solutes which are completely dissociated have a minor affinity for the stationary phase. However, at such a basic pH, it is difficult to fit the data with either linear or quadratic equations, probably on account of the small k' values. Acids that show a linear trend are listed in Table III; regression parameters are also given.

It is known that retention can be affected by ionic strength; such an effect is more pronounced for ionized solutes⁹, and at pH 11 it cannot be neglected. In Table IV capacity factors (k') obtained at constant ionic strength ($I = 0.1$ with sodium sulphate) are compared with those previously obtained. It can be seen that the k' values at constant ionic strength are slightly larger, according to theory^{9,18,19}, but no marked improvement was found in the fit of the retention data vs. mobile phase composition. Hence completely ionized benzoic acids do not allow the column to work under the best operating conditions.

The effect of the pH of the mobile phase on retention was evaluated for some acids and the results are shown in Figs. 4 and 5. The equation relating the capacity factor to pH is¹⁹

$$k' = k_0 / (1 + K_a / [H^+]) + k_{-1} / (1 + [H^+] / K_a) \quad (2)$$

TABLE II

PARAMETERS OF RELATIONSHIP BETWEEN LOGARITHM OF THE RETENTION FACTOR (k') AND VOLUME FRACTION (φ_0) OF METHANOL IN THE MOBILE PHASE AT pH 2.2

$$\text{Log } k' = B - A\varphi_0.$$

Compound	A	B	Correlation coefficient
Benzoic acid	3.3 ± 0.08	2.94 ± 0.05	0.993
2-OH	3.3 ± 0.08	3.16 ± 0.07	0.996
3-OH	2.8 ± 0.1	1.90 ± 0.07	0.993
4-OH	2.7 ± 0.08	1.70 ± 0.06	0.995
2-NH ₂	2.8 ± 0.1	2.40 ± 0.07	0.993
3-NH ₂	2.3 ± 0.3	1.5 ± 0.2	0.948
4-NH ₂	2.4 ± 0.1	1.50 ± 0.08	0.992
2-NO ₂	3.1 ± 0.06	2.50 ± 0.04	0.997
3-NO ₂	3.8 ± 0.2	3.6 ± 0.2	0.988
4-NO ₂	3.5 ± 0.1	3.4 ± 0.1	0.992
2-CH ₃	3.6 ± 0.1	3.4 ± 0.1	0.993
3-CH ₃	4.0 ± 0.2	3.7 ± 0.1	0.993
4-CH ₃	3.5 ± 0.2	3.3 ± 0.1	0.991
2-Cl	3.3 ± 0.1	3.0 ± 0.1	0.988
3-Cl	4.0 ± 0.2	3.8 ± 0.1	0.991
4-Cl	4.0 ± 0.2	3.9 ± 0.2	0.993
2-OCH ₃	2.7 ± 0.1	2.30 ± 0.06	0.995
3-OCH ₃	3.5 ± 0.1	3.3 ± 0.1	0.994
4-OCH ₃	3.6 ± 0.2	3.4 ± 0.1	0.990

where k_0 and k_{-1} are the capacity factors for the undissociated and dissociated form of the weak acid, respectively, and K_a is its ionization constant. The curves in Figs. 4 and 5 are sigmoidal, according to eqn. 2. Although the ionization constants for 3-hydroxybenzoic acid are sufficiently different ($8.7 \cdot 10^{-5}$ and $1.2 \cdot 10^{-10}$)¹⁹, a step-wise k' vs. pH curve is not obtained, while a maximum of retention was found for 2-aminobenzoic acid, suggesting that the intermediate form is not zwitterionic; the same results were obtained by Pietrzyk *et al.*¹⁹ on a column of XAD-2 resin. In Fig. 6, the dependence of k' on the pH of the mobile phase is shown for a laboratory-prepared column of Chromosorb 101, as an example. It can be observed that the retention is lower on Chromosorb 101 than on PRP-1, owing to the lower surface area of Chromosorb 101 (*ca.* 11 m²/g).

In a previous paper³, it was pointed out that the chromatographic behaviour of Chromosorb 101 resembles that of a C₁₈ bonded phase. The comparison between PRP-1, Chromosorb 101 and C₁₈ packings is of interest with respect to acquiring some information about the retention mechanism on polymeric column packings. Such a comparison was made by plotting $\log k'$ vs. $\log k'$ for two classes of compounds, *viz.*, *n*-alkylbenzenes and some substituted phenols, with reference to k'

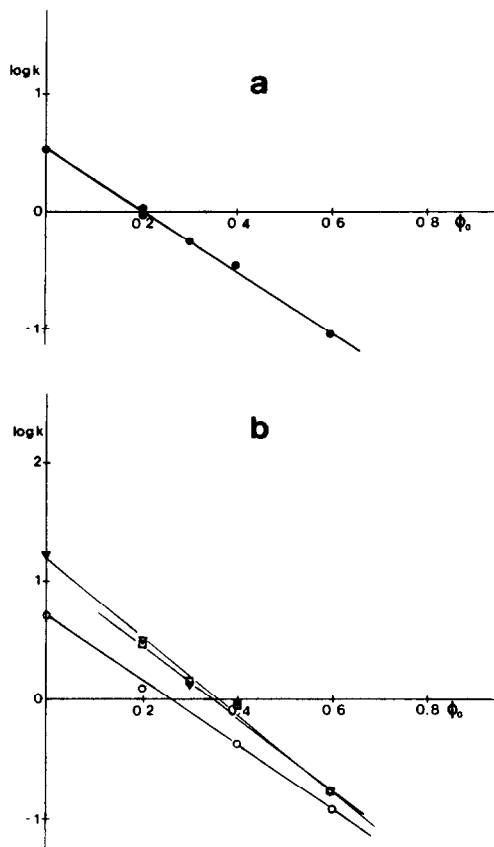


Fig. 3. Plot of $\log k'$ vs. ϕ_0 for (a) benzoic acid and (b) methylbenzoic acids on a PRP-1 column. Mobile phase, water-methanol (pH 11). \circ , *ortho*; \square , *meta*; ∇ , *para*.

values on a C_{18} bonded phase, obtained by Colin and Guiochon²⁰ and Jinno and Kawasaki²¹. Logarithmic plots are shown in Figs. 7 and 8 and regression parameters are given in Table V. According to the method proposed by Melander *et al.*²², the results show that PRP-1 is homoenergetic with C_{18} for the elution of alkylbenzenes whereas it is homeoenergetic for the elution of phenols. Chromosorb 101 displayed the same behaviour³; in particular, the two polymeric columns have similar retention properties, *i.e.*, solvophobic effects could be mainly responsible for solute retention. Plots of $\log k'$ vs. number of carbon atoms (nC) for n -alkylbenzenes on Chromosorb 101 and PRP-1 are reported in Fig. 9 and the regression parameters are given in Table VI. It appears that the increase in retention for a methylene group is similar on the two columns; the interaction for benzene is stronger on PRP-1 owing to its higher surface area.

TABLE III

PARAMETERS OF RELATIONSHIP BETWEEN LOGARITHM OF THE RETENTION FACTOR (k') AND VOLUME FRACTION (ϕ_0) OF METHANOL IN THE MOBILE PHASE AT pH 11

$$\text{Log } k' = B - A\phi_0$$

Compound	A	B	Correlation coefficient
Benzoic acid	2.6 ± 0.07	0.60 ± 0.02	0.996
2-CH ₃	2.7 ± 0.1	0.70 ± 0.04	0.997
3-CH ₃	3.1 ± 0.3	1.1 ± 0.1	0.985
4-CH ₃	3.3 ± 0.2	1.20 ± 0.06	0.995
2-Cl	2.7 ± 0.1	0.60 ± 0.03	0.998
3-Cl	2.9 ± 0.07	1.30 ± 0.03	0.999
4-Cl	3.2 ± 0.2	1.50 ± 0.08	0.992
2-OCH ₃	3.1 ± 0.1	0.51 ± 0.05	0.996
3-OCH ₃	3.2 ± 0.06	0.98 ± 0.02	0.999
4-OCH ₃	3.3 ± 0.2	1.00 ± 0.07	0.993
2-OH	3.2 ± 0.1	1.00 ± 0.04	0.998
3-NO ₂	3.2 ± 0.3	1.2 ± 0.1	0.984

TABLE IV

EFFECT OF IONIC STRENGTH ON THE RETENTION FACTOR

(A) 100% water (pH 11); (B) 20% aqueous methanol (pH 11).

Compound	A		B	
	k'_I	k'	k'_I	k'
Benzoic acid	4.65	3.94	1.14	1.01
2-OH	—	—	2.79	2.45
3-OH	0.09	—	0.03	—
4-OH	—	—	—	—
2-NH ₂	3.67	3.54	0.78	0.65
3-NH ₂	1.02	0.81	0.13	0.13
4-NH ₂	0.38	0.31	0.08	0.07
2-NO ₂	3.59	3.63	0.84	0.69
3-NO ₂	—	—	4.01	3.73
4-NO ₂	—	—	2.11	3.62
2-CH ₃	5.47	5.23	1.42	1.22
3-CH ₃	—	—	3.45	2.92
4-CH ₃	—	—	3.53	3.15
2-Cl	3.93	3.91	1.23	1.02
3-Cl	—	—	—	—
4-Cl	—	—	—	—
2-OCH ₃	3.91	3.23	0.90	0.73
3-OCH ₃	—	—	2.52	2.13
4-OCH ₃	14.0	13.0	2.21	1.98

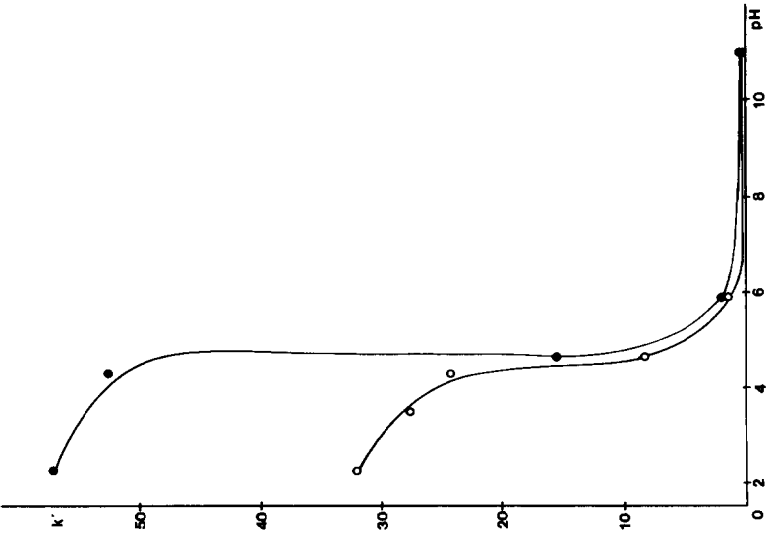


Fig. 4. Plots of k' vs. pH of the mobile phase (60% aqueous methanol) on a PRP-1 column. ●, Benzoic acid; ○, 2-methoxybenzoic acid.

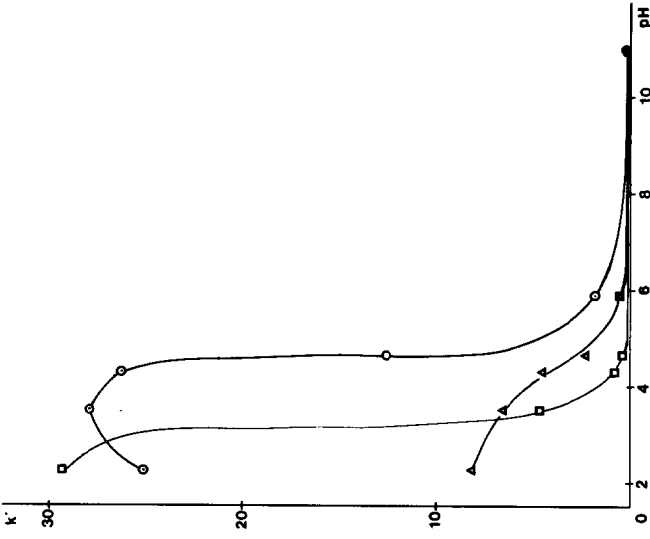


Fig. 5. Plot of $\log k'$ vs. pH of the mobile phase (60% aqueous methanol) on a PRP-1 column. ○, 2-Aminobenzoic acid; □, 2-nitrobenzoic acid; ▽, 3-hydroxybenzoic acid.

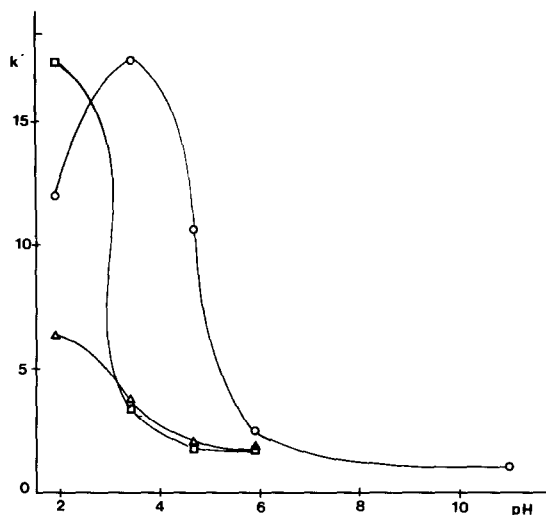


Fig. 6. Plots of k' vs. pH of the mobile phase on a Chromosorb 101 column. Mobile phase, 60% aqueous methanol. Solutes as in Fig. 5.

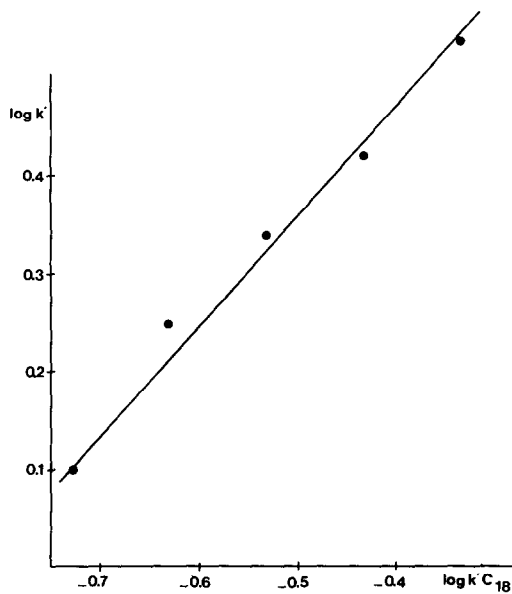


Fig. 7. Plot of $\log k'$ (PRP-1) vs. $\log k'$ (C_{18}) for *n*-alkylbenzenes. Mobile phase, 100% methanol.

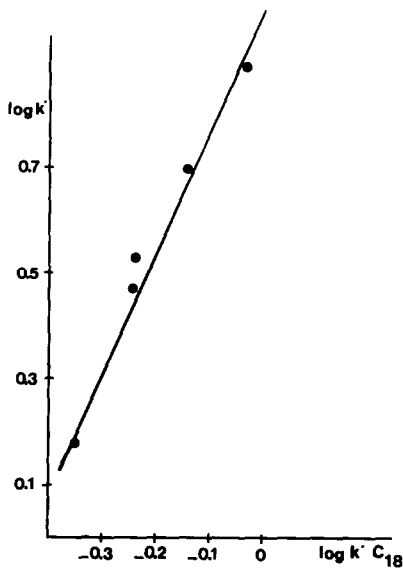


Fig. 8. Plot of $\log k'$ (PRP-1) vs. $\log k'$ (C_{18}) for some chloro- and methylphenols. Mobile phase, 75% aqueous methanol.

TABLE V
LOG k'_{PRP-1} - LOG k'_{C18} REGRESSION PARAMETERS

Compounds	Slope	Intercept	Correlation coefficient
<i>n</i> -Alkylbenzenes*	1.04 ± 0.07	0.88 ± 0.04	0.990
Phenols**	2.2 ± 0.2	0.99 ± 0.04	0.966

* Mobile phase: 100% methanol.

** Mobile phase: 75% aqueous methanol.

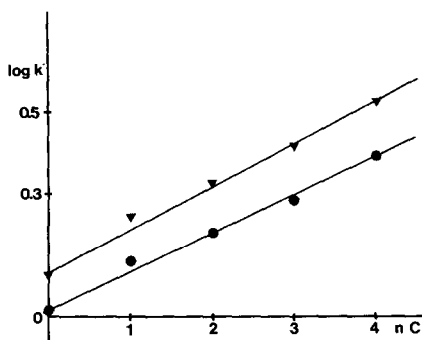


Fig. 9. Plots of $\log k'$ vs. nC for *n*-alkylbenzenes. Mobile phase, 100% methanol. ▼, PRP-1; ●, Chromosorb 101.

TABLE VI
REGRESSION PARAMETERS OF LOG k' vs. nC FOR *n*-ALKYLBENZENES WITH 100% METHANOL AS MOBILE PHASE

Packing	Slope	Intercept	Correlation coefficient
PRP-1	0.104 ± 0.007	0.12 ± 0.02	0.990
Chromosorb-101	0.090 ± 0.006	0.03 ± 0.01	0.990

CONCLUSIONS

The results show that styrene-divinylbenzene column packings, *e.g.*, PRP-1 or Chromosorb 101, can be employed under different pH conditions without compromising the column durability.

Elution of benzoic acids occurs according to the theory of a reversed-phase system, and a linear relationship between $\log k'$ and mobile phase composition was found. This can be useful for the extrapolation and prediction of retention data.

Further, a linear solvent gradient can be used to resolve a complex mixture. Moreover, the selectivity can be enhanced by varying the pH of the mobile phase.

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