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DIFFERENTIAL FRONTAL ANALYSIS OF CARBOXYLIC ACIDS*

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SUMMARY

Frontal analysis of various carboxylic acids was investigated using a differential detector system. Differentiation of the detector analogue signal was achieved with a differentiating loop interposed between sample and reference cells of a UV detector. The theory of this special analytical technique is discussed and optimization of the system based on height and width of the differential response is described. Advantages of frontal development and differential frontal systems in the quantitative determination of trace amounts of carboxylic acids are demonstrated and the use of these techniques in conjunction with microbore columns is examined.

INTRODUCTION

Frontal analysis introduced originally by Tiselius[1] and developed by Claesson[2] has not been used extensively in liquid chromatography. The use of frontal development for quantitative determination of substances received only brief attention in the 1940's[3,4]. This technique was later superseded by elution HPLC and today frontal analytical techniques are largely neglected. This method of analysis, however, exhibits some very unique features which make it very useful for quantitation of asymmetrical peaks and for application in trace analysis. The advantages of frontal analysis for determination of adsorption isotherms were discussed by various workers in the literature[5–7].

In frontal development, the sample is continuously fed on the column and thus constitutes the mobile phase, as opposed to the introduction of a small sample plug as in elution liquid chromatography. The separation of sample components results in the formation of "fronts" rather than solute elution peaks. The analytical interpretation of solute fronts has been difficult, especially in the case of complex mixtures, which limited the use of the technique. Also, the fact that only the least retained sample component of the mixture can be completely recovered in pure form eliminates, in most cases, the use of this technique for preparative purposes.

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By appropriate differentiation of the frontal elution profile, a useful form of elution peak can be obtained. The differential form of the elution curve can be obtained electronically from the detector signal, digitally by using computer data handling systems, or by a simple modification of a conventional UV detector. It is shown in this investigation that, in the special case where asymmetrical solute peaks are obtained and quantitation based on peak areas becomes difficult, differentiation of the frontal signal is advantageous.

The major aim of this work was to investigate frontal analysis of carboxylic acids in conjunction with a differential detection method. The theoretical and experimental aspects of a simple differential detector designed are also described, and the use of the technique with microbore column systems is examined.

THEORETICAL

Differential forms of the frontal curve

Differentiation of the detector analog signal was first carried out by Boeke[8] in gas chromatography and later used by others[9–11]. The advantages expected in using the differential signal are evident in frontal analysis. If we assume frontal development carried out under the conditions of a constant flow-rate of the mobile phase through the column, then the solute concentration, C, in the mobile phase as sensed by the detector at the end of the column, can be expressed from plate theory[12] as

$$C = \frac{C_0}{\sqrt{2\pi N}} \int e^{-\frac{(v-N)^2}{2N}} dv \tag{1}$$

where v is the volume flow of the mobile phase per plate, N is the number of theoretical plates, and C_0 is the original solute concentration placed on the column[13]. Differentiation of the frontal signal should thus yield the elution function:

$$\frac{d}{dv} \int \frac{C_0}{\sqrt{2\pi N}} \cdot e^{-\frac{(v-N)^2}{2N}} dv = \frac{C_0}{\sqrt{2\pi N}} \cdot e^{-\frac{(v-N)^2}{2N}} = f(v) \tag{2}$$

The right-hand side of eqn. 2 corresponds to the familiar Gaussian form of an elution curve which assumes no tailing and symmetrical peak shapes. Since only fronts of elution peaks are obtained in frontal analysis, better resolution and symmetry of sample components on the differential frontal chromatogram would be expected because the tailing parts of peaks are eliminated. This can be indeed experimentally observed, as seen in Fig. 1, where comparison of elution, frontal, differential elution, and differential frontal high-performance liquid chromatographic (HPLC) responses are shown.

Substituting $X = v - N$, the second derivative of the frontal curve, equivalent to the first derivative of the elution concentration profile, $f(v)$, is given by the equation

$$\frac{df(v)}{dv} = \frac{-C_0}{\sqrt{2\pi N}} \cdot e^{-X^2} \cdot \frac{X}{N} \tag{3}$$

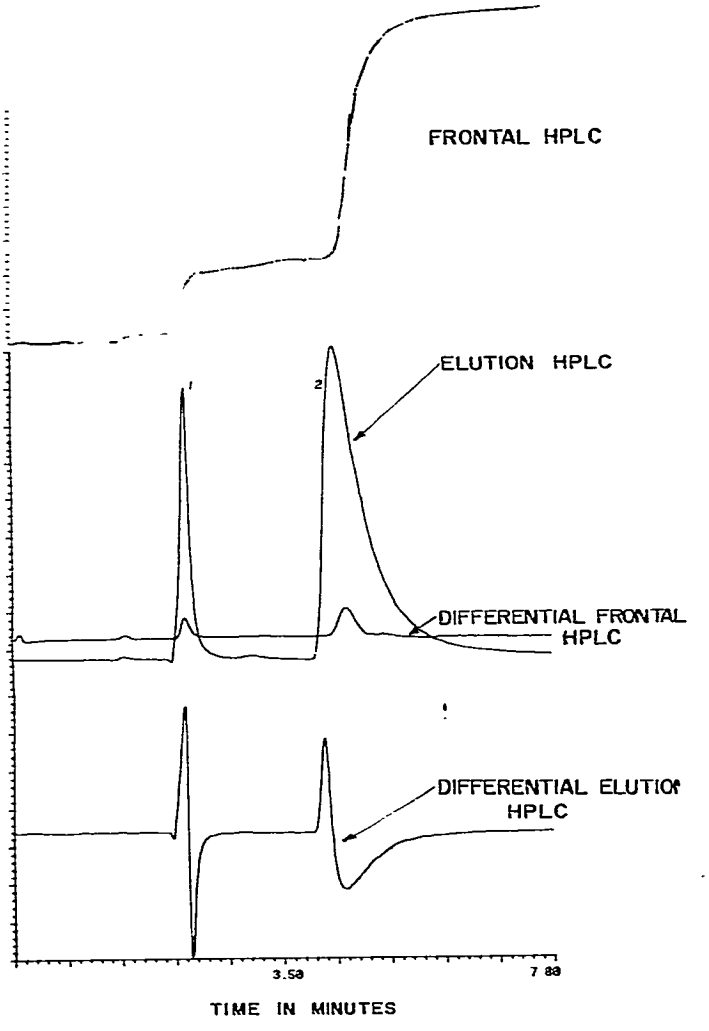

Fig. 1. Comparison of elution, frontal, differential frontal and differential elution chromatograms. Conditions: Partisil SAX column, 25 cm × 4.6 mm I.D.; 0.075 *M* H_3PO_4, pH 1.9, 2 ml/min; Detector attenuation (*S*) = 0.1 ×, 203 nm. Peaks: 1 = acetylsalicylic acid; 2 = 2-fluoro-5-chlorobenzoic acid.

Higher derivatives can be obtained by further differentiation. Computer-simulated functions based on the Gaussian elution curve are shown in Fig. 2; it can be noted that, at the peak maximum of the elution curve, the first derivative goes through zero and, at the points of inflection, the first derivative reaches a maximum and a minimum. In general, more complex curves are obtained by further differentiation.

Since positive and negative peaks of area A_1 and A_2, respectively, are obtained upon differentiation of the elution curve, this can be utilized for determination of solute asymmetry factor *A*

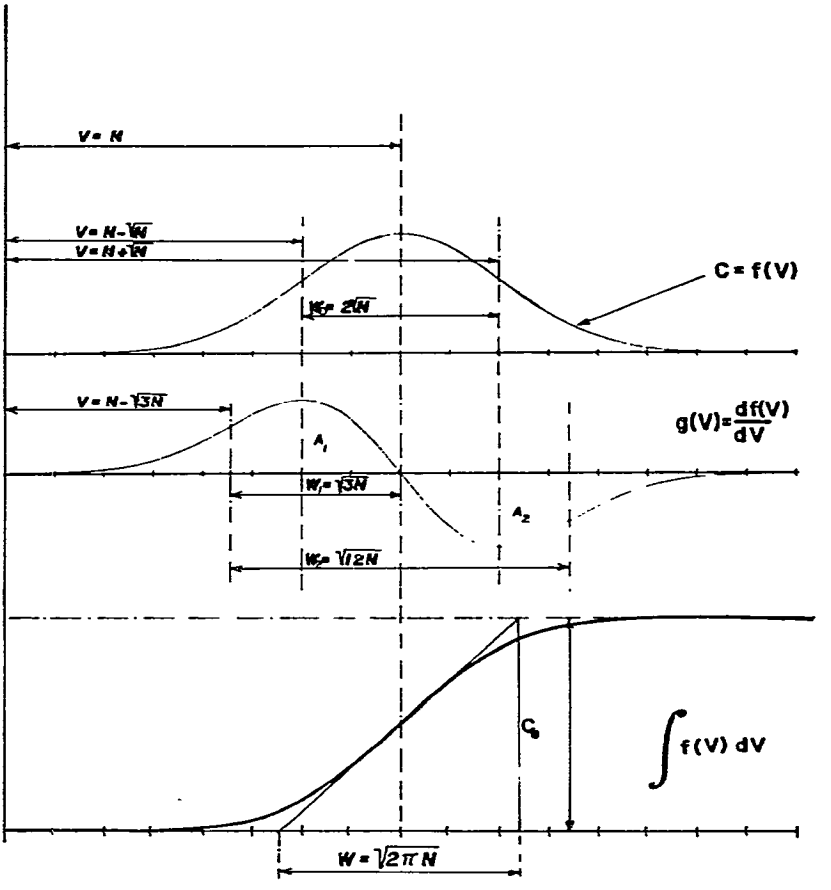

Fig. 2. Integral and differential forms of the elution curve.

where

$$A = \frac{A_2}{A_1} \tag{4}$$

The conditions for a function minimum: $f'(v) = 0$, $f''(v) < 0$; function maximum: $f'(v) = 0$, $f''(v) > 0$; and inflection points: $f'(v) = 0$, $f''(v) = 0$, $f'''(v) \neq 0$ allow one to calculate peak widths and corresponding retention times of the first and higher order derivatives in terms of plate volumes, v. Thus, for the normal elution curve: $v = N$ at the peak maximum. The peak width, w_0, at points of inflection corresponding to two standard deviations (σ) of the elution curve at $e^{-0.5}$ times the peak height, is given as

$$w_0 = 2\sqrt{N} = 2\sigma \tag{5}$$

Similarly, for the first derivative of the elution curve, the width at inflection points w_1 can be calculated as

$$w_1 = \sqrt{3N} = \sqrt{3}\,\sigma \tag{6}$$

The inflection points occur at $\sqrt{3}/e = 0.6372$ times the peak height of the differential signal. Conversion of the plate volumes to real volume in ml, V, can be made via the volume of one theoretical plate, p:

$$v = \frac{V}{p} \tag{7}$$

$$V_R = Np \tag{8}$$

where V_R is solute retention volume.

The above equations allow one to calculate the number of theoretical plates from the differential signal:

$$N = 3\left(\frac{V_R}{w_1}\right)^2 = 4\left(\frac{V_R}{w_0}\right)^2 \tag{9}$$

The widths of the peaks at the base, w_0', w_1', can be determined by tangents drawn through the points of inflection of the differential and the elution curve, respectively; thus, the equation for the column efficiency can be modified to the following forms:

$$N = \frac{27}{4}\left(\frac{V_R}{w_1'}\right)^2 = 16\left(\frac{V_R}{w_0'}\right)^2 \tag{10}$$

The width of the frontal step, w, can also be utilized to determine column efficiency. A tangent drawn through the inflection point of the frontal curve will intersect the plate volume coordinate at point $N - \sqrt{2\pi N}/2$; thus, the width of the frontal step is given as:

$$w = \sqrt{2\pi N} \tag{11}$$

and column efficiency can be calculated as:

$$N = 2\pi\left(\frac{V_R}{w}\right)^2 \tag{12}$$

Other advantages of frontal analytical development seem to be still overlooked. The maximum height of the frontal step can be calculated

$$(C)^{\mathrm{f}}_{\max} = \lim_{v \to \infty} C = \frac{C_0}{\sqrt{2\pi N}} \cdot \int_{-\infty}^{+\infty} e^{-\frac{(v-N)^2}{2N}} dv = \frac{C_0\sqrt{2\pi N}}{\sqrt{2\pi N}} = C_0$$

and corresponds to the original solute concentration, C_0, placed on the column. Thus, the solute dilution resulting from the column dead volume is eliminated and, depending on column efficiency, a significant improvement in solute detection limit can be obtained. This is very advantageous in trace analysis. In elution chromatography, the maximum peak height $(C)^{e}_{max}$ or the solute detection limit is inversely proportional to the solute band width which results in decreased system sensitivity for late eluting components.

$$(C)^{e}_{max} = \frac{4\,C_0 \cdot \sqrt{N} \cdot p}{\sqrt{2\pi} \cdot D^2\pi \cdot L \cdot \varepsilon\,(1 + k')} = \frac{C_0 \cdot \sqrt{N} \cdot p}{\sqrt{2\pi} \cdot V_R}$$

(where D and L are the column diameter and length, respectively, ε is the total column porosity, and k' is the solute capacity factor). This is not true in frontal analysis and represents another advantage for trace applications. The increase in system sensitivity when using frontal analysis on a conventional column, 25 cm × 4.6 mm I.D., can be estimated:

$$\frac{(C)^{f}_{max}}{(C)^{e}_{max}} = \frac{\sqrt{2\pi} \cdot V_0 \cdot (1 + k')}{\sqrt{N} \cdot p}$$

Assume that a peak elutes at column dead volumes (V_0 = 3 ml) and with about 5000 theoretical plates, then the sensitivity of the frontal system should increase with a factor of about 170. It is shown later that about 100 times increase in system sensitivity can be obtained.

The differential detector

The differentiation of the detector response function can be achieved either by connecting a detector system to a differential analog amplifier, by computer means, or by using a differential detector arrangement as shown in Fig. 3. Instead of measuring solute concentration as a function of elution volume at constant flow-rate with the reference cell of the detection system disconnected as it is commonly done today, one can measure concentration difference, $f(v + \Delta v) - f(v)$, as a function of volume. The sample cell then has to be connected with the reference cell using a differentiating loop of volume Δv. The first derivative of the elution curve is given by

$$\frac{df(v)}{dv} = \lim_{\Delta v \to 0} \frac{f(v + \Delta v) - f(v)}{\Delta v}$$

and, at small Δv, can be approximated by the difference quotient, $\Delta f(v)/\Delta v$. The differential response at constant Δv will then be directly proportional to concentration difference. Both sample and reference cell of the detector are a part of the Wheatstone bridge arrangement and, thus, when $\Delta v = 0$, the bridge is balanced and no response is obtained. Increasing the volume of the differentiating loop will result in higher differential response and broader peak because additional band broadening is introduced in the connecting tubing. From the practical point of view, an optimum Δv should be chosen in order to ensure highest peak height and resolution. It has been

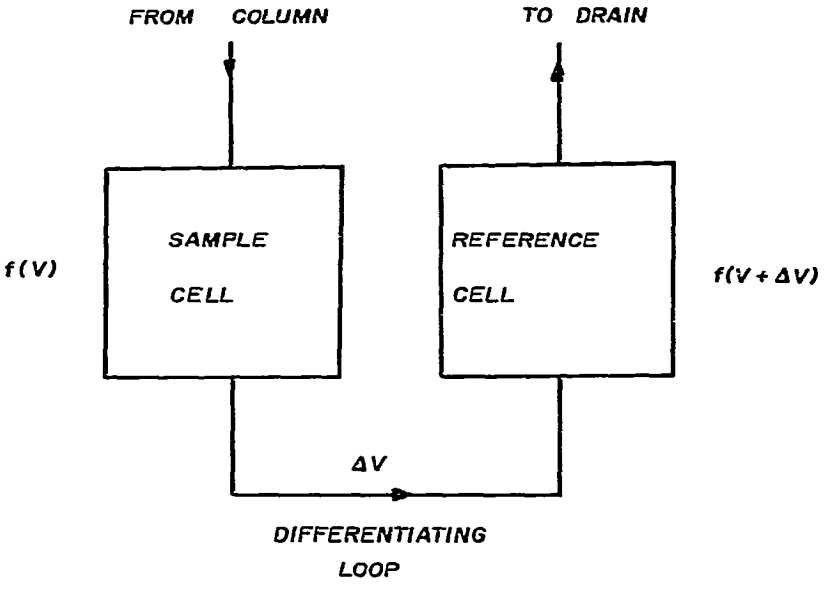

Fig. 3. Experimental arrangement of the differential detector.

shown theoretically that, if the volume of the detector cell (V_d) is equal to about 1/10 of the peak volume (V_p) corresponding to 4σ, the extra column band broadening resulting from the detector cell is insignificant[14].

$$V_p = 10 \cdot V_d = \frac{\pi D^2 \cdot L \cdot \varepsilon \cdot (1 + k')}{\sqrt{N}} \tag{13}$$

For correct differential operation, the volume of the differential detector should obviously be smaller than the volume of the entering peak, otherwise the system would function as two separate detectors,

$$V_p \geqslant 2 \cdot V_d + \Delta v \tag{14}$$

Combination of eqns. 13 and 14 gives

$$\Delta v \leqslant 8 \cdot V_d \tag{15}$$

This can be used as a first approximation to determine Δv for a given system. It is shown later that experimental data are in good agreement with eqn. 15.

EXPERIMENTAL

Apparatus

The HPLC instrument consisted of a Waters Assoc. (Milford, MA, U.S.A.) M6000A solvent delivery system supplied with mobile phase from a glass reservoir, a

Valco (Houston, TX, U.S.A.) sample valve Model ACV-6UHPa-N60 with external loop, and a UV–VIS spectrophotometer with 0.5-μl or 8-μl cell operated at 203 nm (Model SF770; Schoeffel, Westwood, NJ, U.S.A.). Samples were introduced in the sample loop using an Eldex pump, Model B-94 (Eldex Labs., Menlo Park, CA, U.S.A.). The detector was connected to a CIS data handling system (Computer Inquiry Systems, Englewood Cliffs, NJ, U.S.A.) and to a potentiometric recorder. The circuits of the sample valve, sample pump, and computer acquisition system were connected to a programmable microprocessor controller (Model WP 6001; Minarik Electric Co., Los Angeles, CA, U.S.A.) which ensured virtually unattended operation and data handling. The block diagram of the system for frontal analysis with differential detection can be seen in Fig. 4.

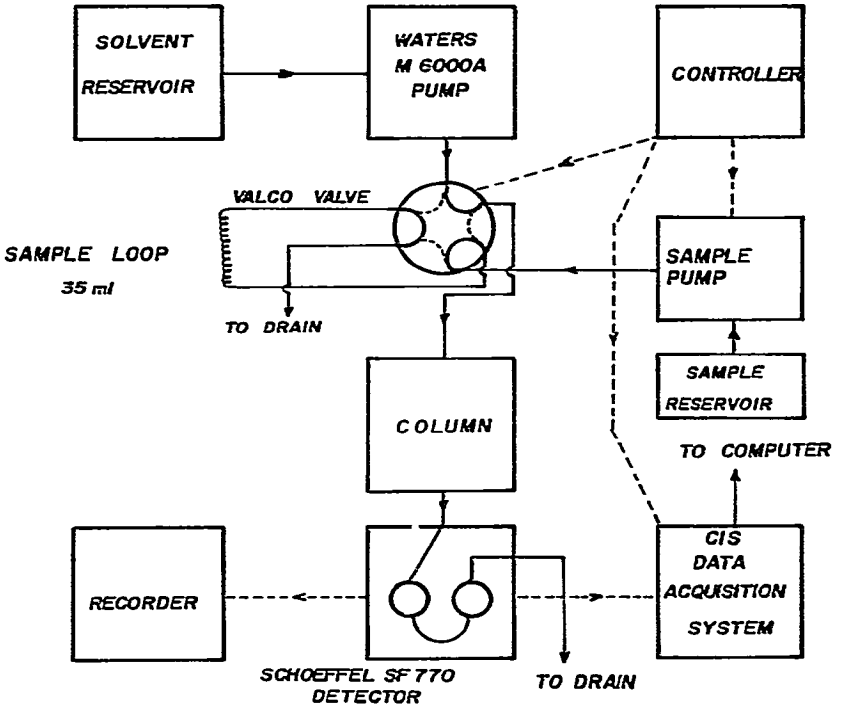

Fig. 4. Block diagram of a frontal analysis liquid chromatograph with the differential detector.

Columns

Either commercially available columns 25 cm × 4.6 mm I.D. packed with chemically bonded anion exchanger Partisil 10 SAX or microbore columns 25 cm × 1 mm I.D. packed with Merck RP-18 reversed phase, 10-μm particle diameter, prepared as reported previously[15] were used.

Mobile phases and samples

Distilled, deionized water and analytical-grade phosphoric acid were used to prepare mobile phases. Sample solutes were from commercial sources and were used

without further purification. The samples were dissolved in the mobile phase and the sample volume placed on the column was controlled by the programmable timer/controller.

RESULTS AND DISCUSSION

Frontal analytical techniques

The separation of a seven-component mixture of six organic acids and hydrochloric acid can be seen in Fig. 5. The concentration of the acids was between 5–50 ppm in the elution mode and volume of sample was 10 μl. Each peak exhibits significant tailing due to slow solute mass transfer in the stationary phase; the resolution between peaks 1, 2 and 6, 7 is poor. Because of the tailing, quantitation of these substances based on peak area would be difficult.

Overloading the column with respect to sample volume (V_s = 35 ml), but using a hundred-fold diluted sample, resulted in the frontal response shown in the center chromatogram of Fig. 5. It can be seen that a clear distinction can be made between the seven components either in the ascending or the descending frontal steps. Differentiation of the frontal response was achieved with a 15 cm × 1.59 mm O.D. × 0.51 mm I.D. PTFE tube (Δv = 30 μl) interposed between the sample and reference cells of the Schoeffel UV–VIS detector; the chromatogram from this experiment can be seen on the bottom of Fig. 5. Significant improvement in peak symmetry and resolution upon differentiation of ascending frontal steps was obtained; the concentration-normalized differential response was higher than in the elution chromatographic mode. The extent of the improvement in apparent column efficiency, peak symmetry, and resolution is summarized in Table I, where data calculated from elution, frontal, and differential frontal chromatograms are presented for two different solutes, one symmetrical (acetylsalicylic acid) and the other with pronounced rear tailing (2-fluoro-5-chlorobenzoic acid).

The height of the differential response was linearly related to total solute mass injected on the column, between 1 and 50 μg, as seen in Fig. 6, indicating that the system is quite suitable for quantitative analysis. It should be pointed out, however, that the positive differential peaks are preferable for quantitation since the negative peaks reflect solute tailing and generally show poor resolution. It was noted during the experimental work that matched UV cells have to be used for the differential detector system otherwise baseline drift and incorrect differentiation may be obtained. Sensitivity of any chromatographic detector system depends in general on signal-to-noise ratio and, since both signal as well as the noise are differentiated during the differential operation, the result is a somewhat decreased sensitivity of the differential system. It was of interest to determine minimum solute concentration which can be placed on the column while retaining at least two times signal-to-noise ratio in different chromatographic modes. The results are shown in Fig. 7 as curves relating height of chromatographic response (either peak height or step height) to solute concentration for 2-fluoro-5-chlorobenzoic acid. The same operating conditions were used in these experiments except that 10-μl sample volumes were employed in elution experiments and 35-ml sample volume in frontal modes. We can see that the use of the frontal technique results in improvement of solute detectability with a factor of about 100, whereas differentiation of the frontal response (Δv = 60

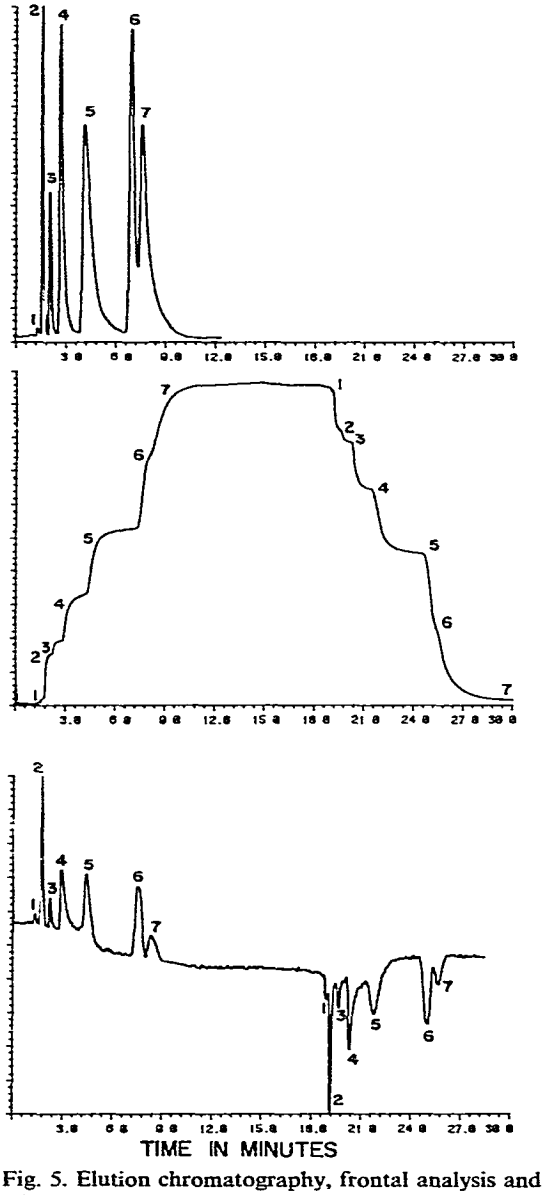

Fig. 5. Elution chromatography, frontal analysis and differential frontal analysis of a seven-component mixture of carboxylic acids. Conditions: column, eluent and flow-rate as in Fig. 1; S = 0.04, 203 nm. Peaks: 1 = hydrochloric acid; 2 = ascorbic acid; 3 = tartaric acid; 4 = (−)-*threo*-hydroxycitric acid; 5 = 2-fluoro-5-chlorobenzoic acid; 6 = garcinia acid; 7 = (−)-*threo*-chlorocitric acid.

µl) yielded only about 13 times improvement in solute detectability. Thus, with the differential detector described, the optimized differential frontal system is more sensitive than methods based on elution chromatography. Increased application of computers for the analysis of digitized HPLC data allows further increase in the sensitivity of the differential frontal system. With the Savitzky–Golay smoothing pro-

TABLE I

EFFICIENCY COMPARISON OF DIFFERENT CHROMATOGRAPHIC MODES

Conditions: column, 25 cm × 4.6 mm I.D. Partisil 10 SAX; mobile phase, 0.075 M H_3PO_4, pH = 1.9; 203 nm. N = Apparent column efficiency in theoretical plates; A = asymmetry factor; R_s = resolution.

Solute	*Elution HPLC*			*Frontal HPLC*			*Differential frontal HPLC*		
	N	A	R_s	N	A	R_s	N	A	R_s
Acetylsalicylic acid	2013	1.03	3.65	2250	1.0	8.42	2160	1.02	8.37
2-Fluoro-5-chlorobenzoic acid	435	3.13		4820	1.0		4865	1.01	

cedure[16], a ten-fold improvement in differential signal-to-noise ratio can be obtained. Frontal and differential frontal analytical techniques are advantageous if the greater amount of sample required is available or if low solubility of sample in the mobile phase prevents the use of normal elution chromatography.

Optimization of the differential detector

An experimental approach for finding an optimum value of Δv was chosen for the purpose of this work. Two solutes, acetylsalicylic acid with a symmetrical peak

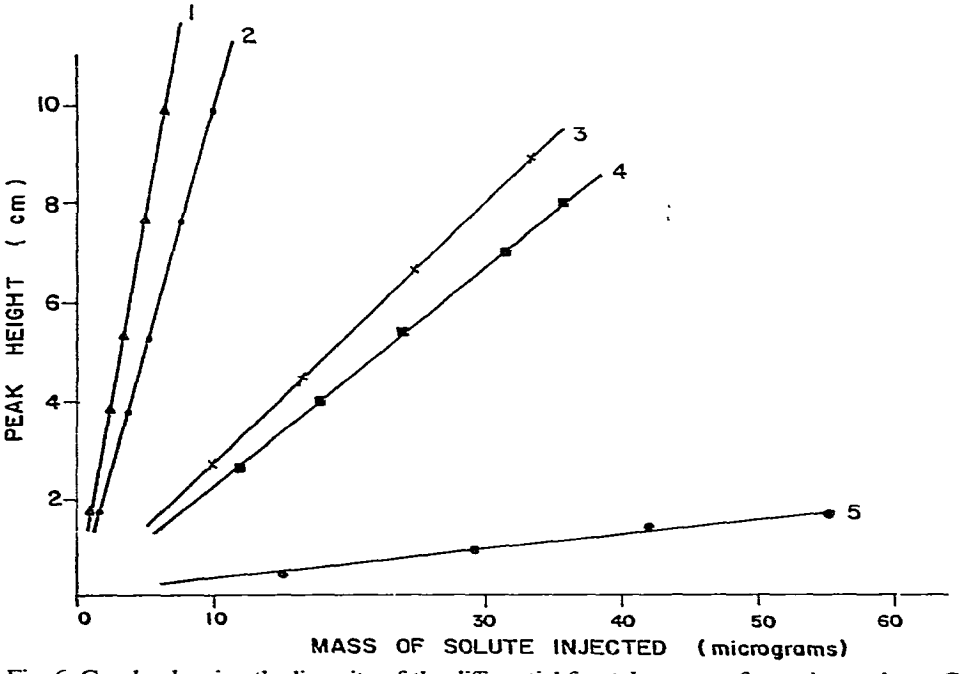

Fig. 6. Graphs showing the linearity of the differential frontal response for various solutes. Conditions: column, eluent and flow-rate as in Fig. 1; S = 0.01, 203 nm; sample size 35 ml. Solutes: 1 = ascorbic acid; 2 = 2-fluoro-5-chlorobenzoic acid; 3 = garcinia acid; 4 = tartaric acid; 5 = chlorocitric acid.

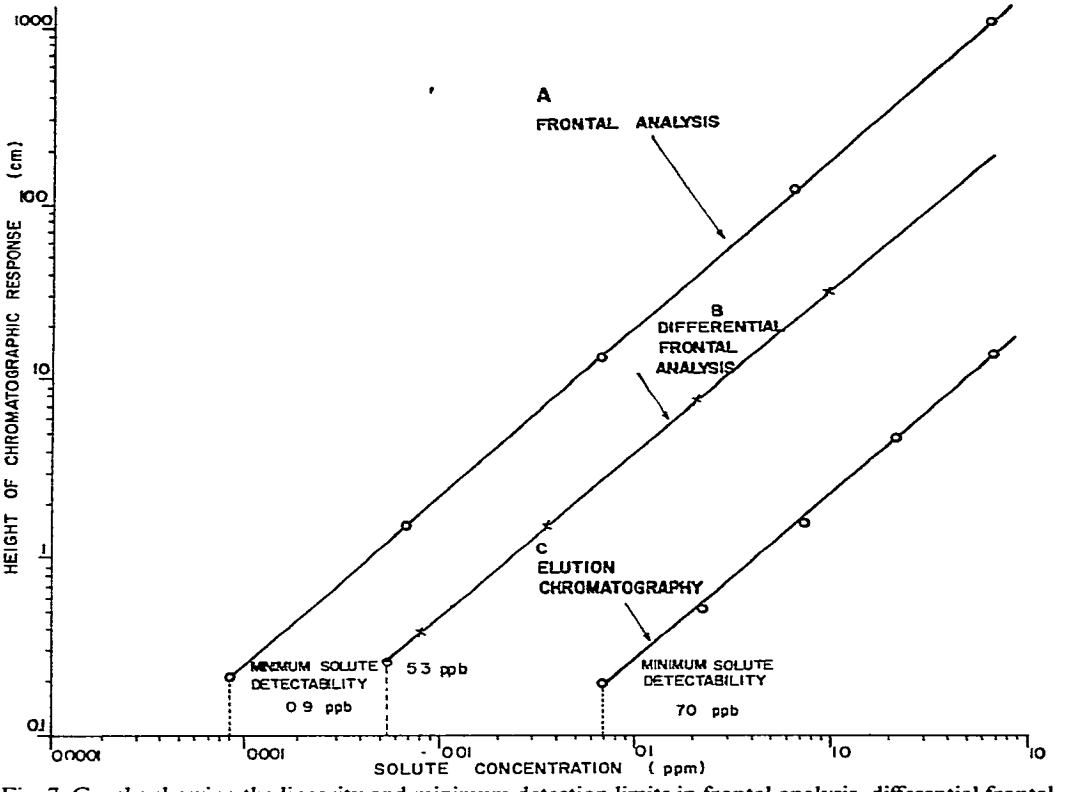

Fig. 7. Graphs showing the linearity and minimum detection limits in frontal analysis, differential frontal analysis and elution chromatography for 2-fluoro-5-chlorobenzoic acid. Conditions: column, eluent and flow-rate as in Fig. 1; $S = 0.01$, 203 nm.

($k' = 0.55$) and 2-fluoro-5-chlorobenzoic acid ($k' = 1.98$) exhibiting serious rear end tailing were used as model compounds. Employing the operating conditions shown in Fig. 8, 10 μl of a mixture of these compounds with 50 ppm concentration of each was injected onto the column connected to the differential detector previously described. The volume of the differentiating loop was varied from 10 μl to 1000 μl and a series of differential elution chromatograms was obtained corresponding to the values of Δv. The heights and widths of the first positive peaks were obtained from the computer data system and efficiency based on previously derived equations was calculated for both peaks. The results are shown in Fig. 9 as graphs relating height, H, and efficiency, N, of the differential peak to Δv. It can be seen that, in the case of detector cell volume of 8 μl, when Δv is smaller than 60 μl, no change in the apparent column efficiency and very similar peak heights are obtained. When Δv exceeds 500 μl, again no change in the peak height can be seen but very poor apparent column efficiency is obtained. Thus, 60 μl can be considered as an optimum value of Δv in this case. This is in good agreement with previously derived eqn. 15. Indeed, the efficiency of the normalized differential elution response for the first peak, $N_d = 1927$, coincided well with that calculated from the elution chromatogram, $N_e = 2013$, whereas improvement in efficiency of the second peak was noted ($N_d = 4934$ and $N_e = 435$). It can be

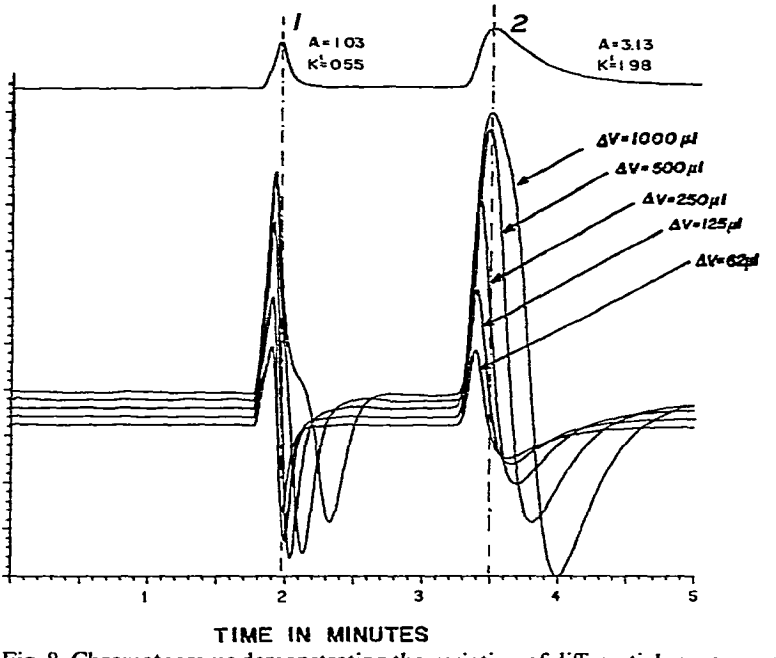

Fig. 8. Chromatograms demonstrating the variation of differential response with volume of differentiating loop. Conditions as in Fig. 1. Peaks: 1 = acetylsalicylic acid; 2 = 2-fluoro-5-chlorobenzoic acid.

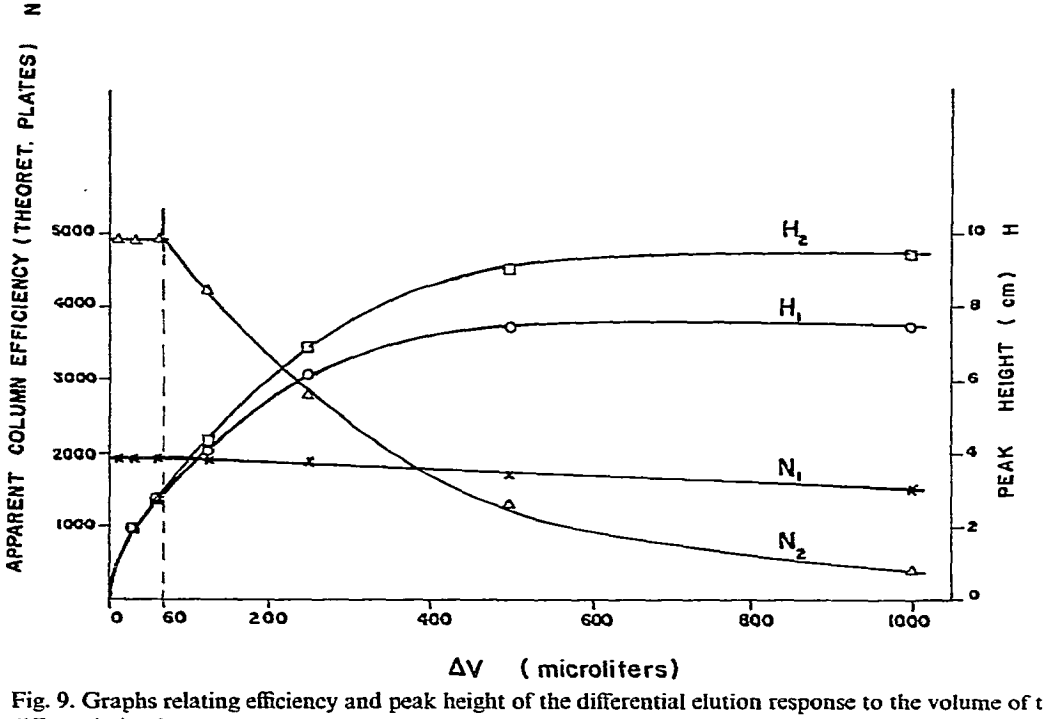

Fig. 9. Graphs relating efficiency and peak height of the differential elution response to the volume of the differentiating loop.

seen that a relatively simple optimization procedure can be carried out in order to attain maximum efficiency and height of differential response.

Applications of frontal techniques using microbore columns

The disadvantage of greater sample volume necessary for frontal analysis can be overcome with the use of microbore column systems. It was shown previously[7] that, when progressively larger sample volumes are placed on the column, distortion of normal elution development towards frontal analysis occurs and at a certain volume of charge, frontal analysis type of development is obtained. The maximum sample volume, V_s, which can be placed on the column while maintaining the resolution between two solutes A and B ($k'_B > k'_A$) is given as

$$V_s = \frac{\pi D^2 L \varepsilon}{4}\left[1 - \frac{2}{\sqrt{N}}\left(\frac{2 + k'_B + k'_A}{k'_B - k'_A}\right)\right]$$

Considering the condition when the difference between the retention volumes of peaks A and B equals or is greater than one half of the sample feed volume, the following equation for the minimal frontal sample volume, V_{sf}, can be derived:

$$V_{sf} \geqslant 2\,(k'_B - k'_A) \cdot \pi \cdot D^2 \cdot L \cdot \varepsilon$$

It can be seen that, in both cases, the sample volume decreases with the square of the column diameter and thus a change from 4.6 mm I.D. to 1.0 mm I.D. microbore column should, under otherwise constant operating conditions, result in a change in V_{sf} by a factor of $(1/4.6)^2 = 0.0473$. An elution chromatogram of several carboxylic acids obtained on a short microbore column 25 cm × 1.0 mm I.D. can be seen in the top of Fig. 10. The sample volume used in the differential frontal system was 1 ml; this allowed total conversion of elution peaks into solute fronts. The differential frontal chromatogram can be seen at the bottom of Fig. 10. The detector cell volume in elution mode operation was 0.5 μl. In accordance with eqn. 15, the volume of the differentiating loop had to be changed to 4 μl to accommodate small band volumes of about 15 μl eluted from the microbore column. All attempts to use greater Δv values resulted in decreased resolution between the sample components on the differential frontal chromatogram. It is seen in the top part of Fig. 10 that all peaks tail to a certain extent; peaks 5 and 6 are not separated, and resolution between peaks 1, 2 and 6, 7 is poor. Operating the system in the differential frontal mode generally improved the resolution, and peak 7 is now uncovered. When using 25-cm microbore columns for elution or frontal analysis, care must be taken that the column is not mass overloaded; the total sample mass placed on the column should not generally exceed about 4 μg. In a multicomponent mixture, the less retained solutes elute from the column while the more retained solutes are still adsorbed on the stationary phase; this will affect loading capacity, peak capacity, and peak symmetry of the system. It has been observed during the course of this work that satisfactory analyses of carboxylic acids at levels between 0.1 to 50 ppm can be obtained with both conventional 4.6 mm I.D. columns as well as with 1 mm I.D. microbore columns of the same length. Operation of the frontal microbore liquid chromatograph is, how-

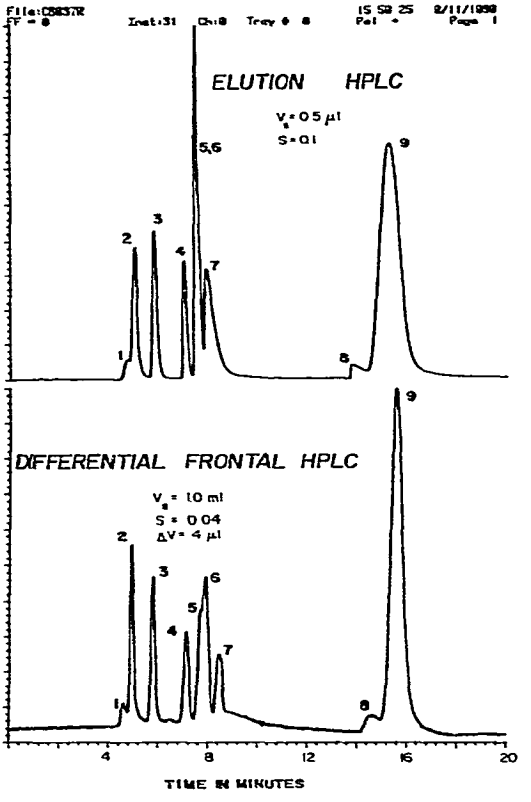

Fig. 10. Elution and differential frontal chromatograms of carboxylic acids on a microbore column. Conditions: RP-18 column, 10 μm, 25 cm × 1 mm I.D.; 0.075 M H_3PO_4, pH 1.9, 40 μl/min; 203 nm. Peaks: 1 = hydrochloric acid; 2 = acetylsalicylic acid; 3 = tartaric acid; 4 = garcinia acid; 5 = ascorbic acid; 6 = pyruvic acid; 7 = lactic acid; 8 = succinic acid; 9 = fumaric acid.

ever, more economical from the point of view of mobile phase, sample consumption, amount of column packing, and associated column hardware used.

CONCLUSION

It has been shown that the special analytical technique of differential frontal analysis has many advantages for trace applications. Better apparent column efficiency, better resolution and symmetry of eluted peaks, and generally increased system sensitivity result from this chromatographic technique. It is demonstrated that sample volume needed for frontal analysis decreases with the square of the column diameter and thus the need for relatively large sample volume can be overcome with the use of microbore columns. Equations are derived which allow calculation of column efficiency from the differential response and the step of the frontal curve. It is shown that a simple and inexpensive instrumentation can be used to optimize the differential detector and to achieve differential frontal results. Various carboxylic acids present in samples at levels of a few ppm can be analyzed using either conventional 4.6 mm I.D. columns or 1 mm I.D. microbore columns.

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REFERENCES

1 A. Tiselius, *Ark. Kemi, Mineral. Geol.*, 14B, No. 22 (1940) 5.
2 S. Claesson, *Ark. Kemi, Mineral. Geol.*, 23A, No. 1 (1946) 133.
3 A. Tiselius, *Ark. Kemi, Mineral. Geol.*, 16A, No. 18 (1943) 11.
4 E. Heftmann (Editor), *Chromatography*, Reinhold, New York, 1950.
5 P. E. Eberly and C. N. Kimberlin, *Trans. Faraday Soc.*, 57 (1961) 1169.
6 P. H. James and C. S. G. Phillips, *J. Chem. Soc.*, (1954) 1066.
7 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 119 (1976) 467.
8 J. Boeke, *Gas Chromatography*, Butterworths, London, 1960.
9 J. M. Essigmann and N. Catsimpoolas, *J. Chromatogr.*, 103 (1975) 7.
10 R. P. W. Scott, *Liquid Chromatography Detectors*, Elsevier, Amsterdam, Oxford, New York, 1977.
11 C. N. Reilly, G. P. Hildebrand and J. W. Ashley, *Anal. Chem.*, 34 (1962) 10.
12 A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, 35 (1941) 1358.
13 J. C. Giddings, *Dynamics of Chromatography*, Marcel Dekker, New York, 1965.
14 J. J. Kirkland, W. W. Yau, H. J. Stoklosa and C. H. Dilks, Jr., *J. Chromatogr. Sci.*, 15 (1977) 303.
15 P. Kucera, *J. Chromatogr.*, 198 (1980) 93.
16 A. Savitzky and M. J. E. Golay, *Anal. Chem.*, 36 (1964) 8.