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SIMPLE DERIVATIVE MODE DETECTOR FOR LIQUID CHROMATOGRAPHY

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SUMMARY

A derivative mode detector is described for liquid chromatography. The derivative mode signal can be obtained by a simple modification of commercially available UV dual-channel detectors. The modification involves a change in the flow pattern of the column effluent in the two optical channels. The resulting derivative signal provides useful information for convenient and precise determinations of peak parameters, such as the retention time and standard deviation of the concentration distribution, which are needed for the estimation of the plate height, resolution, and other chromatographic factors.

INTRODUCTION

High-performance liquid chromatography (LC) has developed in recent years as a useful and versatile separation tool. Its potential has been demonstrated in clinical applications[1], biochemical research[2], pollution analysis[3] and other fields[4–6]. The success of modern LC can be partly attributed to the availability of highly sensitive optical detectors. In general, these detectors consist of two optical channels (flow cells) of small dead volume placed between a filtered or monochromatic light source and a photocell connected to a photometer. The column effluent stream passes through one cell, and its composition is monitored relative to the fluid in the second chamber, which usually is stagnant mobile phase (or air).

The concentration distribution of a solute eluted from the column typically resembles a Gaussian curve, and the analog signal produced is convenient for peak position and area determinations. However, the signal as such (without computer processing or electronic modification) does not provide the inflection points of the curve which are useful in obtaining the standard deviation of the concentration distribution (σ). The values of σ along with the retention time measurements are necessary for precise estimation of important chromatographic factors such as plate height, resolution, skewness, excess and others[7–9]. In the present paper, we report a very simple mechanical modification of commonly used UV dual-channel detectors which provides a derivative mode curve directly on the chart paper of the recorder. This

allows direct measurement of the standard deviation(s) of approximately Gaussian or bi-Gaussian peaks and the retention time. In addition, the derivative mode detector is helpful in the characterization of poorly resolved peaks because of its inherent ability to detect slope changes in the concentration distribution profile. Thus, increased capability in the evaluation of chromatographic data is achieved without extra investment in equipment.

EXPERIMENTAL

Chromatographic system

The liquid chromatograph used in these studies was a Waters Associates ALC 100 (Waters Ass., Milford, Mass., U.S.A.). The column was a 61-cm-long × 2.2-mm-I.D. stainless-steel tube packed with a pellicular cation-exchange resin, Sepcote CA26 (Separation Technology, Cambridge, Mass., U.S.A.). It was eluted at ambient temperature with 0.1 *M* ammonium formate (pH 4.4). The column pressure was 550 p.s.i. and the flow-rate was 0.70 ml/min for the data reported in Table I.

Detector modification

The LC system was originally equipped with an LDC UV detector designed to monitor absorbance at 254 nm (Model 1205 W, Laboratory Data Control, Riviera Beach, Fla., U.S.A.). Fig. 1a shows the geometry of the LDC detector as commonly used in LC (the integral mode) and Fig. 1b illustrates the flow pattern modification introduced in these studies for derivative mode operation.

In the derivative mode, column effluent flows through the sample cell of the detector and into a tube which is connected to what is ordinarily referred to as the reference cell. The holding tube consists of a 21-cm-long × 1.6-mm-O.D. × 0.5-mm-I.D. hollow stainless-steel tube which is held flush to the detector entrance and exit tubes with a short collar of tightly fitting PTFE.

Measurement of peak retention time and variance

Elution times were determined by several different techniques. For certain experiments, peak retention time and standard deviation(s) were measured with a stopwatch as the data were being generated. These measurements were made directly when the detector was operated in the derivative mode. The time interval from the origin to where the derivative curve passes through zero (set at 50% of the recorder chart scale) represents the peak retention time (B in Fig. 2b). The time intervals from the X-intercept to the derivative curve maximum (A) and minimum (C) depict the standard deviations (σ_1 and σ_2) of the front and back sides of the peak, respectively (the maximum (A) and minimum (C) correspond to the inflection points of the Gaussian-like peak in Fig. 2a).

In the integral detection mode only retention times could be measured directly using the maximum of the peak height. Standard deviations (σ_1 and σ_2) were computed from the strip chart recording as the horizontal distances from points on the curve at six-tenths peak height to a perpendicular dropped from the peak maximum.

A digital data acquisition unit consisting of an analog-to-digital converter, variable speed sampler, and a paper tape punch was used in a separate series of comparative experiments for peak retention time and variance measurements. The

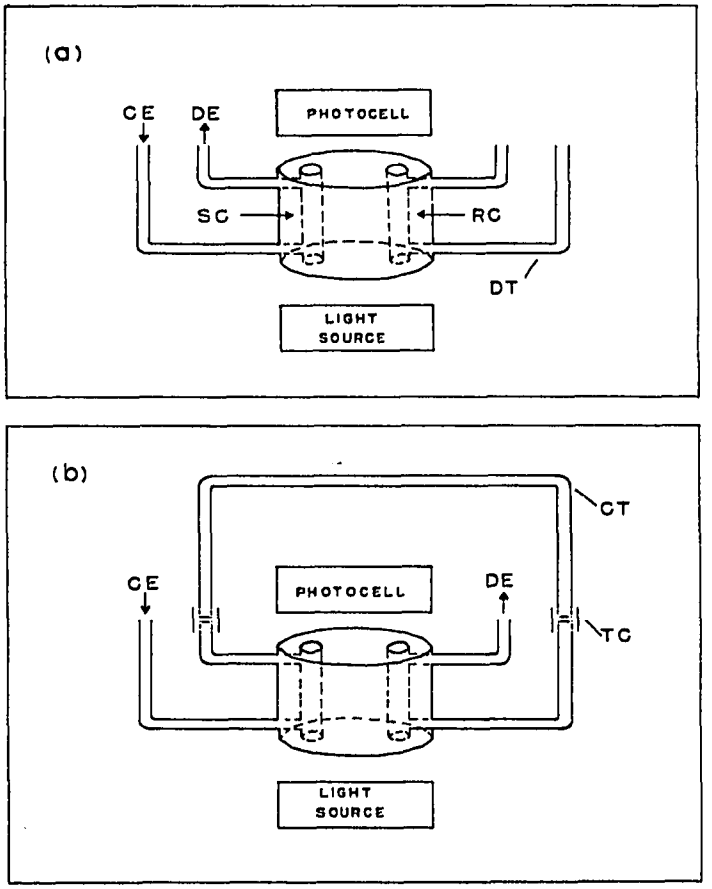

Fig. 1. Diagram of the LDC UV detector as used in the integral mode (1a) and as modified for use in the derivative mode (1b). CE = Column effluent; DE = detector effluent; SC = detector sample cell; RC = detector reference cell; DT = detector tubing; TC = PTFE collar; CT = 21-cm-long × 0.5-mm-I.D. connecting tube.

UV detector output was amplified from 10 mV to 1 V full scale using a d.c. voltage amplifier. The amplified analog signal was fed into both the digitizer and a strip chart recorder. The digitizer produced a punched paper tape record of chromatogram ordinate values at 0.5-sec intervals. The digital values were used to obtain peak retention time and variance data as described above.

RESULTS AND DISCUSSION

An aqueous solution of guanine and adenine was used to compare the derivative and integral modes of detection. The test solution contained 120 and 190 μg/ml of each base, respectively, and 10-μl portions were injected into the LC with a syringe (the nitrogen bases used in these studies were obtained from Sigma, St. Louis, Mo., U.S.A.). Fig. 2 shows a typical separation of these compounds as detected in the

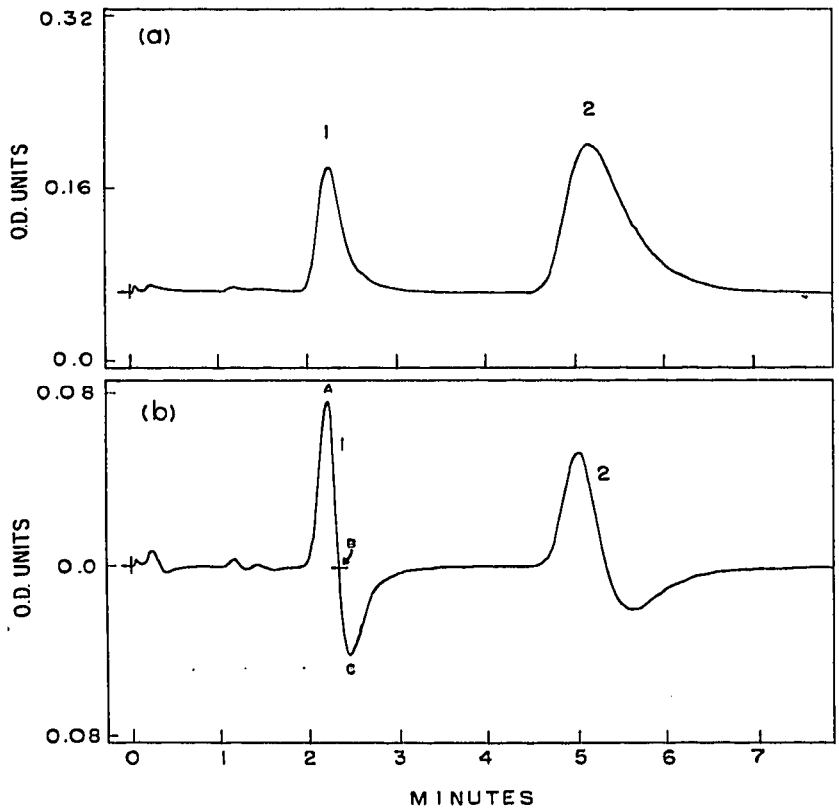

Fig. 2. Separation of guanine (1) and adenine (2) on a 61-cm-long × 2.2-mm-I.D. Sepcote CA26 column eluted with 0.1 *M* ammonium formate (pH 4.4) at ambient temperature. The flow-rate was 0.80 ml/min and the column pressure was 720 p.s.i. Ordinate values represent optical density units at 254 nm. Points A and C in the derivative chromatogram (b) correspond to the inflection points of the guanine peak in the integral chromatogram (a). B corresponds to the peak maximum in (a).

integral mode (2a) and in the derivative mode (2b). Fig. 3 shows the type of chromatogram obtained from a more poorly resolved mixture. In this case, 15-μl (3a) and 20-μl (3b) portions of a mixture of N^7-methylguanine (16 μg/ml) and N^2-methylguanine (51 μg/ml) were injected onto the column.

Peak retention times and front and back side standard deviations (σ_1 and σ_2) were determined for guanine and adenine when the detector was operated in both the integral and derivative modes (Table I). In the traditional (integral) mode of detection, the compound elutes from the column and passes into a short tube which leads to the analytical cell of the detector. However, in the derivative mode the compound continues through tubing connecting the analytical and reference cells. The volume between the two cells was approximately 125 μl (or a lag time of about 11 sec).

The integral mode detector monitors the continuous distribution of absorbances (or concentrations) in the column effluent over time. In the derivative mode, however, the absorbance at one time is measured relative to that at another (ratio measurement). Thus the signal obtained when the detector is operated in the deriva-

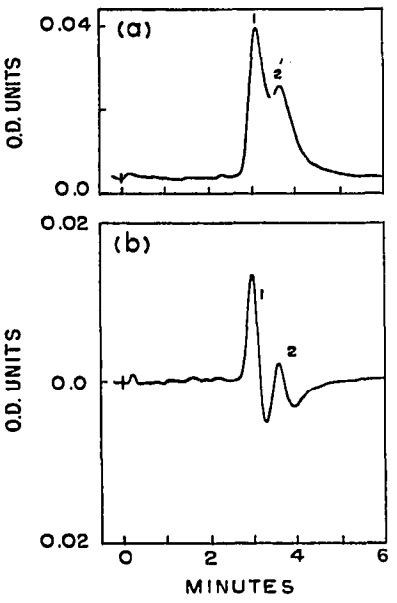

Fig. 3. Separation of N^2-methylguanine (1) and N^7-methylguanine (2) on a 61-cm-long × 2.2-mm-I.D. Sepcote CA26 column eluted with 0.1 *M* ammonium formate (pH 4.4) at ambient temperature. The flow-rate was 0.70 ml/min and the column pressure was 550 p.s.i. Ordinate values represent optical density units at 254 nm. The detector was operated in the integral mode for (a) and in the derivative mode for (b).

tive mode represents the rate of absorbance change with time, which is the first derivative of the signal usually obtained in LC.

The apparent retention time of a given component is longer in the derivative detection mode than in the integral mode (Table I). This discrepancy is the result of

TABLE I

COMPARATIVE COMPUTATION OF PEAK RETENTION TIME AND STANDARD DEVIATION

	"Stopwatch" method		*"Digitizing" method*	
	Derivative	*Integral*	*Derivative*	*Integral*
Guanine				
t_G (sec)*	186.9 ± 1.0	180.5 ± 0.9	183.7 ± 1.3	175.7 ± 0.8
σ_1**	10.6 ± 0.3	(9.1 ± 0.2)***	11.0 ± 0.3	
σ_2	11.0 ± 0.6	(10.9 ± 0.5)***	10.3 ± 0.4	
Adenine				
t_A (sec)	428.6 ± 1.4	418.7 ± 2.0	414.8 ± 1.1	403.5 ± 1.0
σ_1	22.7 ± 0.2	(19.8 ± 0.7)***	22.9 ± 0.4	
σ_2	23.3 ± 0.4	(25.7 ± 0.9)***	20.9 ± 1.4	

* Average of six chromatographic runs.

** σ_1 and σ_2 denote the front and back side standard deviations of a bi-Gaussian distribution.

*** Measured as the time from the peak maximum to points on the curve at six-tenths peak height.

mechanical modifications necessary in the construction of the derivative detector. The retention time of a peak detected in this mode is measured when the signal passes through zero. This occurs when the absorbances of both cells are equal, *i.e.*, when the peak maximum is situated in the exact center of the holding tube between the two detector cells. For a symmetrical peak, therefore, the difference between the apparent retention times of the integral and derivative detectors should be equal to one-half the time required for a molecular species to pass from one cell to the other. This is essentially what was observed.

The data in Table I also show that the difference between the derivative and integral retention time for adenine was slightly longer than for guanine (by approximately 3.5 sec). This occurred probably because the top of the adenine peak was slightly skewed. In this case, the signal would have passed through zero when the center of mass of the concentration distribution in the holding tube, rather than the peak maximum, was half way between the two detector cells. A shorter holding tube would be expected to minimize this effect.

Various time measuring techniques were compared for precision and convenience in peak retention time and variance determinations. Direct measurements of retention time and front and back side standard deviations were possible using a stopwatch when the detector was used in the derivative mode. Standard deviations were easily measured in this way, because they appeared distinctly as maxima and minima on the chromatogram (Fig. 2b). However, it was not possible to make these measurements when the detector was operated in the integral mode because the inflection points of the Gaussian-like curve cannot be visualized directly. It was necessary for comparative purposes, therefore, to compute the peak standard deviations as the horizontal distances from the peak inflection points to a perpendicular dropped from the peak maximum. The peaks obtained in this study were assumed to exhibit bi-Gaussian distributions, so inflection points were measured at six-tenths of the peak height. Determination of peak variance in this way is undesirable because it is time consuming and is inaccurate when poorly resolved components are involved.

The digitizing method was also used for retention time and variance determinations. Precision was comparable with that obtained using the stopwatch method (approximately 0.5% for retention times and less than 5% for variances). This precision is quite satisfactory for most applications.

The derivative mode of detection also permits rapid determination of column efficiency and resolution. These indicators of column performance are easily obtained from the first derivative. Column efficiency, or the number of theoretical plates (N), was calculated by the relation

$$N = \frac{t_R^2}{\bar{\sigma}^2}$$

where t_R is the peak retention time and $\bar{\sigma}^2$ is the variance (in general, $\bar{\sigma} = (\sigma_1 + \sigma_2)/2$). The number of theoretical plates calculated for adenine using the derivative and integral detection modes was 347 and 339, respectively.

Resolution was calculated from

$$R = \frac{t_A - t_G}{2\sqrt{(\bar{\sigma}_A^2 + \bar{\sigma}_G^2)}}$$

where t_A and t_G are the retention times of adenine and guanine and $\bar{\sigma}_A^2$ and $\bar{\sigma}_G^2$ represent their variances, respectively. Resolution was 4.75 using both detection methods.

It is possible to characterize chromatographic peaks by their statistical moments. This approach is particularly valuable where the components of a mixture are not well resolved. The second statistical moment (σ^2), for example, is necessary for peak deconvolution procedures, and the traditional integral chromatogram does not permit accurate peak variance determinations directly. The first derivative of the analog signal, however, is very useful in peak analysis, and the technique presented here provides a simple, inexpensive means to obtain it.

We have reported the basic aspects of the derivative mode detector in the present paper. In future studies, the effect of the volume between the detector cells on the derivative chromatogram will be investigated. The derivative mode of detection also will be evaluated using column systems chosen to produce a range of chromatographic efficiencies commonly encountered in LC.

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