

CHROM. 8185

## Note

---

### **Separation characteristics of a porous styrene–divinylbenzene polymer packing for high-pressure liquid chromatography**

HIDEKUNI TAKAHAGI and SETSUYA SENO

*Product Development Laboratories, Sankyo Company, Ltd., 2-58, 1-chome, Shinagawa-ku, Tokyo (Japan)*

(Received January 10th, 1975)

Rigid porous polystyrene–divinylbenzene beads have excellent chemical and physical durability and large surface areas and give good efficiencies in the separation of organic compounds when used as the packing material in high-pressure liquid chromatography (HPLC). This packing has no active functional groups, yet permits the effective separation of various kinds of compounds. The retention properties of the packing with change in the polarity of the mobile phase have been reported previously<sup>1</sup>.

In this paper, the effect of the pH of the mobile phase on the capacity factor is reported. The results will be of help in finding eluting conditions easily and at the same time may suggest a separation mechanism.

#### EXPERIMENTAL

##### *Equipment*

A Perkin-Elmer 1220 liquid chromatograph with a UV detector was used. The column consisted of a 50 cm × 20 mm O.D. × 5 mm I.D. Pyrex glass tube. The sample injection port was described previously<sup>1</sup>. The column temperature was controlled to within  $\pm 0.2^\circ$  by circulating water through the column jacket.

##### *Materials*

The chromatographic packing used was Hitachi Gel 3010, a porous styrene–divinylbenzene copolymer (Hitachi, Tokyo, Japan). The chemicals used as samples and carrier solvents were of reagent grade (Tokyo Kasei Kogyo, Tokyo, Japan, and Wako Pure Chemicals, Tokyo, Japan). No further purification of these chemicals was required.

##### *Preparation of mobile phases*

Various mobile phases with different pHs in 80% methanol (apparent pH) were prepared by using orthophosphoric acid, acetic acid, boric acid, hydrochloric acid and triethylamine. The compositions of the solvent systems and their apparent pHs are shown in Table I.

TABLE I  
MOBILE PHASE SYSTEMS AND THEIR APPARENT pH VALUES

Component	Proportions (ml)					
0.2 N HCl	20	0	0	0	0	0
0.04 N Mixed acids*	0	20	17.4	14.8	11.4	0
0.2 N Triethylamine	0	0	2.6	5.2	8.6	20
Methanol	80	80	80	80	80	80
pH	1.47	3.35	4.85	7.07	9.30	11.20

\*  $\text{H}_3\text{PO}_4 + \text{CH}_3\text{COOH} + \text{H}_3\text{BO}_3$ .

#### Measuring conditions

Throughout the study, the column temperature and the flow-rate of the mobile phases were maintained at 25° and 2.0 ml/min, respectively.  $T_0$ , the time required for the elution of a non-retained compound, which is necessary for the calculation of the capacity factor,  $k'$ , was determined by measuring the retention time of *m*-amino-benzoic acid, which is probably unretained when eluted with the most basic mobile phase (pH 11.20) of the solvent systems. The  $T_0$  value determined here agrees with that reported previously<sup>1</sup>.

#### RESULTS AND DISCUSSION

Hitachi Gel 3010 often does not give good separation efficiencies in chromatography with polar solvents, as the retention forces of the gel tend to increase with increasing polarity of the mobile phase<sup>1</sup>. The use of polar solvents, such as more than 30% of water in methanol, may cause a loss in accuracy in quantitative determinations and a longer analysis time. In this study, therefore, profiles of  $k'$  against pH for acidic and basic compounds on Hitachi Gel 3010 were determined experimentally, the pH of the mobile phases being varied while the methanol:water ratio was maintained at 4:1.

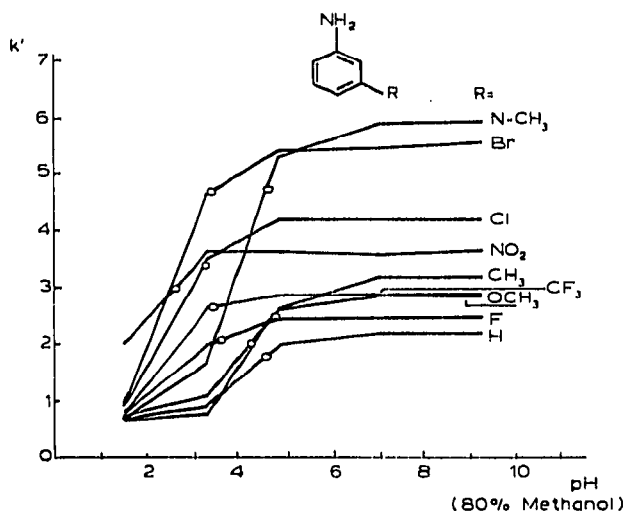


Fig. 1. Effect of pH of the mobile phase (80% methanol) on the capacity factor,  $k'$ , for *meta*-substituted anilines.  $\circ$ ,  $\text{p}K_a$  values.

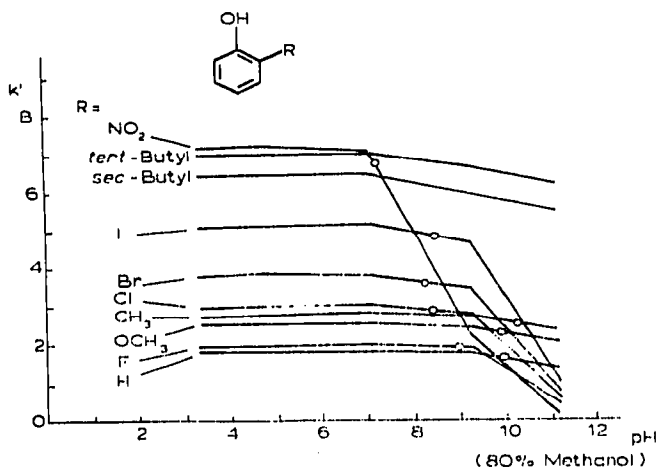


Fig. 2. Effect of pH of the mobile phase (80% methanol) on the capacity factor,  $k'$ , for *ortho*-substituted phenols.  $\circ$ ,  $pK_a$  values.

Figs. 1 and 2 show the  $k'$  values plotted against apparent pH in 80% methanol for various *meta*-substituted anilines and *ortho*-substituted phenols. It can be seen that all of the compounds in ionic forms (anilines in acidic solvents and phenols in basic solvents) showed the least retention and the maximum retentions were obtained with the compounds in their neutral forms. The characteristic features of these graphs are the large breaks that occur in the vicinity of the  $pK_a$  value of each compound.

The capacity factor,  $k'$ , is defined as<sup>2,3</sup>

$$k' = \frac{(\text{volume of solution})(\text{weight of solute on the resin})}{(\text{weight of resin})(\text{weight of solute in solution})} = \frac{T_r - T_0}{T_0} \quad (1)$$

which can be written as

$$k' = \frac{[\text{HA}]_s + [\text{A}^-]_s}{[\text{HA}]_m + [\text{A}^-]_m} \cdot \frac{V_s}{V_m} = \left( \frac{[\text{HA}]_s \cdot \frac{V_s}{V_m}}{[\text{HA}]_m} \right) \left( \frac{1}{1 + K_u/[\text{H}^+]}} \right) + \left( \frac{[\text{A}^-]_s \cdot \frac{V_s}{V_m}}{[\text{A}^-]_m} \right) \left( \frac{1}{1 + [\text{H}^+]/K_u} \right) \quad (2)$$

or

$$k' = \frac{[\text{B}]_s + [\text{HB}^+]_s}{[\text{B}]_m + [\text{HB}^+]_m} \cdot \frac{V_s}{V_m} = \left( \frac{[\text{B}]_s \cdot \frac{V_s}{V_m}}{[\text{B}]_m} \right) \left( \frac{1}{1 + [\text{H}^+]/K_u} \right) + \left( \frac{[\text{HB}^+]_s \cdot \frac{V_s}{V_m}}{[\text{HB}^+]_m} \right) \left( \frac{1}{1 + K_u/[\text{H}^+]}} \right) \quad (3)$$

where  $[\ ]_s$  and  $[\ ]_m$  are the concentrations of the molecular or ionic form of the solutes in the stationary phase and the mobile phase, respectively,  $V_m$  is the total volume of the mobile phase in the column (dead volume or void volume) and  $V_s$  is the volume of the stationary phase (or weight of resin). Eqns. 2 and 3 give the capacity factors for weak organic acids and bases, respectively.

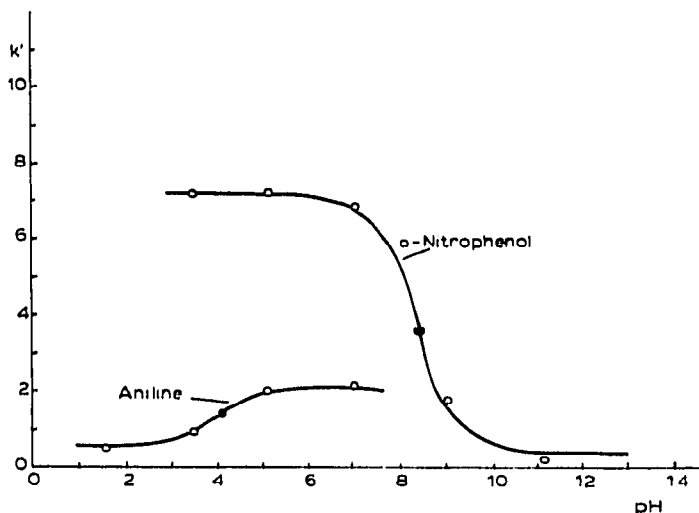


Fig. 3. Capacity factor as a function of pH in 80% methanol. Solid line, calculated curve; ○, experimental values; ●,  $pK_a$  values.

The term  $a$  is approximately equal to the  $k'$  value when most of the solute is in the neutral form and therefore can be determined experimentally in carrier solvents of low pH for acids and in solvents of high pH for bases. The term  $b$  is approximately equal to the  $k'$  value of a solute in an ionic form and can be determined similarly with high pH conditions for acids and low pH conditions for bases when the salt is the major constituent.

The calculated and experimental  $k'$  values for aniline and *o*-nitrophenol as solutes are plotted against apparent pH in Fig. 3. The open circles represent the experimental values and the solid lines were calculated by means of eqns. 2 and 3 based on the experimentally determined  $pK_a$  values in 80% methanol.

The agreement between the experimental and calculated results will enable the  $k'$  value of a sample to be predicted simply by elution with mobile phases with sufficiently higher and lower pH than the  $pK_a$  of the sample.

The retention mechanism between this packing and solute molecules may involve a type of hydrophobic bond or Van der Waals' interactions<sup>1,4-7</sup>. At the same time, however, it might be thought that partition will play a role between the "free" mobile phase and the mobile phase that is held on the surface of the stationary phase, because the  $k'$  values are closely related to the pH of the mobile phase and the  $pK_a$  of the sample, as shown in this paper. Further work is necessary before the separation mechanism can be deduced.

## REFERENCES

- 1 H. Takahagi and S. Seno, *J. Chromatogr. Sci.*, 12 (1974) 507.
- 2 M. D. Grieser and D. J. Pietrzyk, *Anal. Chem.*, 45 (1973) 1348.
- 3 C. Chu and D. J. Pietrzyk, *Anal. Chem.*, 46 (1974) 330.
- 4 R. L. Gustafson, R. L. Albright, J. Heisler, J. A. Lirio and O. T. Reid, Jr., *Ind. Eng. Chem., Prod. Res. Develop.*, 7 (1968) 107.
- 5 D. J. Pietrzyk, *Talanta*, 16 (1969) 169.
- 6 J. J. Paleos, *Colloid Interface Sci.*, 31 (1969) 7.
- 7 A. D. Wilks and D. J. Pietrzyk, *Anal. Chem.*, 44 (1972) 676.