Journal of Chromatography, 107 (1975) 35–42

CHROM. 8123

RECYCLING GEL CHROMATOGRAPHY OF PHENOLIC COMPOUNDS

II. NUMERICAL CALCULATION OF OPTIMAL NUMBER OF CYCLES AND RESOLUTION

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(Received October 2nd, 1974)

SUMMARY

Equations are derived for the calculation of the optimal number of cycles and resolution in recycling gel chromatography. The resolution obtained at the optimal number of cycles is expressed as a function of relative peak distance and number of theoretical plates. The peak distance–relative peak distance function has also been studied and some examples of the recycling separation of phenolic compounds are discussed.

INTRODUCTION

Gel chromatography is a commonly used liquid chromatographic method in which the stable phase is the liquid in the internal part of the gel and the mobile phase moves round the gel particles. The separation is based on the selective permeation of different substances[1–3].

In some instances, the distance between the peaks of different substances is not sufficient for separation. The resolution can be increased by choosing another gel or a longer column or by utilizing a recycling operation. The recycling method has been applied to counter-current distribution[4] and to gas chromatography[5] in order to achieve better separations. Excellent separations were obtained by Porath and Bennich[6], Killander[7] and Chersi[8] with soft gels (Sephadex), and recycling gel permeation chromatography in organic solvents was used by Bombaugh and co-workers, who gave some numerical calculations[9,10]. Bombaugh's equations took into consideration the "increasing length of the column" and the movement of some peaks from others, but did not consider the movement together of some peaks.

In this paper, we derive some equations that were developed in order to distinguish between single and connected columns. We took into consideration the steady separation of the peaks from each other as well as the approach together of the nth and $(n+1)$th peaks as the peak widths gradually increased.

The only assumption made is constancy of the conditions used for chromatography. Thus, the separation process must be identical in the various cycles. Under

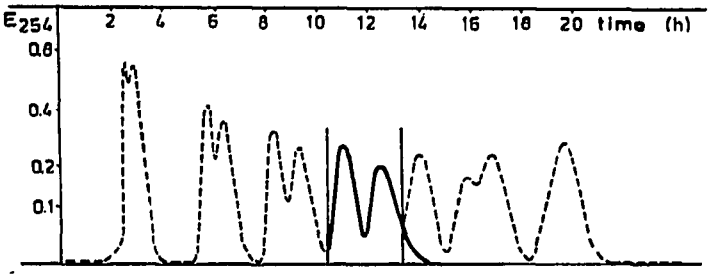

Fig. 1. Separation of two components. When separation occurs, the $(n+1)$th peak of the slower moving substance will be nearing the nth peak of the faster moving substance, and the separation must be stopped at the optimal cycle number.

such circumstances, the equations presented here can be applied to any type of recycling processes, *e.g.*, gel filtration on soft, semi-rigid or rigid gels, in water or in organic solutions. In some instances, they are valid for other types of chromatography, *e.g.*, liquid–liquid partition and counter-current distribution. Naturally, the validity of the peak-widening rule described by Giddings[11] and Bombaugh and coworkers[9,10] is also a necessary condition, *i.e.*, the peak width should be proportional to the square root of the cycle number.

CALCULATION OF OPTIMAL NUMBER OF CYCLES

The equation for the resolution (eqn. 4) means a constant increase in R_n with increase in the number of cycles. Figs. 1 and 2 show the limitations of this equation. After several cycles, besides the separation of peaks of substances A (slower moving) and B (faster moving), the $(n+1)$th peak of the slower moving substance will be nearing the nth peak of the faster moving substance, so the increasing resolution of

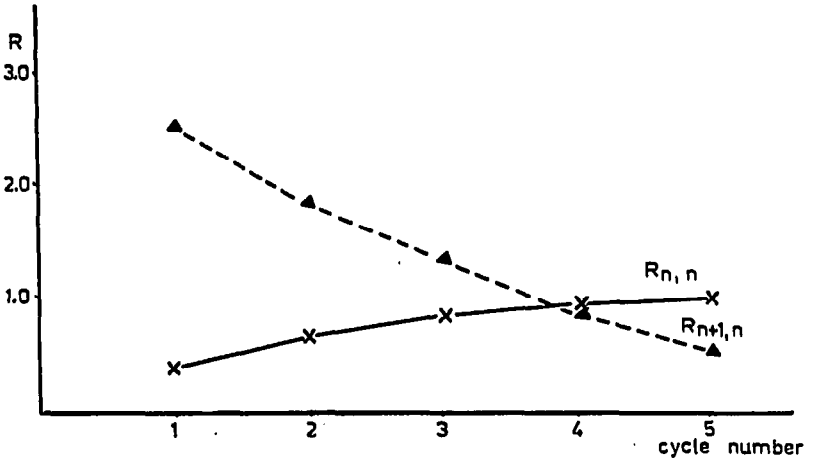

Fig. 2. Plot of eqn. 4. In addition to the resolutions of the nth peaks, the resolutions of the $(n+1)$th and $(n-1)$ th peaks have to be taken into consideration. The increasing values of $R_{n,n}$ occur in parallel with the decreasing values of $R_{n+1,n}$.

the A_n and B_n peaks parallels the decreasing resolution of B_n and A_{n+1} peaks. After the so-called optimal number of cycles, substance B will overtake substance A. Therefore, the separation must be stopped at the cycle number at which B_n is at an equal distance from A_n and A_{n+1}. This stage can be calculated from the elution parameters:

$$\begin{aligned} nt_2 &= (n + \tfrac{1}{2})\, t_1 \\ 2\,nt_2 &= 2\,nt_1 + t_1 \\ n &= t_1/2\,(t_2 - t_1) \end{aligned} \qquad (1)$$

where t_2 and t_1 represent the elution times of the faster and slower moving peaks, respectively, and n is the optimal number of cycles. If we represent the relative peak distance, $(t_2 - t_1)/t_1$, by m, eqn. 1 can be simplified:

$$n = \frac{1}{2m}$$

i.e., the optimal number of cycles is half of the reciprocal of the relative peak distance. For instance, if the relative peak distance is 25% (0.25), the optimal number of cycles is 2, while for a relative peak distance of 5% the optimal number of cycles is 10. In general, of course, we do not obtain integers for the value of n. In these instances, the next highest integer has to be taken because, if the value of $1/2m$ is less than 1.00, the substances must be separated, naturally, in at least one cycle; if the value of $1/2m$ is greater than 1.00, the resolution of the peaks in the $(n-1)$th, nth and $(n+1)$th cycles are taken, and we are seeking the optimal number of cycles, n, when R_n is greater than either R_{n+1} or R_{n-1}. We can observe the $(n-1)$th, nth and $(n+1)$th cycles of the peaks but, having finished the separation at the nth cycle, the $(n+1)$th cycle cannot be observed. Therefore, if n is non-integral, the next highest integer must be used. This procedure of completion up to n integers can be symbolised by the equation

$$n_{\text{integer}} = [n]_{\text{whole part}} + 1 = \left[\frac{t_1}{2\,(t_2 - t_1)}\right]_{\text{whole part}} + 1 = \left[\frac{1}{2\,m}\right]_{\text{whole part}} + 1 \qquad (3)$$

CALCULATION OF RESOLUTION

On the basis of the optimal and limited number of cycles, the maximal and limited resolutions can also be calculated using the equation given by Bombaugh and co-workers[9,10]:

$$R_n = R_0\, n^{1/2} \qquad (4)$$

where R_0 and R_n are the resolutions at the first and nth cycles, respectively. Although eqn. 4 indicates an unlimited increase in R_n with increase in n, the limit of n (*e.g.*, regarding the optimal number of cycles, eqn. 1) determines the maximal value of R_n also. Substituting n values into Bombaugh's equation (eqn. 4), the theoretical limit of R_n can be calculated:

$$R_n = R_0\, n^{1/2} = \frac{2\,(t_2 - t_1)}{w_1 + w_2} \cdot \left(\frac{t_1}{2\,(t_2 - t_1)}\right)^{1/2} \qquad (4a)$$

$$= \frac{t_2 - t_1}{4\,\sigma_t} \cdot \left(\frac{t_1}{2\,(t_2 - t_1)}\right)^{1/2}$$

$$= \frac{1}{4\sqrt{2}} \cdot \frac{t_1}{4\,\sigma_t} \cdot \left(\frac{t_2 - t_1}{t_1}\right)^{1/2} \tag{5}$$

where σ_t is the average standard deviation of the Gaussian curves, assuming that $w_1 + w_2 \approx 8\,\sigma_t$. Using the symbols $N = (4\,t_n/\sigma_t)^2$ (number of theoretical plates) and $m = (t_2 - t_1)/t_1$ (relative peak distance) the equation for R_n can be simplified to

$$R_n = \frac{1}{4\sqrt{2}} \cdot N^{1/2}\, m^{1/2} \tag{6}$$

Thus, the resolution at the optimal cycle number is a function of N and m (*i.e.*, of the number of theoretical plates of the chromatographic column and of the relative peak distance), which determine the maximal resolution that can be obtained in a single column by means of the recycling method.

If a preliminary calculation of R_n made on the basis of eqn. 6 does not give a high enough value, it can be corrected by increasing N or m. N can be increased by increasing the temperature, slowing the rate of elution, decreasing the amount and volume of the sample, increasing the column length, or using a finer porosity (not coarse, but fine or superfine). m can be modified as follows: (a) for large molecules, by choosing a more suitable gel for which the solutes lie in the selective permeation

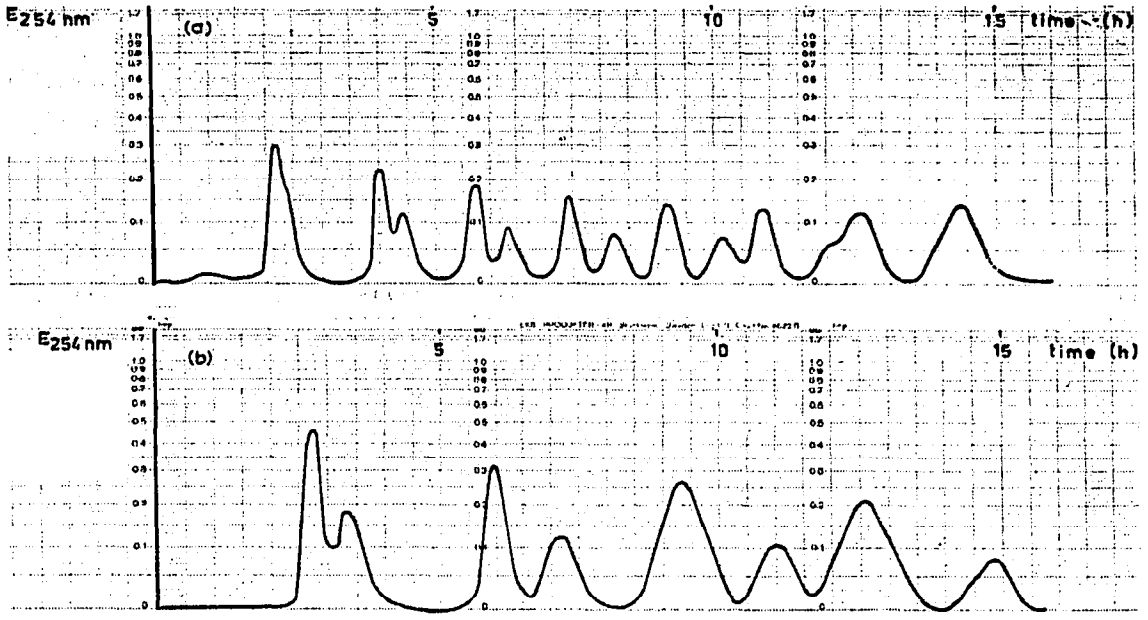

Fig. 3. Separation of phenylacetic acid (20 mg) and acetone (0.3 ml) on Bio-Gel P-2 columns at (a) 50° and (b) 5°. The UV extinction was recorded at 254 nm on a Uvicord II instrument (LKB, Stockholm, Sweden).

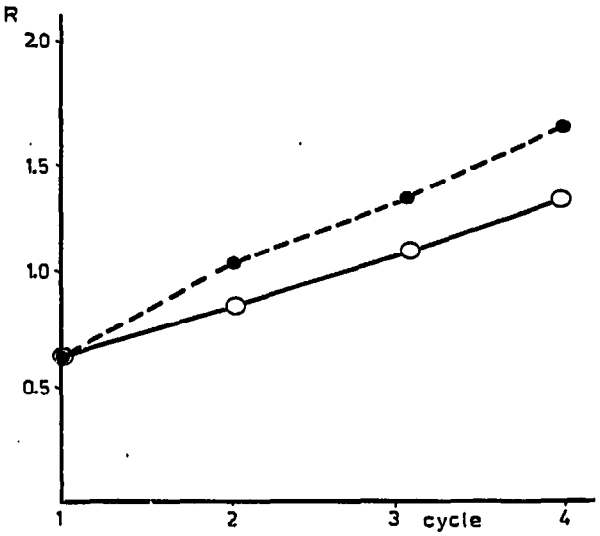

Fig. 4. Resolutions in the separation of phenylacetic acid (20 mg) and acetone (0.3 ml) at 5° (●) and 50° (○).

range, or by changing the type of gel (*e.g.*, sometimes Bio-Gel P gives a higher resolution than Sephadex G for a given pair of substances); (b) for small molecules, by changing the temperature, changing the pH, changing the ionic strength, or increasing the degree of cross-linking of the gel.

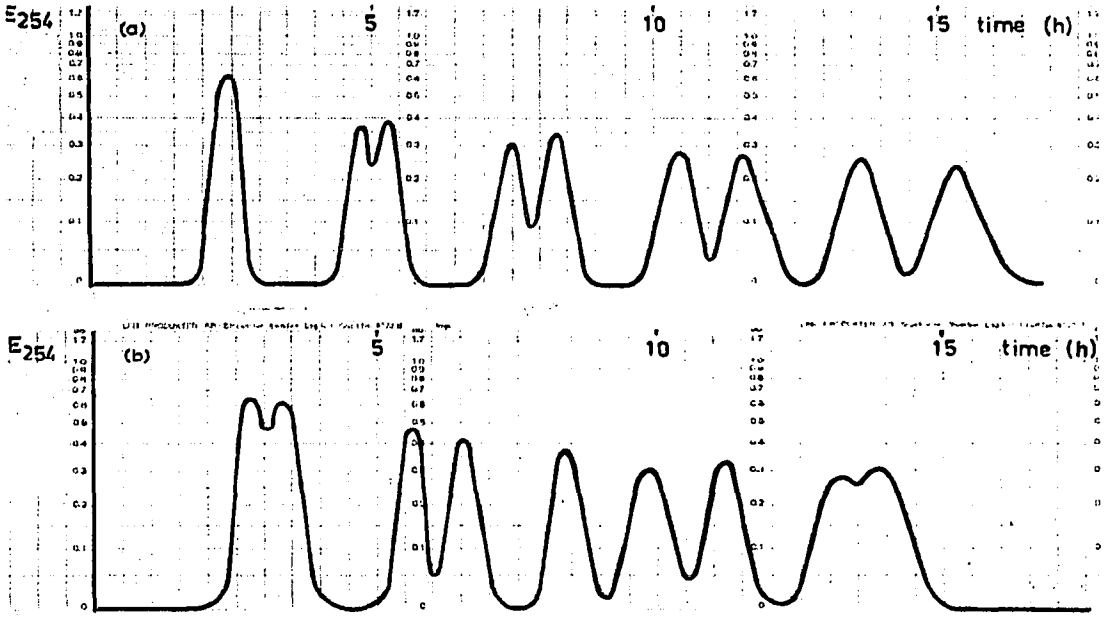

Fig. 5. Separation of tyramine (50 mg) and dopamine (60 mg) on Bio-Gel P-2 columns at (a) 50° and (b) 5°. The UV extinction was recorded at 254 nm on a Uvicord II instrument.

With small molecules, a change in temperature affects both N and m. This effect can be observed in the separation of acetone–phenylacetic acid and of tyramine–dopamine. In the first example, the gel chromatographic behaviour of acetone shows no temperature dependence, but phenylacetic acid has a negative temperature dependence, *i.e.*, its gel chromatographic characteristics (V_e, t_e, K_d, K_{av}.) decrease with increase in temperature. The higher the temperature, the nearer the peaks of acetone and phenylacetic acid approach each other (Fig. 3). At the same time, the widths of the peaks decrease with increasing temperature (*i.e.*, the standard deviations of the Gaussian peaks decrease, and so the number of theoretical plates of the column decreases). In this case, the dual effect of temperature on resolution operates in opposite directions. As the change in peak distance predominates, it is preferable to work at low temperatures (2–5°), when separating substances with different gel chromatographic behaviour (Fig. 4). The separation of tyramine and dopamine (or the separation of any two phenylalkyl substances from each other) represents another type of behaviour. Although the absolute values of the elution parameters change with changes in temperature, the relative elution values undergo only a minor change (Fig. 5). At the same time, the widths of the peaks decrease with increasing temperature, which suggests that it is preferable to work at elevated temperatures (45–60°) (Fig. 6).

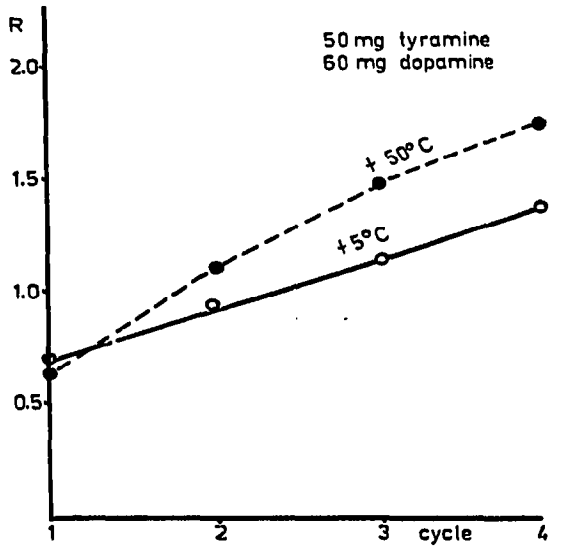

Fig. 6. Resolutions in the separation of tyramine (50 mg) and dopamine (60 mg) at 5° (○) and 50° (●).

CHANGING OF MAXIMAL RELATIVE PEAK DISTANCE

In the first step of the numerical calculations (eqns. 1–3) we took the values $n = 1/2m = t_1/2\,(t_2 - t_1)$ instead of the completed 1, 2, . . ., n values into consideration. However, if we separated the substances by means of recycling gel chromato-

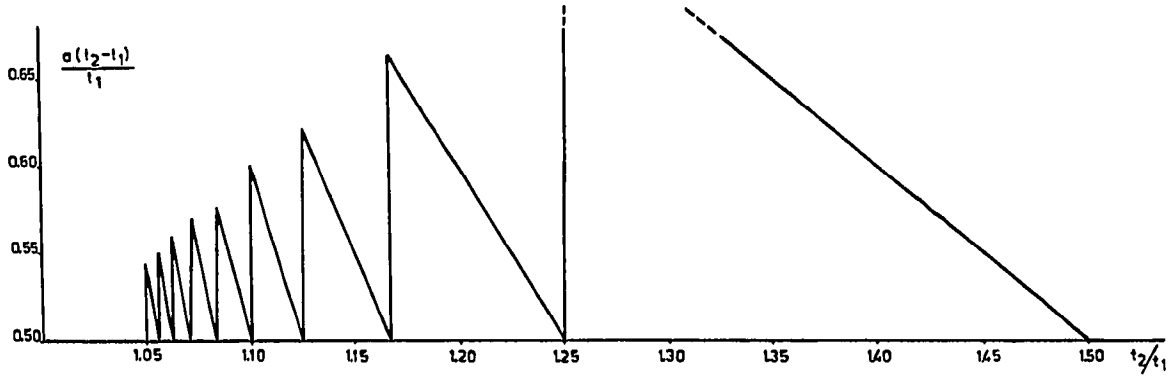

Fig. 7. Relative peak distance *versus* relative elution. A limited, non-monotonous function is obtained.

graphy, only integral cycle numbers could be used. Therefore, the maximal relative peak distances, $(at_2 - at_1)/t_1$, *i.e.*, $a(t_2 - t_1)/t_1$ ($a = 1, 2, \ldots, n$) were investigated as a function of relative elution time, t_2/t_1. In some instances, the number of recycling can be an integer plus a half, *i.e.*, one of the peaks had been cycled n times while the other peak had been cycled $n+1$ times. In this instance the maximal relative peak distances, $[at_2 - (a+1)\,t_1]/t_1 = [at_2 - bt_1]/t_1$ and $a\,(t_2 - t_1)/t_1$ ($a = 1, 2, \ldots, n$) were investigated. A limited, non-monotonous function was obtained (Figs. 7 and 8) in both instances.

In general, the main aim in chromatographic separations is to remove one of the peaks as far as possible from the others, *i.e.*, to obtain the maximal peak distance. In the gel chromatography of phenolic compounds, both the peak distance and the relative peak distance can be increased by changing the temperature, pH or ionic strength, and it is therefore practical to look for t_2/t_1 values at which the above-mentioned function has local maxima. As peak widths increase with increase in the number of cycles, one should use the local maximum belonging to the greatest possible t_2/t_1 value with the minimal cycle number.

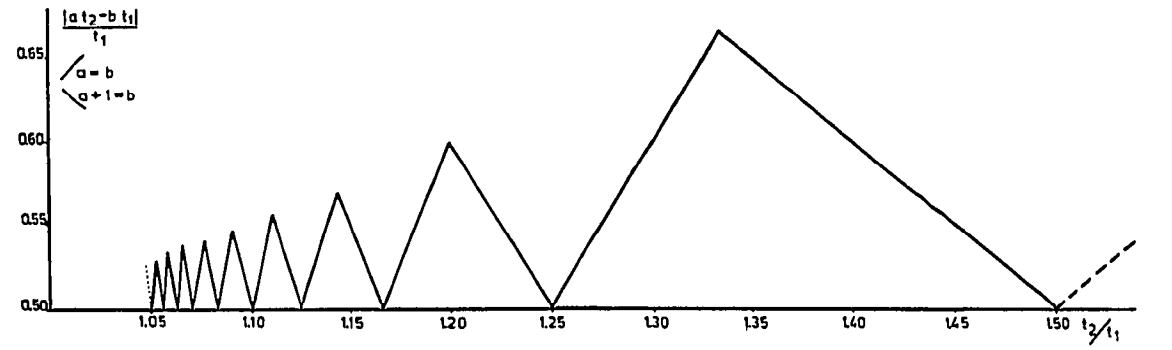

Fig. 8. Relative peak distance *versus* relative elution time taking into account the approach of the peaks.

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