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THEORETICAL INVESTIGATION OF THE POTENTIALITIES OF THE USE OF A MULTIDIMENSIONAL COLUMN IN CHROMATOGRAPHY

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SUMMARY

Two-dimensional column chromatography is a method which combines the advantages of column chromatography (constant, adjustable flow velocity, excellent efficiency, on-line detection) and two-dimensional thin-layer chromatography (successive developments in two perpendicular directions, using two different retention mechanisms).

It is shown that by merely keeping constant the solvent flow velocity during the development of a thin-layer plate, a considerable increase in the spot capacity can be achieved, since plate length and particle size can be selected without any prejudicial influence on the solvent velocity which can be kept constant at the value considered as optimum by the analyst.

Calculations show that two-dimensional column chromatography can generate peak capacities well in excess of 500, up to several thousands, and that the specifications for the equipment are not too stringent. A 10 × 10 cm column would be 1 mm thick, be well packed with 10-μm particles and be operated at a reduced velocity of 10. Such a packing could be expected to be very homogeneous (A = 0.7) and the reduced plate height would be 1.95. The expected spot capacity is 900, while the pressure drop for a compound with a diffusion constant $D \doteq 5 \times 10^{-6}$ cm^2/sec (linear velocity, u, = 0.05 cm/sec) and a solvent with viscosity 1 cP is only 5 atm (flow-rate 3 cm^3/min). The sample spot should be about 1 mm in diameter or less.

Equipment capable of these performances is under construction. Successful operation of this two-dimensional separation scheme, however, relies on the ability to

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find two chromatographic systems operating according to widely different mechanisms.

INTRODUCTION

The one-step chromatographic separation of highly complex mixtures is often impossible. It has been shown that the peak or spot capacity of one-dimensional techniques is insufficient to separate in one run more than a few hundred compounds[1,2]. Moreover, there are problems in achieving a greater capacity.

Two-dimensional thin-layer chromatography offers a much greater capacity than conventional thin-layer chromatography (TLC)[3] but this still is not enough. So, by analogy, we propose the use of a two-dimensional column, eluted successively along two perpendicular directions[4]. The column would be similar to the thin-layer bed used in TLC, just possibly thicker, but instead of relying on the capillarity and surface tension of solvents to develop the chromatogram, a pump would be used to force a stream of solvent across the column, at first parallel to one of its dimensions, until the less strongly retained compounds of interest migrate close to the plate edge opposite to the sampling spot (*cf.*, Fig. 1a,b). This requires that the bed is enclosed in

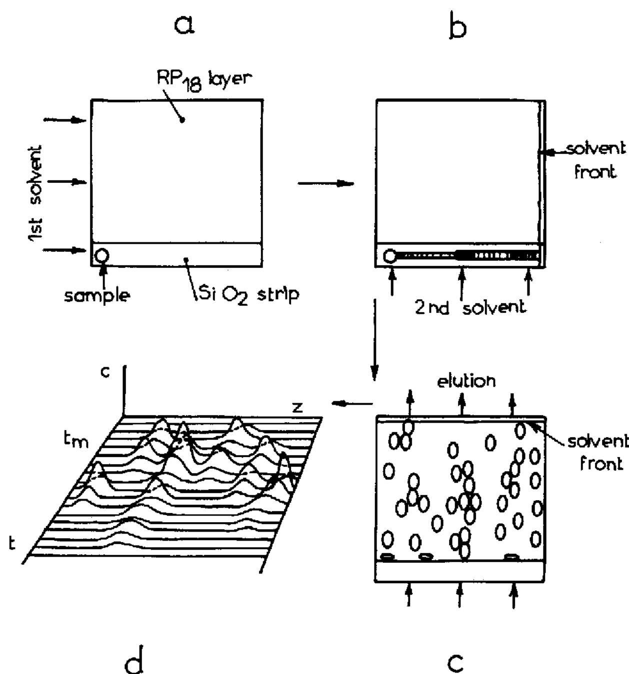

Fig. 1. Principle of two-dimensional column chromatography with development in the first dimension and elution in the second. a, First stage of the separation, development with a first solvent along a narrow-strip of silica, for example, b, Second stage of the separation: the components of the mixture now spread along the silica strip are eluted by a second solvent across a bed of reversed-phase material. c, After the spots are resolved they are eluted across a detector parallel to the column exit slit (not shown). d, Chromatogram obtained.

a leak-proof container. A second solvent stream would then be pumped in the perpendicular direction (Fig. 1c) carrying the partly separated spots through a different chromatographic system.

If the flow of the second solvent were to be stopped when the most rapidly migrating compounds of interest reach the opposite edge, the method would be similar to two-dimensional TLC, with the great advantage that the flow velocity is controlled by the analyst and can be optimized independently from the choice of particle size, plate size and nature of the solvent.

The compounds could also be eluted out of the flat chromatographic bed and detected on-line, as in high-performance liquid chromatography (HPLC), by passing an appropriate beam of UV light perpendicular to the thin solvent stream and focusing it on a diode array. The local changes of absorption of the mobile phase could be recorded and from the signals of the array an x,t,c two-dimensional chromatogram could be derived (Fig. 1d).

Obviously the same principles could be used to carry out three-dimensional separations, with the last step being either a development or preferably an elution. One of the critical problems of multidimensional chromatography is to find two (or three) retention mechanisms which are compatible and sufficiently independent from each other to allow the separation of the mixtures studied and to spread the corresponding zones over the entire chromatogram. This is achieved by using a mobile and/or a stationary phase which are different in the two successive dimensions.

The idea of controlling and adjusting the flow velocity in TLC is not new. Brenner and Niederwieser[5], then Hara and Mibe[6], vaporized the solvent at the top end of the plate to ensure continuous flow, while Saunders and Snyder[7] used a rotating drum. Later Soczewinski and co-workers[8–10] and Siouffi[11] described and used a continuous flow system, similar in principle to the one suggested by Brenner and Niederwieser[5] but permitting the achievement of conditions similar to those prevailing in HPLC, with on-line injection, but with development and not elution. Berezkin *et al.*[12] used the same equipment with an on-line UV detector whose beam crossed the chromatographic bed perpendicularly; the optical path is very short and UV absorption in a porous diffusive medium is more complex than in bulk solution. All these methods suffer from the complexities of the phenomena involved in TLC flow[13].

Finally a more sophisticated approach was developed in which the sorbent layer of a TLC system is covered by a plastic membrane applied under an external pressure[14–22]. The layer makes a porous filling between the support and the membrane, similar to a column and through which the solvent is forced under pressure[14–16]. This method of overpressurized TLC is a kind of column chromatography with a column of unconventional cross-section. The solvent velocity can be adjusted at will, independently of development distance and particle size[17]; the retention data as well as the plate height are very reproducible and independent of development distance, which is not true in TLC[10–13,17,21]. Reduced values of the plate height between 2 and 2.5 were achieved[17] and consequently the separation number increases as the square root of column length instead of going through a maximum as it does in TLC[2,20]. This is in agreement with our independent estimate of TLC plate performances[22]. The drawbacks of the method are that it starts with a dry bed[10] and lacks online detection.

Two-dimensional electrophoresis of proteins[23-26] uses optical scanning to record the thousand separated protein spots. Although this method is difficult to extend to many complex mixtures because of the lack of a general method of staining, at least the software used for data acquisition and handling will be useful for two-dimensional chromatograms.

The method discussed here is different from what has been described in the recent past to be multidimensional chromatography[27] and is rather a multistage process using two chromatographic columns operating via two different retention mechanisms. In the present technique a narrow group of interfering chromatographic zones is selected at the exit of the first column and introduced into the second column on which they will be separated. Only a few selected components of a complex mixture can be analyzed in a reasonable time, however, and any degree of resolution between the transferred bands created during the first analysis is lost during the second one.

The purpose of this work is to calculate what would be the separation power of an instrument operating according to the principles described above using a two-dimensional column, two independent retention mechanisms in the two directions and keeping constant at some optimum value the mobile phase velocities during both the first development and the second one or the elution. Such a calculation would provide the information necessary to optimize the design and operating parameters of the new chromatograph.

THEORETICAL

We shall calculate the spot or peak capacity in two cases, in two-dimensional development column chromatography where development is stopped after one column volume has been pumped through the system successively in both directions and in two-dimensional elution column chromatography when one column volume is pumped through the system in the first direction and then elution is carried out in the second direction as in classical column chromatography. Calculations will also be made in the case of three-dimensional development column chromatography with development in all three dimensions and three-dimensional elution column chromatography with development in the first two dimensions and elution in the last.

Let L_1 and L_2 be the two dimensions of the column used, which does not need to be square, H_1 and H_2 the plate heights in the two directions, σ_i the standard deviation of the sample spot[2,3], n_1 and n_2 the spot (or peak) capacities in the two directions, ${}^2n$ and ${}^3n$ the spot capacities we are looking for in two- and three-dimensional analysis respectively. H_1 and H_2 will be very close to each other as the same particle size must be used to make the entire column, otherwise a systematic trans-column variation of the flow velocity occurs, twists the spot trajectories and leads to poor results. The reduced plate heights, h_1 and h_2, could be expected to be similar since in both directions we have assumed them to be constant, *i.e.*, molecular diffusion and packing heterogeneity control the plate height as in HPLC. As we work at optimum velocity, h for two very similar packings will be very close.

We shall use the law of variance addition to calculate the spot size after its migrations (*cf.*, Fig. 2). After the first development is over, a spot which has migrated a distance z becomes ovoid. Its variance in the axial direction is

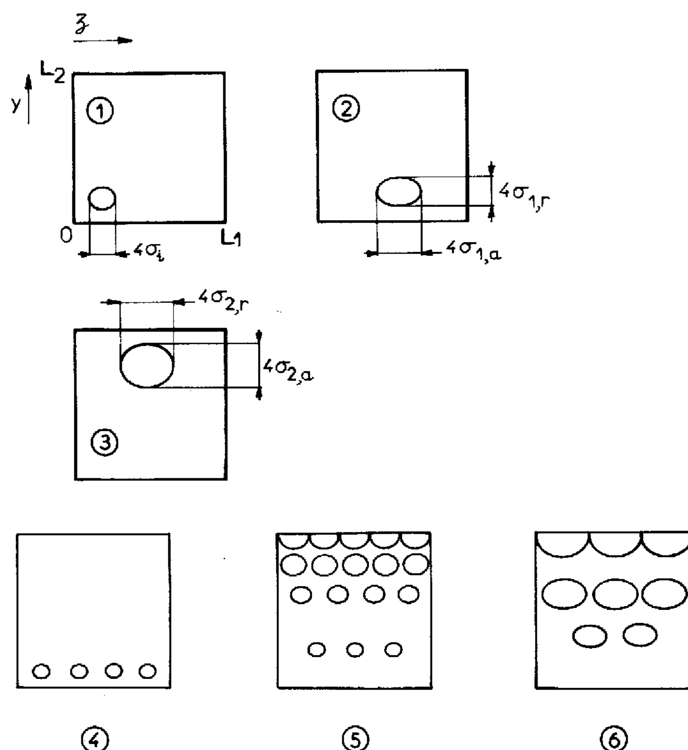

Fig. 2. Symbols, definitions and calculation principles. 1, Sample spot, assumed to be circular. 2, Spot of a compound after migration parallel to the first direction. 3, Spot of a compound after migration successively along the first and second directions. 4, At the end of the first development the resolution of the spots must be larger than 1 since they broaden by diffusion in the z direction during the second development and we want them to be resolved with a resolution 1 at the end of the analysis. 5, In two-dimensional column chromatography with development in the first dimension and elution in the second the compounds are eluted out of the column. Retained compounds have broader spots, hence the spot capacity in the z direction decreases with increasing retention time. 6, Spot capacity in the z direction continues to decrease.

$$\sigma_{1,a}^2 = \sigma_i^2 + z H_1 \tag{1}$$

while in the radial direction, *i.e.*, in the second direction it is

$$\sigma_{1,r}^2 = \sigma_i^2 + 2\gamma D_1 t_1 \tag{2}$$

where t_1 is the development time in the first direction. D_1 is the diffusion constant in the first direction and γ is the obstruction factor for diffusion in a packed column. In eqn. 2 we neglect the contribution to lateral dispersion due to anastomosis of stream splitting, which is approximated by 0.15 $d_p z$ where d_p is the particle diameter[28,29]. This contribution is small compared to the other two in the right-hand side of eqn. 2 at the flow velocities of interest in the present study.

During the second development or the elution along the second direction, the spot variance in the axial direction is

$$\sigma_{2,a}^2 = \sigma_i^2 + 2\gamma D_1 t_1 + y H_2 \tag{3}$$

where y is the distance migrated in the second direction, since the initial variance of the spot, before migration in the second direction begins, is given by eqn. 2. This neglects the narrowing effect on a zone which enters a chromatographic bed in which it is retained, whose length is reduced in proportion to R_F[30]. Thus the spot capacity in two-dimensional development column chromatography (development in two dimensions) will be somewhat underestimated, as well as the peak capacity in two-dimensional elution column chromatography (development in the first dimension, elution in the second) as the bands which leave the column are expanded at elution in the same ratio as their distance. Similarly the spot variance in the radial direction, *i.e.*, direction 1, becomes after the second elution

$$\sigma_{2,r}^2 = \sigma_i^2 + zH_1 + 2\gamma D_2 t_2 \tag{4}$$

since the variance at the beginning of the migration along the second direction was given by eqn. 1 and they expand radially by diffusion. In eqn. 4, t_2 is the time of the second development.

The number of spots dn with resolution unity which can be placed along a distance dz on a chromatographic bed is given by

$$\mathrm{d}n = \mathrm{d}z/4\sigma \tag{5}$$

while the number of peaks dn with resolution unity eluted out of a column with length L in a time dt is given by[31]:

$$\mathrm{d}n = \frac{\mathrm{d}t}{4\sigma_t} = \frac{L}{4}\cdot\frac{\mathrm{d}t}{t\sigma_e} \tag{6}$$

σ_e and σ_t are the zone standard deviations, in length and time units, respectively, and t is the retention time, $L\ (1 + k')/u$ (where k' is the capacity factor and u the flow velocity).

Spot capacity in two-dimensional column chromatography with development in the two dimensions

At the end of the second development the spot standard deviations in the two directions are given by eqns. 3 and 4 respectively, hence:

$$\mathrm{d}(^2n) = \frac{\mathrm{d}z}{4\sigma_{2,r}}\cdot\frac{\mathrm{d}y}{4\sigma_{2,a}} \tag{7}$$

The development times are respectively

$$t_1 = L_1/u_1 \tag{8a}$$

$$t_2 = L_2/u_2 \tag{8b}$$

where u_1 and u_2 are the solvent velocities along the axis z and y respectively (*cf.*, Fig. 2).

Combination of eqns. 3, 4, 7 and 8 gives:

$$d^2n = \frac{dy\,dz}{16\sqrt{(\sigma_i^2 + 2\gamma D_1 L_1/u_1 + yH_2)(\sigma_i^2 + zH_1 + 2\gamma D_2 L_2/u_2)}} \quad (9)$$

Since u_1, u_2, H_1 and H_2 are constants, integration of eqn. 9 is easy[32]. The integration limits are 0 and L_1 for z, 0 and L_2 for y. We obtain:

$$^2n = \frac{1}{4H_1H_2}\left(\sqrt{\sigma_i^2 + 2\gamma D_1 \cdot \frac{L_1}{u_1} + L_2H_2} - \sqrt{\sigma_i^2 + 2\gamma \cdot \frac{D_1L_1}{u_1}}\right)$$

$$\left(\sqrt{\sigma_i^2 + L_1H_1 + 2\gamma D_2 \cdot \frac{L_2}{u_2}} - \sqrt{\sigma_i^2 + 2\gamma D_2 \cdot \frac{L_2}{u_2}}\right) \quad (10)$$

In the particular case where σ_i is negligible and the plate characteristics (H,D,L,u) in both directions can be considered as equal, eqn. 10 simplifies to:

$$^2n = \frac{L}{4H^2}\left(\sqrt{2\gamma \cdot \frac{D}{u} + H} - \sqrt{\frac{2\gamma D}{u}}\right)^2 \quad (11)$$

Using the classical values of the reduced plate height and velocity

$$h = H/d_p \quad (12a)$$

$$v = ud_p/D_m \quad (12b)$$

we obtain

$$^2n = \frac{N}{4h}\left(\sqrt{h + \frac{2\gamma}{v}} - \sqrt{\frac{2\gamma}{v}}\right)^2 \quad (13)$$

where N is the plate number.

At very high velocities, *i.e.*, negligible radial diffusion in both developments, the limit of the spot capacity would be $N/4$. But since N decreases with increasing solvent velocity there is clearly an optimum. It is obtained by eliminating N from eqn. 13 and searching the value of v which makes equal to 0 the derivative of ^2n by respect to v

$$^2n = \frac{L}{4d_p} \cdot \frac{1}{h^2}\left(\sqrt{h + \frac{2\gamma}{v}} - \sqrt{\frac{2\gamma}{v}}\right)^2 \quad (14)$$

with:

$$h = \frac{2\gamma}{v} + Av^{1/3} + Cv \quad (15)$$

where A and C express the contributions of eddy diffusion and mass transfer resistance, respectively. The solution is obtained by a numerical procedure.

In many cases a square plate will be used, with very similar properties in both directions, but the sample spot will not have negligibly small dimensions. Then eqn. 10 simplifies to:

$$^{2}n = \frac{L}{4d_{\mathrm{p}}} \cdot \frac{1}{h^2} \left(\sqrt{\frac{\sigma_{\mathrm{i}}^2}{Ld_{\mathrm{p}}} + \frac{2\gamma}{v} + h} - \sqrt{\frac{\sigma_{\mathrm{i}}^2}{Ld_{\mathrm{p}}} + \frac{2\gamma}{v}} \right)^2 \quad (16)$$

Eqn. 16 shows that the sample spot will not contribute markedly to a decrease of the column performance as long as σ_{i}^2 is small compared to Ld_{p}.

Spot capacity in three-dimensional column chromatography with development in all three dimensions

It is very easy to generalize eqns. 9–11 to the case of a three-dimensional chromatographic medium. In the simple case when the characteristics of the three chromatographic systems used are the same and the sample spot is negligibly small, we have the simplified equation

$$^{3}n = \frac{L^{3/2}}{8H^3} \left(\sqrt{\frac{4\gamma D}{u} + h} - \sqrt{\frac{4\gamma D}{u}} \right)^3 \quad (17)$$

hence:

$$^{3}n = \left(\frac{L}{d_{\mathrm{p}}} \right)^{3/2} \frac{1}{8h^3} \left(\sqrt{h + \frac{4\gamma}{v}} - \sqrt{\frac{4\gamma}{v}} \right)^3 \quad (18)$$

The term $4\gamma/v$ instead of $2\gamma/v$ originates from the fact that now there are three successive developments and the spots enlarge by molecular diffusion in the two directions perpendicular to the direction of their migration.

Numerical calculations have to be made in order to determine the optimum velocity of the solvent.

Peak capacity in two-dimensional column chromatography with development in the first dimension and elution in the second

In this case the compounds are eluted out of the column by the second solvent, to be detected on-line, after a first separation has been made along the first dimensions of the plate, as a development. At instant t there is a number

$$n_1 = \int_0^{L_1} \frac{\mathrm{d}z}{4\sqrt{\sigma_{\mathrm{i}}^2 + zH_1 + 2\gamma D_2 t}} = \frac{1}{2H_1} \left(\sqrt{\sigma_{\mathrm{i}}^2 + L_1 H_1 + 2\gamma D_2 t} - \sqrt{\sigma_{\mathrm{i}}^2 + 2\gamma D_2 t} \right) \quad (19)$$

of spots aligned along the exit edge of the plate. The time, t, during which they have undergone diffusion in the radial direction, *i.e.*, the direction perpendicular to the elution (*cf.*, Figs. 2, 4–6) increases during elution, hence their number decreases.

During a time dt, the number of spots eluted is given by an equation similar to eqn. 6, the time and length standard deviations being related by

$$\sigma_e = \sigma_t \cdot \frac{L}{t} \tag{20}$$

where σ_e is the length standard deviation of the spot at the column exit and is given by eqn. 3 with $y = L_2$. Accordingly:

$$d(^2n) = n_1 \, dn_2 = \frac{(\sqrt{\sigma_i^2 + L_1H_1 + 2\gamma D_2 t} - \sqrt{\sigma_i^2 + 2\gamma D_2 t})\, L_2}{8H_1 \sqrt{\sigma_i^2 + 2\gamma D_1 t_1 + L_2 H_2}} \cdot \frac{dt}{t} \tag{21}$$

In eqn. 21, t_1 is the time of the first development, L_1/u_1, so if we assume that D_1 is approximatively the same for all compounds, we can define for the elution step a plate number

$$N_2 = \frac{L_2^2}{\sigma_i^2 + 2\gamma D_1 t_1 + L_2 H_2} \tag{22}$$

similar to the column plate number but smaller because of the finite size of the spots at the end of the first development when elution is started.

Integration of eqn. 21 can be carried out using the relationship[32]:

$$\int \frac{\sqrt{a + bt}}{t} \, dt = 2\sqrt{a + bt} + \sqrt{a} \ln \frac{\sqrt{a + bt} - \sqrt{a}}{\sqrt{a + bt} + \sqrt{a}} \tag{23}$$

where a and b are constants easy to identify with parameters in eqn. 21. The integration limits are t_R, the retention time at which the analysis is considered as finished, and t_0, the breakthrough time. They are related by the classical equation:

$$t_R = (1 + k')\, t_0 \tag{24}$$

Hence integration of eqn. 21 gives:

$$\begin{aligned} ^2n = \frac{\sqrt{N_2}}{8H_1} \Bigg[& 2\sqrt{\sigma_i^2 + L_1H_1 + 2\gamma D_2 t_R} - 2\sqrt{\sigma_i^2 + L_1H_1 + 2\gamma D_2 t_0} - \\ & 2\sqrt{\sigma_i^2 + 2\gamma D_2 t_R} + 2\sqrt{\sigma_i^2 + 2\gamma D_2 t_0} + \sqrt{\sigma_i^2 + L_1H_1} \times \\ \ln & \frac{(\sqrt{\sigma_i^2 + L_1H_1 + 2\gamma D_2 t_R} - \sqrt{\sigma_i^2 + L_1H_1})(\sqrt{\sigma_i^2 + L_1H_1 + 2\gamma D_2 t_0} + \sqrt{\sigma_i^2 + L_1H_1})}{(\sqrt{\sigma_i^2 + L_1H_1 + 2\gamma D_2 t_R} + \sqrt{\sigma_i^2 + L_1H_1})(\sqrt{\sigma_i^2 + L_1H_1 + 2\gamma D_2 t_0} - \sqrt{\sigma_i^2 + L_1H_1})} \\ & \sigma_i \ln \frac{(\sqrt{\sigma_i^2 + 2\gamma D_2 t_R} + \sigma_i)(\sqrt{\sigma_i^2 + 2\gamma D_2 t_0} - \sigma_i)}{(\sqrt{\sigma_i^2 + 2\gamma D_2 t_R} - \sigma_i)(\sqrt{\sigma_i^2 + 2\gamma D_2 t_0} + \sigma_i)} \Bigg] \end{aligned} \tag{25}$$

Again if we can neglect σ_i, and consider that the plate characteristics are equal in the two directions, eqn. 25 simplifies considerably:

$$^2n = \frac{L}{4H}\left\{\sqrt{1 + \frac{2\gamma(1 + k')}{hv}} - \sqrt{1 + \frac{2\gamma}{hv}} - \sqrt{\frac{2\gamma\,(1 + k')}{hv}} + \sqrt{\frac{2\gamma}{hv}} + \frac{1}{2}\ln\frac{\left[\sqrt{1 + \frac{2\gamma(1 + k')}{hv}} - 1\right]\left(\sqrt{1 + \frac{2\gamma}{hv}} + 1\right)}{\left[\sqrt{1 + \frac{2\gamma(1 + k')}{hv}} + 1\right]\left(\sqrt{1 + \frac{2\gamma}{hv}} - 1\right)}\right\} \cdot \frac{1}{\sqrt{1 + \frac{2\gamma}{v}}} \tag{26}$$

Even in this case the derivation of the optimum velocity is not straightforward and is best carried out by numerical calculations.

Peak capacity in three-dimensional column chromatography with development in the first two dimensions and elution in the third

The sample is placed at the corner of a face of a cube and the separation is first carried out as for two-dimensional column chromatography with development in two dimensions by using two successive developments, parallel to the two edges of the cube; elution is then achieved along the third dimension. The number of spots spread over the exit face at time t is given by

$$^2n = \int_0^{L_1}\int_0^{L_2} \frac{\mathrm{d}y\,\mathrm{d}z}{16\sqrt{(\sigma_i^2 + 2\gamma D_1 L_1/u_1 + yH_2 + 2\gamma D_3 t)\,(\sigma_i^2 + zH_1 + 2\gamma D_2 L_2/u_2 + 2\gamma D_3 t)}} \tag{27}$$

where D_3 is the diffusion coefficient in the third (x) direction. Eqn.27 is similar to eqn. 9, where an additional term has been added to the lateral variances of the zone in the two directions perpendicular to the direction of elution, to account for radial diffusion during elution. Eqn. 27 is integrated in the same way as eqn. 9:

$$^2n = \frac{1}{4H_1H_2}\left(\sqrt{\sigma_i^2 + 2\gamma D_1 \cdot \frac{L_1}{u_1} + L_2H_2 + 2\gamma D_3 t} - \sqrt{\sigma_i^2 + 2\gamma D_1 \cdot \frac{L_1}{u_1} + 2\gamma D_3 t}\right)\left(\sqrt{\sigma_i^2 + 2\gamma D_2 \cdot \frac{L_2}{u_2} + L_1H_1 + 2\gamma D_3 t} - \sqrt{\sigma_i^2 + 2\gamma D_2 \cdot \frac{L_2}{u_2} + 2\gamma D_3 t}\right) \tag{28}$$

The number of spots eluted out of the column during time dt is calculated by using an equation similar to eqn. 6 (*cf.*, eqns. 19–21)

$$\mathrm{d}^3n = {}^2n\,\mathrm{d}n = {}^2n\,\frac{L_3}{4\sigma_e}\cdot\frac{\mathrm{d}t}{t} \tag{29}$$

where ${}^2n$ is given by eqn. 28 and is a function of time, while σ_e is the standard deviation of the zones in the X axis (third direction) and is obtained by the law of variance addition:

$$\sigma_e^2 = \sigma_X^2 = \sigma_i^2 + 2\gamma D_1 \cdot \frac{L_1}{u_1} + 2\gamma D_2 \cdot \frac{L_2}{u_2} + L_3 H_3 \tag{30}$$

If we assume that D_1 and D_2 are approximately equal for all compounds, we can define a plate number for the elution step, N_3, exactly as N_2 (eqn. 22) except that we use now eqn. 30 for σ_e^2. Then:

$$\begin{aligned} {}^3n = \frac{\sqrt{N_3}}{16 H_1 H_2} \int_{t_0}^{t_R} \Bigg(& \sqrt{\sigma_i^2 + 2\gamma D_1 \cdot \frac{L_1}{u_1} + L_2 H_2 + 2\gamma D_3 t} - \\ & \sqrt{\sigma_i^2 + 2\gamma D_1 \cdot \frac{L_1}{u_1} + 2\gamma D_3 t} \Bigg) \Bigg(\sqrt{\sigma_i^2 + 2\gamma D_2 \cdot \frac{L_2}{u_2} + L_1 H_1 + 2\gamma D_3 t} - \\ & \sqrt{\sigma_i^2 + 2\gamma D_2 \cdot \frac{L_2}{u_2} + 2\gamma D_3 t} \Bigg) \frac{\mathrm{d}t}{t} \end{aligned} \tag{31}$$

Development of the integrand function gives four expressions similar to

$$y = \int \frac{\sqrt{a + bt + ct^2}}{t} \,\mathrm{d}t \tag{32}$$

whose integral is[32]:

$$\begin{aligned} Y = {} & \sqrt{a + bt + ct^2} + \frac{b}{2\sqrt{c}} \ln [2\sqrt{c(a + bt + ct^2)} + \\ & 2ct + b] - \sqrt{a} \ln \frac{2\sqrt{a(a + bt + ct^2)} + bt + 2a}{t} \end{aligned} \tag{33}$$

for a positive. Identification with the corresponding terms of eqn. 31 permits its easy but tedious integration. There are four functions similar to Y in the integration of eqn. 31, the coefficients a, b, c being complex expressions of the parameters, *e.g.*:

$$a_1 = \left(\sigma_i^2 + 2\gamma D_1 \cdot \frac{L_1}{u_1} + L_2 H_2\right)\left(\sigma_i^2 + L_1 H_1 + 2\gamma D_2 \cdot \frac{L_2}{u_2}\right)$$

Since three-dimensional column chromatography with development in the first two dimensions and elution in the third is not very well understood yet and still far from

the experimental stage, it has not been found useful to print the complete equation. Calculations have been completed only in the simple case when σ_i is negligible, the column is a cube and its properties identical in the three directions. Eqn. 33 simplifies to:

$$ {}^3n = \frac{\sqrt{N_3}}{16H^2} \int_{t_0}^{t_R} \left(\sqrt{2\gamma \cdot \frac{DL}{u} + LH + 2\gamma Dt} - \sqrt{2\gamma \cdot \frac{DL}{u} + 2\gamma Dt} \right)^2 \frac{dt}{t} \tag{34} $$

In the development of the integrand function, only one expression such as eqns. 32 and 33 is found, with simple coefficients. Integration gives

$$ {}^3n = \frac{L^{3/2}}{16H^{3/2}\sqrt{1+2a}} \Bigg\{ 2ak' + \sqrt{8a(1+2a)} - \sqrt{4a[1+a+(1+2a)(1+k')+a(1+k')^2]} - (1+2a) \cdot \ln \frac{2\sqrt{a[1+a+(1+2a)(1+k')+a(1+k')^2]} + 2a(1+k') + 1 + 2a}{(1+k')[2\sqrt{2a(1+2a)} + 1 + 4a]} + \sqrt{4a(1+a)} \cdot \ln \frac{2\sqrt{(1+a)[1+a+(1+2a)(1+k')+a(1+k')^2]} + (1+2a)(1+k') + 2(1+a)}{(1+k')[2\sqrt{2(1+a)(1+2a)} + 3 + 4a]} \tag{35} $$

with:

$$ a = 2\gamma/h\nu \tag{35a} $$

a is always less than 1 (*cf.*, eqn. 15); it is *ca.* 0.25 at optimum flow velocity and tends toward 0 when the velocity becomes very high.

For further calculations it is often useful to express H as hd_p, as h is also a function of the velocity, while the spot capacity is proportional to $(L/d_p)^{3/2}$. With such a complex expression there is no reason for the maximum spot capacity to be obtained at the same velocity for different values of k'.

DISCUSSION

The simplified equations for spot or peak capacity in the various modes of chromatography discussed here are collected in Table I for easy reference.

Numerical calculations have been carried out in different cases, to investigate the effect of the various parameters of the four different experiments described above, to determine the range of performances which can be expected and to find reasonable values of the parameters permitting the achievement of high resolution while using simple equipment.

TABLE I

SIMPLIFIED EQUATIONS FOR COLUMN PERFORMANCE

$a = 2\gamma/hv$; $h = f(v)$ is given by the plate height equation. $\sigma_i = 0$. Column characteristics (L, D, u, h) identical in all directions.

Development chromatography using a thin column

$$n = \frac{1}{2}\sqrt{\frac{L}{H}}$$

Two-dimensional column chromatography with development in two dimensions

$$^2n = \frac{L}{4H}(\sqrt{1+a} - \sqrt{a})^2 \tag{14}$$

Three-dimensional column chromatography with development in all three dimensions

$$^3n = \frac{1}{8}\left(\frac{L}{H}\right)^{3/2}(\sqrt{1+2a} - \sqrt{2a})^3 \tag{18}$$

Conventional column chromatography

$$n = \frac{1}{4}\sqrt{\frac{L}{H}}\ln(1+k') \tag{38}$$

Two-dimensional column chromatography with development in the first dimension and elution in the second

$$^2n = \frac{1}{8}\cdot\frac{L}{H}\cdot\frac{1}{\sqrt{1+a}}\left\{2\sqrt{1+a(1+k')} - 2\sqrt{1+a} + 2\sqrt{a} - 2\sqrt{a(1+k')} + \ln\frac{(\sqrt{1+a}+1)[\sqrt{1+a(1+k')}-1]}{(\sqrt{1+a}-1)[\sqrt{1+a(1+k')}+1]}\right\} \tag{26}$$

Three-dimensional column chromatography with development in the first two dimensions and elution in the last

$$^3n = \frac{L^{3/2}}{16H^{3/2}\sqrt{1+2a}}\left\{2ak' + \sqrt{8a(1+2a)} - \sqrt{4a[1+a+(1+2a)(1+k')+a(1+k')^2]} - (1+2a)\ln\frac{2\sqrt{a[1+a(1+2a)(1+k')+a(1+k')^2]} + 2a(1+k') + 1 + 2a}{(1+k')[2\sqrt{2a(1+2a)}+1+4a]} + \sqrt{4a(1+a)}\ln\frac{2\sqrt{(1+a)[1+a+(1+2a)(1+k')+a(1+k')^2]} + (1+2a)(1+k') + 2(1+a)}{(1+k')[2\sqrt{2(1+a)(1+2a)}+3+4a]}\right\}$$

Spot capacity in two-dimensional column chromatography with development in two directions

The spot capacity is given by either the general eqn. 10 or the simplified eqn. 14. It is easier to consider first the simple case of a good square plate, developed at the

same speed in the two directions, using retention mechanisms such that the diffusion coefficients in the two solvents are similar. The effect of deviations from this ideal case can then be investigated.

For example, with the typical values $\gamma = 0.7$, $A = 1$ and $C = 0.03$, a numerical calculation shows that the maximum spot number (eqn. 14) is achieved for $\nu = 8.9$, corresponding to $h = 2.50$. For a 10×10 cm column, packed with particles having $d_p = 5$ μm, the maximum spot capacity is 1218, instead of 930 at the optimum flow velocity of 2.6, corresponding to the minimum plate height (1.99). The gain of 30% results from a trade-off between an increase in the axial variance and a marked decrease of lateral diffusion due to a more rapid analysis.

For a typical value of the diffusion coefficient of 5×10^{-6} cm^2/sec, the actual velocity corresponding to $\nu = 8.9$ is 0.089 cm/sec (5-μm particles). The development time is 112 sec in both directions, *i.e.*, less than 4 min total, which is a drastic reduction compared to typical analysis times in TLC or even column chromatography: the chromatograph will have to be fully automated to ensure reproducible results. The corresponding pressure for a solvent whose viscosity is 0.01 poise would be

$$\Delta P = u\eta L/k_0 d_p^2 = 35.6 \text{ atm} \tag{36}$$

where η is the viscosity of the solvent and k_0 is the permeability constant of the column.

Because of leak problems, especially on the side of the column, and the kind of safety problems which originate in equipment designed according to Tyihak and co-workers[14–20] where the membrane would have to be applied on the 10×10 cm column with a pressure exceeding 40 atm, generating a bursting force of more than 4 tons, the first experiments will be made using coarser particles, lower velocities and less viscous solvents.

Eqn. 14 shows that with 10-μm particles and a reduced velocity of 2.6, a spot capacity exceeding 450 can still be achieved with a total analysis time of 26 min and a pressure of 2.6 atm. Performances corresponding to other combinations of parameters are given in Table II where it is seen that extremely large spot capacities can be produced in reasonable times and that the most critical problem will be the design of the mechanical enclosure to contain the pressure. A spot capacity of 1000 could be approached with a pressure drop of 10 atm.

Fig. 3 shows the variation of ${}^2n$ with increasing sample standard deviation as predicted by eqn. 16. A rather small sample spot is an important requirement. The specifications are more drastic than in conventional TLC since the performances are better, *i.e.*, the final spots are smaller. These specifications are comparable to those encountered in column chromatography[33].

As a first approximation we require

$$\sigma_i^2 < 0.1\ Ld_p \tag{37}$$

which leads to reasonable results (Fig. 3). The reduction in spot capacity is then 10–15%. When σ_i^2/Ld_p becomes large, the spot capacity is markedly reduced and eventually tends towards zero. A limited expansion of the right-hand side of eqn. 16 for

TABLE II

PERFORMANCES OF COLUMNS FOR *n*-DIMENSIONAL COLUMN CHROMATOGRAPHY WITH DEVELOPMENT IN *n*-DIMENSIONS

^1n, ^2n, ^3n = Spot capacities of columns for development chromatography using a thin column, two-dimensional column chromatography with development in two dimensions and three-dimensional column chromatography with development in all three dimensions respectively. Column characteristics are the same in all dimensions (L = 10 cm, γ = 0.70, A = 1.0, C = 0.03). ΔP is the pressure drop necessary to pump the solvent at the reduced velocity indicated, with $D_m = 5 \times 10^{-6}$ cm/sec; η = 1 cP; permeability is $d_p^2/1000$. t is the retention time of an inert compound, t_0 or breakthrough time. Analysis time is nt (n = number of dimensions of the column) plus time necessary for intermediate steps like drying. Column efficiency: v = 2.6, h = 1.99 (minimum); v = 5.7, h = 2.20; v = 8.9, h = 2.50; v = 12.6, h = 2.82.

d_p(μm)	v	^1n	^2n*	^3n	t(min)	ΔP(atm)
3	12.6	54	1960	70,050	0.80	233
	8.9	58	2030	67,900	1.12	165
	5.7	61	1960	59,200	1.77	105
	2.6	65	1540	35,000	3.85	48
5	12.6	42	1170	32,500	1.33	50
	8.9	45	*1218*	31,600	1.87	36
	5.7	47	1177	27,500	2.93	23
	2.6	50	*923*	16,280	6.4	10.4
7	12.6	35	840	19,650	1.87	18.4
	8.9	38	870	19,050	2.62	13
	5.7	40	841	16,600	4.1	8.3
	2.6	42	660	9830	9.0	3.8
10	12.6	29	588	11,510	2.6	6.3
	8.9	32	*609*	11,150	3.75	4.5
	5.7	33	589	9720	5.83	2.8
	2.6	36	462	5760	12.8	1.3
15	12.6	24	392	6260	4.0	1.9
	8.9	26	406	6070	5.6	1.3
	5.7	27	392	5300	8.8	0.84
	2.6	29	308	3130	19	0.4

* Italicized values correspond to curves 1–3 in Fig. 3.

small values of Ld_p/σ_i^2 shows that ^2n becomes equivalent to $L^2/16\sigma_i^2$. This upper limit of spot capacity is shown on Fig. 3.

As it has been shown that it is quite possible to apply TLC samples with standard deviations of *ca.* 0.1 mm and to inject samples in column chromatography which are small enough not to contribute significantly to band broadening, there should not be major unexpected difficulties in meeting those specifications and achieving large spot capacities.

Eqns. 14 and 16 show that the spot capacity depends essentially on the column dimension, L, the particle size, d_p, and the reduced plate performances, h,v, exactly the characteristics which determine the peak capacity of a conventional column. From previous discussions on optimization of column performances, we know what to do to increase the spot capacity in two-dimensional column chromatography with development in two dimensions: the packing homogeneity will be critical. For example, with h = 4 at a reduced velocity of 8 the spot capacity of a 10 × 10 cm plate

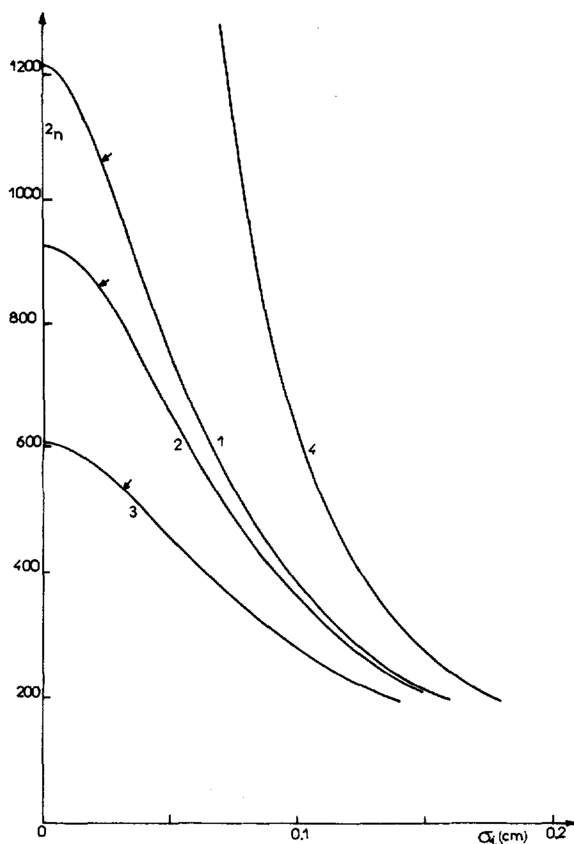

Fig. 3. Variation of the spot capacity in two-dimensional column chromatography with development in two dimensions with the standard deviation of the sample spot. Column: 10 × 10 cm. Other conditions as in Table II. Curves: 1, $d_p = 5$ μm, $\nu \doteq 8.9$; 2, $d_p = 5$ μm, $\nu \doteq 2.6$; 3, $d_p = 10$ μm, $\nu \doteq 8.9$. 4, $n = L^2/16\sigma_i^2$ which is the upper limit to the spot capacity (see text). The arrows correspond to $\sigma_i^2/Ld_p = 0.1$.

made with 5-μm particles is only 825 instead of 1218 if $h = 2.50$. The diffusion coefficient has no effect on the spot capacity, only on the analysis time and pressure.

Spot capacity in three-dimensional column chromatography with development in all three dimensions

Some numerical calculations have been made with the same conditions as above. The optimum velocity is still larger than in two-dimensional column chromatography with development in two dimensions since it is useful to decrease the time during which the spots broaden through lateral diffusion. In the case selected ($A = 1$, $C = 0.03$) the optimum velocity is now 12.6 (*cf.*, Table II).

The spot capacity becomes extremely large with values markedly exceeding 1×10^4 within easy reach, while 100,000 does not seem impossible.

The critical problems in this case are first column packing, as we can foresee the packing problems associated with the manufacture of a 10 × 10 × 10 cm or larger cube, and then to find a suitable detector: it does not appear practical to divide the

cube into 0.1-mm thick slices and scan one by one the 1000 slices with a photodensitometer.

The potential reward is worthy of serious research investment in this area, however.

Peak capacity in two-dimensional column chromatography with development in the first dimension and elution in the second

At a later stage it will probably be necessary to investigate rectangular plates, especially when the two retention mechanisms used have markedly different selectivity for the components of a complex mixture and optimization is complex as the two dimensions of the plate and the order in which the two separation mechanisms are applied may have significant effects on the overall performance. At this stage it is sufficient, however, to consider square columns and assume that their performances are the same in both directions. In fact the largest difference in properties in the two dimensions most probably occurs in the diffusion coefficient of the solutes in the mobile phase and in its viscosity. These parameters determine the analysis time and the pressure drop but have little influence on the spot capacity provided the same reduced velocity is chosen.

The effect of the size of the original sample spot has not been taken into account in this study: the specifications will be similar in two-dimensional column chromatography with development in both dimensions and in that with development in the first dimension and elution in the second, and we have already calculated them and seen that they can be satisfied with a careful instrument design. Keeping σ_i^2/Ld_p less than 0.1 will result in a loss in peak capacity not exceeding about 10%.

It is seen in eqn. 26 that the peak capacity depends separately on the ratio of column length to particle size, on the range of k' scanned during elution and on the column bed performance, *i.e.*, the reduced plate height at the reduced velocity at which the column is operated. The influence of these three factors can be studied separately.

The peak capacity increases steadily with increasing range of k' (Fig. 4). We know that in conventional column chromatography the time optimum range is 0–6.4: the highest peak capacity in a given time, t_A, is obtained by adjusting column length and solvent strength so that the compound with $k' = 6.4$ is eluted at time t_A (ref. 1). This is because the peak capacity in conventional column chromatography is given by

$$n = \frac{\sqrt{N}}{4} \ln (1 + k') \tag{38}$$

and that selecting $k' = 6.4$ [$\ln(1 + k') = 2$] achieves the best compromise between the effect on analysis time of an increase in column length and in the k' range scanned. Eqn. 26 is much more complicated than eqn. 38 and it is not possible to obtain a general result in two-dimensional column chromatography with development in the first dimension and elution in the second.

Nevertheless we have chosen the value $k' = 7$ as the end of the analysis when such a choice is necessary in the following discussion. The dotted line on Fig. 4 shows the variation of the peak capacity of a column (eqn. 38) having 4 million theoretical

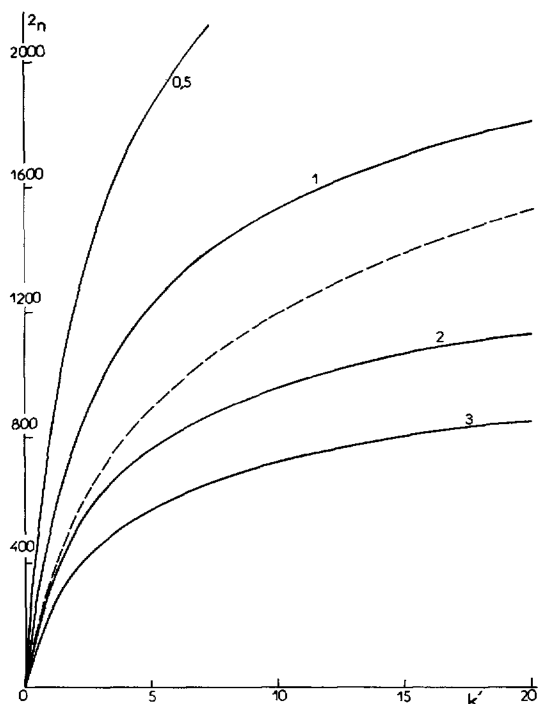

Fig. 4. Plot of the peak capacity in two-dimensional column chromatography with development in the first dimension and elution in the second *versus* the value of k' at which the analysis is stopped. The value on the curve is the coefficient of packing homogeneity, A, in the plate height equation (eqn. 15). The dotted line gives the peak capacity of a conventional column having 4×10^6 plates. Columns are 10×10 cm; $d_p = 5$ μm; reduced velocity, $v = 7$.

plates, for sake of comparison. The curve appears steeper than those corresponding to two-dimensional column chromatography with development in the first dimension and elution in the second, which saturate faster. Consequently is would probably not be worthwhile to continue development after $k' = 5$ or so in two-dimensional column chromatography with development in the first dimension and elution in the second, if we could optimize the plate dimensions and solvent compositions. On the other hand, if 1200 spots are eluted out of the column ($A = 1$) between $k' = 0$ and $k' = 5$, 300 are still eluted between $k' = 5$ and $k' = 10$ and that capacity could appear useful when it is difficult to increase the size of the two-dimensional column chromatography with development in the first dimension and elution in the second equipment[33]. This explains why the value $k' = 7$ is assumed to be a good compromise. Furthermore, if we compare eqns. 14 and 26 (Table II) we see that the value of k' at which the spot capacity of two-dimensional column chromatography with development in two dimensions is equal to the peak capacity of two-dimensional column chromatography with development in the first dimension and elution in the second depends only on a, and thus on the plate height coefficients. With the numerical values selected here ($A = 1.0$, $C = 0.03$), we obtain the same resolution power for $k' = 6.5$, a value very close that for which the peak capacity of a conventional column is equal to the spot capacity of an overpressurized TLC plate ($k' = 6.4$).

The peak capacity is a function of the plate characteristics which determine both $a = 2\gamma/h\nu$ and h. There is of course no reason for the maximum peak capacity be achieved at the velocity which gives the minimum plate height: a higher velocity results in a larger variance contribution in the direction of the solvent migration but, because the residence timer is shorter, in a smaller variance contribution in the perpendicular direction.

Fig. 5 shows that there is an optimum velocity markedly larger than the one corresponding to the minimum plate height, as in two-dimensional column chromatography with development in two dimensions, as in Table II. This optimum velocity increases slightly with the range of k' scanned during the analysis and under the conditions given in the figure is around $\nu \doteq 6.5$. The peak capacity is almost 20% larger than at $\nu_0 = 2.6$. We note in passing that these results are independent of the plate size and particle diameter. The peak capacity is merely proportional to the length of the column side and inversely proportional to d_p. On the other hand, the results shown in Fig. 5 depend on the values of γ, A and C in eqn. 15. There is little we

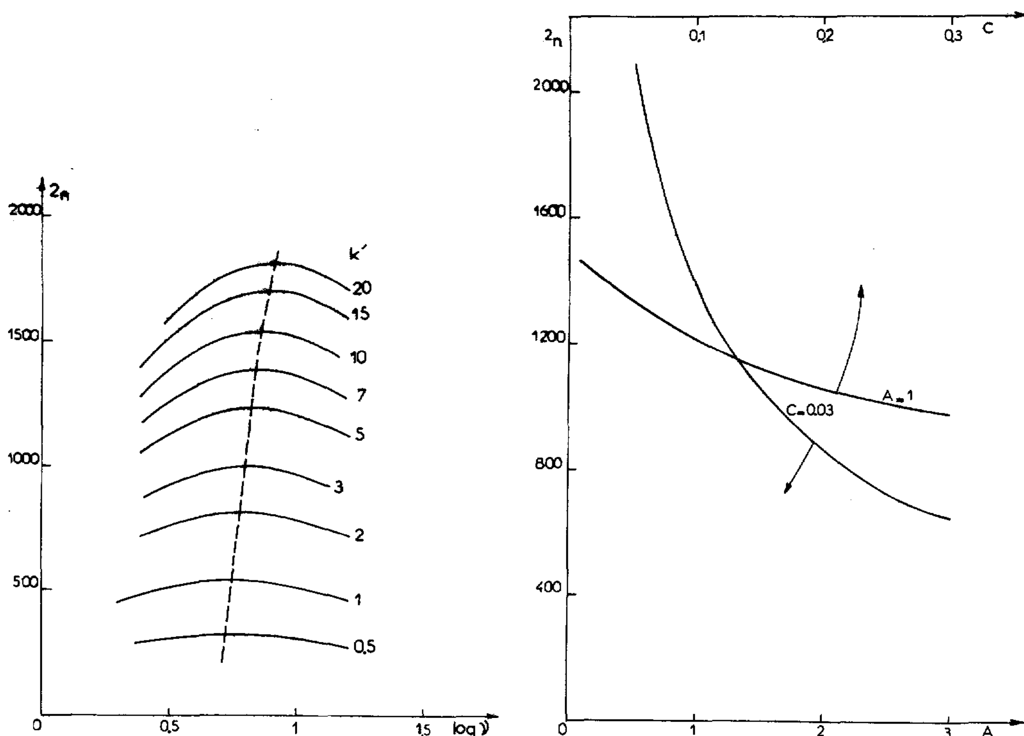

Fig. 5. Plot of the peak capacity in two-dimensional column chromatography with development in the first dimension and elution in the second *vs.* the reduced velocity. Column: 10 × 10 cm. $d_p = 5$ μm. The number on each curve is the value of k' at which the analysis is stopped. The dotted line indicates the maximum. $\gamma = 0.70$; $A = 1.0$; $C = 0.03$.

Fig. 6. Plot of the peak capacity in two-dimensional column chromatography with development in the first dimension and elution in the second *vs.* the value of the constants A (steeper curve, obtained with $C = 0.03$) and C (obtained at $A = 1$). In both cases: $k' = 7$; $\nu = 7.0$; 10 × 10 cm column packed with 5-μm particles.

can do about γ[28,29]. A depends on the homogeneity of the bed packing and for a thin-layer bed it should not be too difficult to obtain values of A less than 1. C describes the mass transfer in the particle and is certainly a function of k'[34,35] but as a first approximation can be assumed to be constant.

Fig. 6 shows the variation of the peak capacity with the constants A and C, for a 10 × 10 cm column packed with 5-μm particles; values well in excess of 1500 can be expected at $k' = 7$. The reduced velocity is 7, corresponding to the maximum peak capacity for $A = 1$, $C = 0.03$, but certainly not for the other values of these parameters. It can be expected to increase slowly with decreasing values of A and C.

Accordingly we can expect to be able to generate a peak capacity close to 1000 by using a 10 × 10 cm column, packed carefully ($A = 0.7$) with 10-μm particles as typically used in conventional column chromatography, with a reduced velocity close to 7, a pressure drop around 5 atm (Table II) and recording the chromatogram for values of k' up to *ca.* 10.

Peak capacity in three-dimensional column chromatography with development in the first two dimensions and elution in the third

In view of the difficulties encountered in the design and construction of a chromatograph for two-dimensional column chromatography with development in the first dimension and elution in the second it seems too early to discuss in detail the potential performances of a chromatograph for three-dimensional column chromatography with development in the first two dimensions and elution in the third. Furthermore, eqn. 35 is similar to eqn. 26, the parameters L and d_p are separated from the column characteristics and the consequences will be similar to those encountered in

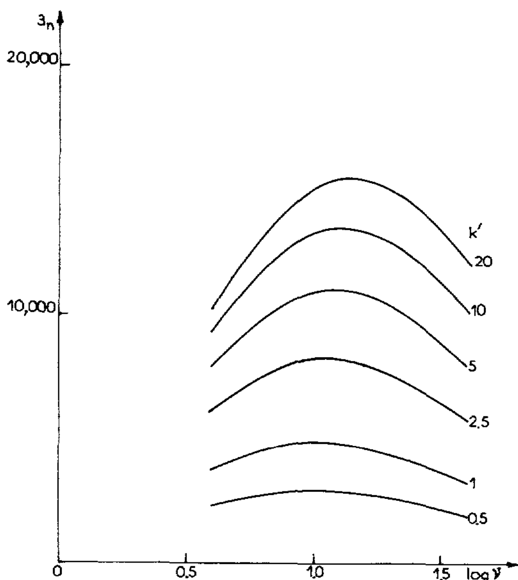

Fig. 7. Plot of the peak capacity in three-dimensional column chromatography with development in the first two dimensions and elution in the last *vs.* the reduced velocity. Column: 10 × 10 × 10 cm. d_p = 10 μm. The number on each curve is the value of k' at which the analysis is stopped. $\gamma = 0.70$; $A = 1.0$; $C = 0.03$.

two-dimensional column chromatography with development in the first dimension and elution in the second. The numbers, however, are now gigantic.

The peak capacity is maximum for a relatively large value of the reduced velocity, which would be quite convenient for the analysis of high-molecular-weight compounds such as biopolymers. The optimum reduced velocity is around 14, but would be markedly smaller should the performance of the three-dimensional packed bed be less satisfactory than assumed in the calculations (Fig. 7).

The peak capacity increases very rapidly with increasing k' at the beginning, but levels off at values above 10. It is clear from eqn. 35 that there is a limit at large k'. A peak capacity equal to the spot capacity of the same column used in three-dimensional column chromatography with development in all three dimensions is achieved for $k' = 6.8$ with the numerical values chosen here ($A = 1.0$, $C = 0.03$). The limit at very large k' is about 50% larger so in practice it is not easy to achieve better separation efficiency in three-dimensional column chromatography with development in the first two dimensions and elution in the last than in three-dimensional column chromatography with development in all three dimensions.

The peak capacity increases very rapidly with increasing ratio of column length to particle size (*cf.*, Fig. 8). As in three-dimensional column chromatography with development in all three dimensions, peak capacities of several tens of thousands

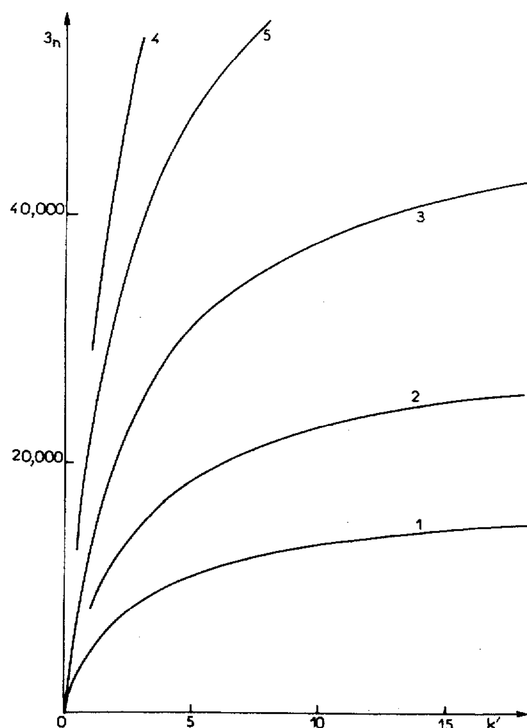

Fig. 8. Plot of the peak capacity in three-dimensional column chromatography with development in the first two dimensions and elution in the last *vs.* the value of k' at which the analysis is stopped. Columns: 10 × 10 × 10 cm. Curves: 1, d_p = 10 μm; 2, d_p = 7 μm; 3, d_p = 5 μm; 4, d_p = 3 μm, A = 1.0, C = 0.03; 5, d_p = 5 μm, A = 0.70, C = 0.01. Reduced velocity ν = 14.

would be easy to achieve while a capacity of 100,000 is a theoretical possibility, for example with a 12 × 12 × 12 cm cube well packed with 3-μm particles!

The potential performances of one-, two- and three-dimensional column chromatography are compared in Table III. It must be understood that the peak capacities are not obtained at the same reduced velocities so the ratios ${}^2n/n$ and ${}^3n/{}^2n$ have been calculated just to show that the peak capacity increases more slowly than with the power of the space dimension used.

TABLE III

COMPARISON BETWEEN THE PERFORMANCES OF COLUMNS FOR CONVENTIONAL COLUMN CHROMATOGRAPHY, TWO-DIMENSIONAL COLUMN CHROMATOGRAPHY WITH DEVELOPMENT IN THE FIRST DIMENSION AND ELUTION IN THE SECOND AND THREE-DIMENSIONAL COLUMN CHROMATOGRAPHY WITH DEVELOPMENT IN THE FIRST TWO DIMENSIONS AND ELUTION IN THE LAST

L = 10 cm; d_p = 5 μm; A = 1.0; C = 0.03; γ = 0.70. Conventional column chromatography: $v \doteq 2.6$, h = 1.99. Two-dimensional column chromatography with development in the first dimension and elution in the second: $v \doteq 7$; h = 2.32. Three-dimensional column chromatography with development in the first two dimensions and elution in the last: $v \doteq 14$; h = 2.93.

k	${}^1n$	${}^2n$	${}^2n/{}^1n$	${}^3n$	${}^3n/{}^2n$
1	17	500	29	13,400	27
3	34	950	27	25,000	26
5	44	1180	26	30,800	26
7	52	1320	25	34,400	26
10	60	1470	24	37,900	26
20	76	1720	23	38,500	22

CONCLUSIONS

Several of the possible separation schemes described here raise considerable experimental difficulties, for example detection in three-dimensional column chromatography with development in all three dimensions or three-dimensional column chromatography with development in the first two dimensions and elution in the last. On the other hand two-dimensional TLC is very easy to carry out. It seems that while it offers markedly improved performances over two-dimensional TLC, two-dimensional column chromatography with development in the first dimension and elution in the second could be reduced to practice without excessive experimental difficulties.

Certainly the combination of a peak capacity several times greater than in two-dimensional TLC, a fast and straightforward data acquisition scheme and easy, accurate quantitative analysis would represent a major step forward in the field of complex mixture analysis, provided two different retention mechanisms can still be combined effectively, resulting in retention patterns with weak or no correlation. The equipment specifications described above do not appear too stringent.

Work is in progress in our laboratory on the design of a suitable instrument. This and the performances achieved will be reported elsewhere.

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REFERENCES

1 G. Guiochon, *J. Chromatogr.*, 185 (1979) 3.
2 G. Guiochon and A. M. Siouffi, *J. Chromatogr.*, 245 (1982) 1.
3 G. Guiochon, M. F. Gonnord, A. Siouffi and M. Zakaria, *J. Chromatogr.*, 250 (1982) 1.
4 L. A. Beaver and G. Guiochon, *Patent pending*, U.S.A., 1981.
5 M. Brenner and A. Niederwieser, *Experientia*, 17 (1961) 237.
6 S. Hara and K. Mibe, *J. Chromatogr.*, 66 (1972) 75.
7 D. L. Saunders and L. R. Snyder, *J. Chromatogr. Sci.*, 8 (1970) 706.
8 E. Soczewinski, *J. Chromatogr.*, 138 (1977) 443.
9 E. Soczewinski and T. Wawrzynowicz, *Chromatographia*, 11 (1978) 466.
10 E. Soczewinski and T. Wawrzynowicz, *J. Chromatogr.*, 218 (1981) 729.
11 A. M. Siouffi, unpublished results.
12 V. G. Berezkin, R. G. Vinogradova, O. A. Rysiev, V. N. Chechevichkin and F. I. Romanov, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 93.
13 A. L. Ruoff, G. H. Stewart, K. Shinzh and J. C. Giddings, *Kolloid.-Z.Z. Polym.*, 173 (1960) 14.
14 E. Tyihak, H. Kalasz, E. Mincsovics and J. Nagy, *Proc. Hung. Annu., Meet. Biochem.*, 17 (1977) 183.
15 E. Tyihak, E. Mincsovics and H. Kalasz, *J. Chromatogr.*, 174 (1979) 75.
16 E. Tyihak, F. Körmendi, A. Gulyas, H. Kalasz, L. Kulacsy, J. Nagy and J. Knoll, *Hung. Pat.*, 173,749 (1976).
17 E. Mincsovics, E. Tyihak and H. Kalasz, *J. Chromatogr.*, 191 (1980) 293.
18 E. Tyihak, E. Mincsovics, P. Tetenyi, I. Zambo and H. Kalasz, *Herba Hung.*, 19 (1) (1980) 113.
19 H. Kalasz, E. Tyihak and E. Mincsovics, in A. Frigerio and M. McLamish (Editors), *Recent Developments in Chromatography and Electrophoresis*, Vol. 10, Elsevier, Amsterdam, 1980, p. 289.
20 E. Tyihak, E. Mincsovics, H. Kalasz and J. Nagy, *J. Chromatogr.*, 211 (1981) 45.
21 G. Guiochon and A. Siouffi, *J. Chromatogr. Sci.*, 16 (1978) 598.
22 G. Guiochon and A. Siouffi, *J. Chromatogr. Sci.*, 16 (1978) 470.
23 N. L. Anderson, J. Taylor, A. E. Scandora, P. B. Coulter and N. G. Anderson, *Clin. Chem.*, 27 (1981) 1807.
24 A. K. Thorsrud, H. F. Haugen and E. Jellum, in *Electrophoresis 79*, W. de Gruyter, New York, 1980, p. 425.
25 P. F. Lemkin and L. E. Lipkin, *Comp. Biomed. Res.*, 14 (1981) 272.
26 M. F. Gonnord, M. Zakaria and G. Guiochon, *J. Chromatogr.*, in preparation.
27 J. F. K. Huber, E. Kenndler and G. Reich, *J. Chromatogr.*, 172 (1979) 15.
28 J. H. Knox and J. F. Parcher, *Anal. Chem.*, 41 (1969) 1599.
29 D. S. Horne, J. H. Knox and L. McLaren, *Separ. Sci.*, 1 (1966) 531.
30 T. H. Jupille, *J. Amer. Oil Chem. Soc.*, 54 (1977) 179.
31 E. Grushka, *Anal. Chem.*, 42 (1970) 1142.
32 R. C. Weast and S. M. Selby (Editors), *Handbook of Chemistry and Physics*, CRC Press, Cleveland, OH, 60th, ed., 1979, eqns. 238, 258, 262.
33 G. Guiochon, M. F. Gonnord, G. Preau and A. M. Siouffi, in preparation.
34 J. F. K. Huber, *Ber. Bunsen-Ges.*, 77 (1973) 179.
35 Cs. Horváth and H.-J. Lin, *J. Chromatogr.*, 149 (1978) 43.