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Note

Column loadability and particle size in preparative liquid chromatography

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In a previous article[1] the specific loadability, defined as the mass of solute per gram of packing material above which the column plate number tends to decrease sharply, was found to depend on the particle size of two different fractions obtained from one batch of silica material. A similar effect was found by Bombaugh and Almquist[2]. An explanation for this dependence was not given, but changes in the silica arising from the grinding and sieving procedures were mentioned as a possible cause. In this paper we present some more experimental results pertaining to the relation between column plate number, particle size and loadability for a given solute and a discussion of this relation.

EXPERIMENTAL

Apparatus

A home-made liquid chromatographic system was used comprising a high capacity pump (V 410; Burdoza, Giessen, G.F.R.), a flow-through Bourdon-type manometer serving also as a pulse dampener and a variable-wavelength UV detector (PM 2 ALC; Zeiss, Oberkochen, G.F.R.). The following columns of stainless-steel 316 tubing were used: 10 cm × 17 mm O.D. × 10.8 mm I.D., honed to a smooth inner surface and filled with 5–8 μm Si 60; 25 cm × 17 mm O.D. × 10.8 mm I.D., honed and filled with 20–25 μm Si 60; 50 cm × 17 mm O.D. × 10.8 mm I.D. and 50 cm × 16 mm O.D. × 10 mm I.D. used as delivered and both filled with 20–25 μm Si 60. The two 50-cm columns were coupled[3] to give a 100-cm column. A central injection system was used on all columns. The bottom terminators for the two 50-cm columns had a 150° top angle conical shape with an embedded 6-μm frit (5 mm diameter), while the bottom terminator for the 10-cm column had a 150° top angle complete conical shape connected to the outlet capillary tubing (the packing material in this case was retained by a plug of PTFE wool). Samples were introduced by means of a sampling valve (70-10; Rheodyne, Berkeley, CA, U.S.A.) fitted with a 50-, 100- or 1000-μl loop. All connections between the valve, the column(s) and the detector consisted of Swagelok 1/16 in. zero dead-volume unions.

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Materials

Acetone, tetrabromoethane and chloroform (E. Merck, Darmstadt, G.F.R.) were used in the column packing procedure. Dichloromethane (Baker, Deventer, The Netherlands) was used as solvent. Silica gel Si 60 (E. Merck) was used as the packing material; it was ground and classified by means of an air classifier (Alpine M.Z.R., Augsburg, G.F.R.) to particle size ranges of 5–8 µm and 20–25 µm. The tested solute was 2,4-dimethylphenol.

Procedures

All columns were filled using a balanced density slurry technique as described previously[1]. Two methods were used for the calculation of the standard deviation of an elution profile: (a) the standard deviation was taken as half of the peak width at 0.607 of the maximum plate height (denoted by the subscript 0.6); (b) the standard deviation was taken as 1/4.29 of the peak width at 0.1 of the maximum peak height (denoted by the subscript 0.1). Column plate numbers were calculated from the retention time of the peak maximum, t_{Ri}, and the standard deviations $\sigma_{0.6}$ and $\sigma_{0.1}$ using:

$$N_{0.6} = (t_{Ri}/\sigma_{0.6})^2 \quad \text{and} \quad N_{0.1} = (t_{Ri}/\sigma_{0.1})^2$$

The $N_{0.1}$ values are given since it is thought[1] that they give a better insight into the separation capabilities of a column for preparative purposes.

RESULTS AND DISCUSSION

The processes involved in the dilution of a large mass of solute from the top to the end of a column are the isotherm non-linearity broadening and the column dispersion. How these two broadening effects interact and add up to give the final profile cannot be described analytically, only by approximate calculation[4] or by simulation. For dispersionless chromatography (infinite plate number) and where the sample is injected as a spike, Huber and Gerritse[6] showed that it is only the solute outlet concentration which determines the final peak shapes

$$t_R(c_{i,m}) = t_{R,0}\left(1 + q\cdot\frac{dc_{i,s}}{dc_{i,m}}\right)$$

where $t_R(c_{i,m})$ is the retention time for the concentration $c_{i,m}$, $c_{i,m}$ and $c_{i,s}$ are the concentrations of solute i in the mobile and the stationary phase respectively, $t_{R,0}$ is the retention time of an unretained component, q is the phase ratio, and $dc_{i,s}/dc_{i,m}$ is the derivative of the isotherm at the mobile phase concentration $c_{i,m}$. For other input profiles in dispersionless chromatography this relation still holds[5]. Also for real chromatography (finite plate number) it can be shown[7] that different input profiles (*e.g.*, variation of injection volume, but avoiding volume overload) result in identical elution profiles. So it is understood that it is only the outlet concentration which determines the magnitude of the broadening due to isotherm non-linearity (see also ref. 8).

When loadability studies are carried out by increasing the mass of a solute in a constant injection volume, one is searching for the mass of solute which yields the maximum allowable increase of peak width over the normally observed peak width for a very low mass of solute (and consequently the maximum allowable loss of resolution). Above this mass, the isotherm non-linearity broadening starts to dominate the column dispersive broadening, giving rise to a sharp decrease in column efficiency for higher loading. This effect, however, is in itself related to concentration, rather than to mass. When the isotherm non-linearity broadening is not operative (*e.g.*, for low loading), it is the column dispersion which determines the maximum concentration of the elution profile. Consequently, the column dispersion determines the loading above which the isotherm non-linearity broadening becomes effective, as a specific maximum outlet concentration may not be surpassed: for columns of the same size, a greater mass of solute must be introduced into a column with a low plate number than into a column with a high plate number.

A further "advantage" of the higher dispersion in a column of low plate number is the effect of a high sample load on the peak shape. A deviation in the $dc_{i,s}/dc_{i,m}$ value from the infinite dilution value will occur when a specific (high) outlet concentration is obtained. This deviation can severely affect the peak shape on a column of high plate number while only a small change in peak shape may occur on one of low plate number.

Following this reasoning we can now understand:

(a) Why columns of different plate numbers, filled with the same amount of packing material, must have quite different specific loadabilities. This is shown in Fig. 4 in ref. 1: for columns of the same size, but different particle size, different specific loadabilities are found.

(b) Why for columns of different tube size and different particle size but equivalent plate number, the same specific loadability should be found. This aspect is shown in Fig. 1A and B, where two different columns with approximately the same plate number are compared. Indeed, the specific loadabilities for the packing materials (5–8 *µ*m and 20–25 *µ*m fractions from one batch of silica Si 60 material) are

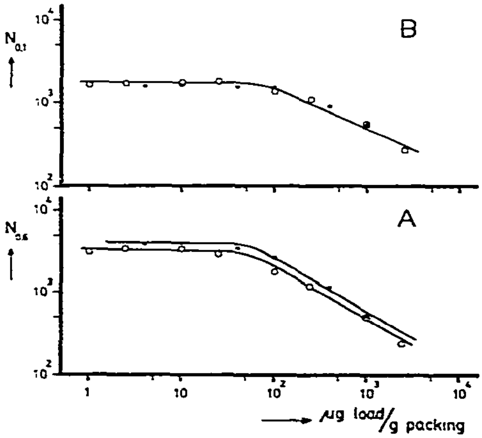
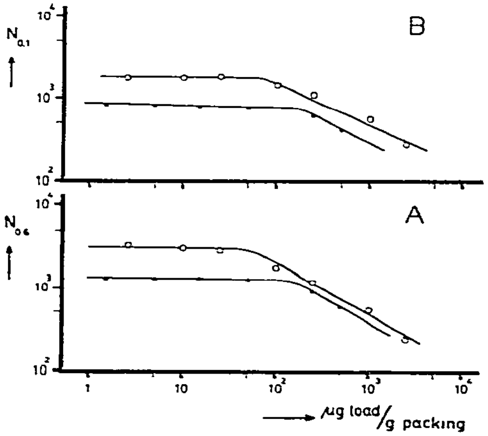


Fig. 1. Plots of column plate number $N_{0.6}$ (A) and $N_{0.1}$ (B) *versus* specific loading for 2,4-dimethylphenol (capacity factor, $\kappa = 3$) on columns of approximately equal plate numbers. Solvent: dichloromethane. Columns: ○, 100 cm (50 cm × 10.8 mm I.D. + 50 cm × 10 mm I.D.), 39.5 g of 20–25 *µ*m Si 60; ●, 10 cm × 10.8 mm I.D., 4.9 g of 5–8 *µ*m Si 60.

equivalent. This aspect was also reported by Wehrli[9], but the columns compared by him have quite different plate numbers for low solute loadings, and also no comparison was made on the basis of the amount of packing material in the columns.

Fig. 2A and B demonstrate the different specific loadabilities found for 2,4-dimethylphenol on the 20–25 μm silica, when different column sizes, and consequently different plate numbers, are used. Different solvents were also used in these two columns, but the capacity factor was approximately the same for 2,4-dimethylphenol. It is clearly useless to ascribe a specific loadability value to a column packing material if the plate number is not specified. Therefore, in Fig. 3A and B the observed column plate numbers are plotted against the total column loading. It is seen that the loadability increases with decreasing plate number on a given column. Although no other columns and packing materials have been tested in this respect, it appears possible, that a loadability borderline can be drawn for each column, into which all the different lines merge. This borderline represents the situation where column dispersion no longer plays the major rôle: the elution profile is determined by isotherm non-linearity broadening. The loadability borderline is entirely determined by the distribution isotherm of the solute in the phase system. Thus, the effect of the loading on the separation efficiency can be visualized from a graph of the observed plate number, *N*, *versus* the specific loading.



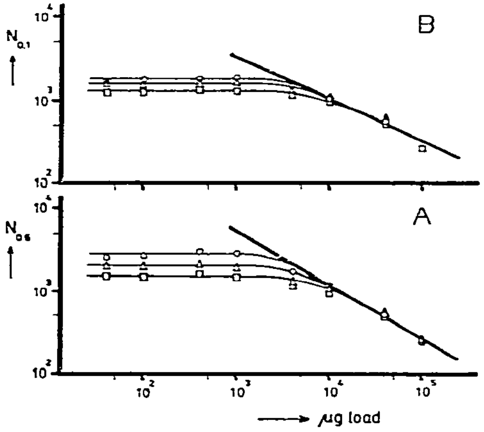


Fig. 2. Plots as in Fig. 1 but for columns of different plate numbers. Columns and phase systems: ○, 100 cm (50 cm × 10.8 mm I.D. + 50 cm × 10 mm I.D.), 39.5 g of 20–25 μm Si 60, solvent, dichloromethane; ●, 25 cm × 10.8 mm I.D., 9.6 g of 20–25 μm Si 60, 1% butanol in 2,2,4-trimethylpentane as solvent (see ref. 1).

Fig. 3. Plots of column plate number $N_{0.6}$ (A) and $N_{0.1}$ (B) *versus* loading for 2,4-dimethylphenol ($\kappa = 3$) using different solvent flow-rates. Column and phase system: 100 cm (50 cm × 10.8 mm I.D. + 50 cm × 10 mm I.D.); 20–25 μm Si 60; solvent, dichloromethane. Linear velocities: ○, 1.8 mm/sec; △, 5.5 mm/sec and □, 8.4 mm/sec.

In Fig. 4A and B the specific loadability results are compiled for the above 10- and 100-cm columns. Such a graph, made for various columns of different plate numbers, again shows an envelope or borderline, which characterizes the particular combination of solute and phase system. For an actual separation, where a certain plate number is required, one can never use a specific loading higher than the value indicated by the borderline at the point corresponding to this required plate number. Therefore, such a loadability borderline for a given solute and a given phase system gives an indication of the preparative utility of the phase system and may be a helpful guide to the choice of column and flow-rate. The borderline is in principle predictable from the distribution isotherm of the solute.

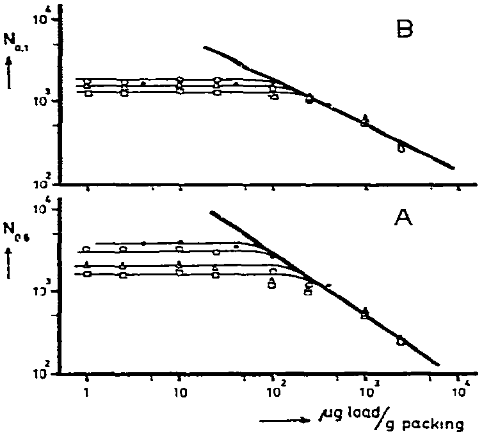


Fig. 4. Plots as in Fig. 1 for the phase system silica Si 60 (E. Merck)–dichloromethane. Columns and linear velocities: ●, 10 cm × 10.8 mm I.D., 5–8 µm, 2.1 mm/sec; ○, △, □, 100 cm × ±10 mm I.D., 20–25 µm, 1.8 mm/sec (○), 5.5 mm/sec (△) and 8.4 mm/sec (□).

CONCLUSIONS

The reporting of specific loadabilities for a solute on a given packing material must be accompanied by specification with respect to the column and particle size and/or column plate number (*i.e.*, the operating conditions).

A borderline, observed in graphs of the observed plate number, *N*, *versus* the specific loading, is a useful aid to selecting optimum conditions in preparative liquid chromatography.

The higher loadabilities found for coarser particles, reflected in a relative increase in plate height (as compared to the loadability for smaller particles in the same column), are a necessary result of this testing procedure and have nothing to do with the different distribution parameters found for the coarser particles.

REFERENCES

1 A. W. J. de Jong, H. Poppe and J. C. Kraak, *J. Chromatogr.*, 148 (1978) 127.
2 K. J. Bombaugh and P. W. Almquist, *Chromatographia*, 8 (1975) 109.
3 A. W. J. de Jong, *Thesis*, University of Amsterdam, Amsterdam, 1980.
4 T. S. Buys and K. de Clerk, *J. Chromatogr.*, 67 (1972) 1.
5 J. C. Smit, H. C. Smit and E. M. de Jager, *Anal. Chim. Acta*, 122 (1980) 1.
6 J. F. K. Huber and R. G. Gerritse, *J. Chromatogr.*, 58 (1971) 137.
7 A. W. J. de Jong, J. C. Smit, H. Poppe and J. C. Kraak, *Anal. Proc.*, 17 (1980) 508.
8 A. W. J. de Jong, J. C. Kraak, H. Poppe and F. Nooitgedacht, *J. Chromatogr.*, 193 (1980) 181.
9 A. Wehrli, *Z. Anal. Chem.*, 277 (1975) 289.