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CHARACTERIZATION OF EXTRACOLUMN AND CONCENTRATION-DEPENDENT DISTORTION OF CHROMATOGRAPHIC PEAKS BY EDGEWORTH-CRAMÉR SERIES

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SUMMARY

The Edgeworth-Cramér series peak handling method has been used to detect the onset of small variations of chromatographic peak shape when the mass of solute injected is increased. The effect of extracolumn distortion on statistical peak parameters and on the resultant peak shape is theoretically considered. Extracolumn distortion factors have been characterized on experimental peaks.

INTRODUCTION

The Edgeworth-Cramér (EC) series is of fundamental importance in chromatographic theory^{1,2}, in fitting skewed peaks obtained under conditions of infinite dilution, and in determining statistical peak parameters, such as area, A , standard deviation, σ , skewness, S and excess, E , from experimental peaks³⁻⁵. However, only those asymmetry effects that are classified as non-ideality effects have been considered⁵.

Concentration-dependent asymmetry effects and extracolumn peak distortions, which often work in chromatographic practice^{6,7}, are treated here by the EC series. None of the previously reported peak handling methods is, in fact, completely valid in characterizing these effects. Some of them, such as the characterization of small peak variations by the distribution function method or by setting the maxima in coincidence, give only a qualitative description^{8,9}, whereas other peak-fitting methods or the numerical computation of the statistical moments are of limited applicability^{3,10}.

THEORY

A chromatographic peak, $f(x)$, normalized to a unit area and expressed as a function of the standardized time variable $x = (t - m)/\sigma$, is approximated by the EC series, developed up to the k th order, as follows:

$$f(x) = Z(x) + \sum_{v=1}^k Q_v (-Z) + R_k(x) \quad (1)$$

where $Q_v(-Z)$ is a linear aggregate of the derivatives of the normal frequency function $Z(x)$, of maximum order $3v$, and contains cumulant coefficients of a given frequency function $f(x)$ of maximum order v . $R_k(x)$ is the remainder. The detailed expressions of the $Q_v(-Z)$ and $Z(x)$ terms are reported elsewhere^{3,5}.

Extracolumn band broadening phenomena can be considered either as convoluting factors of the chromatographic peak, or as independent random variables that sum to the original peak shape, considered as probability distribution functions¹¹. Thus the extracolumn phenomena are mathematically equivalent to the addition of random variables, with different distribution functions.

The cumulants, which are linear combinations of the statistical moments and which completely characterize a particular distribution function¹², both when this is a chromatographic peak and when it is a distortion factor, have the following general property:

$$\chi_{\text{tot}}^{(v)} = \chi_c^{(v)} + \chi_e^{(v)} \quad (2)$$

where $\chi_{\text{tot}}^{(v)}$, $\chi_c^{(v)}$ and $\chi_e^{(v)}$ are respectively the cumulants of order v of the distribution function of the resultant peak (tot), of the original peak (c) and of the extracolumn factor (e). The first two cumulants are, respectively, the mean (m) and the variance (σ^2). Eqn. 2, which holds true whatever the order v of the cumulant considered, is therefore an extension of the well-known theorem of the variance additivity.

The peak skewness and excess are:

$$S_{\text{tot}} = \frac{\chi_{\text{tot}}^{(3)}}{\sigma_{\text{tot}}^3} = \frac{\chi_c^{(3)} + \chi_e^{(3)}}{(\sigma_c^2 + \sigma_e^2)^{3/2}} \quad (3)$$

$$E_{\text{tot}} = \frac{\chi_{\text{tot}}^{(4)}}{\sigma_{\text{tot}}^4} = \frac{\chi_c^{(4)} + \chi_e^{(4)}}{(\sigma_c^2 + \sigma_e^2)^2} \quad (4)$$

The cumulants of important extracolumn distortion factors^{6,7} and of theoretical peak shape can be evaluated^{1,13}, so the resultant peak skewness and excess are easily computed from eqns. 3 and 4. For example, an exponential decay (one of the most important extracolumn factors) has the following cumulants:

$$\begin{aligned} \chi_e^{(1)} &= \beta + \tau \\ \chi_e^{(2)} &= \sigma_e^2 = \tau^2 \\ \chi_e^{(3)} &= 2\tau^3 \\ \chi_e^{(4)} &= 6\tau^4 \quad \text{etc.} \end{aligned} \quad (5)$$

where τ is the time constant and β is the starting point of the exponential decay¹³.

The resultant chromatographic peak shape arising from such processes can be approximated by the EC series, because it is a function of the peak parameters m , σ , S and E . The approximating properties of this series have been proved to hold true even in the case of the addition of several random variables with different distribution functions¹². Therefore, the EC series is a theoretically correct tool for de-

TABLE I
PEAK PARAMETERS VALUES AND PRECISIONS OBTAINED WITH SYRINGE INJECTION
Cyclohexane on SE-30, 100°C.

| q (nmol) | S | E | m (sec) | σ^2 (sec ²) | k_{max} | $C.V.$ (%) |
|------------|-------------|-------------|--------------|--------------------------------|-----------|------------|
| 14.07 | 0.96 ± 0.01 | 1.44 ± 0.01 | 83.16 ± 0.01 | 24.62 ± 0.08 | 5 | 1.14 |
| 9.37 | 0.96 ± 0.01 | 1.45 ± 0.01 | 83.53 ± 0.02 | 19.14 ± 0.04 | 5 | 1.08 |
| 3.87 | 0.57 ± 0.04 | 1.70 ± 0.05 | 82.02 ± 0.18 | 13.76 ± 0.26 | 3 | 1.41 |
| 2.60 | 0.69 ± 0.03 | 0.95 ± 0.09 | 83.47 ± 0.01 | 9.33 ± 0.14 | 3 | 0.81 |
| 1.26 | 0.51 ± 0.02 | 0.89 ± 0.09 | 83.25 ± 0.02 | 8.77 ± 0.19 | 2 | 0.74 |
| 0.53 | 0.53 ± 0.01 | 0.92 ± 0.02 | 82.96 ± 0.01 | 8.59 ± 0.05 | 2 | 0.71 |

scribing the extracolumn distortion of the chromatographic peak. However, the approximation degree is not provided by the mathematical theory and it must be verified in practice.

Conversely, different hypotheses about the nature of extracolumn distortion factors can be checked on the basis of experimentally determined m , σ , S and E values. In this case, extracolumn distortion factors are controlled one at a time and their cumulants are extracted from series of peaks with and without any specified effect.

On the contrary, no theoretical evidence exists as to whether concentration-dependent effects can be accounted for by the EC series.

EXPERIMENTAL

A gas chromatographic instrument equipped with a flame ionization detector, sampling valve (Bimatic, Carlo Erba, Milan, Italy, 0.3-ml loop) and apparatus for generation of standard gaseous mixture (diffusion/injection), similar to that described elsewhere^{14,15}, was employed. The detector linearity was controlled through the log-log plot of the detector current (A) versus the mass flow-rate (mol sec⁻¹) of cyclohexane (Spectrograde). The slope, in the range of 10⁻¹⁰ to 10⁻⁸ A used in the present work, was found to be 1.01 ± 0.02. The sensitivity was 0.2 C mol⁻¹.

Cyclohexane vapour was injected in the range 0.2-20 nmol by syringe (10 μ l, Hamilton) or by sampling valve fed by the apparatus for generation of the standard

TABLE II
PEAK PARAMETERS VALUES AND PRECISIONS OBTAINED WITH VALVE INJECTION
Cyclohexane on SE-30, 100°C.

| q (nmol) | S | E | m (sec) | σ^2 (sec ²) | k_{max} | $C.V.$ (%) |
|------------|-------------|-------------|--------------|--------------------------------|-----------|------------|
| 8.08 | 0.90 ± 0.01 | 1.27 ± 0.01 | 88.66 ± 0.01 | 18.90 ± 0.01 | 5 | 1.40 |
| 3.34 | 0.73 ± 0.01 | 1.52 ± 0.02 | 89.38 ± 0.01 | 12.99 ± 0.02 | 2 | 1.12 |
| 1.51 | 0.63 ± 0.02 | 1.34 ± 0.09 | 88.70 ± 0.02 | 10.59 ± 0.21 | 2 | 0.91 |
| 0.81 | 0.58 ± 0.04 | 1.09 ± 0.14 | 89.30 ± 0.03 | 9.58 ± 0.30 | 2 | 0.53 |
| 0.41 | 0.60 ± 0.01 | 1.18 ± 0.05 | 89.75 ± 0.01 | 10.05 ± 0.07 | 2 | 0.87 |
| 0.21 | 0.57 ± 0.02 | 1.06 ± 0.08 | 89.68 ± 0.02 | 9.64 ± 0.16 | 2 | 0.83 |

gaseous mixture. The sampling valve was inserted outside the standard injection point. The total dead connection volume between the sampling valve and the injection point of the syringe (just above the packing) was *ca.* 4 ml. The temperature of the injector was 120°C. The connection (to the injector and to the detector) were standard parts of the Fractovap (Carlo Erba, Model 2350).

The chromatographic column was 1.5 m × 4.5 mm I.D. stainless steel, packed with Chromosorb W 100–120 mesh DMSC coated with 10% (w/w) SE-30 (0.92 g of liquid phase in column) and was kept in a Colora Ultrathermostat HT 13 (100 ± 0.05°C). The flow-rate was 1 ml sec⁻¹. The concentration ranges to the peak maximum, computed according to ref. 8, were 0.1–10 nmol cm⁻³ and 5–500 nmol cm⁻³, respectively, in the gas and in the liquid phase.

The detector signal was digitized by a Solartron 7055 (5½ digits, 1 μV sensitivity) and stored on a Digital Minc 11. A digitization rate of *ca.* 2 data sec⁻¹ was employed. For each peak *ca.* 60 points were collected in order to obtain the previously established conditions for the non-linear least-squares minimization procedure by the EC series³. The signal-to-noise ratio⁵ (S/N) was slightly lower than 100. The maximum requested expansion order of the series (k_{\max}), peak parameters values and precisions, variation coefficient CV% of the fitting (Tables I and II) and plots (Fig. 3) were obtained as previously explained^{3,5}. The three-dimensional plot was performed by using the Calcomp software.

All the calculations were run on a CDC Cyber 76 computer (Cineca, Casalecchio, Bologna, Italy).

RESULTS AND DISCUSSION

Several peaks obtained with different injected amounts (by valve) are compared in Fig. 1. These peaks have been reduced at the same area and displayed on a three-dimensional plot (the *x*-axis is the logarithm of the amount injected). The peak parameters obtained from the best fits are listed in Tables I and II.

The most important variation observed with the amount injected is not in the peak mean, which remains almost constant, but in the apparent peak variance (Tables I and II and Fig. 2). The overall peak shape changes in a complex way, as one can observe visually in Fig. 1. This change is also revealed in a substantial increase of the number of the EC series terms (k_{\max} from 2–3 to 5) necessary for attaining the best fit for the greatest amounts injected (Fig. 3 and Tables I and II).

The onset of the concentration-dependent phenomena is located at *ca.* 2 nmol for cyclohexane under these experimental conditions, on the basis of the σ^2 values reported in Tables I and II (see also Fig. 2). This value corresponds to *ca.* 1 nmol cm⁻³ and 50 nmol cm⁻³ of concentration in the gas and the liquid phases, respectively (values computed according to ref. 8). When the amounts are lower than these figures, the peak shape remains almost unchanged and the peak parameters are practically constant, within experimental error (Tables I and II). In addition, the minimization pattern (fitting degree and expansion order of the EC series) agrees with the results of the previous simulation study and with the experimental precision of the present work.

When the amount injected is more than 2 nmol, the degree of fit is poorer (Fig. 3) and nothing can be said about the significance of the peak parameters obtained.

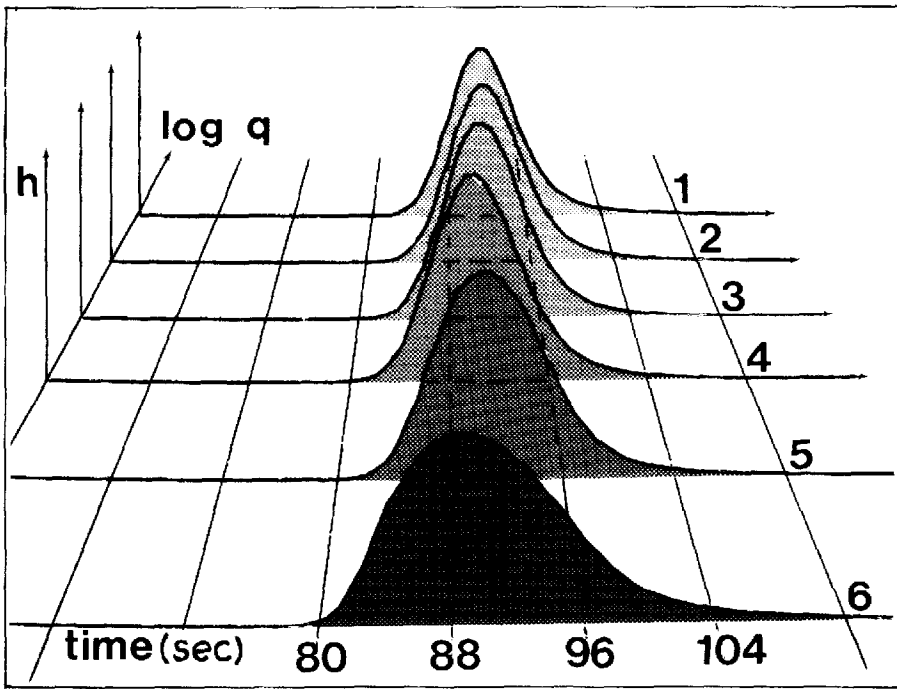


Fig. 1. Three-dimensional plot of chromatographic peaks. Cyclohexane on SE-30, 100°C; valve injection. Peaks: 1, $q = 0.21$ nmol; 2, $q = 0.41$ nmol; 3, $q = 0.81$ nmol; 4, $q = 1.51$ nmol; 5, $q = 3.34$ nmol; 6, $q = 8.08$ nmol.

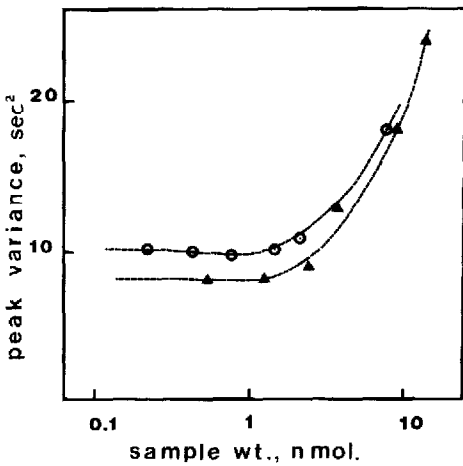


Fig. 2. Peak variance as function of the amount injected. Cyclohexane on SE-30, 100°C. \odot = valve injection; \blacktriangle = syringe injection.

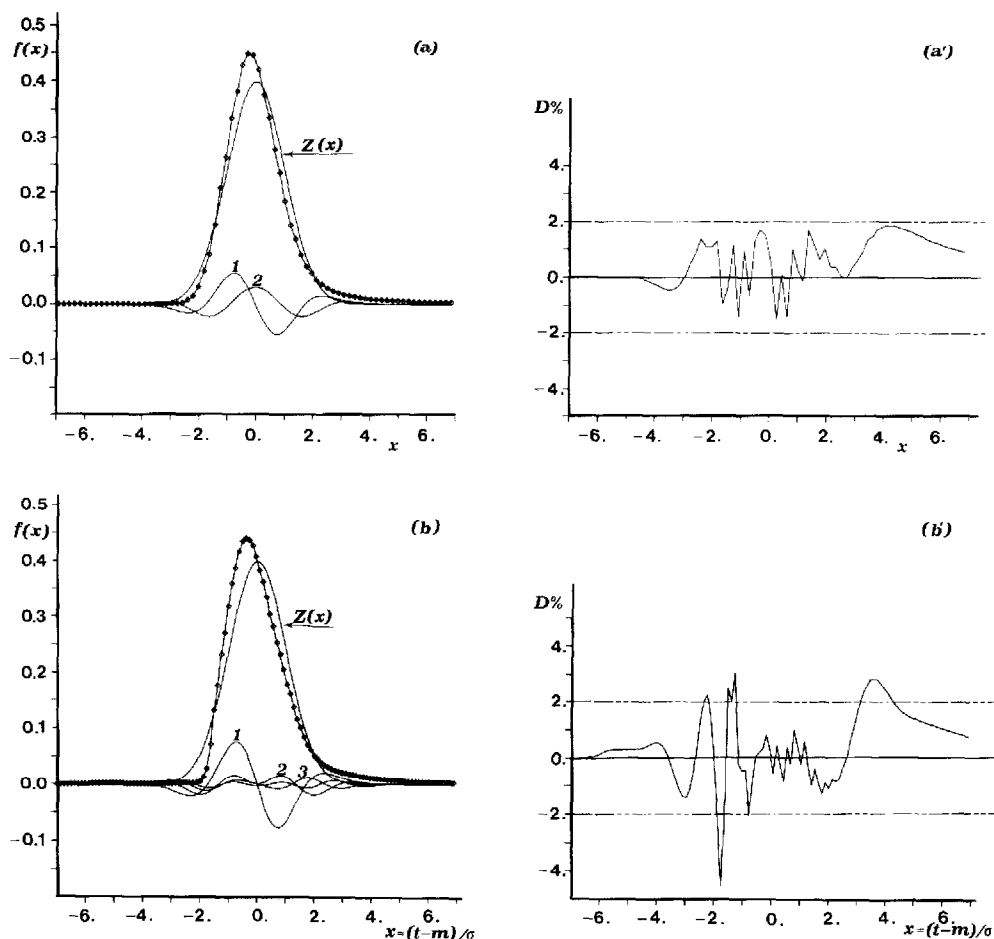


Fig. 3. EC series fitting of experimental peaks. Cyclohexane on SE-30, 100°C. (a, a') Valve injection: amount injected, 0.41 nmol. (b, b') Syringe injection: amount injected, 14.07 nmol. (a, b) Peak fitting; (a', b') differences between experimental and fitted peaks. \diamond = experimental peak: — = terms of the EC series: 1 = $Q_1(-Z)$, 2 = $Q_2(-Z)$, 3 = $Q_3(-Z)$.

However, when the amount injected is less than 2 nmol, from the experimental values of the peak parameters found with syringe and valve injection one can evaluate the extracolumn distortion effect determined by that part of the apparatus between the two injection points. Partial cumulants were computed according to eqns. 2-4 and different time constants τ from these quantities were evaluated by employing eqn. 5, under the hypothesis of exponential decay. The values obtained were in the range 1-1.5 sec, and thus the hypothesis proved to be acceptable. It must be noted that the difference between the two means is *ca.* 6 sec (see Tables I and II), and therefore not all the dead volume acts as an exponential dilution flask.

The conclusion is that the EC series can be used in fitting experimental peaks even when strong extracolumn distortion factors are present. From the peak parameters of the fitting, the hypothesis about the nature of the distortion factor can be

checked. Only the onset of concentration-dependent distortion phenomena can be controlled in this way.

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REFERENCES

- 1 F. Dondi, *Anal. Chem.*, 54 (1982) 473.
- 2 V. A. Kaminskii, S. F. Timashev and N. N. Tunitskii, *Russ. J. Phys. Chem.*, 39 (1965) 1354.
- 3 F. Dondi, A. Betti, G. Blo and C. Bigli, *Anal. Chem.*, 53 (1981) 496.
- 4 A. Betti, F. Dondi, G. Blo, S. Coppi, G. Cocco and C. Bigli, *J. Chromatogr.*, 259 (1983) 433.
- 5 F. Dondi and F. Pulidori, *J. Chromatogr.*, 284 (1984) 293.
- 6 J. R. Conder, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 341.
- 7 J. R. Conder, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 397.
- 8 J. L. Excoffier, A. Jaulmes, M. C. Vidal-Madjar and G. Guiochon, *Anal. Chem.*, 54 (1982) 1941.
- 9 J. E. Oberholtzer and L. B. Rogers, *Anal. Chem.*, 41 (1969) 1590.
- 10 M. C. Vidal-Madjar and G. Guiochon, *J. Chromatogr.*, 142 (1977) 61.
- 11 J. C. Giddings, *Dynamics of Chromatography*, Dekker, New York, 1965, Ch. 2.
- 12 H. Cramér, *Random Variables and Probability Distributions*, Cambridge University Press, Cambridge, 1962, Ch. 3, 16 and 17.
- 13 M. Abramowitz and I. Segum, *Handbook of Mathematical Functions with Formulas, Graphs and Mathematical Tables*, Dover, New York, 1965, p. 930.
- 14 F. Dondi, M. F. Gonnord and G. Guiochon, *J. Colloid Interface Sci.*, 62 (1977) 303.
- 15 F. Dondi, M. F. Gonnord and G. Guiochon, *J. Colloid Interface Sci.*, 62 (1977) 316.