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Experimental Characterization of Strongly Nonlinear Elution Peaks in Liquid Chromatography

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Abstract

The shape of nonlinear elution peaks was studied experimentally in various chromatographic modes and a wide sample size range encompassing the case where the distribution isotherm curvature is the prevailing factor of band broadening. It is shown that as sample size is varied, the tailing part of all peaks adjoins a common curve that can be fitted with an exponential function whose parameters are independent of mobile phase linear velocity and stationary phase particle size. The effect of solute capacity factor on these parameters was also investigated. These findings provide valuable help for the optimization of linear velocity, particle size, as well as sample size in preparative liquid chromatography under mass overload conditions.

INTRODUCTION

In the last few years the interest in preparative liquid chromatography (PLC) has grown widely, as evidenced by the emergence of one book (*1*) and the holding of two international meetings especially devoted to this field. Simultaneously, efficiently packed preparative columns have become commercially available in diameters of up to 60 cm, and several manufacturers have proposed standard or customized apparatus to implement them (*2*). This situation is, in part, the result of a great deal of technological and in depth theoretical work accomplished in the last decade. In particular, the theory of linear PLC, assuming a linear distribution isotherm between the two phases and leading to the commonly called volume overload conditions, is currently well established (*3-7*). However, this approach obviously does not allow full

advantage to be taken of the separating capacity of a given amount of mobile and stationary phases, and thereby of the related investments. On the other hand, the theory of nonlinear PLC, taking into account the isotherm curvature, is not as well documented. Although a number of nonlinear theoretical models have been described in the literature, very few of them are directly suitable to PLC. An exception is the treatment of Poppe and Kraak (*8*), who used either a Langmuir or a quadratic isotherm. This latter approach was further developed by Cretier and Rocca (*9*, *10*), but it was devised and experimentally verified only for slight deviations from linearity. Very recently, Knox and Pyper developed an attractive theoretical framework for maximizing PLC throughput allowing for the isotherm curvature (*11*). Of course, a more precise knowledge of PLC nonlinear profiles could be obtained from a rigorous determination of distribution isotherms, but this approach is cumbersome and little realistic because of the mutual interactions of the various mixture components at high loading. In fact, PLC often needs merely a rough understanding of the nonlinear behavior of the compounds to be separated in order to reduce the number of preliminary injections necessary for optimizing the injected volume and concentration of the sample. So far, a rather limited number of papers dealing with the overall characterization of nonlinear experimental elution peaks is available (*12–14*). One of the authors of this paper published earlier results of this type derived from ion-exchange experiments (*15*). Our purpose now is to report on the strongly nonlinear peak shapes obtained from additional experiments in adsorption and reversed-phase chromatography in a column loading range for which the isotherm curvature becomes progressively the major source of band broadening.

EXPERIMENTAL

The experiments were made with either a Varian 8500 chromatograph or an Orlita MS 15/7 reciprocating pump (Orlita, Giessen, GFR). The samples were injected with a Rheodyne 7120 six-way valve equipped with sample loops (for sampling volumes lower than 10 mL) or connected to an auxiliary Orlita DMP 15/15 reciprocating pump (for sampling volumes in excess of 10 mL). The sampling loops were either purchased from Rheodyne (10, 20, 50, 100, 200 μL volumes) or homemade from open 0.5 mm i.d. × 1/16 in. stainless steel tubing (0.54, 1.11, 2.04 mL volumes) or 2.1 mm i.d. × 1/8 in. stainless steel tubing (injections of volumes between 3 and 10 mL). When a loop made from 2.1 mm i.d. tubing was used, the sampling valve was always turned back to its load position

before the total volume of the loop had been injected in order to avoid additional band spreading (*3*, *16*). The volume actually injected was calculated from the time spent in the inject position. Detection was performed with a L.D.C. Spectromonitor II spectrophotometer fitted with 3 mm path length preparative cells in order to increase its linear range at high concentrations.

The solvents used as the mobile phase were of chromatographic-grade quality and the solutes of analytical reagent grade. All of them were used as received from the suppliers. The column dimensions, stationary phase specifications, and mobile phase compositions used throughout this study are given in Table 1 unless otherwise specified. The column dead volumes were determined from the hold-up volume of carbon tetrachloride, potassium nitrate, and cupric tetramine ion on the adsorption, reversed phase, and ion-exchange columns, respectively. All the experiments were performed at room temperature.

For each of the three chromatographic modes studied, the test solutes and their analytical chromatographic data under the operating conditions of Table 1 are given in Table 2. For the present purpose, retention volumes V_R were simply measured at peak maximum, and standard deviations σ, in volume units, were derived from measurements of peak width at half peak height, assuming a Gaussian shape. For each injected amount, three or four different volume-concentration pairs of the test solutes were realized. The injected volumes varied over at least a factor of 10. The spectrophotometric detector was standardized by injecting the test solutes directly into the cell. The standardization curves were used to correct the elution profiles for detector nonlinearity.

RESULTS AND DISCUSSION

Characterization of Peak Shape

The quantitative measurements achieved by varying the sample size Q_0 injected to the three columns studied are given in Table 3. In order to compare the elution peak shapes observed with different chromatographic modes, one has to account for both the differences in column dimensions and in the intrinsic sample capacities of the stationary phases. Hence, in Table 3, the sample sizes were normalized on the basis of the maximum available capacity (MAC), Q_A of each column (*19*). This concept was introduced by us in order to provide any stationary phase with a capacity parameter roughly independent of operating conditions and solute nature, and to generalize the idea of exchange capacity of the

TABLE 1
General Experimental Operating Conditions

Chromatographic mode	Adsorption	Reversed phase	Ion exchange
Column:			
Length (cm)	20	25	47
Inner diameter (cm)	0.48	0.76	1.05
Stationary phase	Partisil 5 (Whatman) Specific surface area: 400 m^2/g Silanol group concentration:[a] 3.4 mmol/g Water content: 2.7%	Lichroprep RP 8 (Merck) Carbon content: 11.6% Bonded chain concentration:[b] 1.2 mmol/g	Dowex AG1 × 8 (Bio-Rad Laboratories) Exchange capacity: 3.8 meq/g
Particle diameter	5 μm	25–40 μm	−400 mesh (<37 μm)
Mass (g)	1.9	5	19
Mobile phase	Hexane–dichloromethane (80–20)	Water–methanol (75–25)	Ammonium acetate 1 *M* buffered at pH 9 with ammonia
Flow rate (mL/min)	2	4.5	16.7
Linear velocity (cm/s)	0.27	0.23	1.1
Dead volume (mL)	2.5	8.0	12.0

[a]Assuming 5 silanol groups/nm^2 (*17*).
[b]Assuming a monomeric bonding (*18*).

TABLE 2
Analytical Chromatographic Data of the Test Solutes (operating conditions: see Table 1)

Chromatographic mode	Adsorption	Reversed phase	Ion exchange
Test solute	*o*-Phenyl phenol	Phenol	Maleate ion
Retention volume V_R (mL)	14.0	49.6	330
Capacity factor	4.6	5.2	26.5
Standard deviation σ (mL)	.29	1.7	35

ion exchangers. The MAC of a stationary phase can be determined from the amount of a monofunctional compound, injected at a high concentration (~1 *M*), which causes the saturation of 1 g of stationary phase under high capacity factor conditions (~10). The MAC of the stationary phases investigated here were determined (*19*) experimentally as 1.2 mmol/g for Partisil 5, 1.8 mmol/g for Lichroprep RP 8, and 3.8 mmol/g for Dowex AG1 × 8. It should be noted that these figures are of the same order of magnitude as the concentrations of the functional moieties of the supports (Table 1).

The elution peak shapes studied here were in a sample size range corresponding to about 0.5 to 14% of the MAC of the various columns. Typical profiles are shown in Fig. 1. As the sample size is increased, the front boundary moves toward shorter retentions and the elution volume of the peak maximum decreases. The peak asymmetry was assessed from the measurement of the ratio of the total peak area, Q_0, to the area of the part of the peak eluted after its maximum, Q_0' (Fig. 1). Table 3 shows that this ratio decreases rapidly to a value of the order of 1.1. More asymmetrical peaks were observed in adsorption and reversed-phase chromatography than in ion-exchange. Another relevant parameter to discuss is the elution volume, V_F, at which the trailing edge of the peak returns to the baseline. A criterion of 2% peak height was chosen. The results shown in Table 3 show that $V_F - V_0$ is almost independent of sample size and volume, and remain very close to the value of $V_R + 3\sigma$, as can be calculated from the data of Table 2. This latter result means that the return to baseline of strongly nonlinear peaks is identical to that of the linear one under the same elution conditions. This fact is very important in PLC applications since it enables one to set the cycle time for repeated injections.

All these statements are in agreement with recently reported remarks on strongly nonlinear peak shapes (*12–14*). However, the results are different if the elution peaks were replotted with the "end of injection" as their common abscissa origin. First of all, Fig. 1 shows that the peaks

TABLE 3
Characteristics of Strongly Nonlinear Elution Peaks in Adsorption, Reversed-Phase, and Ion-Exchange Chromatography[a]

	Q_0 (mmol)	Q_0/Q_A (%)	$V_{max} - V_0$ (mL)	Q_0/Q_0'	$V_F - V_0$ (mL)	$V_R + 3\sigma$ (mL)	Q_0/C_{max} (mL)
Adsorption	0.009	0.4	12.0	1.25	15.0		1.4
	0.018	0.8	11.0	1.18	14.7	14.9	1.7
	0.045	2	9.6	1.13	14.7		2.0
	0.090	4	8.2	1.11	14.7		2.4
Reversed phase	0.07	0.7	43.8	1.30	55		6.1
	0.26	3	33.5	1.17	53	54.7	11.2
	1.03	12	22	1.10	53		10.6
Ion exchange	0.4	0.6	296	1.68	410		85
	2	3	239	1.36	400	435	87
	10	14	139	1.21	380		94

[a] Q_0 = sample size. Q_A = MAC of the column (the stationary phase masses are given in Table 1). V_{max} and C_{max} = volume and concentration at the apex of the elution peak. Q_0' = sample amount eluted after the peak apex (see Fig. 1). V_F = elution volume, on the peak trailing edge, for which the sample concentration becomes lower than ~2% C_{max}. V_0 = sample volume. V_R and σ = analytical retention volume and standard deviation. Operating conditions as in Table 1 and test solutes as in Table 2.

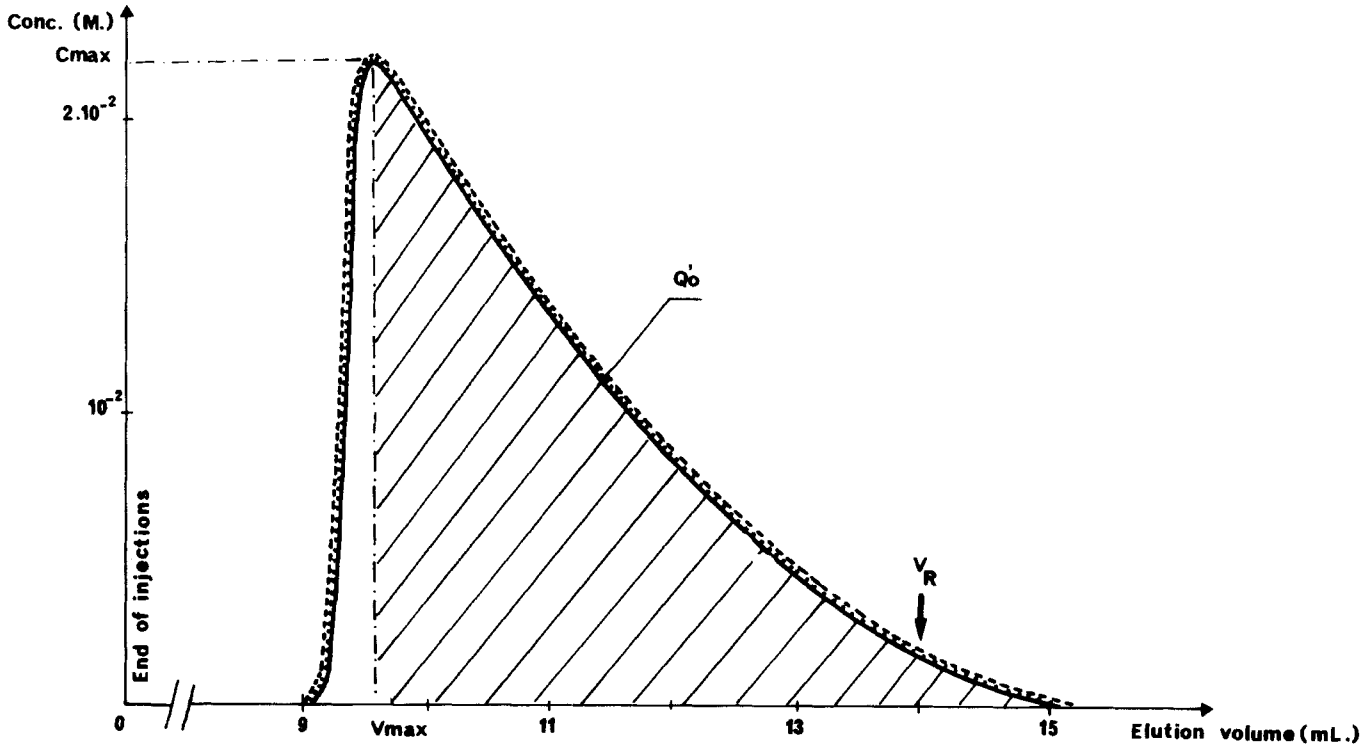

FIG. 1. Nonlinear elution peaks of *o*-phenyl phenol in adsorption chromatography. Sample size: 45 μmol. Volumes and concentrations: (dashed line) 0.05 mL, 0.9 *M*; (dotted line) 0.1 mL, 0.45 *M*; (solid line) 0.5 mL, 0.09 *M*. Other operating conditions: see Table 1.

resulting from the injection of constant sample size but having different volume-concentration relationships are identical in shape. This property, also mentioned by Knox (*11*), was all the better verified over a larger range of sample volumes because the sample size is higher, i.e., the nonlinearity stronger. Then, when the sample size is varied, the rear edges of the peaks were fairly superimposable. Figures 2A, 2B, and 2C show that this behavior holds irrespective of the chromatographic mode. To a first approximation, the common trailing edge $C(v)$ can be fitted satisfactorily with an exponential curve, such as

$$C(v) = C_m \exp\left[-\frac{v - V_m - V_0}{\tau}\right] \tag{1}$$

where V_0 and V_m are the sample and dead volumes, respectively; v is the elution volume variable; and C_m and τ are adjustable parameters in concentration and volume units, respectively. The best experimental fittings to the experimental nonlinear elution peaks studied are represented in Figs. 2A, 2B, and 2C (mixed line).

The near-exponential shape of the rear edge of nonlinear elution peaks could be anticipated by considering the ratio of sample size to concentration at peak maximum, Q_0/C_{max} (Table 3). Figure 3 illustrates the variation of this ratio with Q_0 for the case of adsorption chromatography. With Gaussian analytical peaks, this ratio is independent of sample size and equal to $\sigma\sqrt{2\pi}$. As the sample size increases, Q_0/C_{max} increases toward a quasi-constant limit value (Fig. 3). A well-known mathematical property of exponential curves states that the area beneath the curve, noted here by Q'_0, is related to the exponential constant τ by

$$Q'_0 = C_{max}\tau \tag{2}$$

Therefore, it can be concluded that

$$\tau = \frac{Q'_0}{C_{max}} = \frac{Q'_0}{Q_0}\frac{Q_0}{C_{max}} \tag{3}$$

Table 4 shows that the values obtained from curve fitting were in good agreement with those derived from Eq. (3) using the data taken from Table 3. All these results support the findings that the tails of strongly nonlinear peaks can be described by an exponential decay.

The preceding peak shape was observed experimentally provided that the sample volume remains lower than a limiting value, $V_{N\,lim}$. Starting

from Eq. (3) and noting that C_{max} cannot exceed the injected concentration C_0, it can be readily shown that

$$V_{N\,lim} = \tau \frac{Q_0}{Q_0'} \# \tau \tag{4}$$

The existence of a limiting volume was also described by Knox et al. (*11*). Using a different approach, that volume was estimated to be of the order of twice the standard deviation of the analytical peak.

Effect of Linear Velocity

Figure 4 shows the effect of the linear velocity of the mobile phase on the exponential constant τ and on the ratio Q_0/C_{max} at high loading (10 mmol) in ion-exchange chromatography. For each linear velocity the injections were done in triplicate by varying the sample volume (5, 10, and 20 mL). A two-factor variance analysis showed that the sample volume and linear velocity have no significant influence on τ and Q_0/C_{max}. The variation of the ratio Q_0/C_{max} with linear velocity for the corresponding analytical peak (i.e., $\sigma\sqrt{2\pi}$) is also shown in Fig. 4 (broken line). Figure 5 represents the variation of the ratio Q_0/C_{max} in reversed-phase chromatography as a function of sample size Q_0 in a wide range of linear velocities. It is shown that the effect of linear velocity on the Q_0/C_{max} value tends to vanish as the sample size increases. For sample sizes above ~3.5% of the MAC, the peak shape is independent of the linear velocity of the mobile phase.

Effect of Stationary Phase Particle Diameter

Figure 5 also shows the effect of particle diameter on the ratio Q_0/C_{max} as the sample size is increased. As expected, differences clearly appear for low sample sizes, the smallest particle size affording the best efficiency and therefore the lowest ratio Q_0/C_{max}. However, for sample sizes in excess of ~1% of the MAC, it was ascertained that there is no significant variation in peak shape as a function of particle diameter. This finding was recently reported by Eisenbeiss et al. (*14*).

Effect of Solute Capacity Factor

The above results indicate that the shape of strongly nonlinear peaks is independent of the linear velocity and of the particle diameter. Therefore

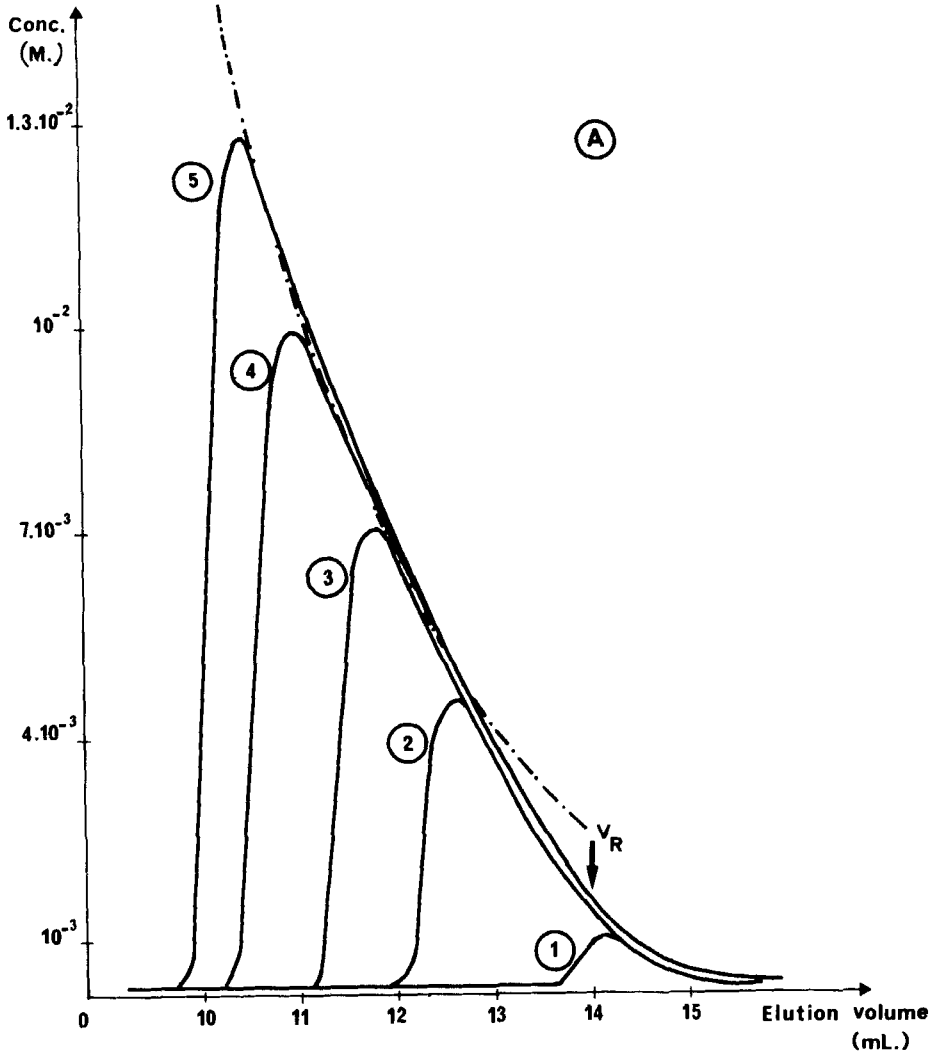

FIG. 2A. Experimental nonlinear elution peaks as a function of sample size (solid line) and their near-exponential envelope curve of the rear edge (mixed line). *o*-Phenyl phenol in adsorption chromatography. Sample size: (1) 0.45 μmol, (2) 4.5 μmol, (3) 9 μmol, (4) 18 μmol, (5) 22.5 μmol. Exponential parameters: $C_m = 0.52$ *M*, $\tau = 2.2$ mL. Other operating conditions: See Table 1.

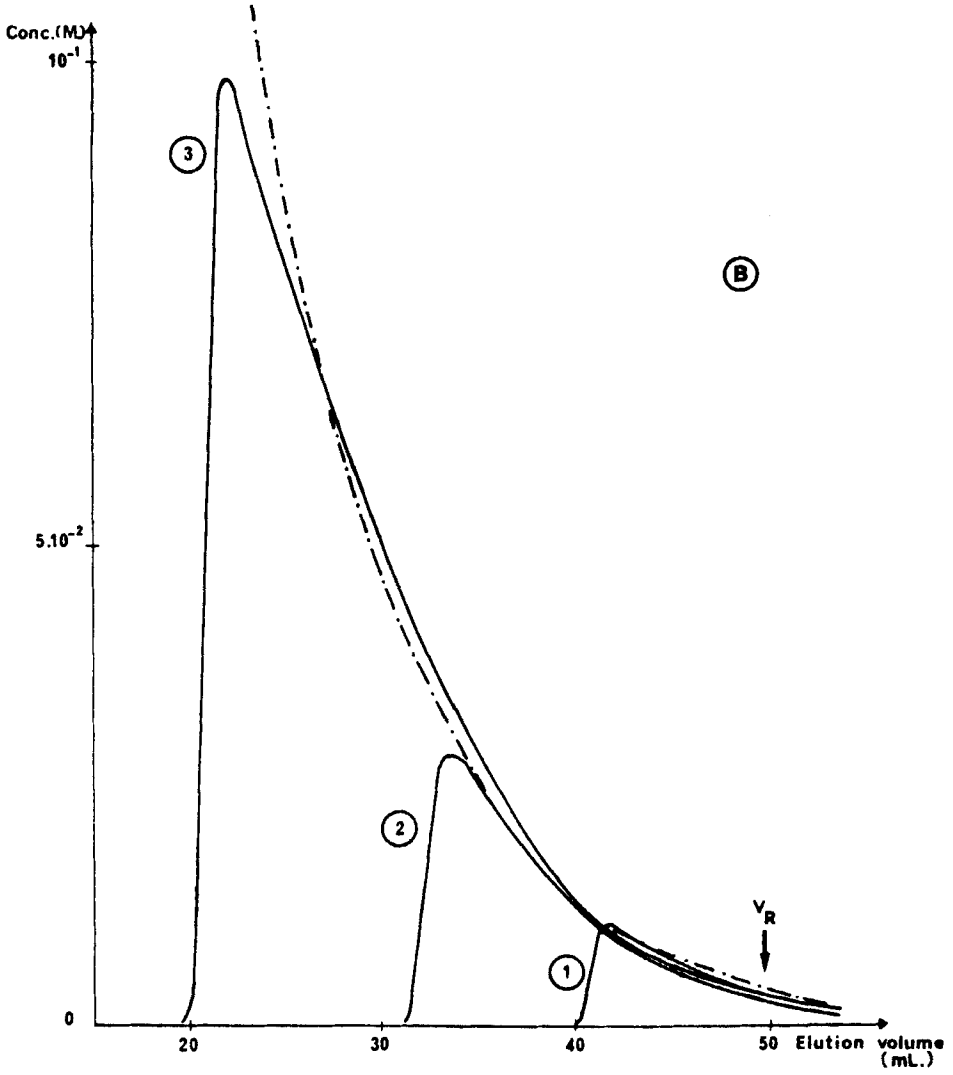

FIG. 2B. As for Fig. 2A. Phenol in reversed phase chromatography. Sample sizes: (1) 73 μmol, (2) 260 μmol, (3) 1030 μmol. Exponential parameters: $C_m = 0.75\ M$, $\tau = 8.0$ mL. Other operating conditions: See Table 1.

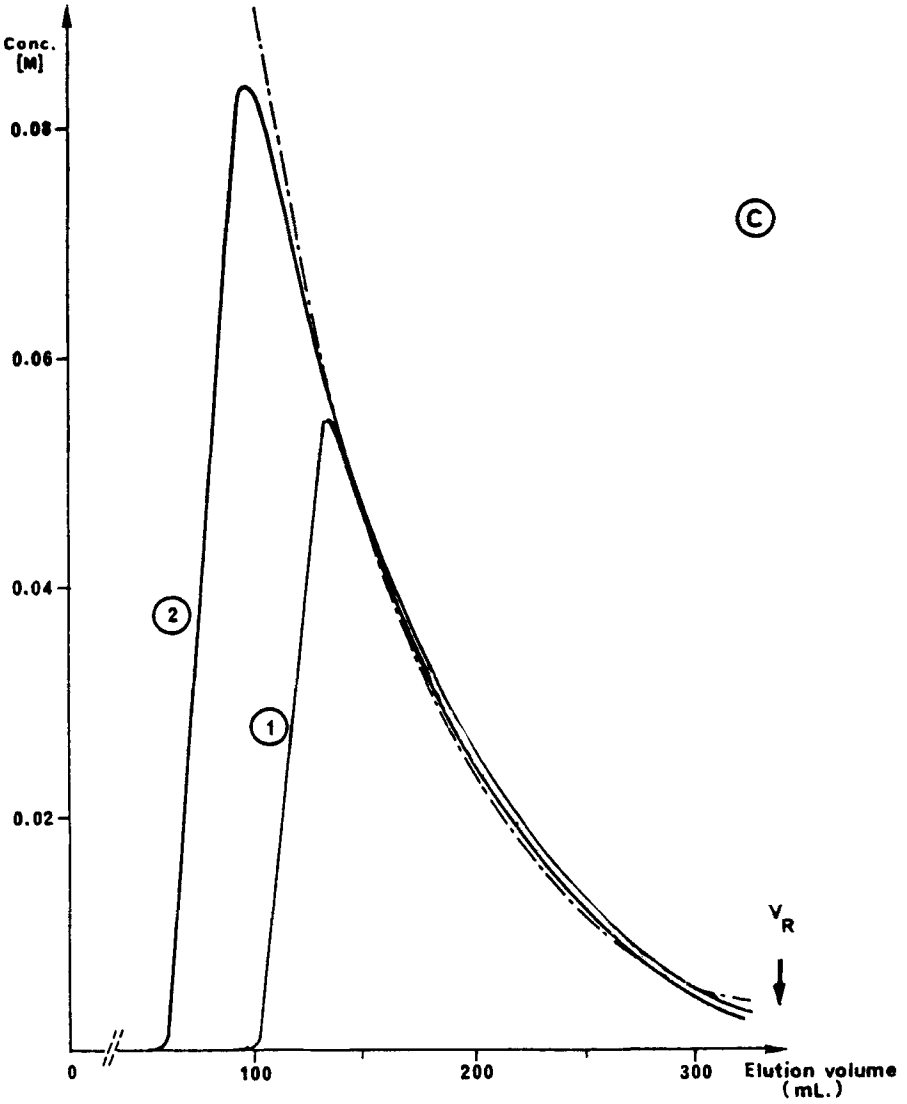

FIG. 2C. As for Fig. 2A. Maleate ion in ion-exchange chromatography. Sample sizes: (1) 10 mmol, (2) 15 mmol. Exponential parameters: $C_m = 0.32\ M$, $\tau = 75$ mL. Other operating conditions: See Table 1.

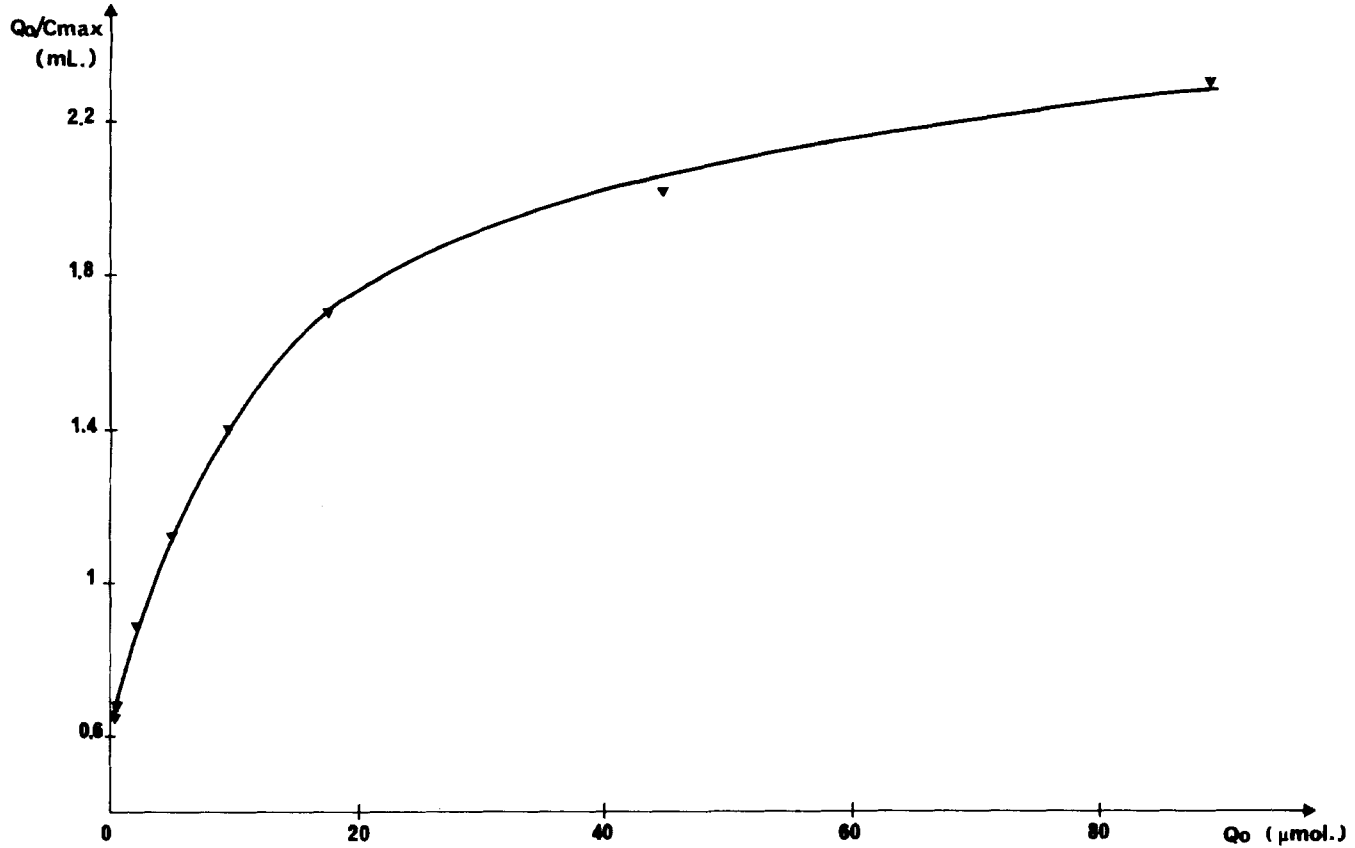

FIG. 3. Variation of the ratio of sample size to concentration at peak apex, Q_0/C_{max}, with sample size Q_0 in adsorption chromatography. Operating conditions: See Tables 1 and 2.

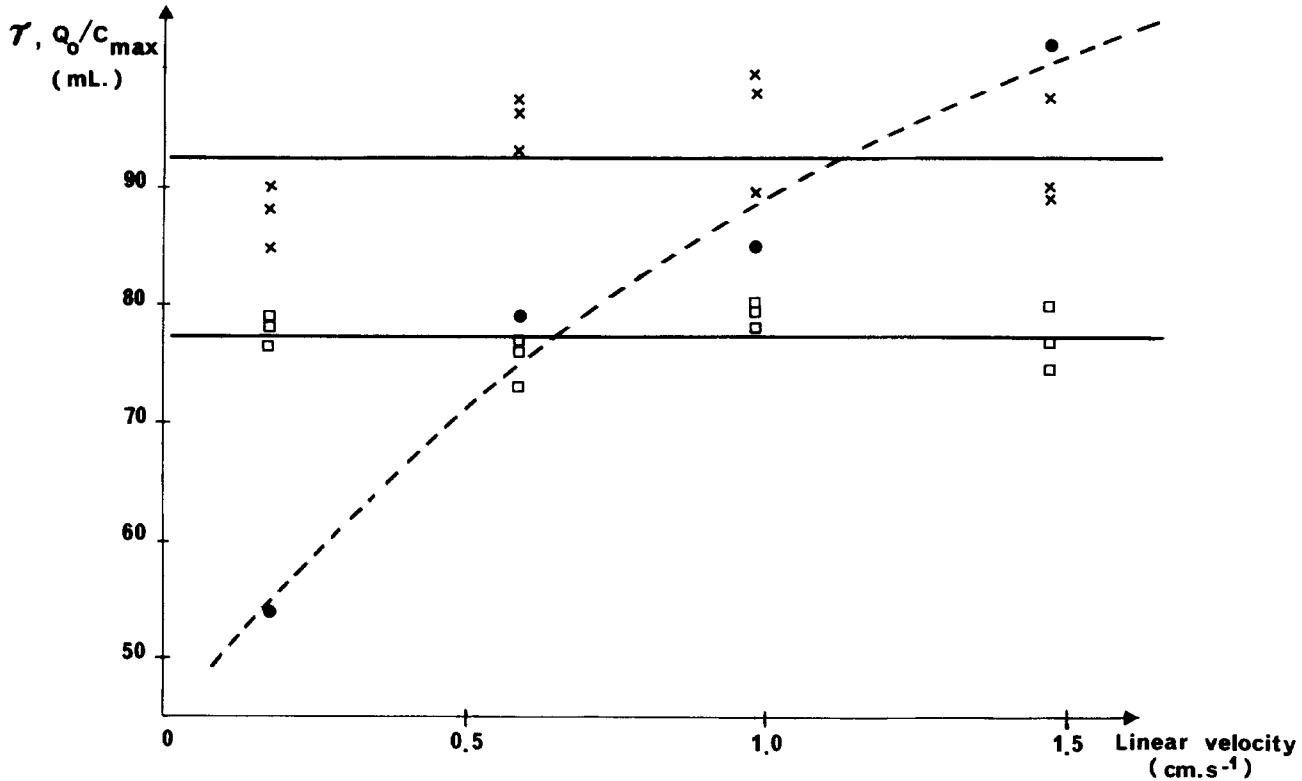

FIG. 4. Effect of mobile phase linear velocity on the exponential constant τ and the ratio Q_0/C_{max} in ion-exchange chromatography. Sample size: 10 mmol. Injected volumes: 5, 10, and 20 mL. Other operating conditions: see Tables 1 and 2. (□) τ; (×) Q_0/C_{max} for 10 mmol injections; (●) Q_0/C_{max} for analytical injections.

TABLE 4
Comparison between τ Values Derived from Curve Fitting and Eq. (3)

	τ Values (mL)	
	Estimated from curve fitting (Fig. 2)	Calculated from Eq. (3) and data of Table 3
Adsorption	2.2	2.2
Reversed phase	8.0	9.6
Ion exchange	75	77

C_m and τ are also independent of these two parameters. The question remains: What factors do influence C_m and τ? Table 5 gathers the numerical values of C_m and τ determined from the experiments reported here, as well as from additional experiments in which the mobile phase composition was varied. In spite of the relatively low precision of these determinations (about 10% for C_m and 5% for τ), τ seems to be approximately proportional to the analytical retention volume of the compound, i.e., to $1 + k'$. No other correlation has been identified so far. In particular, the influence of solute nature seems to be small. As for the parameter C_m, values between 0.2 and 0.8 M were observed. A more advanced interpretation of these values according to solute nature and/or capacity factor is hazardous.

CONCLUSION

The experimental characterization of strongly nonlinear elution peaks provides useful help for the choice of stationary phase particle diameter and mobile phase linear velocity in PLC. If the separation at issue is sufficiently easy to allow working under mass overload conditions, or, more precisely, if the sample size is such that isotherm curvature becomes the major source of band broadening, then the above results show that it is not necessary to choose very fine particles. The best compromise is probably the range 20 to 40 μm. Similarly, the linear velocity can be set at a rather high value without detrimental effect on the separation quality. In that case it is also possible to implement a longer preparative column than the analytical one, because the pressure drop is moderate. This situation is the opposite of that of a more difficult separation. In the latter case, mass overloading is limited, and the best choices are fine particles (~10 μm) and moderate linear velocity in order to take full advantage of the high resolving power of the column. As a result, the preparative

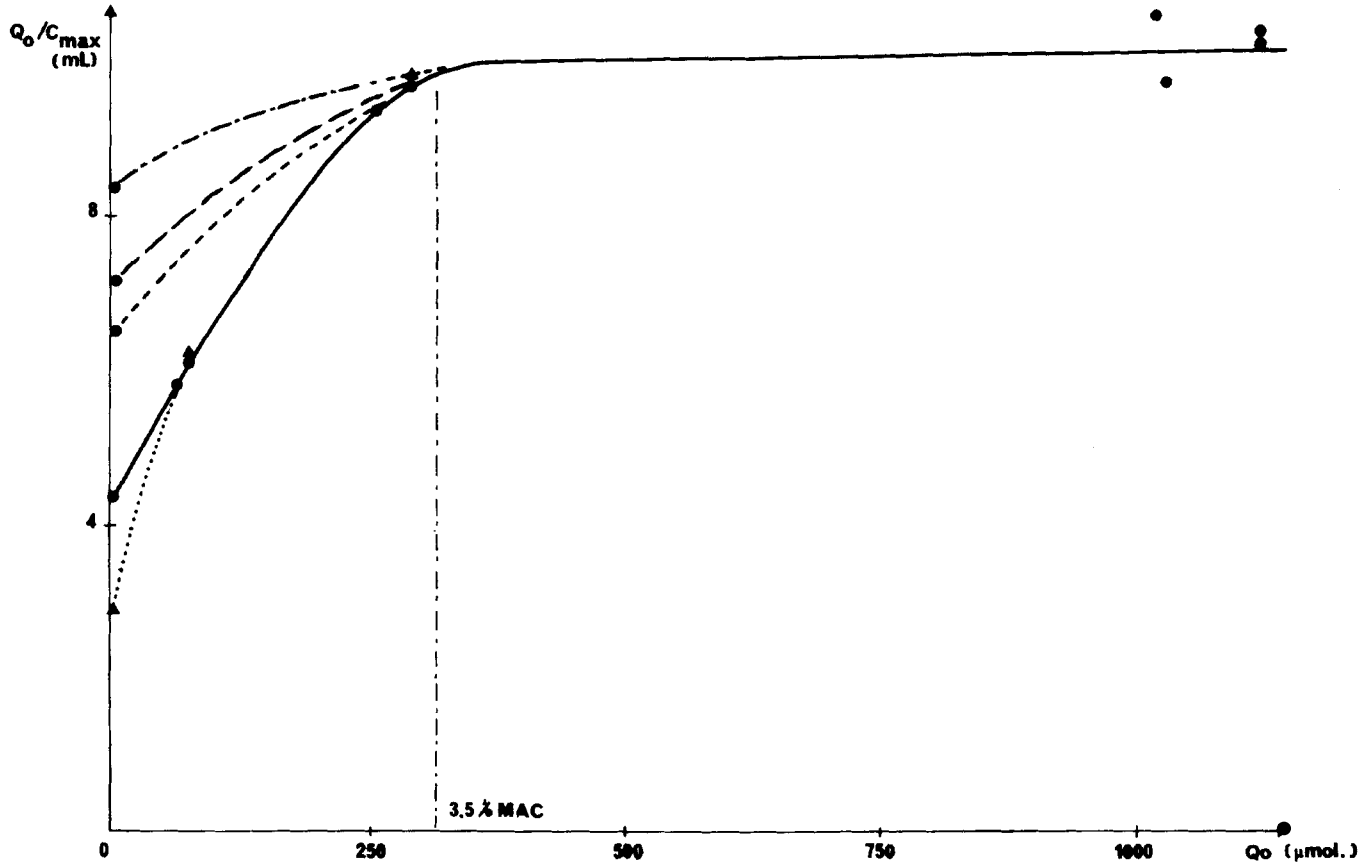

FIG. 5. Variation of the ratio Q_0/C_{max} with sample size Q_0 for various mobile phase linear velocities, u, and stationary phase particle diameters, d_p, in reversed-phase chromatography. (——): d_p, 25–40 µm; u = 0.23 cm/s. (- -): d_p, 25–40 µm; u = 0.89 cm/s. (— —): d_p, 25–40 µm; u = 1.26 cm/s. (· —): d_p, 25–40 µm; u = 1.79 cm/s. (· · ·): d_p, 5–20 µm; u = 0.19 cm/s. Other operating conditions: See Tables 1 and 2.

TABLE 5
Various Determinations of the Parameters τ and C_m of the Exponential Peak Tailings at High Sample Size[a]

Chromatographic mode	Test solute	Capacity factor	Retention volume V_R (mL)	τ (mL)	τ/V_R	C_m (M)
Adsorption	*o*-Phenyl phenol	4.6	14	2.2	0.16	0.52
	o-Phenyl phenol	10	26.4	4.3	0.16	0.60
Reversed phase	Phenol	5.2	49.6	8.0	0.16	0.75
Ion exchange	Maleate ion	26.5	330	75	0.23	0.32
	Maleate ion	8.3	120	30	0.25	0.60
	Fumarate ion	40	500	95	0.19	0.21

[a]Operating conditions: see Table 1, except mobile phase for maleate ion ($k' = 8.3$); ammonium acetate, 1.5 M, pH 9; and for *o*-phenyl phenol ($k' = 10$); hexane–dichloromethane (92:8).

column length will be about the same as in analytical scale. Of course, in many practical situations the allowed sample size is in between the two cases discussed, and optimization of particle diameter and linear velocity requires more preliminary experiments.

Another practical result of the work presented here is that it can serve as a model to assess the optimum sample size quantitatively under mass overload conditions (*20*). This approach is based on the quasi-exponential shape of the tailing part of nonlinear peaks and requires numerical values for C_m and τ. Table 5 can serve as a practical guideline. It was tested with several real cases from our laboratory and the literature. Although rough and simple, it enables one to decrease considerably the number of preliminary injections leading to the determination of optimum sample size. The only case for which it quantitatively failed dealt with an enantiomeric separation on a chiral silica-based stationary phase (*21*). This phase involved two consecutive bondings, and we feel that its resulting low capacity was responsible for this particular nonlinear behavior.

REFERENCES

1. M. Verzele and C. Dewaele, *Preparative High Performance Liquid Chromatography. A Practical Guideline,* Alltech, Gent, Belgium, 1986.
2. "Preparative and Process Liquid Chromatography," *Anal. Chem., 57,* 998A (1985).
3. R. P. W. Scott and P. Kucera, *J. Chromatogr., 119,* 467 (1976).

4. A. Wehrli, V. Hermann, and J. F. K. Huber, *Ibid., 125,* 59 (1976).
5. B. Coq, G. Cretier, and J. L. Rocca, *Ibid., 186,* 457 (1979).
6. L. Personnaz and P. Gareil, *Sep. Sci. Technol., 16,* 135 (1981).
7. B. Coq, G. Cretier, and J. L. Rocca, *Anal. Chem., 54,* 2271 (1982).
8. H. Poppe and J. C. Kraak, *J. Chromatogr., 255,* 395 (1983).
9. G. Cretier and J. L. Rocca, *Chromatographia, 18,* 623 (1984).
10. G. Cretier and J. L. Rocca, *Ibid., 21,* 143 (1986).
11. J. H. Knox and H. M. Pyper, *J. Chromatogr., 363,* 1 (1986).
12. J. R. Conder, *J. High Resolution Chromatogr. Chromatogr. Commun., 5,* 341 (1982).
13. M. Verzele, C. Dewaele, J. Van Dijck, and D. Van Haver, *J. Chromatogr., 249,* 231 (1982).
14. F. Eisenbeiss, S. Ehlerding, A. Wehrli, and J. F. K. Huber, *Chromatographia, 20,* 657 (1985).
15. P. Gareil, L. Personnaz, J. P. Feraud, and M. Caude, *J. Chromatogr., 192,* 53 (1980).
16. B. Coq, G. Cretier, J. L. Rocca, and M. Porthault, *J. Chromatogr. Sci., 19,* 1 (1981).
17. K. K. Unger, *Porous Silica* (Journal of Chromatography Library Series, Vol. 16), Elsevier, Amsterdam, 1979.
18. E. Grushka and E. J. Kikta Jr., *Anal. Chem., 49,* 1004A (1977).
19. P. Gareil, L. Semerdjian, M. Caude, and R. Rosset, *J. High Resolution Chromatogr. Chromatogr. Commun., 7,* 123 (1984).
20. P. Gareil, C. Durieux, and R. Rosset, *Sep. Sci. Technol., 18,* 441 (1983).
21. A. Tambute, P. Gareil, M. Caude, and R. Rosset, *J. Chromatogr., 363,* 81 (1986).