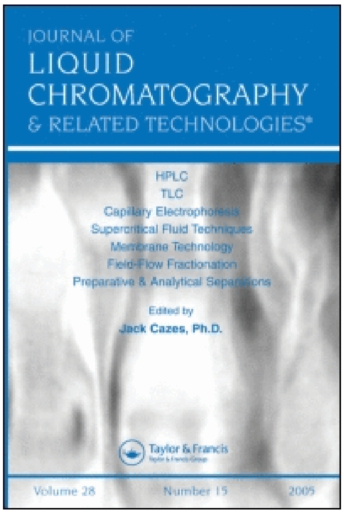

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RECYCLING TECHNIQUE IN PREPARATIVE LIQUID CHROMATOGRAPHY

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ABSTRACT

A theoretical and practical study of recycling technique is developped for preparative liquid chromatography. The optimal cycle number, n_{opt}, is determined for the separation of two solutes in such a way that the resolution between the second peak of cycle (n-1) and the first one of cycle n is equal to the resolution between the peaks of cycle n. A simple relationship is propounded to determine n_{opt} which only depends on retention volumes and external volume, but it does not depend on band spreading. With this recycling technique it is shown that the maximum injection volume under optimal recycling conditions is greater than n_{opt} times the maximum injection volume for one cycle. So the use of recycling can be opportune and beneficial to increase the throughput in preparative liquid chromatography.

INTRODUCTION

Preparative liquid chromatography is probably one of the separation methods that requires much effort in order to optimize its peculiar parameters such as recovery ratio, throughput, costs .... For the separation and collection of a component by liquid chromatography, the method to be used must fit the purpose of the work, the available apparatus and staff. For routine work, it is necessary to optimize the method. On the other hand, for an occasional preparative work, the chosen method will be the faster one and will use an available device. For the recycling the column eluent (mobile phase and sample) is flowed through the column head until the resolution between the components is sufficient. From an industrial point of view, such a separation has the advantage to be more economical, with respect to the consumption of solvent and stationary phase, than an identical one obtained with a single elution. For an occasional operation, recycling may allow an immediate work with the available column even if its dimensions seem not to be appropriate for the preparative problem without recycling. However recycling is chiefly limited by two factors : firstly, the sample is not always a pure binary mixture but it often is a much more complex solution for which interferences may occur between the various compounds. In this case a pretreatment of sample makes it possible to isolate the two compounds of interest and to eliminate the other solutes. Secondly, the increase of the difference between the retention volumes resulting from the n passages of the compounds through the column is counterbalanced by the bandspreading originating in the external volume. So the recycling technique is not always possible and, in this work, we try to emphasize the conditions under which it can be used for a preparative work and its advantages in comparison with successive injections on the same column.

Up to now, many investigations dealt with the advantages of this technique in analytical chromatography and in preparative chromatography as well.

Martin (1) made an exhaustive study of the parameters that affect the quality of recycling and particularly the bandspreading in connecting tubes and pump. This latter phenomenon had not been taken into account by Lesec (2,3) and Kalash (4) because it was probably negligible in their cases. This interesting simplification is quite justified by using columns packed with large particles,but it is no longer valid in modern preparative liquid chromatography, with the use of microparticles for which chromatographic dispersion decreases.

THEORY

If the column is volume overloaded and the phase system operates in the linear part of the solute partition isotherm, the maximal sample load, $Q_{i,max}$, depends on two terms : the maximal injection volume, $V_{i,max}$, and the maximal injection concentration,$C_{i,max}$. The maximal injection volume, $V_{i,max}$, is given by the next relationship (5) :

$$V_{i,max} = V_o \left[k_1' (\alpha - 1)/R_{s,mini} - 1.25 \; (2 + k_1' + \alpha k_1')/\sqrt{N} \right] \quad (1)$$

where V_o is the column dead volume, N is the theoretical plate number of the column, k_1' is the capacity factor of the first eluted solute, α is the selectivity factor between the two solutes, and $R_{s,mini}$ is the minimal resolution at preparative scale to obtain the recovery ratio and the purity desired. To a first approximation $V_{i,max}$ can be estimated with $R_{s,mini} = 1$.

The purpose of recycling is, on the one hand, the increase of the apparent dead volume V_o, on the other hand, the increase of the apparent plate number, N.

Equation 1 shows that the maximal injection volume, $V_{i,max}$, as well increases under these conditions. In order to examine how such aims can be reached, it is necessary to describe the recycling problem in terms of chromatographic parameters and apparatus. For this study the sample is assumed to contain two solutes and the flow rate is kept constant. The resolution between peaks 1 and 2 after a single passage, R_1, can be written :

$$R_1 = (V_{R2} - V_{R1})/2\ (\sigma_{c,1} + \sigma_{c,2}) \qquad (2)$$

where V_{R1} and V_{R2} are the retention volumes of peaks 1 and 2 after a single passage i.e. the volumes of mobile phase flowing through the detector between sample injection and passage of the maxima of the peaks 1 and 2 in the detector respectively. $\sigma^2_{c,1}$ and $\sigma^2_{c,2}$ are the volume variances of these peaks after a single passage. Let V_A be the "recycling volume" i.e. the volume of the liquid phase in the apparatus between the outlet of the detector cell and the inlet of the injection device, and σ^2_A the volume variance of the peak broadening originating in this volume V_A. The "pseudo retention volumes" after n cycles for peaks 1 and 2 are respectively :

$$V_{1,n} = n\ V_{R1} + (n - 1)\ V_A \qquad (3)$$

$$V_{2,n} = n\ V_{R2} + (n - 1)\ V_A \qquad (4)$$

and the volume variances are :

$$\sigma^2_{1,n} = n\ \sigma^2_{c,1} + (n - 1)\ \sigma^2_A \qquad (5)$$

$$\sigma^2_{2,n} = n\ \sigma^2_{c,2} + (n - 1)\ \sigma^2_A \qquad (6)$$

These preceeding parameters are summarized in Figure 1 (for n = 2).

If we assume that, for two adjacent peaks, the contribution of the column to the volume variance does not depend on the retention :

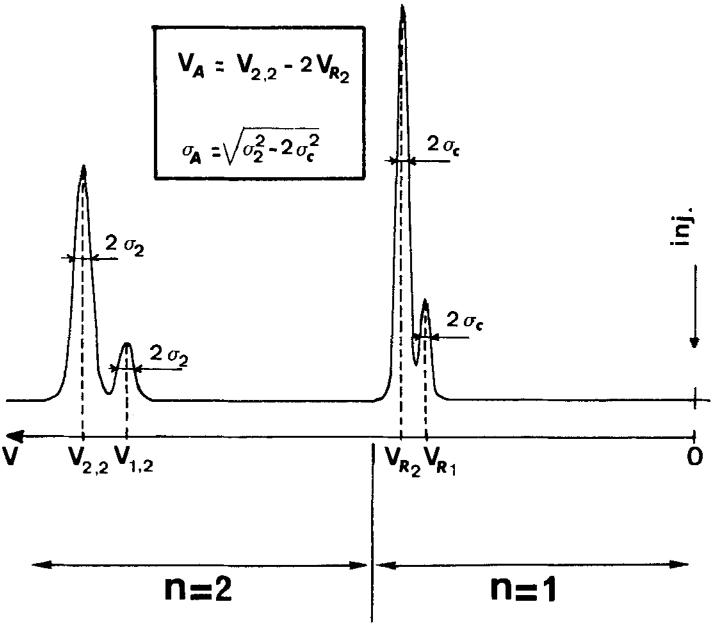

Figure 1 : Principal parameters of interest in recycling technique.

$$\sigma_{c,1} = \sigma_{c,2} = \sigma_c \qquad (7)$$

and therefore :

$$\sigma_{1,n} = \sigma_{2,n} = \sigma_n \qquad (8)$$

with $\lambda = \sigma_A^2/\sigma_c^2$, Martin (1) showed that the resolution after n cycles is given by :

$$R_n = R_1 \sqrt{n} \,/\, \sqrt{1 + \frac{n-1}{n}\lambda} \qquad (9)$$

R_n is larger than R_1 if n is larger than λ; under such conditions the recycling technique becomes profitable.

The resolution between the peak 2 of cycle (n-1) and the peak 1 of cycle n, R_m, decreases while the cycle number n increases because the total volume of the system is limited and the second peak of cycle (n - 1) tends to overtake the first peak of cycle n. In the same way as Equation 1, R_m can be written (for n > 1) :

$$R_m = (V_{1,n} - V_{2,n-1})/2\ (2\sigma_n + \sigma_{n-1}) \qquad (10)$$

or :

$$R_m = \frac{V_{R2} + V_A - n\ (V_{R2}-V_{R1})}{2\sigma_c \left(\sqrt{n}\ \sqrt{1 + \frac{n-1}{n}\lambda} + \sqrt{n-1}\ \sqrt{1 + \frac{n-2}{n-1}\lambda}\right)} \qquad (11)$$

For a value of n greater than 5, Equation 11 can be simplified by using the next approximation :

$$\sqrt{\frac{n-2}{n-1}} \simeq \sqrt{\frac{n-1}{n}} \simeq 1 - \frac{1}{2\ n} \qquad (12)$$

and Equation 11 becomes :

$$R_m = \frac{V_{R2} + V_A - n\ (V_{R2} - V_{R1})}{2\sigma_c\ \sqrt{n}\ \sqrt{1 + \frac{n-1}{n}\lambda}\ \left(2 - \frac{1}{2\ n}\right)} \qquad (13)$$

The optimal cycle number, n_{opt}, can be defined from a given value of R_m (1) or by the cycle number for which R_m is equal to R_n (4). For a preparative purpose, the latter criterion is better to define n_{opt}, and Equations 2, 11 and 13 give :

$$n_{opt} = (5\ V_{R2} - V_{R1} + 4\ V_A)\ /\ 8\ (V_{R2} - V_{R1}) \qquad (14)$$

The value of n_{opt} differs from the one proposed by Kalash (4). This difference originates in the fact that we take into account the volume and the band broadening in external region out of the column. n_{opt} can be expressed in terms of the chromatographic parameters (k_1' and α) and $\mu = V_A/V_o$:

$$n_{opt} = \frac{4\ (1 + \mu) + (5\alpha - 1)\ k_1'}{8\ k_1'\ (\alpha - 1)} \qquad (15)$$

When the capacity factor, k_1', increases the optimal cycle number, n_{opt}, decreases down to a minimal value, $(5\alpha - 1)/8(\alpha - 1)$, which only depends on the selectivity factor, α.n_{opt} increases while α decreases. Futhermore n_{opt} increases with μ i.e., for a given column, the external volume, V_A, acts as a reservoir for the peaks. But, from Equation 9, it is difficult to know the variation of the final resolution, $R_{n_{opt}}$, with V_A because V_A and σ_A^2 (i.e. λ and μ) are each other dependent ; the relationship between V_A and σ_A^2 depends on the geometrical characteristics of the external hydraulic circuit. Recycling is interesting only if :

$$n_{opt} > \lambda \qquad (16)$$

For the calculation of the maximum injection volume in recycling technique, (n_{opt} V_o) and N_{opt} are substituted for V_o and N respectively in Equation 1 :

$$(V_{i,max})_{n_{opt}} = n_{opt}\ V_o \left[k_1'(\alpha-1)/R_{S,mini} - 1.25\ (2+k_1' + \alpha k_1')/\sqrt{N_{opt}}\ \right] \quad (17)$$

with :

$$N_{opt} = N \frac{n_{opt}}{1 + \dfrac{n_{opt} - 1}{n_{opt}}\ \lambda} \qquad (18)$$

(n_{opt} V_o) and N_{opt} are the apparent dead volume of the column and the apparent plate number respectively after the optimal cycle number, n_{opt}. If Inequality 16 is verified, the combination of Equations 1, 17 and 18 enables to deduce :

$$(V_{i,max})_{n_{opt}} > n_{opt} V_{i,max} \tag{19}$$

The result is very interesting : the maximal injection volume with the recycling technique under optimal conditions is larger than n_{opt} times the maximal injection volume with a single passage ; moreover, the operation times for n_{opt} cycles and for n_{opt} successive injections are roughly equivalent, so the recycling technique allows the increase of throughput in comparison with successive injections.

EXPERIMENTAL

The liquid chromatograph consists of the following components :

- a Waters M 6000 A reciprocating pump (Waters, Paris, France) as elution pump which allows recycling operation by means of a special manifold.
- An Orlita AE 10 - 4 diaphragm pump (Touzart et Matignon, Vitry, France) to supply the sample solution.
- A Rheodyne 7010 valve (Touzart et Matignon) as commutation valve between the two pumps.
- A stainless steel column, 100 cm x 0.47 i.d., slurry packed with silica gel Partisil 20 (Whatmann, Ferrières, France), 19 µm mean particle size.
- A Pye Unicam LC 55 spectrophotometer (Pye Unicam, Paris, France) or an Altex model 153 analytical UV photometer (Touzart et Matignon) operating at 254 nm.

The scheme of chromatograph is given in Figure 2. The solvents and chemicals are of high purity and were purchased from various suppliers.

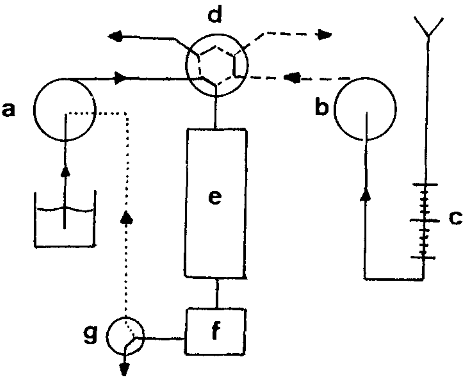

Figure 2 : Scheme of the chromatograph.
a : elution pump ; b : injection pump ; c : precision burette ; d : 6 way valve ; e : column ; f : detector ; g : recycling manifold.

RESULTS AND DISCUSSION

The validity of Equation 15 is examined for different values of the parameters k_1', α and μ. For this study the samples are equimolar mixtures of butyl phtalate and isobutyl phtalate at about 10^{-4} mg/ml dissolved in the mobile phase. The mobile phases are binary mixtures of isooctane and ethyl acetate (from 98/2 v/v up to 90/10 v/v) in order to vary the capacity ratio values of solutes. The flow rate was 2.5 ml/mn. Experimental determinations give μ values of 0.40 and 0.23 when the experiments are performed with the Altex photometer and the Pye Unicam spectrophotometer respectively. The n_{opt} values are experimentally determined by checking the variation of R_n and R_m versus n. Figure 3 examplifies such a determination. We can notice the rapid decrease of R_m during the first cycles. On the other

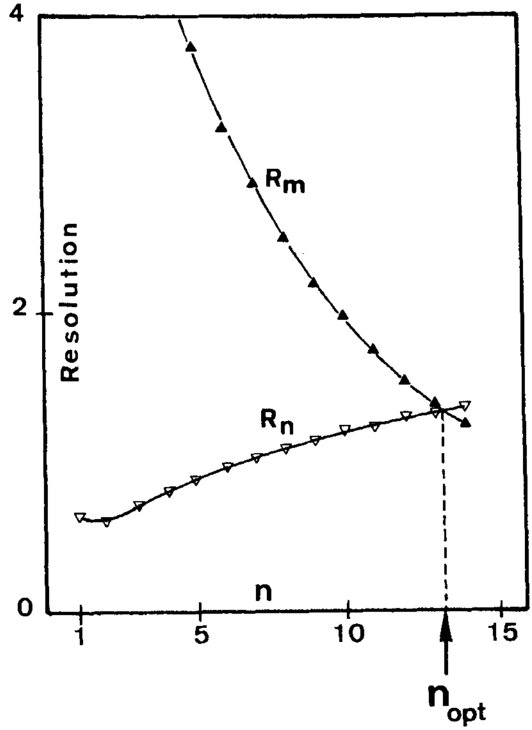

Figure 3 : Resolutions R_n and R_m versus the cycle number n.
$\lambda = 2.09$
$\mu = 0.23$
$k'_1 = 1.23$
$\alpha = 1.08$

hand, R_n slowly increases during the same time, and even keeps constant until the third passage ; it is quite logical because λ is slightly larger than 2 and so R_n must become larger than R_1 only after the second passage. The optimal cycle number determined experimentally in this way is 13.6 ; it is in good agreement with the one calculated from Equation 15 i.e. 13.9.

Experimental and theoretical values of n_{opt} and $R_{n_{opt}}$ for different values of α , k'_1, μ and λ are summarized in Table 1.

TABLE 1

Optimal cycle number under different experimental conditions.

	Experiment N°	1	2	3	4	5	6
	α	1.08	1.10	1.07	1.11	1.08	1.47
	k'_1	4.39	2.88	1.22	5.05	1.25	0.88
	μ	0.40	0.23	0.23	0.40	0.23	0.23
	λ	0.41	0.36	0.40	0.21	2.09	2.06
	R_1	1.28	0.95	0.60	1.21	0.65	1.78
n_{opt}	Experimental Value	8.6	7.6	15	5.9	13.6	4.4
	Theoretical Value	8.8	7.8	16	6.4	13.9	3.2
$R_{n_{opt}}$	Experimental Value	2.9	2.2		2.5	1.4	2.2
	Theoretical Value	2.7	2.0		2.6	1.4	2.3

Experimental measurements generally agree very well with theoretical values, the latter are always larger than the former except for the experiment N°6 in which the optimal cycle number is smaller than 5 ; in this case the approximations of Equation 12 is no longer valid.

So Equation 15 allows the determination of the optimal cycle number, n_{opt}, and consequently the calculation of resolution, $R_{n_{opt}}$, and standard deviation $\sigma_{n_{opt}}$, for this cycle number.

In the second part of this work, the validity of Equation 17 which gives the maximal injection volume after n_{opt} cycles, is illustrated with an example of separation : the two solutes are nitrated isomers of o-terphenyl : x-nitro o-terphenyl and 4-nitro o-terphenyl (ponderal ratio : 1/4), eluted with a binary mixture of isooctane and dichloromethane (80/20 v/v) at a flow rate of 2.1 ml/mn. The concentration of the injected samples dissolved in the mobile phase is 1.1 mg/ml. The operating conditions determined after two passages are : x-nitro o-terphenyl retention volume $V_{R1}=$ 30.5 ml, 4-nitro o-terphenyl retention volume $V_{R2}=$ 32.9 ml, column standard deviation $\sigma_c=$ 0.57 ml, external volume $V_A=$ 3.48 ml, external standard deviation $\sigma_A=$ 0.47 ml, resolution after a single passage $R_1=$ 1.05. Under these conditions the calculated optimal cycle number is equal to 7.7 ; practically we chose the nearest smaller integer. After 7 cycles and for a small injected volume, the resolution, R_7, and the standard deviation, σ_7, are respectively equal to 2.2 and 1.83 ml. The maximum injection volume calculated from Equation 17 with $R_{s,mini} = 1$, is $(V_{i,max})_{n_{opt}} = 12.3$ ml i.e. 6.7 σ_7. This sample volume is injected on the column and the solutes are collected : the end of the collection of the first peak and the beginning of the collection of the second one is performed at the valley between the two peaks. Analysis of the second collected fraction shows a 98 % purity for the 4-nitro o-terphenyl and demonstrates the validity of the maximum feed volume in recycling calculated according to Equation 17.

For the comparison between recycling technique and successive injection technique, sample volumes varying from $V_i = 0.5\ \sigma_c$ up to $V_i = 3.08\ \sigma_c$ are injected on the column and solute collection at the valley is performed after a single passage. The 98 % purity for the 4-nitro o-terphenyl is obtained for $V_i = 1.2\ \sigma_c$ which corresponds to $V_i = 0.68$ ml. 7 successive injections of the latter volume give an overall injected volume of 4.75 ml i.e. 2.6 times smaller than the maximum injected volume with the recycling operation. The recycling technique (7 cycles) and the successive technique (7 injections) require 266 ml and 240 ml respectively for the complete elution of the two solutes. So, in this case and under identical flow rate conditions, the throughput reached in recycling is 2.3 times larger than in usual elution.

CONCLUSIONS

For an occasional preparative work, the recycling technique can be used under volume overload conditions. There is an optimal cycle number, n_{opt}, for which the injection volume is maximum. This optimal cycle number only depends on the retention volumes of the two solutes and on the external volume out of the column. Furthermore the recycling technique under optimal conditions (after n_{opt} cycles) allows the increase of the throughput in comparison with n_{opt} successive injections.

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