CONTRIBUTION TO THE CHOICE OF OPTIMAL GEOMETRIC CONDITIONS FOR PREPARATIVE LIQUID CHROMATOGRAPHY

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SUMMARY

Column technology for preparative liquid chromatography is discussed. The choice of the optimal particle size is considered. A derivation is presented that shows that operation in the rising part of the plate height curve results in an improved throughput when the plate number and pressure drop are specified.

The influence of the injection mode on the preparative performance is also discussed. Experimental results pertaining to three injection modes are given. A proposed flow-surrounded conical disperser system was found to be superior for difficult separations.

INTRODUCTION

General interest in preparative column liquid chromatography has increased remarkably since the introduction of columns with highly improved performance, brought about by the application of totally porous microparticulate material and suitable packing techniques. The success of modern column technology in the analytical field may lead to its exploitation for preparative purposes, as the columns concerned have high efficiencies and high specific loading capacity (amount of sample per unit amount of packing).

Although the choice of optimal conditions in analytical liquid chromatography is now clear, as a result of both theoretical considerations and practical experience, we feel that for preparative applications a number of questions remain.

Bombaugh and Almquist[1] and Wehrli[2] introduced the throughput as the main performance parameter for a preparative liquid chromatography system (the amount of pure material collected per unit time). The factors that determine the maximum obtainable throughput are:

(a) Geometric and kinetic parameters, such as column and particle dimensions, injection mode and velocity of the eluent. These aspects are more or less universal.

(b) The composition of the original mixture and the requirements with respect to yield and purity of the product. These aspects are specific to the problem at hand.

(c) Parameters of the phase system chosen, such as the selectivity factor and

the usable range of the distribution isotherm. These aspects are specific to the compounds in the mixture and the phase system chosen.

The factors mentioned under (b) and (c) were discussed by Wehrli[2] and Wehrli et al.[3]. They showed that high selectivity factors, when suitably exploited, can increase the throughput considerably. Selectivity adjustment is therefore of great value.

Difficult situations arise, however, if one has to separate a complex mixture on a preparative scale and there is no possibility of influencing the selectivity or, as usually occurs with complex mixtures, the selectivity for one pair can be improved only at the expense of the selectivity for another pair. High numbers of theoretical plates are then necessary in order to avoid contamination of products with closely eluting compounds. Improvement of the throughput in those instances can be obtained only by adjusting the general geometric and kinetic factors, taking into account such limitations as the available pressure drop, the required plate number and the maximal cross-section of the column that which is practically feasible.

A number of workers have considered these general factors: the column diameter and efficiency were studied by Wolf[4], Godbille and Devaux[5,6] and Wehrli[2]; the effect of particle size was discussed by Wehrli[2] and Scott and Kucera[7]; and injection mode and recycling were studied by Wehrli[2] and Conroe[8].

The aim of this study was to investigate further the effect of two factors, the particle size and the injection mode, on the performance with respect to efficiency, loadability and throughput, because we felt that existing discussions in the literature left a number of crucial questions unanswered or reached conflicting conclusions. Particular attention was paid to the effect of peak tailing, caused by irregularities in the packing at the column wall, (local) overloading or the injection profile, as this effect often diminishes the throughput obtainable at a specified purity of the product.

The work was carried out using a normal silica adsorption system, as this is the most popular for preparative work.

THEORETICAL

Of decisive importance in both production-scale and analytical work is the amount per unit time. It may seem that in semi-preparative applications, such as structure elucidation, it is the amount per load which is the important parameter, but the time required to process one load determines how many samples can be treated per day or per week, so that the ultimate characteristic is the same in both cases instances, i.e., the throughput (number of grams of material of a specified purity obtained per second).

An optimal choice of conditions has to be made with respect to the phase system and phase ratio, column dimensions, particle diameter, specific load (grams of material per gram of column packing) on the column and volume of the sample. Whatever phase system is chosen, it will have a limited linear range in the distribution isotherm; the concentration in the mobile phase corresponding to a specified deviation from this linearity will be denoted by $c_{l,m}^{max}$. The concentration of the eluate must certainly be less than $c_{l,m}^{max}$, as the solution in the first part of the column can only be more concentrated. An upper limit to the eluting concentration, $\alpha c_{l,m}^{max}$, α being dependent on the dispersive processes within the column, and having a value between 0 and 1, will exist.
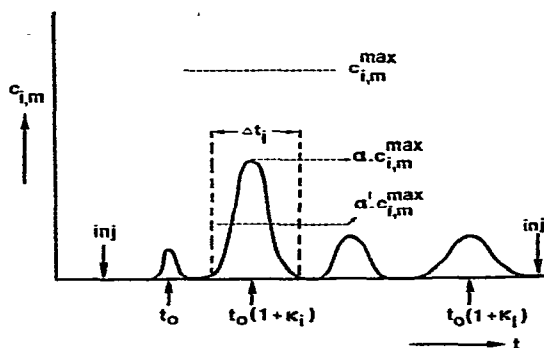
Fig. 1. Chromatogram, collection and injection interval.

At the time of elution of the peak maximum, we will obtain a momentary production rate of $wac_{i,m}^{max}$. However, averaged over the peak collection time of Δt_i sec, for compound i, the production rate, T_i', will be (see Fig. 1):

$$T_i' = \frac{wa \cdot \int_{\Delta t} \frac{c_{i,m}}{ac_{i,m}^{max}} \cdot dt}{\Delta t_i} \cdot c_{i,m}^{max} \tag{1}$$

For a gaussian peak and a collection time corresponding to 4 σ_t (95% yield), this "peak-averaged" production rate would be

$$T_i' = \frac{\sqrt{2\pi}}{4} \cdot wa \cdot 0.95 \cdot c_{i,m}^{max} \tag{2}$$

The production rate, averaged over a long period, will depend on the injection frequency. For a complicated mixture, which completely fills the chromatogram, an injection is possible every $t_0 (1 + \kappa_l)$ sec, where t_0 is the unretained retention time and κ_l is the capacity ratio of the last eluting compound. In that case, the "long-time-averaged" production rate or throughput will be

$$T_i = \frac{a \cdot \int_{\Delta t_i} \frac{c_{i,m}}{ac_{i,m}^{max}} \cdot dt}{t_0 (1 + \kappa_l)} \cdot wc_{i,m}^{max} \tag{3}$$

The integral expression is a time, which in general will not deviate more than a factor of two or three from Δt_i itself.

If a gaussian time distribution is observed, this results in

$$T_i = \frac{a \sqrt{2\pi} \cdot 0.95 \cdot \sigma_{t,i}}{t_0 (1 + \kappa_l)} \cdot wc_{i,m}^{max} = \frac{\sqrt{2\pi}}{\sqrt{N}} \cdot 0.95 \cdot a \cdot \frac{1 + \kappa_i}{1 + \kappa_l} \cdot wc_{i,m}^{max} \tag{4}$$

where N is the number of theoretical plates. In both equations, the important product $wc_{i,m}^{max}$ plays the dominant role; the other factors are either fixed numerical values,

such as $\sqrt{2\pi}$, or are dictated by the phase system and mixture at hand (α, κ_i and κ_1 for instance). We now include numerical factors such as $\sqrt{2\pi}$ and 0.95 in α and obtain

$$T_i = \alpha'N^{-\frac{1}{2}} \cdot \frac{(1 + \kappa_i)}{(1 + \kappa_i)} \cdot wc_{i,m}^{max} \tag{5}$$

For simple mixtures, the sample injection repetition frequency can be higher, as pointed out by a number of workers[2,8,9]. The simplest case is that of a binary mixture, in which a sample can be injected every $\Delta t_i + \Delta t_j$. For a gaussian peak shape, this results in

$$T_i = \frac{\alpha \cdot \int\limits_{\Delta t_i} \frac{c_{i,m}}{\alpha c_{i,m}^{max}} \cdot dt}{\Delta t_i + \Delta t_j} \cdot wc_{i,m}^{max} = \alpha' \cdot \frac{(1 + \kappa_i)}{(2 + \kappa_i + \kappa_j)} \cdot wc_{i,m}^{max} \tag{6}$$

which shows that in that case the required plate number has no influence.

The aim of optimization in preparative liquid chromatography, a maximum production rate or throughput, can now be simplified to maximum $c_{i,m}^{max}$ and w. The constraints within which we must realize this are, of course, set by the specified purity of the product; there should be a possibility of choosing the Δt_i interval for the collection of compound i in such a way that the compound produced is of sufficient purity and with expensive products, in an acceptable yield. Optimization towards large w values should, of course, be effected while observing this constraint.

The allowable width or the collection interval, Δt_i, while specifying the yield or purity, is determined by (a) peak broadening due to dispersive effects in the column, in the simplest and mostly used description fully characterized by the plate number; (b) peak broadening due to non-linearity of the distribution isotherm; and (c) external peak broadening, particularly the volume and injection profile of the feed solution. The second effect will not be discussed here, mainly because a general quantitative description of the effect of this phenomenon, when operating together with dispersion and external broadening, is not available. In fact, we already took this effect into consideration in a crude manner while assuming a $c_{i,m}^{max}$ value that should not be surpassed. This crude and somewhat arbitrary simplification may raise some doubts, but can be made plausible in the following way.

In the absence of dispersion and external broadening effects, the shape of the elution curve can be completely described by the distribution isotherm:

$$t_{elution}(c_{i,m}) = t_0 \left(1 + q \cdot \frac{dc_{i,s}}{dc_{i,m}}\right) \tag{7}$$

where q is the phase ratio. With two or more compounds chromatographed together, the situation is more complicated, because different solutes will influence each other's distribution behaviour, and without careful detailed study nothing can be said about the elution function that can be expected. However, also in this instance a $c_{i,m}^{max}$ value will also undoubtedly exist; when we operate the column with ever-increasing loads of i and j, there will be a case where the $q \cdot (dc_{i,s}/dc_{i,m})$ value of one compound will be equal to the infinite dilution value of another one.

With the combined effects of non-linearity and dispersion, the $c_{i.m}^{max}$ value will be lower, but the extent cannot be calculated with theories available at present, and this in any event will require detailed knowledge of the distribution isotherms of i and j in the presence of each other. A liberal choice of a high $c_{i.m}^{max}$ value would require a high plate number and low injection volumes. Nevertheless, for the present discussion, which aims at the elucidation of the role of dispersion and external volumes, we can take $c_{i.m}^{max}$ as chosen, and consider its effect in the throughput via eqn. 1 and its effect on the maximum allowable other dispersive effect as given a priori.

Whatever the concentration load on a column may be, a certain plate number will be required in order to obtain sufficient purity. On the other hand, a certain maximum pressure will be given. With reasoning similar to that presented by Knox and Saleem[10] and Kraak et al.[11], we have

$$T_i = \alpha' \, N^{-\frac{1}{2}} \cdot \frac{1 + \kappa_i}{1 + \kappa_i} \cdot wc_{i.m}^{max} = \alpha' \, N^{-\frac{1}{2}} \cdot \frac{1 + \kappa_i}{1 + \kappa_i} \cdot \frac{vD}{d_p} \cdot \varepsilon A c_{i.m}^{max} \tag{8}$$

where $v = vd_p/D$, D is the diffusion coefficient, v is the migration velocity of the mobile phase, d_p is the particle diameter, ε is the porosity of the bed with respect to the mobile phase, and A is the area of the cross section of the column.
On the other hand, we have a pressure limitation, giving

$$\Delta p = \varphi \cdot \frac{vL\eta}{d_p^2} = \varphi \, D \, \eta \, d_p^{-2} \, N \, hv \tag{9}$$

where Δp is the pressure drop, φ is the shape factor of permeability, L is the column length, η is the viscosity of the mobile phase, $h = H/d_p$, and H is the plate height. Solving for d_p:

$$d_p = \varphi^{+\frac{1}{2}} \, D^{\frac{1}{2}} \, \eta^{\frac{1}{2}} \, \Delta p^{-\frac{1}{2}} \, N^{\frac{1}{2}} \, h^{\frac{1}{2}} \, v^{\frac{1}{2}} \tag{10}$$

Substituting into eqn. 8, we obtain

$$T_i = \alpha' \varphi^{-\frac{1}{2}} \, D^{\frac{1}{2}} \, \eta^{-\frac{1}{2}} \, \Delta p^{\frac{1}{2}} \, N^{-1} \left(\frac{v}{h}\right)^{\frac{1}{2}} \varepsilon A \, c_{i.m}^{max} \cdot \frac{1 + \kappa_i}{1 + \kappa_i} \tag{11}$$

Thus, while adapting d_p and L to the required plate number and the pressure limitation, T_i will be dependent on a number of physical parameters, which we can regard as constants, as soon as the phase system and column diameter are chosen, but proportional to the expression $v^{\frac{1}{2}}/h^{\frac{1}{2}}$, giving the effect of the choice of the operating point in the plate height curve.

Clearly, we must work under conditions such that v/h is as high as possible. All liquid chromatography dispersion theories give $h(v)$ expressions which for large changes of v can be approximated by

$$h = 2 \, \alpha/v + \beta + \gamma v$$

The throughput, T_i, is therefore expressed by a proportionality as follows:

$$T_i \propto \frac{v^{\frac{1}{2}}}{(2 \, \alpha/v + \beta + \gamma v)^{\frac{1}{2}}} = t(v)$$

Fig. 2 gives $t(v)$ as a function of v, for $\alpha = 0.75$, $\beta = 3$ and $\gamma = 0.03$. Optimal kinetic conditions for preparative liquid chromatography are therefore obtained on the ascending branch of the H/v curve, in the region where the major part of the plate height is caused by intra-particle mass transfer. This suggests particle sizes in the range 30–100 μm, plate heights of millimetres and column lengths of several metres. In analytical columns, this leads to long retention times, as is universally known. In preparative liquid chromatography this drawback is more than compensated for by an increase in the allowable sample load, keeping the concentration of the solute constant.
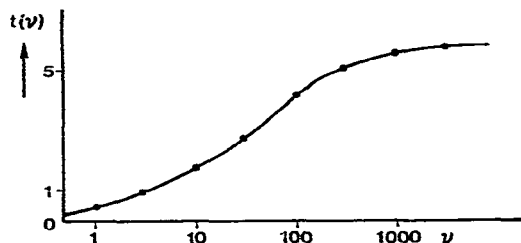

Fig. 2. Dependence of the factor $t(v) = (v/h)^{\frac{1}{3}}$ on the reduced velocity, v. Assumed parameters for the plate height curve: $\alpha = 0.75$, $\beta = 3$ and $\gamma = 0.03$.

A similar discussion to that presented above was given by Scott and Kucera[7]. However, they assumed *a priori* that the working point is in the ascending part of the H/v curve, and therefore did not compare this point with other points nearer to the minimum in the H/v curve. Some results of their theoretical discussion do not agree with ours, *e.g.*, the effect of the phase ratio and the effect of using a longer column with increased particle sizes. In our treatment, "solvent economy" is not affected by this process, as $c_{l,m}^{max}$ remains the same.

From eqn. 11, it can be seen that the cross-sectional area of the column has a direct proportional influence on the throughput. The above discussion, given for a fixed value of this area, therefore may seem useless, as an increase in the throughput can always be obtained by simply increasing the diameter of the column. The efficiency will be maintained because column performances in this respect do not change provided that the linear velocity is not changed[2,12,13]. The following limitations exist, however, for increase in column diameter:

· (a) the realization of sufficient mechanical strength of the column, necessary for operating at high pressures, becomes increasingly difficult;

(b) heat dissipated within the column by friction cannot be conducted to the walls in columns with large cross-sections. Wider columns, especially when filled with small particles, are practically adiabatic.

EXPERIMENTAL

Apparatus

The experiments were carried out on a home-made liquid chromatograph constructed from a double-headed reciprocating pump (Orlita Type AE-10-4.4), a flow-through Bourdon-type manometer serving as a pulse damper and a variable-wavelength UV detector (Zeiss PM 4). The columns had a length of 25 cm and were

made of stainless-steel 316 tubing of I.D. 10–20 mm and O.D. 16–36 mm. The inside surfaces of the tubes were smoothed by honing. Three types of column-top terminators were used in combination with a sampling valve (Valco HPSV CV-6 UHPa) for introduction of the sample. In one injection mode (FSCD) a second pump (Orlita Type ZB-De) was used for feeding the injection flow. The column-end terminators had a conical shape with a top angle of 150°; a disc of PTFE wool prevented transport of packing material into the outlet tubing. All connections between the different parts of the chromatograph were made of 0.3–0.5-mm I.D. capillary tubing and 1/16-in. zero dead volume Swagelock connectors.

Materials

The materials used were 2,2,4-trimethylpentane, acetone, tetrabromoethane, chloroform, butanol-1 (Merck, Darmstadt, G.F.R.), silica gel SI 60 (Merck), ground and classified in particle size (d_p) ranges of 5–8 and 20–25 μm by means of an air classifier (Alpine MZR, Augsburg, G.F.R.). The test solutes were toluene, *o*-nitrotoluene and 2,4-dimethylphenol.

Procedures

The columns were filled by a balanced-density slurry technique. About 20% (w/w) of silica slurry in a mixture of chloroform and tetrabromoethane of sp. gr. 2.10 was placed into the metal stock tube (20 mm I.D., 100 cm length, wall thickness of 8 mm) to which the column was connected. The slurry was pumped into the column at a very high fluid velocity with a high-pressure, high-capacity pump (Burdoza, Giessen, G.F.R., Type V410) with 2,2,4-trimethylpentane at an ultimate pressure of 800 bar. After packing, the columns were washed with several column volumes of acetone and finally eluted with the eluent (trimethylpentane containing 1% of butanol-1) until the capacity ratios became constant.

For the calculation of the column efficiencies two methods for measuring the standard deviation were used: (i) half of the peak width at 0.607 of the maximal peak height (denoted by $H_{0.6}$ and $N_{0.6}$) and (ii) the peak width at 0.1 of the maximal height divided by 4.3 (denoted by $H_{0.1}$ and $N_{0.1}$).

In the loadability experiments the wavelength was adapted to the amount of injected sample in such a way that a linear response was obtained.

RESULTS AND DISCUSSION

Sample introduction

As was shown by Wehrli[2], the method of sample introduction significantly influences the column loadability. He showed that for large-diameter columns the loadability increases if the sample is simultaneously injected at different points on the cross-section of the column. In order to measure the effect of the method of sample introduction more extensively, three injection modes were tested with respect to band spreading, peak symmetry and column loadability. In all three modes a sample valve was used in combination with different column-top terminators (Fig. 3).

The in-column syringe injection as used by Knox and Parcher[14], creating conditions for the so-called infinite-diameter column, was omitted from the experiments because a lower loadability can be expected as only a fraction of the total bed is used by the sample. This simplest way of introducing the sample is shown in Fig.
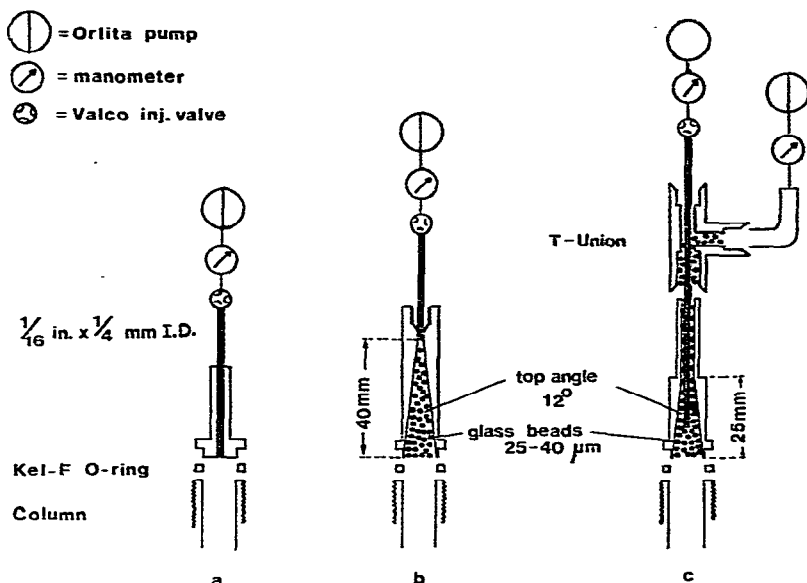
Fig. 3. Schematic representation of three column-top terminators.

3a and involves a spot injection in the middle of the cross-section of the column top. Although column efficiencies were found to be in close agreement with the values expected theoretically, all compounds investigated appear as peaks with some tailing in the 5–10% peak height range. The asymmetry factor, defined as the ratio of the peak width at 0.1 of the peak height of the front and back of the peak, decreases with increasing fluid velocity (Fig. 4). The asymmetry can be caused by a number of effects. Firstly, as a result of the forced stream lines in the first part of the column packing some stagnant mobile phase areas exist (*i.e.*, dead corners). During the injection, mass transport from the moving eluent to these stagnant areas occurs by diffusion or (perhaps) by reflection of the injection plug against the column packing as a result of the jet stream-like injection induced by the interruption of the flow during the switching of the valve. The release of the compounds from the stagnant areas into the moving eluent is slow as it is a diffusion-controlled process. This consequently leads to an asymmetric injection profile and to tailing peaks. At very low and high flow-rates, however, the asymmetry caused by the diffusion of the compounds into the stagnant areas must be small, as at low flow-rates the mass transport by diffusion is large enough to obtain equilibrium while at very high flow-rates the injection plug moves so fast along the stagnant areas that no significant mass transfer can occur.

The second reason for peak tailing might be the irregularities in the packing near the wall (*i.e.*, the so-called wall effect), which causes differences in flow-rates across the column. Another reason, column overloading, could be excluded in our experiments as no significant overloading occurred by injection the same amount of sample into an analytical column.

To avoid stagnant eluent areas, a conical column top, filled with glass beads, was installed between the column packing and injection valve (Fig. 3b). The glass-bead filled conical top first prevents the occurrence of stagnant eluent areas and
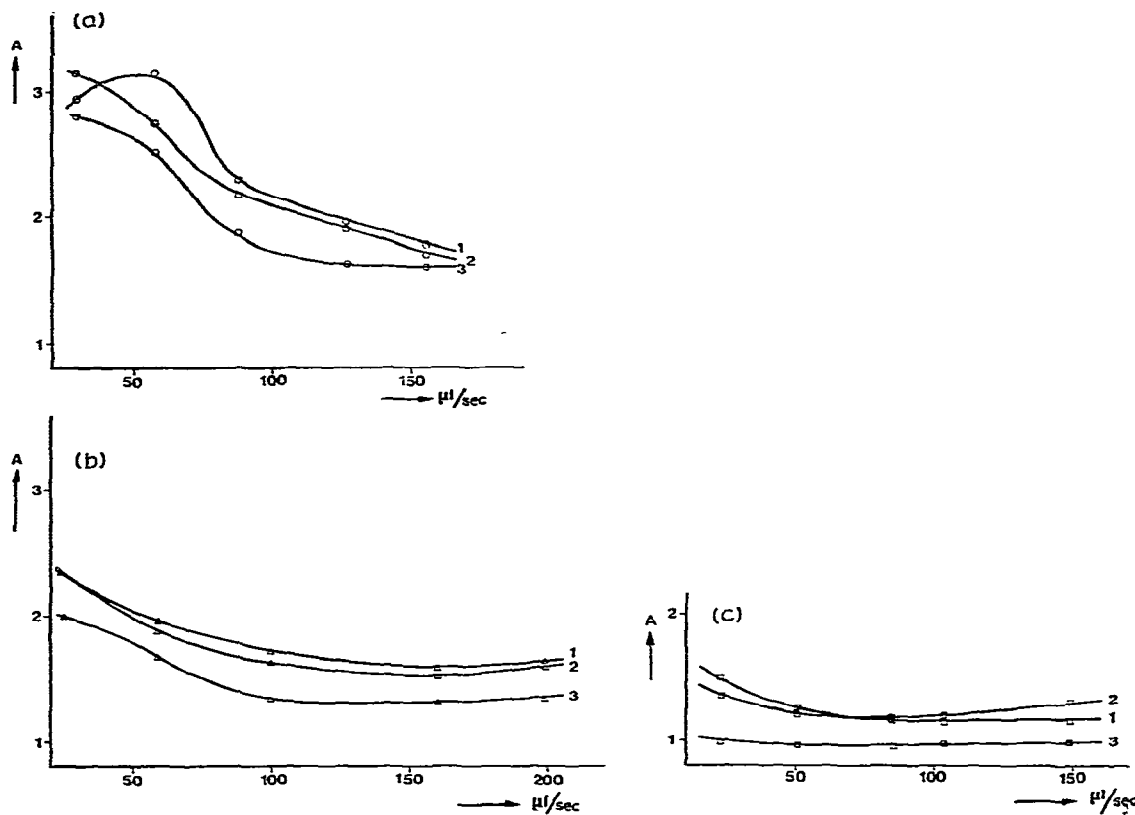
Fig. 4. Asymmetry (*A*) as function of the flow-rate for different injection modes. Column: 250 × 10 mm I.D., SI 60, d_p = 5–8 μm. Eluent: 2,2,4-trimethylpentane + 1% (v/v) butanol-1. Injection volume: 50 μl. Solutes: (1) toluene (κ = 0); (2) *o*-nitrotoluene (κ = 0.30); (3) 2,4-dimethylphenol (κ = 3.26). (a) Central injection; (b) conical disperser; (c) flow-surrounding conical disperser.

secondly might distribute the sample more equally across the separation column, diminishing local sample overloading at higher sample loads. As can be seen from Fig. 4, the asymmetry factors are indeed significantly smaller with the conical injection mode than with the flat injection mode. This experiment confirms that the stagnant eluent areas and inhomogeneous sample introduction act as a source of peak tailing.

Despite the improvement in the symmetry, tailing is still observed with the conical injection mode. The remaining tailing must be attributed to the so-called wall effects. In order to diminish the wall effects, a flow-surrounding injection system, similar in principal to that described by Webber and McKerrell[12], was tested (Fig. 3c). The system described by Webber and McKerrel is comparable with syringe injection in the eluent stream as eluent flows continuously through the column and forces the injection plug to move in certain flow lines away from the wall (*i.e.*, the surrounding flow injection leads to an infinite-diameter column). Thus, in this injection mode one can expect small loadabilities as only a small part of the packing is effectively used by the samples. To improve the loadability, the flow-surrounding system was combined with the conical glass-bead filled column inlet. By this means the sample

is more spread out when injected into the column packing, diminishing the risk of local overloading. Primary experiments with this injection mode showed that smaller asymmetry factors were obtained than with the other injection modes (Fig. 4c). It was found, however, that the ratio of the flow-rates of the injection and surrounding flow can influence the asymmetry significantly (Fig. 5). As was noticed with the other injection modes, smaller asymmetry factors were found at higher total flow-rates. For the retained compound 2,4-dimethylphenol almost perfect gaussian peaks were found at flow-rates above 50 μl/sec. The less retained compounds have some larger asymmetry factors (*ca.* 1.2) but are still much better than those obtained with the flat and conical injection system. The small remaining tailing even with the conical flow-surrounding system indicates that other effects, such as the geometrical shape of the column-end terminator, contribute. A more systematic investigation in this respect will be necessary in future.
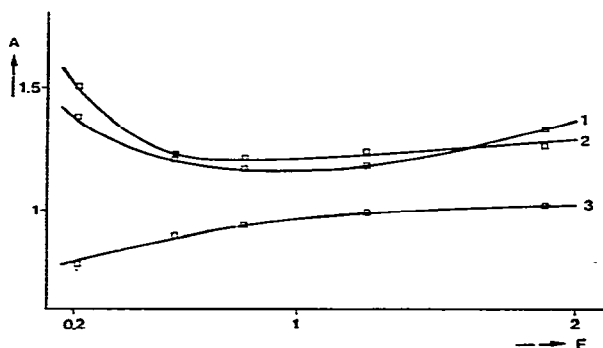

Fig. 5. Effect of the ratio of injection- and flow-surrounding flow (F) on the asymmetry factors (*A*) of the flow-surrounding conical disperser injection mode at constant total flow-rate. Conditions as in Fig. 4; total flow-rate, 85 μl/sec.

Column performance

The large-diameter columns were packed by a balanced-density slurry technique, as previously described[11], and *H* versus *v* plots were constructed for the three injection systems (Fig. 6a). The theoretical plate heights of the test compounds were calculated from the peak width at 0.607 of the peak height and their retention times. As can be seen from Fig. 6a, highly efficient 1-cm I.D. columns can be packed and there is no significant difference between the three injection modes. Such *H* versus *v* plots, based on the peak width measurements at 0.607 of the peak height or by drawing tangents, are valuable for characterizing roughly the performance of analytical columns. In preparative work, however, where one wishes to collect pure samples within the shortest collection interval, Δt_i, possible, these plots are less accurate as they ignore to a large extent the effect of tailing in the lower regions of the peak height. *H* versus *v* plots based on the measurement of peak standard deviations in the lower region of the peak heights (*i.e.*, 5–10%) are more realistic as they incorporate the surplus of resolution needed as the result of tailing to separate two compounds in such a way that pure fractions can be collected. Fig. 6b shows the *H* versus *v* plots for the three injection modes, calculated with peak widths measured at 0.1 of the peak height. The plots demonstrate clearly that significant differences in column efficiency exist if the
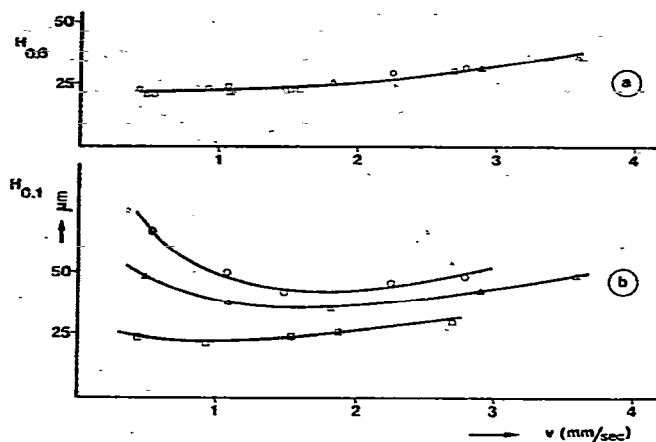
Fig. 6. H versus v curves with the three injection modes. Conditions as in Fig. 4; solute, 2,4-dimethyl-phenol. (a) H calculated with σ_t measurements at 0.607 of the peak height ($H_{0.6}$); (b) H calculated with σ_t measurements at 0.1 of the peak height ($H_{0.1}$). $\bigcirc$, Central injection; $\triangle$, conical disperser; $\square$, flow-surrounding conical disperser.

effect of tailing is included in the calculations. At these low sample concentrations the conical flow surrounding system is by far superior. The FSCD system gives an increase of about 20–100% in the effective resolution (taking into account the effect of the lower parts of the peak curves compared with the other injection modes).

In order to investigate if an increase in the column diameter influences the column efficiency, columns of I.D. 1, 1.5 and 2 cm were packed using the balanced-density slurry technique described and H versus v plots were constructed (Fig. 7). No significant decrease in efficiency was observed indicating that the packing procedure used is very suitable and gives reproducible packing characteristics.

Loadability aspects

In all experiments so far, very low sample concentrations were used in order to characterize the injection modes and column efficiency. For preparative work, however, a high production rate is necessary. One way of increasing the production
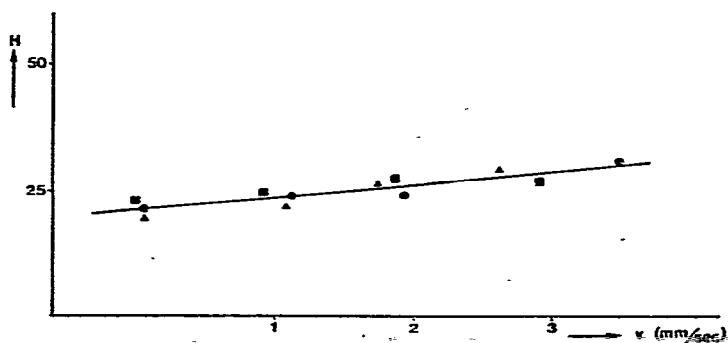

Fig. 7. Influence of the inner diameter of the column on the efficiency. Conditions as in Fig. 6a; central injection. I.D.: ●, 10 mm; ▲, 16 mm; ■, 20 mm.

rate is to increase the amount of sample injected or to increase the injection volume with a fixed sample concentration. The effect of sample loading on the number of theoretical plates measured with the three injection modes described and calculated from standard deviation measurements at 0.607 of the peak height is shown in Fig. 8a. Up to a certain concentration (amount) no significant decrease in column efficiency (i.e., $H_{0.6}$) is found. At larger concentrations, however, the column is highly overloaded and the efficiency decreases drastically. As can be seen from Fig. 8a, no remarkable difference in the loadability curve is found for the flat and conical injection systems when calculated at 0.607 of the peak height. With the conical surrounding-flow injection system, however, the loadability (i.e., maximum amount of sample that produces no change in N) is a factor of 2–3 smaller. This indicates that the sample injected with the conical surrounding-flow system uses only a fraction of the column packing and is close to an infinite-diameter column as obtained if syringe injection into the packing is used. If one includes, however, the effect of the lower parts of the peak curves in the N calculations by measurement of the peak width at 0.1 of the peak height (i.e., $N_{0.1}$), the loadability curve up to 1 mg on a 1-cm I.D. column with the
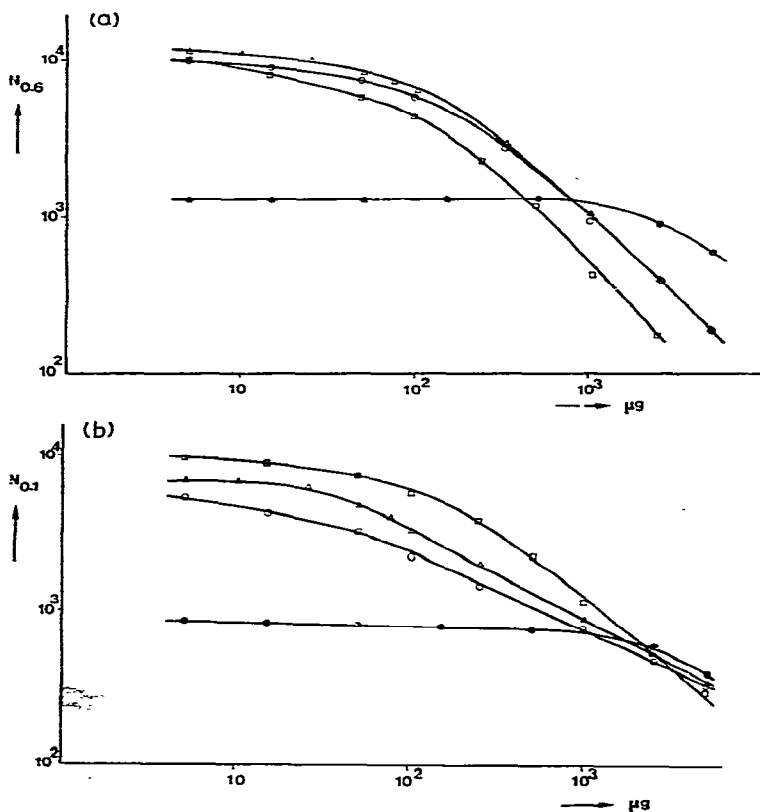

Fig. 8. Column loadability *versus* column efficiency. Conditions as in Fig. 4a; flow-rate, 85 μl/sec; solute, 2,4-dimethylphenol. (a) N calculated with σ_t measurements at 0.607 of the peak height ($N_{0.6}$); (b) N calculated with σ_t measurements at 0.1 of the peak height ($N_{0.1}$). $\bigcirc$, $\bullet$, Central injection; $\triangle$, conical disperser; $\square$, flow-surrounding conical disperser. d_p: $\square$, $\triangle$, $\bigcirc$, 5–8 μm; $\bullet$, 20–25 μm.

FSCD system is significantly better than those of the other injection systems, as can be seen in Fig. 8b. Only at larger sample concentrations is the performance of the FSCD system lower. The effect of the injection volume, containing a fixed amount of sample (250 μg), on the column efficiency calculated from the peak width at 0.6 ($N_{0.6}$) and 0.1 ($N_{0.1}$) of the peak height is shown in Fig. 9. The number of theoretical plates (i.e., $N_{0.6}$) measured with the FSCD system is lower compared to the other injection systems as the column is highly overloaded when this amount of sample is injected. Fig. 9a shows that no decrease in efficiency occurs up to an injection volume of 1 ml. At larger injection volumes the peak shape is completely determined by the injection profile and the efficiency decreases drastically. With respect to peak symmetry, the injection systems perform in the order FSCD > conical > flat. Taking into account the effect of the peak tailing (i.e., $N_{0.1}$), the FSCD system seems to be significantly better than the others, despite the fact that the column is overloaded, as is shown in Fig. 9b.

  Another way of increasing the production rate is to increase the speed of separation as more sample can be injected in the same period of time. In practice, however, the pressure is limited and hence so is the speed of separation. As was derived
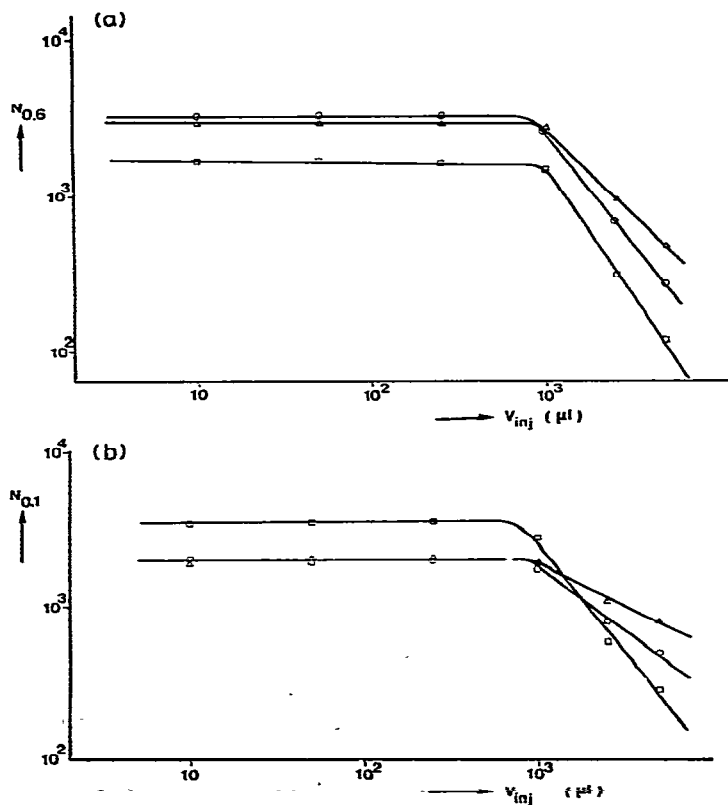

Fig. 9. Dependence of the column efficiency on the injection volume. Conditions as in Fig. 8; solute, 250 μg of 2,4-dimethylphenol. (a) N calculated with σ_t measurements at 0.607 of the peak height ($N_{0.6}$); (b) N calculated with σ_t measurements at 0.1 of the peak height ($N_{0.1}$). For designation of symbols, see Fig. 8.

theoretically, the production rate is dependent on the particle size, and is determined by the amount of sample injected and the speed of separation (i.e., proportional to v/h). The result of the theoretical derivation of the production rate at limited pressure drop predicts higher production rates with long columns filled with large-diameter particles than on shorter columns filled with small particles. : :

In order to verify this result in practice, v/h versus v plots were constructed with small (5–8 μm) and larger (20–25 μm) particles (Fig. 10). This plot shows that if the pressure drop is limited and a fixed number of theoretical plates is required in order to obtain a complete separation, indeed a larger production rate is obtained on longer columns with larger particles, as v/h is largest with the large-diameter particles.

The loadability for the test compound on the column packed with 20–25-μm particles was found to be significantly larger than on the small particle diameter columns (see Fig. 8). An explanation for this effect has not yet been found.
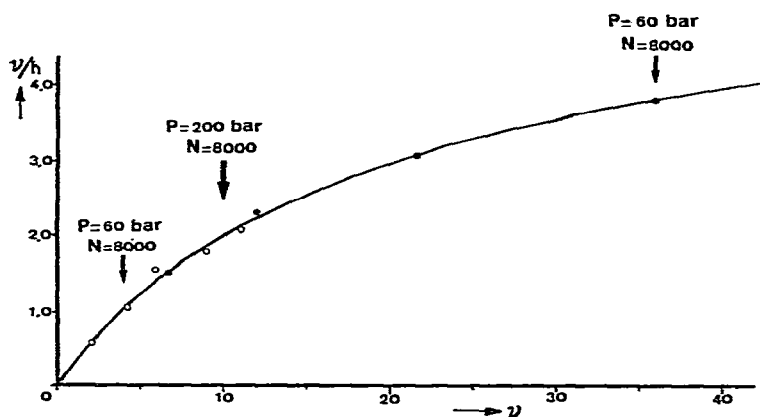

Fig. 10. v/h versus v curve. Conditions as in Fig. 6a. d_p: ○, 5–8 μm; ●, 20–25 μm.

CONCLUSIONS

(i) The production rate or throughput and solvent economy depends firstly on the usable range of the distribution isotherm. Further work, characterizing different phase systems, is necessary.

(ii) Theoretically, very long columns packed with relatively large particles give an improved throughput. The realization of this concept, together with the use of coupled columns, should be investigated.

(iii) The choice of the injection system will depend on the separation problem at hand. For difficult separations, the proposed FSCD system is to be preferred.

(iv) Columns with high efficiency and suitable for high pressures with an I.D. of 20 mm can be constructed.

(v) The characterization of column performance by measuring the peak width at 0.6 of the peak height or via tangents gives no insight into the performance of columns for preparative work.

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