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Short communication

Differential signal detection system for improved analysis of overlapping signals in liquid chromatography

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Abstract

We have developed a differential signal detection system for the analysis of liquid chromatographic signals using two or more detectors and a differential amplifier circuit. The proposed detection system is an improvement on conventional chromatography in which signals are detected by means of a single detector. The differential signal detection system eliminates difficulties of isolating minor components of the signal which are masked by the major component, as well as difficulties of separating two components of the signal having similar retention times.

Keywords: Differential signal detection; Overlapping signals; Peak overlap

1. Introduction

Liquid chromatography is widely used for analyzing the quality and the quantity of organic and inorganic compounds. Trace analysis by liquid chromatography invariably requires the use of highly sensitive detection techniques, to produce an enhanced response in a conventional detector; such as in conductivity detection, voltammetric detection, potentiometric detection, UV–Vis absorbance detection, fluorescence detection and atomic spectroscopic detection, as well as in chromatography in combination with mass spectrometry. Many researchers have advanced different applications for each detection technique [1–7]. The signals generated by the above detection techniques are recorded as a function of the elution time (retention time) for the identification of different components of a sample. The intensity of the signals are used for determining the quantitative value of each component. In conventional chromatography, the signals sensed by the detector and recorded in the recorder are a direct indication of the concentration of the solute, which naturally varies as a function of the retention time. However, using a conventional system, a signal can be difficult to detect when a small component of the signal is hidden in a larger component of the signal, or when a component of the signal has a similar retention time to the larger component of the signal.

Several approaches to overlapping signal separation, such as the deconvolution method and the multivariate method, can be utilized when a small signal of a trace component contaminates the large signal of the principal component, or when two signals are inseparably overlapping. Some methods involve the transformation of the elution profile to its first, second or derivative using mathematical techniques [8–11].

The differential signal technique is an alternative technique, which is suitable when a small signal of a

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trace component contaminates the large signal of the principle components, or when two signals are inseparably overlapping. The proposed detection system is a simple and easy technique for obtaining a differential signal, eliminating the need for computerized programs that are necessary in mathematical techniques.

2. Conventional liquid chromatography

Conventional liquid chromatography is summarized in Fig. 1. The eluant solvent is introduced into the chromatographic system from the eluant reservoir 1, by means of a metering pump 2. The solvent carries the sample S introduced into the system at the sample inlet port 3, toward the separation column 4, which is filled with the stationary phase. Thereafter, the adsorbed sample components are eluted out at different rates by the eluant, while being continuously introduced into column 4. The eluate solution containing the successively eluted solutes is passed through the detector 5, where the signals of an analytical parameter corresponding to the amounts of the respective solutes are detected. It is recorded in recorder 9, as a function of the retention time.

3. Proposed differential signal detection system

A signal in a conventional chromatographic system is generated by the quantity of the analyte and the elution time. On the other hand, a signal in the differential system is generated by changes in the quantity of the analyte and the elution time. Therefore, a differential signal is obtained by subtracting the intensity of a signal at a certain moment in time by the intensity of the signal at an earlier moment in time. Fig. 2 is a block diagram of the proposed detector used in a chromatographic system. This detection system is constructed by using three detectors 5, 5′, 5′ arranged in series. The solution containing analytes passing through the separation column 4 are simultaneously detected in these detectors, which generate the signals A1, A2, A3. The signals A1 and A2 from the detectors 5 and 5′ are generated with a time lag Dt, and with a phase difference. The signal A2 generated simultaneously from the second detector 5′ is identical to the signal A1 generated from the first detector 5, except that the signal A2 from the second detector 5′ is delayed by the time Dt.

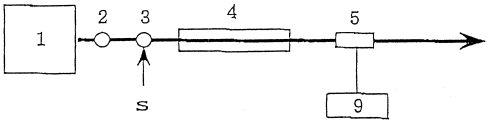

Fig. 1. Block diagram illustrating the conventional chromatographic apparatus. (1) Eluant reservoir, (2) metering pump, (3) sample inlet port, (4) separation column, (5) detector, (R) recorder.

The voltage values of the signals A1 and A2 are processed in a differential amplifier 6, which generates the differential as a signal B1, which is the first-order differential of the signals A1 and A2. Similarly, the voltage values from the detectors 5′ and 5′ are processed in the second differential amplifier 6′, which generates the first-order differential B2 of the signals A2 and A3.

In the next stage, two first-order differential signals B1 and B2 are obtained in the above described manner and are processed in another differential amplifier 7, which generates the second-order differential signal C1. In this way, *n*th-order of differential signals of voltage values can be obtained by means of $(n+1)$ detectors arranged in series and n th number of differential amplifiers in a cascade connection. The final differential voltage signal is recorded in recorder 9.

4. Experimental

A liquid chromatographic system was constructed from a metering pump (Model 576, manufactured by GL Science Co. Ltd.), an injector with a 20 ml loop (Model 7125, manufactured by Rheodyne Co. Ltd.), an octadecylsilylated silica gel column of 4.6 mm diameter and 50 mm length filled with adsorbent particles of 5 mm diameter and 10 nm pore diameter (manufactured by Fuji Serial Chemical Co.), two ultraviolet detectors (Model SPD6AV, manufactured by Shimadzu Co. Ltd.), a differential amplifier and a recorder (Model U228, manufactured by Shimadzu Co. Ltd.). The differential amplifier, which was

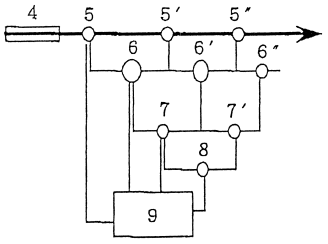

Fig. 2. Block diagram illustrating the proposed chromatographic apparatus. 5, 5′, 5′′ – detectors, 6, 6′, 6′′, 7, 7′, 8 – differential amplifiers, R – recorder.

assembled by the author, had a time constant of about 1 ms and was capable of working with a voltage range of 1 mV to 10 V.

Liquid chromatography was performed at room temperature using a sample of pure benzene and a benzene mixture containing an impurity (2, 5, 10% by weight). The eluant contained a mixture of water and acetonitrile (1:1 by volume). The eluant was introduced into the column at a flow-rate of 1 ml/minute. The eluted components were detected by ultraviolet-spectrophotometry using a wavelength of 254 nm.

In this experiment, it was assumed that the impurity component had a retention time which was longer than the retention time of benzene by 15 s. Two solutions (a 10 ml and either a 0.2, 0.5 or 1.0 ml solution of benzene in acetonitrile/water in a concentration of 5 mg/ml) were introduced into the two serial sample inlet ports. The second inlet port was opened 15 s after opening the first sample inlet port and the signal of the impurity components was detected.

5. Results and discussion

Figs. 3A and 3B show the signals generated by benzene with 5 and 10% impurity components, respectively, obtained by using a conventional chromatographic system with a retention time of approximately 3 min and 30 s. It can be seen from Fig. 3A that the 5% impurity component in the sample is not visible at all in the signal. It can be seen from Fig. 3B that the 10% impurity component is displayed only as a small shoulder in the signal, indicating that the conventional system cannot measure impurity

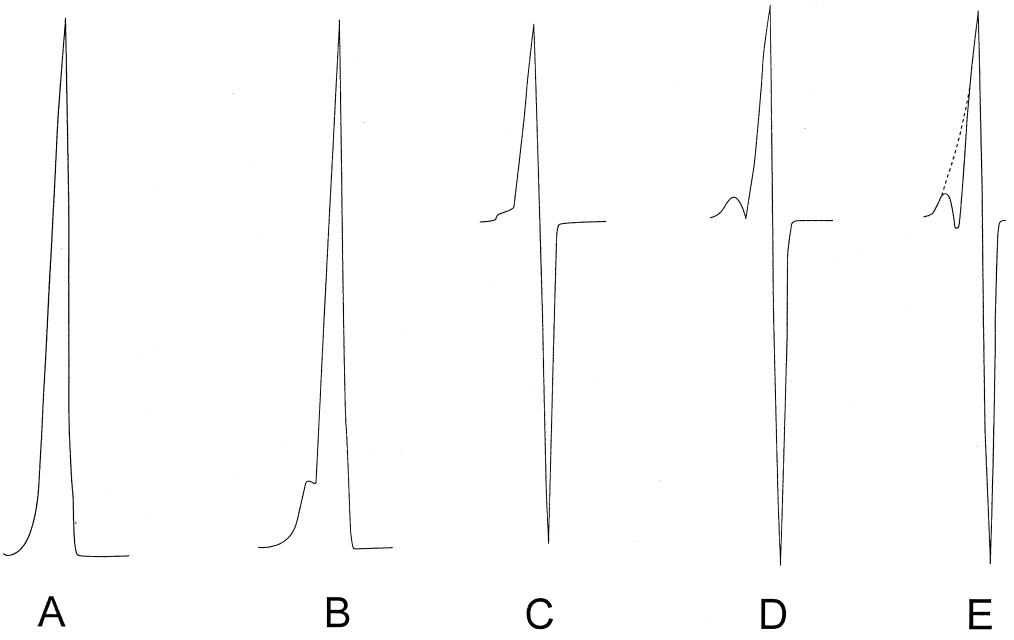

Fig. 3. Signals generated by benzene containing 2, 5 and 10% of an impurity. 3A and 3B are signals generated by benzene containing 5 and 10% of an impurity obtained by the conventional system. 3C, 3D and 3E are differential signals generated by benzene containing 2, 5 and 10% of an impurity, respectively, obtained by the proposed technique.

components less than 5% of the sample when the retention time is 15 s.

Figs. 3C, 3D and 3E show the first-order differential signals generated by solutions with impurities of 2%, 5% and 10%, respectively, recorded by the proposed system. It is clear that the signals of samples which have an impurity component of 5% (Fig. 3D) and 10% (Fig. 3E) generate a signal which clearly display the impurity component in the sample. A small shoulder is visible in the signal generated by samples containing only 2% impurity component (Fig. 3C). Obviously, the signal corresponding to an impurity can be obtained and more clearly isolated from the signal of the principal component using the proposed technique, in comparison to the conventional system. It is possible to separate the impurity signal from the main signal for each of the three impurity levels by extending the hypothetical curve of the main signal (dashed line) over the impurity dip until the peak of the main signal. Then, a vertical line can be drawn through the peak point of the impurity signal. The distance from the peak point of the impurity signal to the hypothetical curve of the main signal was measured for each of the impurity levels and transformed to Fig. 4. The calibration curve in Fig. 4 illustrates that a good linear relationship between the amount of impurity and signal intensity can be obtained using the proposed method.

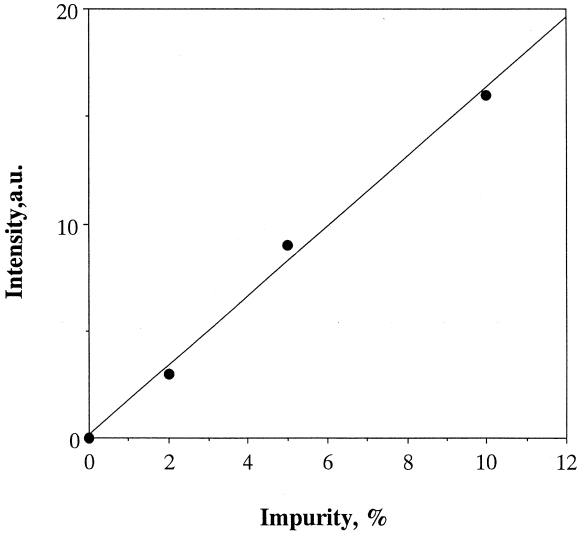

Fig. 4. Calibration curve for the impurity component generated by using the proposed system.

6. Conclusion

We have developed a differential signal detection system for liquid chromatography using two or more detectors, which does not require mathematical techniques. An experiment indicated that the proposed technique is clearly an improvement in detection and recording compared to conventional chromatographic systems, in which the signals are detected by means of a single detector. This experiment demonstrated that a small component of the signal compared to the principle component was more clearly detectable using the proposed system than the conventional system.

It is suggested that this technique is specially suitable for the detection of trace impurities in a high purity sample, e.g., impurity elements in highly concentrated solvent and high purity acids. The proposed system is, however, not suitable for testing samples containing many different elements. This is because in the proposed system two signals are generated for each element; cluttering the display and making separation difficult. This is particularly true when using the proposed system with gas chromatography.

Various types of conventional chromatographic equipment can easily be modified for measuring signals using the differential technique by transforming signals obtained from detectors into voltage signals from which a differential signal can be generated by means of differential amplifiers.

Note: The differential signal apparatus described in this communication has been patented under application number 08/887,646 on June, 10, 1998, United States Department of Commerce, Patent and Trademark Office. A comparable detector to the one described here has been patented by Afeyan [12]. However, in this detector phase difference was not used.

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