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THEORETICAL AND EXPERIMENTAL STUDY OF RECYCLING IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Optimal recycling would demand zero extra-column band broadening connections, but it can be shown that under realistic conditions, even far from the optimal, resolution is always increased if the cycle number is sufficiently large. However, this cycle number is limited by the condition that the first eluted peak should not re-mix with the last one. For this reason, recycling of complex mixtures without fraction collection is very limited, if not impossible, but the potential of recycling a mixture of two closely eluted compounds is excellent.

In such a case, recycling shows well known advantages in pressure and column length over one-cycle systems giving identical resolution and analysis time. It also displays interesting advantages in analysis time and dilution over one-cycle systems working at the same pressure. Experimental characteristics of recycling under analytical conditions with a Waters M6000 pump and under preparative conditions with an Orlita MS 15-7-7 pump are given and compared with calculated results.

INTRODUCTION

Recycling was originally suggested for use in gas chromatography[1], where it was never used in practice because of technical difficulties and lack of incentive. It was introduced for the first time in liquid chromatography by Porath and Bennich in 1962[2] as a new principle for increasing the effective bed height without the use of additional packing material. In this technique, the column eluent is re-pumped through the column instead of mobile phase taken from the reservoir, and the sample is thus analyzed several times on the same column, so that the resolution between its components can be increased.

Since its introduction, the advantages of recycling have been widely used in gel filtration and gel permeation chromatography. In this case, the span of retention is very narrow and long columns are therefore necessary in order to achieve separations, but it is impossible to use long columns at relatively large mobile phase veloci-

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ties because the gel packings cannot stand large pressure drops, even if the pressure gradient is moderate. Thus recycling offers a practical solution. This technique has also been found useful in high-pressure size-exclusion chromatography[3–14], and even in high-pressure liquid–solid chromatography[6,15].

Recycling has generally been used to achieve the large number of theoretical plates required to obtain a satisfactory resolution of closely eluted components such as diastereoisomers[12,14]. Today, however, the development of efficient techniques for packing columns with very small particles (5–20 μm) makes possible the achievement, in a reasonable time, of large plate numbers (several tens of thousands or even several hundred thousand plates[16] while using relatively moderate pressure drops. Hence, it is questionable whether recycling is needed any longer in common practice. One of the aims of this paper is to help to clarify this point.

Recycling is frequently applied to preparative chromatography[2,5–7,11,13]. In this instance, only a fraction of the sample of major interest is recycled while the other fractions are collected when they leave the column. It appears that this technique has great flexibility and can be adapted for each different sample. The total performance of a recycling separation will be affected by this succession of recycling and collection operations. A model taking into account all of these possibilities would be too complex, and therefore in this paper we consider only continuous recycling, fraction collection being carried out only at the end of the recycling process. Such a model is mainly applicable to analytical recycle chromatography, or to the preparative separation of closely eluted isomers. The essential differences between preparative and analytical recycle chromatography will be indicated.

Before considering the application of recycling in liquid chromatography, we shall discuss its main characteristics and describe two types of recycling techniques.

EXPERIMENTAL TECHNIQUES FOR RECYCLING CHROMATOGRAPHY

The simplest recycling technique[2,3,11] consists in connecting the detector output with the pump inlet, as shown in Fig. 1. The sample, introduced in the injection port, percolates through the column and the detector and is then recycled via the four-port valve through the pump into the column. For recycle operation, the valve is in the position of the dotted line and for collection in the position of the full line. The scheme illustrated is very simple. Several other multi-port valves can be used for flushing, disposal to waste and other purposes, but in these instances the basic principle of recycling remains the same: it is the closed-loop pumping approach or the direct pumping approach.

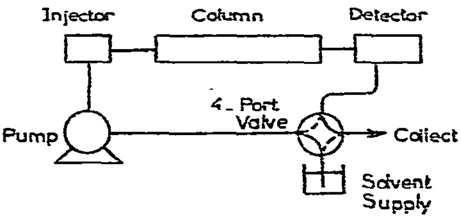

Fig. 1. Schematic diagram of a direct pumping (closed-loop) recycling apparatus.

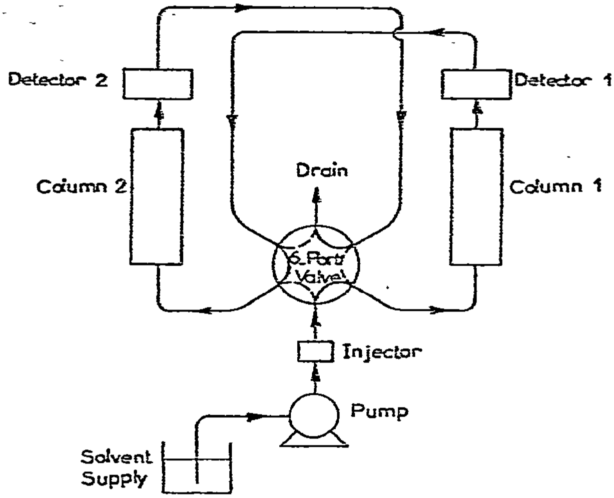

Fig. 2. Schematic diagram of an alternate pumping recycling apparatus.

The other method was presented by Biesenberger and co-workers[8-10], and adapted to high-performance liquid chromatography (HPLC) by Henry *et al.*[15]. Fig. 2 shows a schematic diagram of this version, termed "alternate pumping" recycling. In this configuration, two nearly identical columns, two detectors or one dual-cell detector and one six-port, two-position valve are needed. In the actual full-line configuration in Fig. 2, the sample flows from the injector to column 1, detector 1 and through the valve to column 2 and detector 2. When all of the sample is in column 2, the valve is switched so that column 2 becomes upstream of column 1 and the sample can return to column 1. To minimize the contribution of the injection to band broadening, the injector can be set between the valve and the column, near the last one.

In recycle operation, the extra-column contribution to the peak broadening must be kept low for optimal performance. From this point of view, as the pump contributes much more to band spreading than do connection tubes and valves[17], alternate pumping recycling is interesting compared with closed-loop recycling as the pump is not included in the sample circuit. It should be noted that for this reason, all types of chromatographic pumps are compatible with alternate pumping recycling, whereas in closed-loop recycling only alternative pumps with low chamber volumes can be used because of their relatively small peak broadening contribution. On the other hand, there are two detector cells and longer tubing in alternate pumping recycling. Furthermore, for identical performances, each of the two columns, should be similar to that used in closed-loop recycling, hence the pressure is twice as large.

In the following, we discuss mainly closed-loop recycling, but the theoretical considerations and conclusions can also be applied to alternate pumping recycling with only minor amendments.

CHARACTERISTICS OF RECYCLING

Resolution after n cycles

The aim of recycling is to increase the resolution between the components of a sample and it is therefore interesting to calculate the variation of the resolution as a function of the cycle number. For this purpose, we shall consider a two component system.

Let V_{R1} and V_{R2} be the retention volumes of peaks 1 and 2 after a single passage through the column, *i.e.*, the volumes of the mobile phase flowing through the detector between sample injection and passage of the maxima of peaks 1 and 2 in the detector, respectively; let σ_{c1}^2 and σ_{c2}^2 be the volume variances of these peaks after a single passage, *i.e.*, the time variance multiplied by the flow-rate, which is assumed to be constant throughout the analysis. The resolution, R_1, between peaks 1 and 2 after their first passage through the detector is[18,19]

$$R_1 = \frac{V_{R2} - V_{R1}}{2\,(\sigma_{c1} + \sigma_{c2})} \tag{1}$$

We shall neglect the contribution of injection to peak width and assume that σ_{c1}^2 and σ_{c2}^2 are the sums of the contributions of the column and detector only to the peak variance. Let V_A be the extra-column volume, that is, the volume of liquid phase in the apparatus between the outlet of the detector and the inlet of the column, and σ_A^2 the contribution of this extra-column part of the apparatus to the volume variance of the peaks (it is the same for the two peaks). The retention volumes after n cycles, *i.e.*, after n passages through the column and $n - 1$ passages through the extra-column volume, for peaks 1 and 2 are, respectively

$$V_{1,n} = nV_{R1} + (n - 1)\,V_A \tag{2}$$

$$V_{2,n} = nV_{R2} + (n - 1)\,V_A \tag{3}$$

and the volume variances are

$$\sigma_{1,n}^2 = n\sigma_{c1}^2 + (n - 1)\,\sigma_A^2 \tag{4}$$

$$\sigma_{2,n}^2 = n\sigma_{c2}^2 + (n - 1)\,\sigma_A^2 \tag{5}$$

From eqns. 2 and 3, the difference between the retention volumes of the two peaks increases proportionally to the cycle number, as the two peaks spend the same time in the extra-column part of the apparatus. This is shown on a schematic illustration of a recycling chromatogram in Fig. 3, where it appears also that, because of the increase in the peak widths, the maximum peak concentration decreases as the cycle number increases.

The resolution R_n after n cycles is then

$$R_n = \frac{n\,(V_{R2} - V_{R1})}{2\,[\sqrt{n\sigma_{c1}^2 + (n - 1)\,\sigma_A^2} + \sqrt{n\sigma_{c2}^2 + (n - 1)\,\sigma_A^2}]} \tag{6}$$

Variation of resolution with n

Whether R_n is greater than R_1 or not depends on the actual values of n, σ_A^2, σ_{c1}^2, σ_{c2}^2. One can show, however, that R_{n+1} is always greater than R_n, provided that n

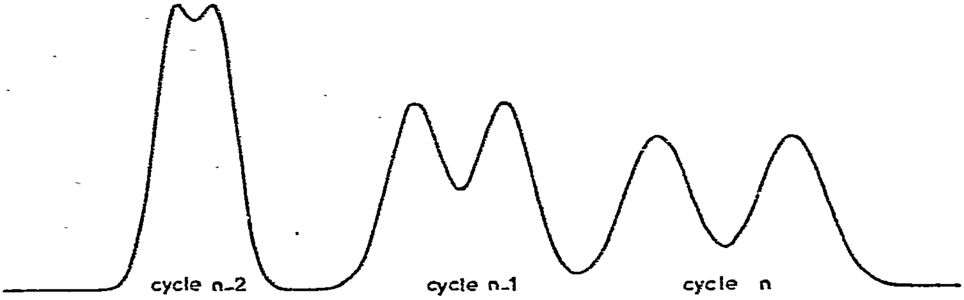

Fig. 3. Schematic illustration of a recycling chromatogram.

is greater than 1. This means that although R_2 can be smaller than R_1, the resolution increases continuously after the second cycle. Comparison of eqns. 1 and 6 shows that R_n is greater than R_1 if the following condition is fulfilled:

$$\sigma_A^2 < \frac{1}{4}[(n-1)(\sigma_{c1}+\sigma_{c2})^2 + 4\sigma_{c1}\sigma_{c2}] \tag{7}$$

The derivation of this condition is lengthy but straightforward.

These results can be applied to alternate pumping recycling if n represents the number of passages through any of the two columns assumed to be identical, and σ_A^2 the contribution to the peak volume variance of the connecting tubes and the valve tubings between the outlet of one detector and the inlet of the following column, provided that the two circuits (from detector 1 to column 2 and from detector 2 to column 1) are identical.

Eqns. 1, 6 and 7 can be simplified if we assume that the contributions of the column to the volume variance of the two peaks are identical, so that

$$\sigma_{c1} = \sigma_{c2} = \sigma_c \tag{8}$$

Then eqns. 1 and 6 become, respectively:

$$R_1 = \frac{V_{R2} - V_{R1}}{4\sigma_c} \tag{9}$$

$$R_n = \frac{\sqrt{n}\,(V_{R2} - V_{R1})}{4\sigma_c\sqrt{1 + \frac{n-1}{n}\cdot\frac{\sigma_A^2}{\sigma_c^2}}} \tag{10}$$

If λ is the ratio of the extra-column to column detector contributions to the volume variance of the peaks:

$$\lambda = \frac{\sigma_A^2}{\sigma_c^2} \tag{11}$$

then R_n is related to R_1 by the expression

$$\frac{R_n}{R_1} = \frac{\sqrt{n}}{\sqrt{1 + \frac{n-1}{n}\lambda}} \tag{12}$$

and the condition (eqn. 7) to have R_n greater than R_1 becomes

$$\lambda < n \tag{13}$$

Fig. 4 shows the variation of R_n/R_1 *versus* the cycle number n for different values of the variance ratio λ. Obviously, n must be a whole number.

The smaller λ, the better is the efficiency of recycling. The best results are obtained in the ideal case when λ is zero; then R_n/R_1 is proportional to $\sqrt{n}$ and the resolution is multiplied by 2 after 4 cycles and by 5 after 25 cycles, as if the column length were multiplied by the cycle number. When λ is between 0 and 1, the resolution increases continuously, but more slowly. When λ is greater than 1, the resolution after the second cycle is smaller than R_1, but then it increases with the cycle number and becomes larger than R_1 when n is greater than λ. Hence Fig. 4 shows that even when the extra-column sources of peak broadening are relatively important, the resolution between two peaks can be improved by recycling.

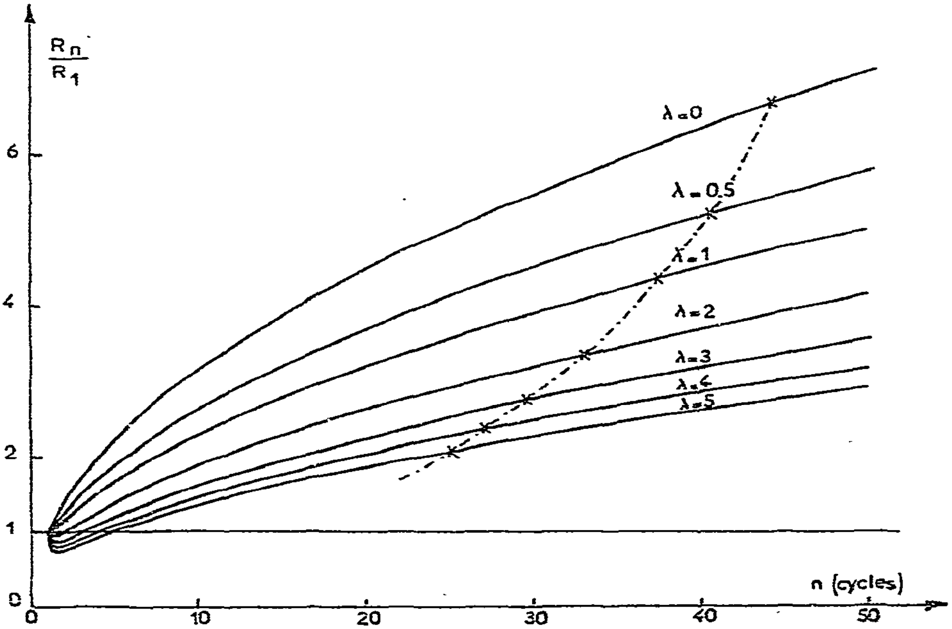

Fig. 4. Variation of the ratio of the resolution after n cycles (R_n) to the resolution after the first cycle, R_1, *versus* the cycle number, n, for different values of the extra-column to column variance ratio, λ. The broken line relates the values in each case of the final to initial resolution ratio and of the limit cycle number. $N_1 = 5000$; $R_1 = 0.25$; $\mu = \sqrt{\lambda/N_1}$ (mixing chamber).

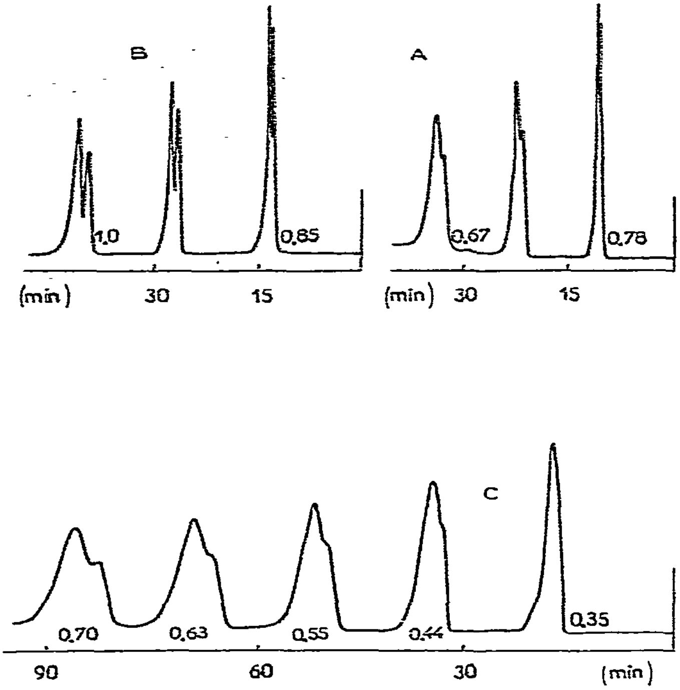

Fig. 5. Direct pumping recycling of an anthracene–phenanthrene mixture. A. Pump: modified Orlita MS 15-7-7 without damping device. Column: 50 cm × 10.7 mm; $N_1 = 6200$; $k'_2 = 1.7$; $Q = 9.4$ cm^3/min; $\sigma_A = 2.75$ cm^3; $V_A = 6.6$ cm^3; $\lambda = 4.6$. B. Pump: Waters M6000. Column: 50 cm × 10.7 mm; $N_1 = 6200$; $k'_2 = 1.7$; $Q = 7.6$ cm^3/min; $\sigma_A = 1.70$ cm^3; $V_A = 2.0$ cm^3; $\lambda = 1.75$. C. Pump: modified Orlita MS 15-7-7 without damping device. Column: 50 cm × 32 mm; $N_1 = 500$; $k'_2 = 4.5$; $Q = 97$ cm^3/min; $\sigma_A = 41.3$ cm^3; $V_A = 9.8$ cm^3; $\lambda = 0.31$. The values next to each pair of peaks give the resolution. The difference in σ_A between cases A and C results from the use of different tubings and fittings, and of largely different flow-rates.

The influence of the column and apparatus performances are illustrated in Fig. 5, which shows three direct pumping recycling chromatograms of a sample of anthracene and phenanthrene obtained under different conditions. This influence is better understood if λ is written as a function of the column parameters: the mean plate number for the two peaks after the first passage in the detector, N_1, the mean capacity factor, k', the volume fraction of mobile phase in the column, ε_m, the column length, L, and the column internal diameter, d_c:

$$\lambda = \frac{4\,\sigma_A^2\,N_1}{\pi\,\varepsilon_m\,d_c^2\,L^2\,(1+k')^2} \tag{14}$$

Chromatogram A was obtained with a 50 cm long, 1 cm I.D. column, using a two-head Orlita pump modified so as to have a smaller chamber volume. After three cycles the resolution is lower than after one, but better than after two cycles. In this instance, $\lambda = 4.6$. Chromatogram B shows the same separation on the same column, but using a Waters M6000 pump at a slightly different flow-rate. Because of the improved extra-column characteristics, owing to the change of the pump, $\lambda = 1.75$ and the resolution is slightly improved after three cycles. With chromatogram C, the separation was carried out using the modified Orlita pump and a preparative column, 50 cm long and 3.2 cm I.D. The plate number is smaller in this instance than with chromatogram A, so that the initial resolution is also smaller, although the capacity factors are increased (different silica gel). Owing to the decrease in the plate number and to the increase in the capacity factors and the internal diameter of the column, the λ ratio with chromatogram C is much smaller ($\lambda = 0.31$) than with A, so that the resolution increases by a factor of 2 after five cycles.

The strong dependence of λ on the internal diameter of the column explains why recycling is particularly interesting in preparative chromatography. It should be noted that the previous equations also apply to preparative chromatography provided that the column peak broadening is independent of the concentration of the compounds.

Fig. 4 indicates that any given resolution could be achieved, even with very poor apparatus, provided that the cycle number is sufficiently large. Unfortunately, it is not possible to increase the cycle number indefinitely.

Limit cycle number and final resolution

In Fig. 3, one can see that the resolution between peak 2 at cycle $n - 1$ and peak 1 at cycle n always decreases when the cycle number increases. This effect occurs because the total volume of the system is limited and the first peak will eventually overtake the second. For instance, in Fig. 3, the resolution between peak 2 at cycle $n - 2$ and peak 1 at cycle $n - 1$ is about 2, while this resolution after one more cycle is only 1.25. Thus, re-mixing of the two peaks occurs after a given cycle number. The corresponding resolution will be termed "re-mixing resolution".

We shall consider that it is not possible to recycle further the sample if the re-mixing resolution becomes smaller than a given value, R_{rem}. This defines the limit cycle number. There are some other possible definitions of this number. For example, we can consider that this limit is achieved when the sum of the volumes of the peak-half widths and of the peak separation corrected for the retention of the solutes is equal to the volume of the system[3,12,14]. This definition is similar to the precedent for the particular case where $R_{\text{rem}} = 1$. Another possibility is to define the limit cycle number by the condition that the re-mixing resolution becomes equal to the resolution of the two peaks in the same cycle[20]. When this generally increases the limit cycle number for difficult separations, it also leads to a large re-mixing, which is generally not wanted.

Hence the limit cycle number is defined as the maximum cycle number for which the following condition is fulfilled (*cf.*, eqns. 2–5):

$$\frac{V_{1,n} - V_{2,n-1}}{2(\sigma_{1\,n} + \sigma_{2\,n-1})} \geqslant R_{\text{rem}} \tag{15}$$

If μ is the ratio of the extra-column volume of the apparatus, V_A, to the retention volume of the second peak, V_{R2}:

$$\mu = \frac{V_A}{V_{R2}} \tag{16}$$

the inequality 15 can be written using eqns. 2–5, 8, 9 and 11, so the limit cycle number is the solution of

$$\sqrt{n}\left[\sqrt{(1+\lambda)-\frac{1+2\lambda}{n}}+\sqrt{(1+\lambda)-\frac{\lambda}{n}}\right]=\frac{V_{R2}(1+\mu)}{2\sigma_c R_{\mathrm{rem}}}-2n\frac{R_1}{R_{\mathrm{rem}}} \tag{17}$$

If we assume that n is sufficiently large, so that $[1+2\lambda]/[n(1+\lambda)]$ is negligible compared with 1, eqn. 17 can be solved and the limit cycle number, n_{lim}, is given by

$$n_{\mathrm{lim}}=\frac{R_{\mathrm{rem}}^2}{4R_1^2}(1+\lambda)\left[\sqrt{1+\frac{R_1\sqrt{N_1}}{R_{\mathrm{rem}}^2}\cdot\frac{1+\mu}{1+\lambda}}-1\right]^2 \tag{18}$$

The final resolution, $R_{n_{\mathrm{lim}}}$, at the limit cycle number is calculated by using eqns. 12 and 18:

$$R_{n_{\mathrm{lim}}}=\frac{R_{\mathrm{rem}}}{2}\left[\sqrt{1+\frac{R_1\sqrt{N_1}}{R_{\mathrm{rem}}^2}\cdot\frac{1+\mu}{1+\lambda}}-1\right] \tag{19}$$

From eqns. 18 and 19, the greater the value accepted for the re-mixing resolution, the smaller are the limit cycle number and the final resolution. If all other things remain unchanged, n_{lim} and the corresponding resolution are larger if the column plate number is larger, if the initial resolution is smaller, if the extra-column to column variance ratio is smaller and if the extra-column to column volume ratio is greater. In other words, the smaller the contributions of the column and of the apparatus to the whole sample peak width (not the width of the individual component peaks) compared with the volume of the system, the greater is the limit cycle number and hence the final resolution. It should be noted, however, that the column plate number, the initial resolution and the ratios λ and μ are generally not independent of each other. For instance, a change in the plate number of a column results in changes in the initial resolution and λ. For identical column characteristics, λ and μ are generally not independent, as an increase in V_A often causes an increase in σ_A^2. In direct pumping recycling, the main contribution to V_A and σ_A^2 is due to the pump. The relationship between V_A and σ_A^2 is a function of the pump type; in our experiments, it was also found to be a function of the flow-rate. Depending of the flow-rate, the pump can behave better or worse than a complete mixing chamber for which $\sigma_A = V_A$. A similar phenomenon occurs for connecting tubing[17].

However great the limit cycle number, the recycling will be efficient only if the final resolution is greater than the initial resolution, that is, after eqns. 12 and 19, if the following condition is valid:

$$\frac{1+\mu}{1+\lambda}>\frac{4}{\sqrt{N_1}}(R_1+R_{\mathrm{rem}}) \tag{20}$$

In this instance, the limit cycle number is greater than λ.

The dotted line in Fig. 4 joins the points corresponding to the final to initial

TABLE I

LIMIT CYCLE NUMBER AND FINAL RESOLUTION AS A FUNCTION OF THE INITIAL RESOLUTION (R_1), THE COLUMN PLATE NUMBER (N_1) AND THE ACCEPTED RE-MIXING RESOLUTION (R_{rem}) IN AN IDEAL CASE ($\lambda = \mu = 0$)

N_1	Parameter	$R_1 = 0.25$		$R_1 = 0.5$		$R_1 = 0.75$		$R_1 = 1$
		$R_{rem} = 1$	$R_{rem} = 1.5$	$R_{rem} = 1$	$R_{rem} = 1.5$	$R_{rem} = 1$	$R_{rem} = 1.5$	$R_{rem} = 1.5$
500	n_{lim}	9.8	6.8	6.2	4.7	4.6	3.6	3.0
	R_{fin}	0.78	0.65	1.24	1.08	1.61	1.43	1.73
1000	n_{lim}	15.7	11.4	9.6	7.6	7.0	5.7	4.7
	R_{fin}	0.99	0.84	1.55	1.37	1.99	1.80	2.16
2500	n_{lim}	28.6	21.9	16.8	13.8	12.0	10.3	8.2
	R_{fin}	1.34	1.17	2.05	1.86	2.60	2.40	2.86
5000	n_{lim}	44.1	35.1	25.3	21.4	17.9	15.6	12.4
	R_{fin}	1.66	1.48	2.51	2.32	3.17	2.97	3.52
10,000	n_{lim}	67.2	55.4	37.7	32.8	26.4	23.6	18.5
	R_{fin}	2.05	1.86	3.07	2.86	3.86	3.64	4.30
25,000	n_{lim}	115.2	98.6	63.2	56.5	43.9	40.0	31.2
	R_{fin}	2.68	2.48	3.97	3.76	4.97	4.75	5.58
50,000	n_{lim}	171.2	150.1	92.6	84.3	63.9	59.2	45.7
	R_{fin}	3.27	3.06	4.81	4.59	5.99	5.77	6.76

resolution ratio and the limit cycle number, on the different curves (various values of λ), assuming an initial resolution of 0.25, a column plate number of 5000 and that the extra-column part of the apparatus behaves like a mixing chamber. The final resolution varies from 1.66 to 0.52 and the limit cycle number from 44 to 25 when λ increases from 0 to 5.

Table I shows the values of n_{lim} and $R_{n_{lim}}$ obtained for different values of the initial resolution, the column plate number and the accepted re-mixing resolution in the ideal case where λ and μ are zero. These values were calculated from eqns. 18 and 19. Obviously, only whole number for n_{lim} are meaningful and the condition 15 will be satisfied if the recycling is stopped after the whole numbers in Table I, ignoring the decimal part. It should be noted that in all instances, even for the small values of the limit cycle number, when the approximation made to solve eqn. 17 is not too good, the values calculated using eqn. 18 do not differ by more than 0.3 from numerical solutions of eqn. 17 and are always smaller. As only whole numbers for n_{lim} are expected, eqn. 18 is satisfactory in all instances.

The values in Table I are larger than those achievable in real situations, where λ and μ are different from zero. Table II gives the values obtained for two extra-column systems representing well designed configurations for alternate pumping (system A: $\sigma_A = 0.05$ cm^3, $V_A = 0.1$ cm^3) and closed-loop recycling (system B: $\sigma_A = 0.5$ cm^3, $V_A = 2$ cm^3). The column dimensions are 50 cm × 4 mm, the total porosity ε_m is 0.85, the initial resolution is 0.25 and the capacity factor of the second peak is 2.

TABLE II

LIMIT CYCLE NUMBER AND FINAL RESOLUTION FOR REAL SYSTEMS

$R_1 = 0.25$; $R_{rem} = 1.5$. Column: 50 cm × 4 mm; $\varepsilon_m = 0.85$; $k'_2 = 2$. System A: $\sigma_A = 0.05$ cm^3; $V_A = 0.1$ cm^3. System B: $\sigma_A = 0.5$ cm^3; $V_A = 2$ cm^3.

N_1	*Parameter*	*System A*	*System B*
1000	n_{lim}	11	9
	R_{fin}	0.83	0.55
10,000	n_{lim}	54	21
	R_{fin}	1.76	0.35

System A behaves like an ideal system for a plate number of 1000 and similarly for 10,000 plates. On the contrary, for system B, the limit cycle number and the final resolution are much smaller than for an ideal system. It should be observed that the value of the final resolution is smaller for the 10,000 than for the 1000-plate column, although the limit cycle number is greater for the former column. This is because the relative contribution of the apparatus to the peak broadening is much greater in the 10,000-plate column, in spite of the fact that the components to be separated are more closely eluted on the column with the largest plate number, as the initial resolution and the capacity factor of the second peak are assumed to be constant. The values of λ and μ for the two systems are given in Table III for the instances discussed in Table II.

For alternate pumping recycling, it is meaningless to speak of a re-mixing resolution, as the components cannot re-mix. When the total sample width becomes too large, however, a fraction of the sample cannot be recycled and it has to be drained.

TABLE III

EXTRA-COLUMN TO COLUMN VARIANCE (λ) AND VOLUME (μ) RATIOS FOR REAL SYSTEMS

The columns and extra-column systems are described in Table II.

N_1	*Parameter*	*System A*	*System B*
1000	λ	0.00974	0.974
	μ	0.00624	0.125
10,000	λ	0.0974	9.74
	μ	0.00624	0.125

Hence, if we wish to recycle all of the sample, the sum of the peak-half widths and of the peak separation corrected for the retention of the solutes must be kept smaller than a fraction of the half-volume of the total system. The smaller this fraction, the larger the amount of sample recycled. Such a condition can be written in terms very similar to those of a re-mixing resolution. Thus, keeping this fact in mind, the previous equations can be applied to alternate pumping recycling.

In preparative chromatography, the situation is much better than that described above for both direct pumping and alternate pumping recycling. When pure fractions are collected at both ends of the mixture zone, the sample width is shortened and the limit cycle number can be increased accordingly. The final resolution is brought to a higher value, which is favourable.

Minimum initial resolution

The aim of a recycling process is to bring the resolution between the two components to a given final value, R_{fin}, without re-mixing the compounds. This is not possible if, for a given plate number, the initial resolution is too small because the re-mixing appears before the desired final resolution is reached. The values of the minimum initial resolution, $R_{1\text{min}}$, is obtained after eqn. 19:

$$R_{1\,\text{min}} = 4\,\frac{R_{\text{fin}}}{\sqrt{N_1}}\cdot\frac{1+\lambda}{1+\mu}\,(R_{\text{fin}} + R_{\text{rem}}) \tag{21}$$

and the necessary cycle number, n_{nec}, which is the limit cycle number, is calculated using eqns. 12 and 21 with the same previous approximation:

$$n_{\text{nec}} = \frac{N_1}{16\,(R_{\text{fin}} + R_{\text{rem}})^2}\cdot\frac{(1+\mu)^2}{1+\lambda} \tag{22}$$

The minimum value of the relative retention, α_{min}, of the two components is calculated using eqn. 21 and the Purnell equation for the resolution[21]:

$$\alpha_{\text{min}} = \frac{k_2'}{k_2' - \dfrac{4\,(1+k_2')\,R_{1\text{min}}}{\sqrt{N_1}}} \tag{23}$$

TABLE IV

MINIMUM VALUES OF THE INITIAL RESOLUTION (R_{1min}) AND RELATIVE RETENTION (α_{min}) TO OBTAIN A GIVEN FINAL RESOLUTION (R_{fin}) AFTER A NUMBER OF CYCLES EQUAL TO THE LIMIT NECESSARY CYCLE NUMBER (n_{nec}) IN AN IDEAL CASE ($\lambda = \mu = 0$)

The α_{min} values are tabulated for $k'_2 = 2$.

N_1	*Parameter*	$R_{fin} = 1$		$R_{fin} = 1.5$
		$R_{rem} = 1$	$R_{rem} = 1.5$	$R_{rem} = 1.5$
500	R_{1min}	0.358	0.447	0.805
	α_{min}	1.106	1.136	1.276
	n_{nec}	7.8	5.0	3.5
1000	R_{1min}	0.253	0.316	0.569
	α_{min}	1.050	1.064	1.121
	n_{nec}	15.6	10.0	6.9
2500	R_{1min}	0.160	0.200	0.360
	α_{min}	1.020	1.025	1.045
	n_{nec}	39.1	25.0	17.4
5000	R_{1min}	0.113	0.141	0.255
	α_{min}	1.0097	1.012	1.022
	n_{nec}	78.1	50.0	34.7
10,000	R_{1min}	0.080	0.100	0.180
	α_{min}	1.0048	1.0060	1.011
	n_{nec}	156	100	69.4
25,000	R_{1min}	0.051	0.063	0.114
	α_{min}	1.0019	1.0024	1.0043
	n_{nec}	391	250	174
50,000	R_{1min}	0.036	0.045	0.080
	α_{min}	1.0010	1.0012	1.0022
	n_{nec}	781	500	347

Typical results are given in Table IV in the ideal case ($\lambda = \mu = 0$) for different values of the column plate number and of the final resolution. The α values are tabulated for a capacity factor of the second peak, k'_2 equal to 2. Fig. 6 shows the variation of the minimum value of the relative retention and of the initial resolution *versus* the column plate number in the ideal case for R_{fin} and R_{rem} equal to 1. Very closely eluted compounds can be separated if the plate number is sufficiently large, but the necessary cycle number is also very large.

Higher minimum values of R_1 and α are generally obtained in real situations, as can be seen in Table V, which gives the minimum values of the initial resolution and of the relative retention and the necessary cycle number using the same two systems A and B and the same column geometry as previously described (*cf.*, Tables II and III).

The final resolution and the accepted re-mixing resolution are equal to 1. The values obtained with system A, designed for alternate pumping recycling, are very similar to those obtained with an ideal system. With system B, designed for closed-loop recycling, however, the values of α_{min} and R_{1min} are much larger, especially with the 10,000-plate column, as the limit cycle number is smaller.

Recycling and concentration

As the cycle number increases, the peak width increases and hence the maxi-

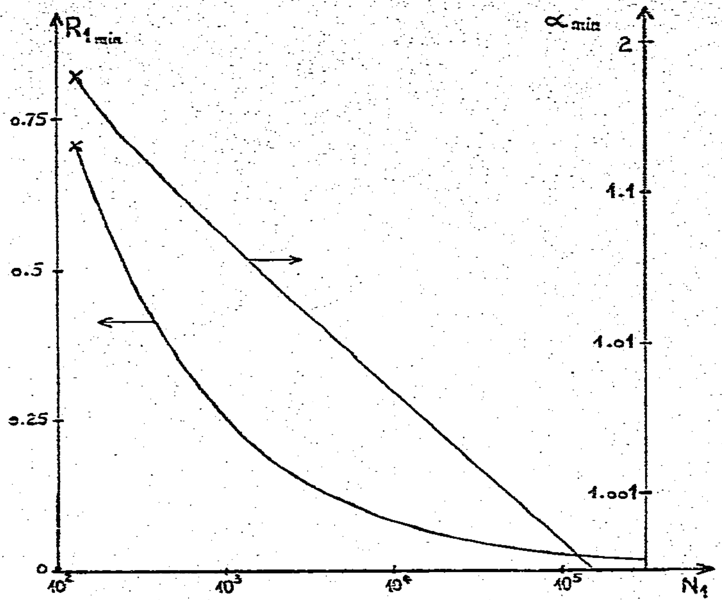

Fig. 6. Variation of the minimum selectivity factor and initial resolution to obtain a final resolution of 1 with the column theoretical plate number in an ideal case ($\lambda = \mu = 0$). $R_{rem} = 1$; $k'_2 = 2$. The crosses correspond to a cycle number of 2.

mum concentration of the peak decreases. After the first passage through the detector, the maximum concentration of the peak, $C_{max,1}$ is given by[22]

$$C_{max,1} = \frac{m}{\sigma_c \sqrt{2\pi}} \tag{24}$$

if m is the amount of this compound injected. After n cycles, this concentration becomes

$$C_{max,n} = \frac{m}{\sigma_n \sqrt{2\pi}} \tag{25}$$

TABLE V

MINIMUM VALUES OF THE INITIAL RESOLUTION (R_{1min}) AND RELATIVE RETENTION (α_{min}) TO OBTAIN A FINAL RESOLUTION OF 1 AFTER THE LIMIT NECESSARY CYCLE NUMBER (n_{nec}) IN REAL CASES

The α_{min} values are tabulated for $k'_2 = 2$. $R_{rem} = 1.5$. The columns and extra-column systems are described in Table II.

N_1	*Parameter*	*System A*	*System B*
1000	R_{1min}	0.32	0.55
	α_{min}	1.06	1.12
	n_{nec}	10	6
10,000	R_{1min}	0.11	0.95
	α_{min}	1.007	1.06
	n_{nec}	92	11

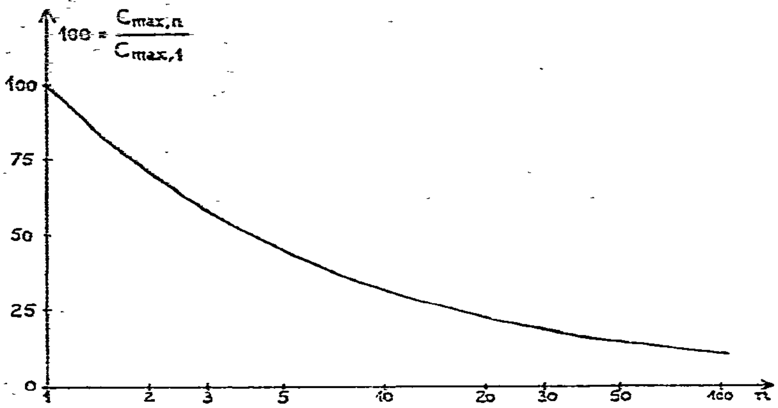

Fig. 7. Variation of the ratio of the peak maximum concentration after n cycles, $C_{max,n}$, to the maximum concentration after the first cycle, $C_{max,1}$, with the cycle number n in the ideal case ($\lambda = 0$).

The combination of eqns. 4, 8, 24 and 25 gives

$$\frac{C_{max,n}}{C_{max,1}} = \frac{1}{\sqrt{n\left(1 + \frac{n-1}{n}\lambda\right)}} \tag{26}$$

The larger $C_{max,n}$ values are obtained in the ideal case ($\lambda = 0$). The variation of the ratio $C_{max,n}/C_{max,1}$ *versus* n in the ideal case are plotted in Fig. 7. This variation is mainly important for the first cycles, as for the resolution.

In fact, the product of the resolution and the maximum concentration remains constant.

Peak number

The previous equations for the final resolution and the limit cycle number are valid for two compounds. The situation is more difficult for a complex mixture with p components, a, b, ..., p, of capacity factors k'_a, k'_b, ..., k'_p.

The limit cycle number depends only on the behaviour of the extreme peaks, a and p. In this instance, we shall no longer assume that the peak variances are equal (*cf.*, eqn. 8), but that the column plate numbers of these two peaks are the same, N_1. Let β be the velocity ratio of these two peaks in the column:

$$\beta = \frac{V_{R,a}}{V_{R,p}} = \frac{1 + k'_a}{1 + k'_p} \tag{27}$$

and let

$$\lambda_p = \frac{\sigma_A^2}{\sigma_{c,p}^2} \tag{28}$$

and

$$\mu_p = \frac{V_A}{V_{R,p}} \tag{29}$$

where $\sigma^2_{c,p}$ and $V_{R,p}$ are the column contribution to the variance and the retention volume of the last peak, respectively.

With the slight approximation that $\lambda_p/(\beta^2 + \lambda_p)n$ and $(1 + 2\lambda_p)/(1 + \lambda_p)n$ are negligible compared with 1, the limit cycle number is given by

$$n_{\text{lim}} = \frac{R^2_{\text{rem}}}{N_1 (1 - \beta)^2} \left[\sqrt{(\sqrt{\beta^2 + \lambda_p} + \sqrt{1 + \lambda_p})^2 + \frac{N_1 (1 - \beta)(1 + \mu_p)}{R^2_{\text{rem}}}} - (\sqrt{\beta^2 + \lambda_p} + \sqrt{1 + \lambda_p}) \right]^2 \tag{30}$$

This expression is similar to eqn. 18 if one takes into account that for two peaks

$$R_1 = \frac{\sqrt{N_1}}{4} (1 - \beta) \tag{31}$$

The final resolution for a pair of adjacents compounds is always given by eqn. 12, n being the limit cycle number given by eqn. 30 and λ being the extra-column to column variance ratio for this pair of compounds. Hence, eqn. 14 shows that the λ values for the more retained compounds are smaller than the values for the first-eluted peaks. Then the resolution of the more retained compounds increases slightly more rapidly than the resolution of the first-eluted compounds.

Eqn. 30 is complicated, but it shows that, at least in the ideal case ($\lambda_p = \mu_p = 0$), the limit cycle number does not depend on the number of components, p, but only on the characteristics of the extreme peaks. It also does not depend on the individual capacity factors of these peaks, but only on their velocity ratio, β. The smaller this value, the smaller is the limit cycle number. In the ideal case, the minimum value of this velocity ratio, which is necessary to recycle the sample n times while keeping a re-mixing resolution of greater than R_{rem}, is calculated after eqn. 30:

$$\beta_{\min} = \frac{(n - 1)\cdot\dfrac{\sqrt{N_1}}{2R_{\text{rem}}} + \sqrt{n - 1}}{n\cdot\dfrac{\sqrt{N_1}}{2R_{\text{rem}}} - \sqrt{n}} \tag{32}$$

The consideration of two extreme cases can help to give a picture of the possibilities of multi-component recycling. The first unrealistic case occurs if there is no resolution between the first and the last peaks. The final resolution is, of course, zero and the cycle number is limited by the re-mixing of the head of the peak with its tail.

This maximum cycle number is calculated by solving eqn. 17 with $R_1 = 0$:

$$n_{\max} = \frac{R^2_{\text{rem}}}{N_1 (1 + \mu_p)^2 (1 + \lambda_p)} \left\{ \left[\frac{N_1 (1 + \mu_p)^2}{4 R^2_{\text{rem}}} + (1 + 3\lambda_p) \right]^2 - 4\lambda_p (1 + \lambda_p) \right\} \tag{33}$$

This number is 28 in the ideal case ($\lambda_p = \mu_p = 0$) if the column plate number is 1000 and the accepted re-mixing resolution is 1.5.

The other extreme case is that for which the first and the last peaks are completely re-mixed, so that their maxima occur at the same time and their re-mixing resolution is therefore zero. The cycle number and the β value corresponding to this case are given by the following expressions:

$$n = \frac{1 + \mu_p}{1 - \beta} \tag{34}$$

$$\beta = \frac{n - (1 + \mu_p)}{n} \tag{35}$$

Hence, in the ideal case ($\mu_p = 0$), if $\beta = (n - 1)/n$ the first and last peaks are completely re-mixed after n cycles. For example, if $\beta = 0.67$ (maximum k' range, k'_a to k'_p: 0 to 0.5 or 2 to 3.5 or 5 to 8 or 10 to 15.5), this cycle number is only 3; if $\beta = 0.91$, this cycle number is 11 but the maximum k' range is very small (0–0.1 or 2–2.3 or 5–5.6 or 10–11.1).

In practice, these cycle numbers are smaller, as one wants to keep a given re-mixing resolution, R_{rem}, between the last and the first peaks. This picture shows that the possibilities of continuous recycling chromatography for multicomponent samples are very limited, because the limit cycle number rapidly becomes very small when the k' range increases. As mentioned earlier, the situation is much better in preparative chromatography, as it is possible to collect pure fractions when necessary, and to increase the cycle number by reducing the sample width during the separation.

ADVANTAGES OF RECYCLING

When comparing the advantages and drawbacks of recycling systems over those of one-cycle systems, one wishes to look at the relationship between resolution, analysis speed and pressure drop for these systems. For one-cycle systems, the resolution is unambiguously related to the theoretical plate number by the Purnell equation[21], when the α and k' values are determined:

$$R = \frac{\sqrt{N}}{4} \cdot \frac{\alpha - 1}{\alpha} \cdot \frac{k'_2}{1 + k'_2} \tag{36}$$

and the required relationship is given by the following equation[23]:

$$\frac{t_R \Delta P}{N^2} = \frac{\eta}{k_0} (1 + k') h^2 \tag{37}$$

where ΔP is the pressure drop, t_R the analysis time (retention time of the last peak), k' the capacity factor of the last peak (second peak for a two-component system), η the viscosity of the mobile phase, k_0 the dimensionless permeability coefficient and h the reduced plate height. The smallest and best value of $t_R \Delta P/N^2$ is obtained for the minimal value of h. Thus, analyses carried out with systems working at the minimum of the efficiency curve, $H(u)$, are obtained with a minimum pressure drop[24].

Recycle and one-cycle systems must therefore be compared under optimized conditions, giving the same resolution for the same pair of components using the same phase systems at the same temperature. Under these conditions, η, k'_2 and α are constant; k_0, which depends on the packing structure and $h_{\min}$ (the minimum reduced plate height), can reasonably be considered as constant.

In recycle systems, the relationship between the theoretical plate number and the resolution is not as clear as eqn. 36 for one-cycle systems, as the apparent k' value depends on the cycle number. Indeed, if V_m is the volume of mobile phase in the column, V_n and $V_{0,n}$ the retention volumes of the retained and inert peaks, respectively, after n cycles, the apparent value of k' (k'_{app}) is related to the true value (k'_{true}) in the column by the following equation:

$$k'_{\mathrm{app}} = \frac{k'_{\mathrm{true}}}{1 + \frac{n-1}{n} \cdot \frac{V_A}{V_m}} \tag{38}$$

A similar phenomenon can appear when coupling a large-volume refractive index (RI) detector after a UV detector. The theoretical number measured with the RI detector can be greater than that measured with the UV detector, because the relative increase of the elution time due to the addition of the second detector is greater than the relative increase of the standard deviation of the peak. But of course, the resolution is greater in the UV detector. Such a change in k' and hence in the theoretical plate number occurs in programming techniques[25].

One therefore has to use an efficiency value related to the resolution, independent of the k' value, such as the effective plate number. This number after one cycle, $N_{\mathrm{eff},1}$, is related to the theoretical plate number and to the resolution by the following equations:

$$N_{\mathrm{eff},1} = N_1 \left(\frac{k'}{1+k'}\right)^2 \tag{39}$$

$$R_1 = \frac{\sqrt{N_{\mathrm{eff},1}}}{4} \cdot \frac{\alpha - 1}{\alpha} \tag{40}$$

The relative retention, *i.e.*, the ratio of the k' values of the two peaks, does not depend on the cycle number.

The theoretical and effective plate numbers, N_n and $N_{\mathrm{eff},n}$, obtained after n cycles are:

$$N_n = N_1\, n \frac{\left(1 + \frac{n-1}{n} \cdot \mu\right)^2}{\left(1 + \frac{n-1}{n} \cdot \lambda\right)} \tag{41}$$

$$N_{\mathrm{eff},n} = N_{\mathrm{eff},1} \cdot \frac{n}{1 + \frac{n-1}{n} \cdot \lambda} \tag{42}$$

From eqns. 12 and 42 we obtain

$$\frac{R_n}{R_1} = \sqrt{\frac{N_{\mathrm{eff},n}}{N_{\mathrm{eff},1}}} \tag{43}$$

Four systems are compared: one recycling system and three different one-cycle systems. The first one (system A) is the direct pumping system, recycling the sample through a column while working under optimized conditions so that, after n cycles, $N_{\mathrm{eff},n}$ effective plates are obtained in a time t_n, with a pressure drop ΔP. The second system (system B) is a one-cycle optimized system, giving the same effective plate number in the same time; the third (system C) is also an optimized one-cycle system and gives the same effective plate number with the same pressure drop; and the fourth is a one-cycle optimized system (D) and gives the same effective plate number using particles of identical size. The analysis time, pressure drop, column length, particle diameter, maximum concentration of the peak at the end of the analysis, flow-rate (Q) and volume V of mobile phase used during the analysis necessary to obtain the separation with the three last systems are compared with the values of the closed-loop recycling system.

For this calculation the following relationships have been used[23]:

$$\Delta P = C_1 \cdot \frac{N_{\mathrm{eff}}^2}{t_R} \tag{44}$$

$$L = C_2 \sqrt{N_{\mathrm{eff}}\, t_R} \tag{45}$$

$$d_p = C_3 \sqrt{\frac{t_R}{N_{\mathrm{eff}}}} \tag{46}$$

$$C_{\max} = C_4 \sqrt{\frac{1}{t_R}} \tag{47}$$

$$Q = C_5 \sqrt{\frac{N_{\mathrm{eff}}}{t_R}} \tag{48}$$

$$V = V_m (1 + k') \tag{49}$$

where C_1–C_5 are the following proportionality constants:

$$C_1 = \frac{\eta}{k_0} \cdot \frac{(1 + k')^5}{k'^4} \cdot h_{\min}^2 \tag{50}$$

$$C_2 = \sqrt{h_{\min}\, \nu_{\mathrm{opt}} \cdot \frac{D_m (1 + k')}{k'^2}} \tag{51}$$

$$C_3 = \sqrt{\frac{\nu_{\mathrm{opt}}}{h_{\min}} \cdot D_m \cdot \frac{k'^2}{(1 + k')^3}} \tag{52}$$

TABLE VI

COMPARATIVE PERFORMANCES OF n-CYCLE AND ONE-CYCLE SYSTEMS

Parameter	*System A*	*System B*
Effective plate number or resolution	1	1
Theoretical plate number	$\left(1+\frac{n-1}{n}\cdot\mu\right)^2$	1
Analysis time	1	1
Pressure drop	1	$\frac{n}{\left(1+\frac{n-1}{n}\cdot\lambda\right)^2\left(1+\frac{n-1}{n}\cdot\mu\right)}$
Particle diameter	1	$\left(1+\frac{n-1}{n}\cdot\lambda\right)^{1/2}\left(1+\frac{n-1}{n}\cdot\mu\right)^{1/2}$
Column length	1	$n\,\frac{\left(1+\frac{n-1}{n}\cdot\mu\right)^{1/2}}{\left(1+\frac{n-1}{n}\cdot\lambda\right)^{1/2}}$
Maximum concentration at the end of the analysis	1	$\frac{\left(1+\frac{n-1}{n}\cdot\lambda\right)^{1/2}}{\left(1+\frac{n-1}{n}\cdot\mu\right)^{1/2}}$
Flow-rate	1	$\frac{1}{\left(1+\frac{n-1}{n}\cdot\lambda\right)^{1/2}\left(1+\frac{n-1}{n}\cdot\mu\right)^{1/2}}$
Volume of mobile phase used during the analysis	1	$\frac{1+k'}{1+\frac{\mu}{1+k'}}\cdot n\cdot\frac{\left(1+\frac{n-1}{n}\cdot\mu\right)^{1/2}}{\left(1+\frac{n-1}{n}\cdot\lambda\right)^{1/2}}$

$$C_4=\frac{4m}{\pi\,\varepsilon_m\,d_c^2\,\sqrt{2\pi}}\sqrt{\frac{1}{h_{\min}\,\nu_{\text{opt}}\,D_m\,(1+k')}} \tag{53}$$

$$C_5=\frac{\pi\,\varepsilon_m\,d_c^2}{4}\sqrt{h_{\min}\,\nu_{\text{opt}}\,D_m\cdot\frac{(1+k')^3}{k'^2}} \tag{54}$$

where ν_{opt} is the optimum reduced velocity, D_m the diffusion coefficient of the second peak, m the injected mass of the component of interest, assumed constant, ε_m the

tem C	System D	System E
	1	1
	1	1
$\frac{n}{+\frac{n-1}{n}\cdot\lambda)^2\left(1+\frac{n-1}{n}\cdot\mu\right)}$	$\frac{1}{\left(1+\frac{n-1}{n}\cdot\lambda\right)\left(1+\frac{n-1}{n}\cdot\mu\right)}$	1
	$\frac{n}{\left(1+\frac{n-1}{n}\cdot\lambda\right)}$	2
$\frac{n^{1/2}}{+\frac{n-1}{n}\cdot\lambda)^{1/2}}$	1	1
$\frac{n^{3/2}}{+\frac{n-1}{n}\cdot\lambda)^{3/2}}$	$\frac{n}{\left(1+\frac{n-1}{n}\cdot\lambda\right)}$	1
$\frac{+\frac{n-1}{n}\cdot\lambda)^{3/2}}{n^{1/2}}$	$\left(1+\frac{n-1}{n}\cdot\lambda\right)$	1
$\frac{+\frac{n-1}{n}\cdot\lambda)^{1/2}}{n^{1/2}}$	1	1
$\frac{+k'}{\frac{\mu}{1+k'}}\cdot\frac{n^{3/2}}{\left(1+\frac{n-1}{n}\cdot\lambda\right)^{3/2}}$	$\frac{1+k'}{1+\frac{\mu}{1+k'}}\cdot\frac{n}{\left(1+\frac{n-1}{n}\cdot\lambda\right)}$	$\frac{1+k'}{1+\frac{\mu}{1+k'}}\cdot n\left(1+\frac{n-1}{n}\cdot\mu\right)$

total porosity and d_c the column internal diameter, considered as constant. For closed loop recycling, the analysis time, t_n, is related to the elution time after the first cycle, t_1, using eqn. 3:

$$t_n = t_1\, n\left(1+\frac{n-1}{n}\cdot\mu\right) \tag{55}$$

The results of this comparison obtained using eqns. 42, 44–49 and 55 are reported in Table VI. Of course, the relative values depend on the ratios λ and μ.

The results in Table VI also contain the performances of the alternate pumping recycling (system E), which are similar to those of direct pumping recycling (system A), except for the pressure, which must be multiplied by two owing to the presence of two identical columns (neglecting the pressure drop in the connecting tubing and in the valve) and for the volume of mobile phase used, which in this instance is equal to the retention volume of the second peak, that is after eqns. 3 and 16:

$$V_{(\text{alternate pumping})} = n\, V_m\,(1 + k')\left(1 + \frac{n-1}{n}\cdot\mu\right) \qquad (56)$$

when this volume is only $V_m\ (1 + [\mu]/[1 + k'])$ for direct pumping recycling.

Note that for alternate pumping recycling, n is the number of passages of the sample through either one or the other of the two columns.

The advantages of the recycling system A over system B are well known: recycling the sample n times through a column can give an analysis with the same resolution and in the same time, at least for an ideal system, as does a column n times longer packed with the same particles but needing a pressure drop n times higher. In this instance, recycling is advantageous mainly from the points of view of pressure and packing material. System D is identical with system B for an ideal system and these two systems differ only in real cases when λ and μ differ markedly from zero.

When comparing an n-cycle recycling system with a single column passage giving the same resolution with the same pressure drop (systems A and C), it appears that the ideal recycling uses a column $n^{3/2}$ shorter, packed with $n^{1/2}$ smaller particles. Moreover, the analysis time by recycling is divided by n and the maximum concentration of the peaks at the end of the analysis is $n^{1/2}$ greater. These results are illustrated in Fig. 8, which is a logarithmic plot of the effective plate number *versus* the analysis time for different inlet pressures. For one-cycle chromatography, these curves are straight lines with slopes of 0.5 because, after eqn. 44, for a given pressure the analysis time is proportional to the square of the plate number. Of course, achieved in a given time, the higher the pressure drop, the greater is the plate number. These lines correspond to typical values of the parameters in eqn. 50[24] of $k_0 = 8.46\cdot 10^{-4}$, $\eta = 0.4$ cP, $k'_2 = 2$ and $h_{\min} = 3.15$. If one recycles the sample through one column for which the elution time is, for example, 5 min, the effective plate number achieved with an ideal system increases in proportion of the cycle number (eqn. 42) or to the analysis time (eqn. 55), as indicated by the full straight lines with slopes equal to 1. Of course, these lines are limited by the limit cycle number, which depends on the initial resolution and the initial plate number.

In Fig. 8 is plotted in the ideal case, the limit cycle number obtained with an initial resolution such that the final resolution be 1, while the re-mixing resolution is 1.5 (eqn. 22 with $\lambda = \mu = 0$). This is the full straight line with a slope equal to 2. From Fig. 8, 200,000 effective plates can be obtained with a pressure of 100 atm in more than 3 days by one-cycle chromatography and in less than 3 h by ideal recycling chromatography.

It should be noted that the reason for the difference in slope of the lines corresponding to systems A and C is that in this last instance, the characteristics of the column (d_p, L) change while the number of plates achieved increases.

Direct pumping recycling is also greatly advantageous in preparative chroma-

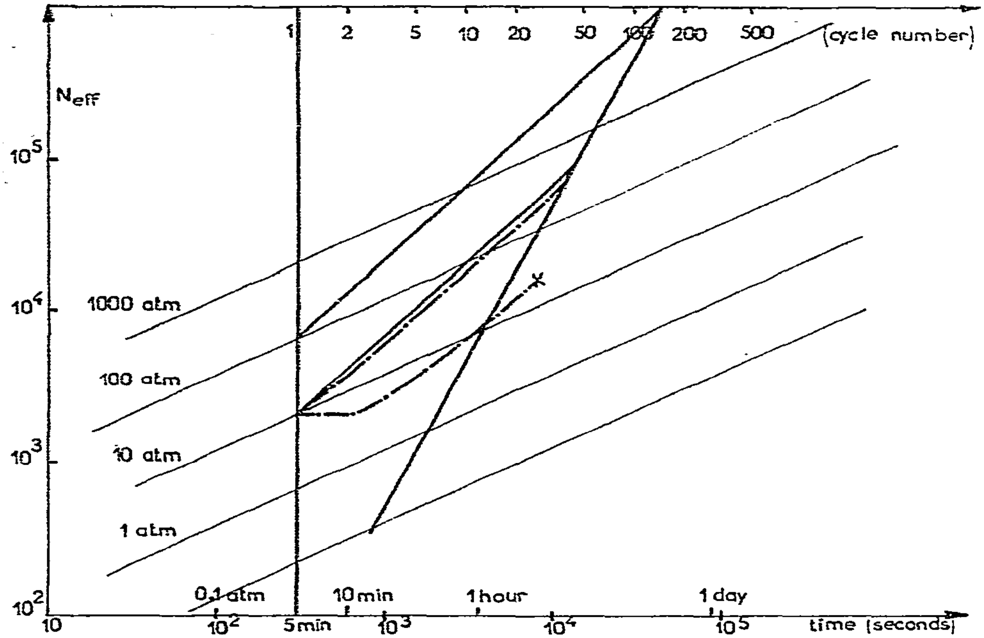

Fig. 8. Comparison of the performances of recycle and one-cycle systems working under optimized conditions (at the minimum reduced plate height). Variation of the effective plate number with the analysis time (or the cycle number for recycling). The straight lines with slope 0.5 represent the performances of one-cycle systems for different available inlet pressures. The full straight lines with slope 1 represent the performances of ideal recycling through a column for which the elution time is 5 min, with different inlet pressures. The full straight line with slope 2 corresponds in the ideal case ($\lambda = \mu = 0$) to the limit cycle number obtained with an initial resolution such that the final resolution is 1. The dotted lines represent real recycle systems; for the upper one, $\lambda = 0.1$ and $\mu = 0.0047$; for the lower one, $\lambda = 1$ and $\mu = 0.0148$. $R_{rem} = 1.5$; $k_0 = 8.46 \cdot 10^{-4}$; $\eta = 0.4$ cP; $k'_2 = 2$; $h_{min} = 3.15$.

tography because, independent of the fact that preparative systems are nearly ideal, the volume of mobile phase used during the recycling is much smaller than that used in one-cycle systems (Table VI). Even for fraction collection, recycling is interesting as the dilution is lower. Another possible advantage of recycling in preparative work comes from the reduced flow-rate (*cf.*, Table VI).

Of course, the advantages of recycling are less for real systems, as shown by the dotted lines on Fig. 8. The upper dotted line corresponds to an alternate pumping recycling system with a λ value of 0.1 and the lower dotted line to a closed-loop recycling system with a λ value of 1, with a pressure drop of 10 atm through both columns. The two extra-column systems are assumed to act as mixing chambers (in this instance $\lambda = N_1\mu^2$). If the first system is close to ideal, the second system becomes better than the one-cycle system only after about 10 cycles. The cross on the line for the second system indicates the limit cycle number, as described above.

From this diagram, it is found that the smaller the elution time for the first cycle, the shorter the time necessary to reach a given effective plate number with a given pressure. However, this procedure cannot be extended to very small values of the first cycle time and very high cycle numbers, because on the one hand the limit cycle number becomes small (for a given analysis time, the line with slope equal to 2 is displaced to higher effective plate numbers and, on the other hand, for a given apparatus λ and μ would become relatively important. Hence a compromise exists which depends on the particular case involved.

CONCLUSION

Recycling chromatography permits the achievement of high-performance analyses more rapidly than with one-cycle chromatography and thus extends the ultimate limits of liquid chromatography. We know that any effective plate number can be achieved, at least theoretically, in one-cycle chromatography provided that sufficient time is spent; recycling permits a marked decrease of this time.

It is desirable, however, to design extra-column volumes that behave similarly to ideal systems, with zero contributions to retention and to peak broadening. Such systems are feasible in alternate pumping recycling, although two identical columns are needed, or in direct (closed-loop) pumping recycling with low-volume alternative pumps, especially if columns of large internal diameter are used. Then the column diameter is such that the maximum flow-rate delivered by the pump corresponds to the optimum flow-rate. Although this study was carried out under linear elution conditions, the general conclusions can also be applied in preparative chromatography, in which case a net increase in productivity can be obtained.

Recycling could also be used successfully in trace analysis with possible collection of the major peaks, because firstly, as mentioned above, the time necessary to separate the trace peak sufficiently is less than for a one-cycle system (*cf.*, Table VI) and secondly, the dilution factor is smaller, so the detection limit of this compound is decreased.

An example of recycling is shown in Fig. 9. It shows a separation of anthracene

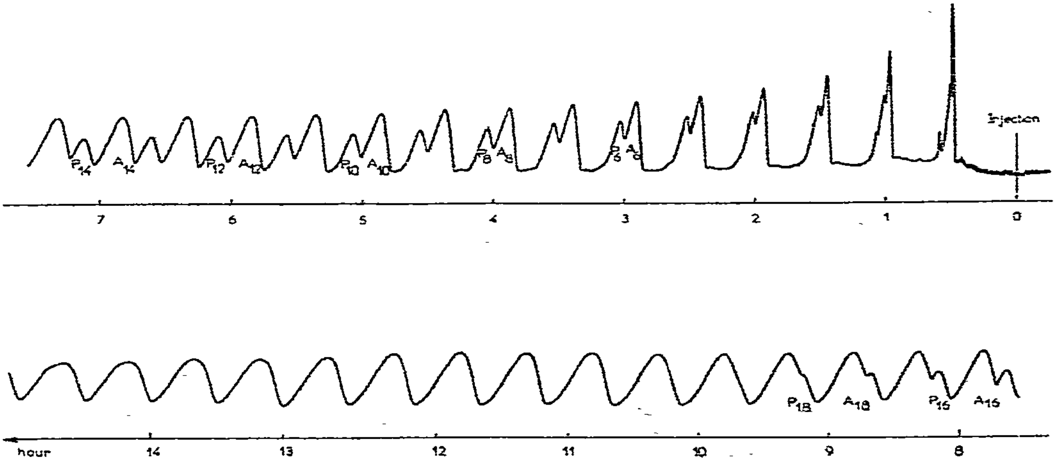

Fig. 9. Direct recycling chromatogram of an anthracene(A)–phenanthrene(P) mixture. Pump: modified Orlita 15-7-7 without damping device. Loop-valve injection (716 μl). Column: 50 cm × 10.7 mm; k'_1 (anthracene) = 3.58; k'_2 = 3.83; $\alpha = 1.07$; V_A = 6.6 ml; Q = 5.7 ml/min.

and phenanthrene under conditions similar to those in Fig. 5A, but with a smaller flow-rate. In spite of the tailing of the peaks, satisfactory resolution of the two peaks can be obtained without significant re-mixing after 12 or 13 cycles.

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