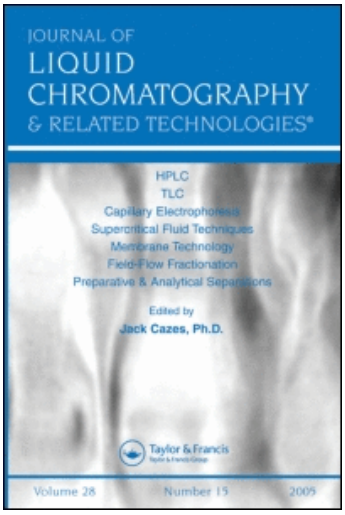

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Concentration Gradient Detector in Capillary Separation Technology

Janusz Pawliszyn[a]
[a] Utah State University, Lagan, Utah

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CONCENTRATION GRADIENT DETECTOR IN CAPILLARY SEPARATION TECHNOLOGY

Janusz Pawliszyn
Assistant Professor
Utah State University
Lagan, Utah 84322-0300

Abstract

The physical principle and properties of the concentration gradient method based on Schlieren optics are described and discussed. Simple, low volume and inexpensive sensors can be designed based on this principle. This technique is applied as a detection scheme in high efficiency capillary separation technology. The advantages of frontal analysis compared to the elution method in high efficiency separations are discussed.

Recent advances in capillary separation technology have produced dramatic improvement in efficiency of these methods (1). For example, capillary liquid chromatography (2) or capillary zone electrophoresis (3) are known to achieve efficiencies close to a million of theoretical plates. In these methods, due to favorable geometry of the capillary system, the injected sample is not dispersed significantly during the migration process. Therefore, "sharp" peaks characterized by high concentration gradients are produced at the detector (See Figure 1b).

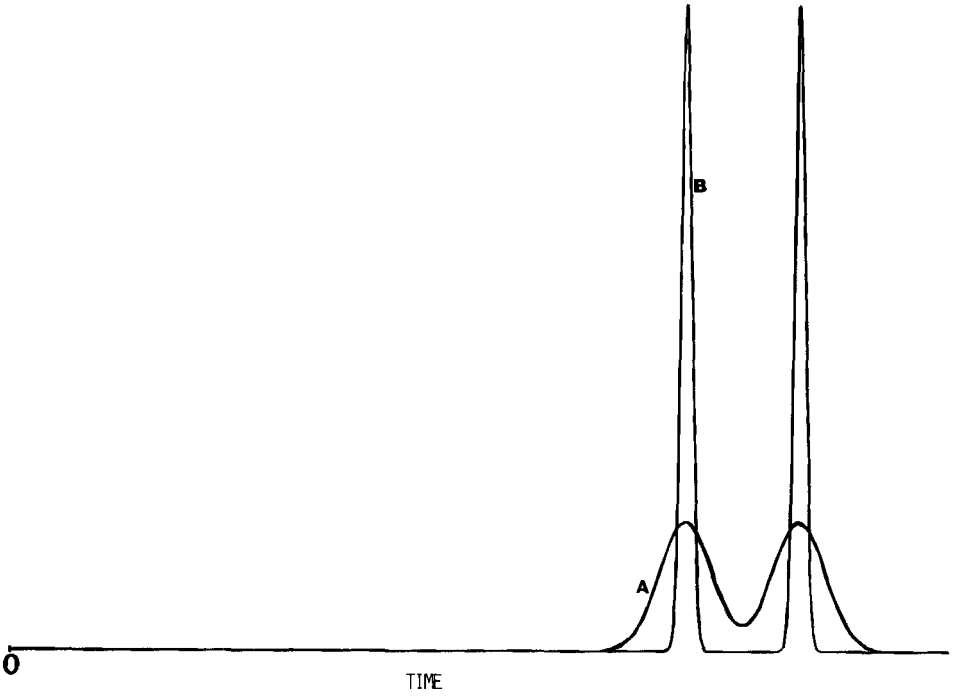

Figure 1

Results of high and low efficiency separation ($N_h/N_L \approx 25$) under same conditions:

(a) low efficiency method

(b) high efficiency method

The concentration profile detected at the end of the column can be described in the linear-ideal case by the Gaussian distribution:

$$C(t) = \frac{M}{F}\frac{1}{\sigma_t(2\pi)^{1/2}} \exp\left[-\frac{1}{2}\left(\frac{t_r - t}{\sigma_t}\right)^2\right] \qquad (1)$$

where M is the total mass injected, F is the flow rate, σ_t is the standard deviation of the distribution in time units and t_r is the retention time.

The efficiency of the column is represented by a number of theoretical plates $N=(t_r/\sigma_t)$ and describes, primarily, the kinetic contribution to the band broadening. The sensitivity of small volume chromatographic detection is proportional to the concentration of the solute at $t=t_r$ (Figure 2b):

$$C_{max} = C(t_r) = \frac{M}{F}\frac{1}{\sigma_t(2\pi)^{1/2}} = \frac{M}{F}\frac{N^{1/2}}{t_r(2\pi)^{1/2}} \tag{2}$$

Therefore, the detector with a linear reponse to concentration will produce a signal proportional to the mass of any component of the sample. The sensitivity improvement of $N^{\frac{1}{2}}$ is expected with efficiency increase (Figure 1).

On the other hand, sensitivity of the detection method based on the concentration gradient measurement will be proportional to the gradients at two inflection points at $t_r+\sigma_t$ and $t_r-\sigma_t$ (Figure 2a):

$$\left(\frac{dC}{dt}\right)_{max} = \left|\frac{dC}{dt}(t_r \pm \sigma_t)\right| = \frac{M}{F}\frac{e^{1/2}}{\sigma_t^2(2\pi)^{1/2}} \tag{3}$$

Therefore

$$\left(\frac{dC}{dx}\right)_{max} = \frac{M}{F}\frac{e^{1/2}N}{t_r^2(dx/dt)(2\pi)^{1/2}} \tag{4}$$

where $\frac{dx}{dt}$ is a linear flow rate. The detection method based on this principle will also give a signal proportional to the total mass of solute injected. However, improvement in detection is now proportional to column efficiency, N, which has a larger enhancement than the magnitude of concentration method.

The ratio of these two sensitivies (4) and (2) may be expressed as a function of chromatographic parameters:

$$\frac{(dC/dx)_{max}}{C_{max}} = \frac{e^{-\frac{1}{2}}}{\sigma_t\,(dx/dt)} = B_o \tag{5}$$

indicating that the sensitivity advantage of the concetration gradient method becomes larger for very narrow peaks (if σ_t becomes small B_o becomes

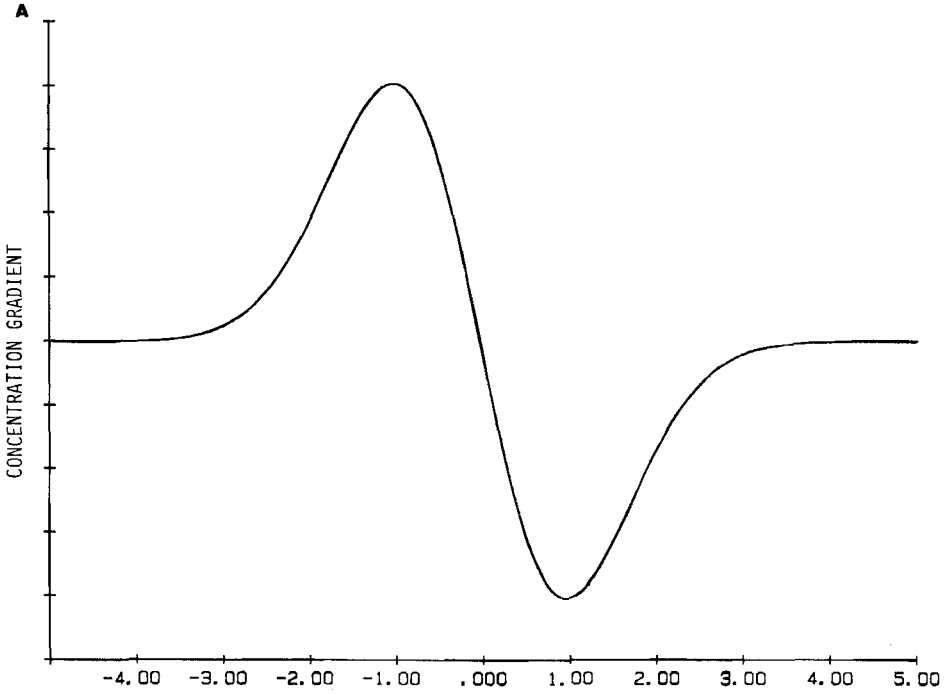

Figure 2

(a) Derivative of a Gaussian peak

(b) A Gaussian peak

(c) Integral of a Gaussian peak

(continued)

large). In other words, increased resolution results in enhanced sensitivity.

The signal produced by the concentration gradient detector has the shape of a Gaussian derivative rather than the Gaussian peak. Naturally integrating this signal will provide the Gaussian peak. But the derivative will offer more accurate information about the retention since it will correspond to the time when the chromatographic trace will cross the base

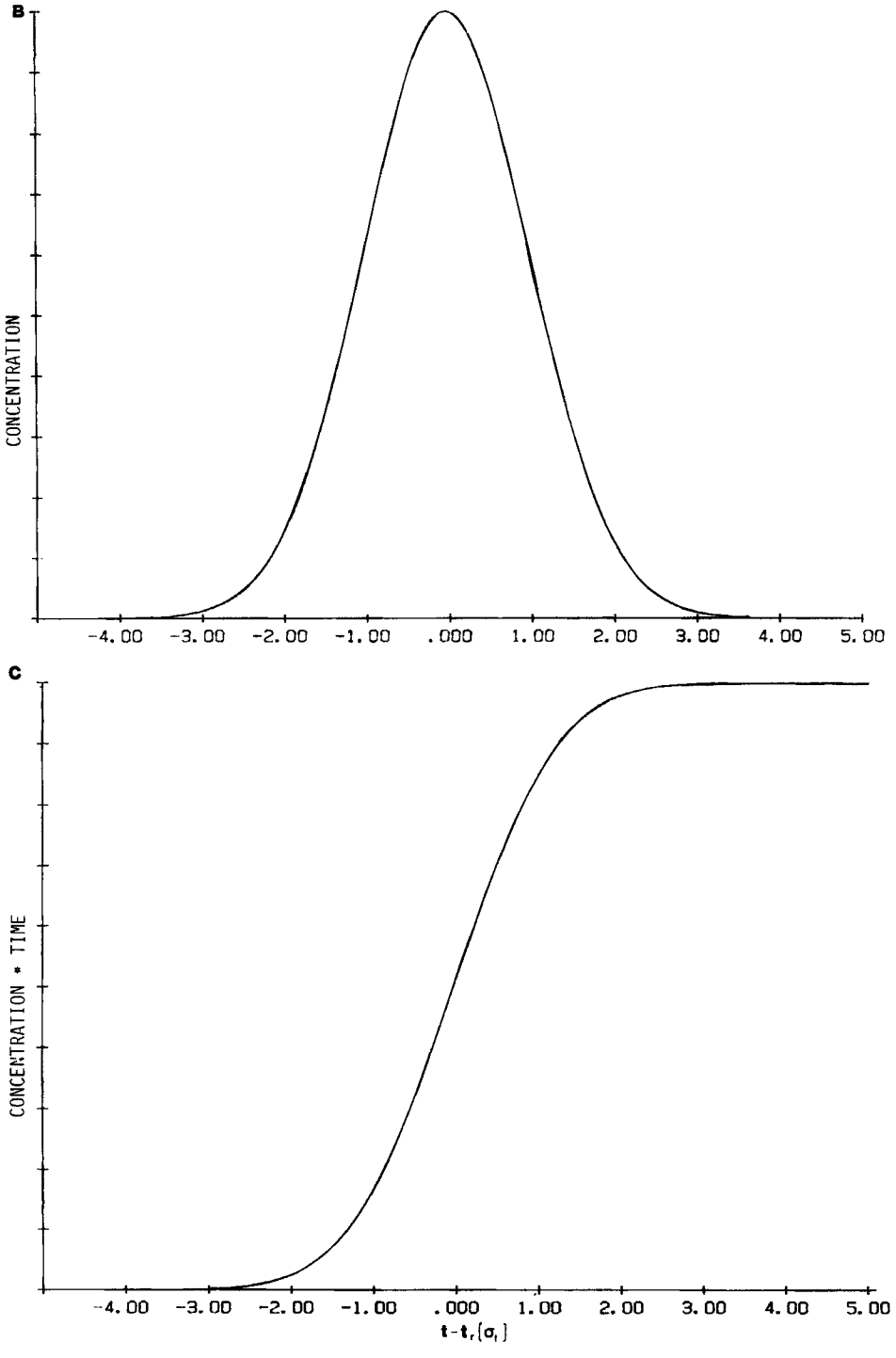

Figure 2 (continued)

line. Also the difference between the positive and negative optima can be measured instead of the single peak value. This increases the sensitivity of the method by the factor of 2. The difference between the optima in vertical "x" direction will correspond to $2\sigma_t$.

Any detection scheme to be useful in capillary separation techniques needs to be of low volume. Sharp peaks produced in the high efficiency method (Figure 1b) requires smaller sample volumes in order to correctly describe its shape compared to broad bands produced in low efficiency techniques (Figure 1a). Schlieren Optics, a concentration gradient method (4), appears to be an ideal choice since it requires only a single probing light beam to propagate through the detection volume in order to measure the concentration gradients present.

Physical Principle of Schlieren Optics

The optical effect associated with Schlieren Optics is well known and is observable on an everyday basis. It is called "a streak" in English or "schliere" in German. A typical example is when sugar is dissolved in water producing streaks.

Figure 3 outlines the physical process behind the Schlieren Optics method. It is well known that the presence of a solute in a medium changes the refractive index of the mixture (5). This is a physical principle of widely used refractive index detectors (6). Similarly, concentration gradients formed by the solute will generate corresponding refractive index gradients: $\frac{dn}{dx} = \frac{dn}{dc}\frac{dc}{dx}$. The wavefront I of the probing light beam which passes through the detection volume encounters the refractive index gradient associated with a solute at a given time t. The different sections of this wavefront experience different refractive indexes. Therefore various parts of the probe beam will propagate through the gradient with

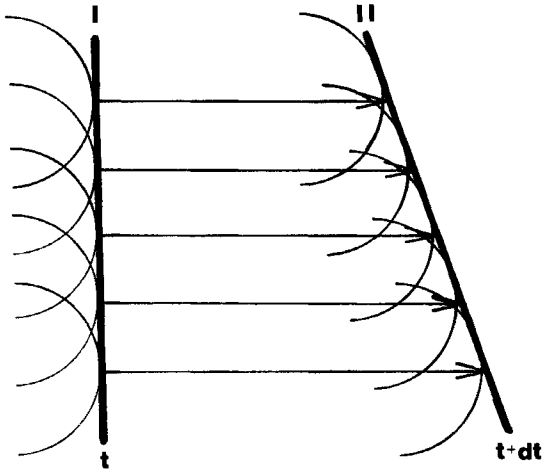

Figure 3

Physical principle of the Schlieren optics method

different velocities since the light velocity v is inversely proportional to the refractive index $v=\frac{c}{n}$, where c is the velocity of light in a vacuum (n=1). After the time increment dt, the parts of the beam which experience a lower refractive index will propagate further than other regions. This will result in a wavefront II formed at time $t+dt$, being tilted relative to the wavefront I. The net effect will be a deflection of the probe beam towards higher refractive indexes since light similar to other waves always propagates perpendicular to its wavefront.

The quantitative relationship between the deflection angle θ and the detector cell dimention d (Figure 4) can be derived from the Fermat Principle (the light path through the medium is such that the time necessary for its traversal is a minimum). Assuming a uniform refractive index gradient $\frac{dn}{dx}$, normal to the probe beam direction and small d and θ this relationship can be expressed simply as: $\theta=\frac{d}{n}\frac{dn}{dx}$, where n is a refractive index of the medium.

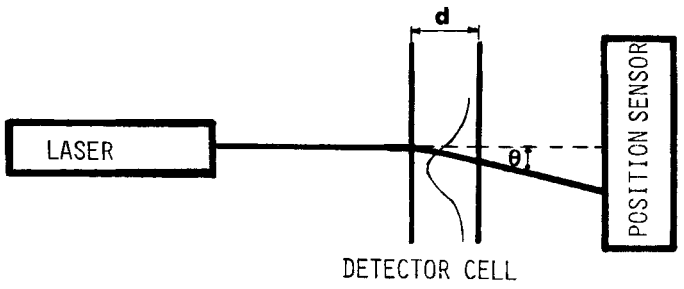

Figure 4

Modern experimental arrangement associated with Schlieren optics.

Experimental Arrangement

Schlieren optics techniques have been known for over a century (8) but have not been widely accepted as an analytical tool, due to their low sensitivity in low efficiency applications, difficulty in quantitative analysis and highly non-selective responce (7). Combining this detection scheme with capillary separation methods (high concentration gradients) will increase sensitivity. Additional improvement in sensitivity and precision in quantitative analysis can be achieved by replacing traditionally used incandescent light sources and photographic plates with new developments in optical and communication technologies such as lasers, with their collimated beams (Figure 4), NIR light sources (LED's and laser diodes) and silicon position sensors (9). These components also allow design of low cost integrated devices.

Properties of the Concentration Gradient Detector

The last limitation of this method associated with its universal response is in fact a significant asset in separation applications. The

sample is devided into its single components during the migration process in the capillary tube. The ideal detector should have a non-selective response since it is only then that all components of the sample mixture can be detected.

A number of interesting properites of this detector are associated with its response which is proportional to the refractive index gradient and not to the absolute value of the refractive index as in the refractive index detector. For example, this method exhibits high sensitivity towards "sharp" peaks associated with the sample components, but broad variations due to dissolved gas, temperature changes, and most importantly due to the gradient elution condition common in liquid chromatography, are not easily detected (10). The baseline slope present during gradient elution, limits the application of the refractive index detector. However, in concentration gradient detection this slope is "seen" as an offset from the "true baseline" by a constant value which is easily corrected to "zero" before integration and analysis. Also, the temperature variations which can be expected in capillary zone electrophoresis methods do not effect the response of the concentration gradient detector during analysis. Only "sharp" temperature changes, which are present just after the high voltage is turned on, are observed. Temperature gradients become undetectable for longer times due to large heat diffusivities (the heat diffusivities are about a hundred times larger than mass diffusivities in liquid media).

The deflection of the probing beam in the Schlieren method is proportional to the concentration gradient by over four orders of magnitude. The detection limits of the method for a given detector design are correctly expressed in the units of the concentration gradient: mol/(L·m). However, in separation applications it can be expressed in the concentration units since the concentration gradient at the inflection point of the Gaussian peak is proportional to its height (see Formula 5). The experimental detection limit is below 10^{-6}M for few nanoliter volume detectors.

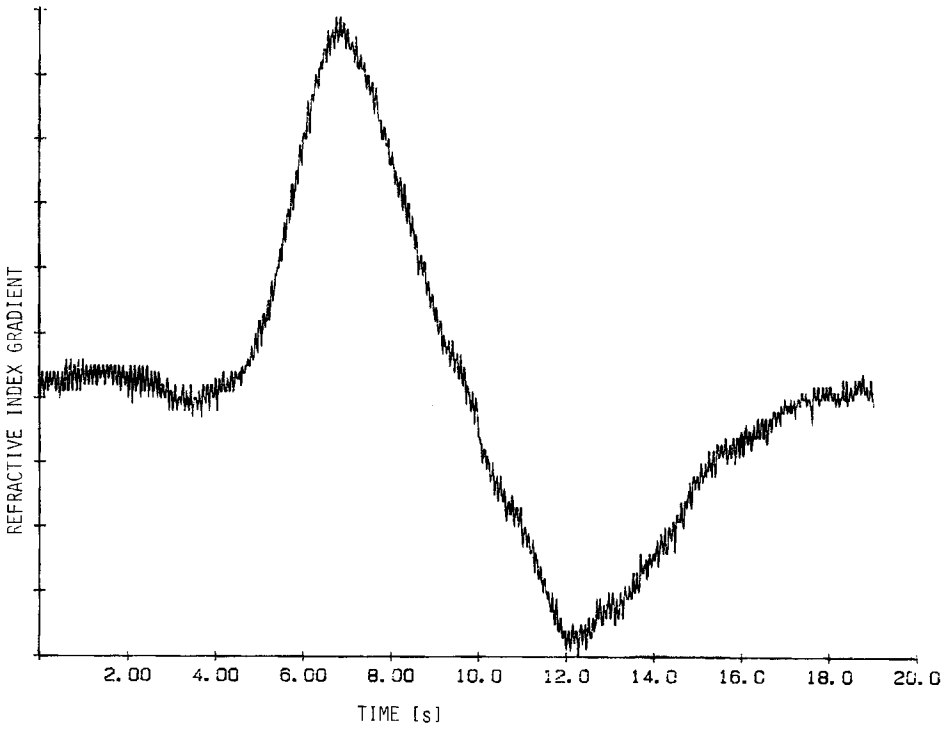

Figure 5

A concentration gradient signal produced by injecting 4nL of 10^{-4} M L-lysine solution (about 100 pg of L-lysine).

Therefore, the total amount of the sample injected can be a few picograms or less (10). Figure 5 shows the signal produced by injection of about 100 pg of lysine.

The simplicity of optical arrangements (a single probe beam passing through the detector volume) permits the design of robust, compact and inexpensive sensors. For example, a light emitting diode and silicon detector coupled to optical fibers and chrometographic cross connection can be used to build a detector for a total cost of below $100 (10).

Applications

The concentration gradient method can find application in any system where high concentration gradients are present. In analytical investigations these systems can involve electrochemistry (11), flow injection analysis (10), field flow fractionation and capillary separation methods (12). In the last category, a lot of interest has been recently focussed on capillary zone electrophoresis (3). This type of electrophoresis has significant advantages compared to the more established approaches. Efficient cooling of the capillary permits the use of high voltages which result in high resolution separation in a short period of time. Efficiency of these separations can reach in excess of a million theoretical plates and therefore generate narrow bands with high concentration gradient. Also, a recently reported, new differential migration technique based on electrophoretic migration and strength of interaction between neutral molecules and migrating species (13) significantly enhances the capability of the method. This allows not only a separation of neutral molecules (13), but also DL-isomers of amino acids (14). Below I will limit my discussion to the capillary zone electrophoresis application of this detection method.

Figure 6a shows the typical electropherogram obtained for three underivatized animo acids. The separation efficiency is only about 50.000 due to an experimental limitation put on high voltage (10kV). But a fraction of ng of the sample introduced can be easily detected. Figure 6b shows the integral of the electropherogram from Figure 6a. This procedure converts the refractive index gradient into the refractive index on the vertical scale. Now the refractive index drifts due to temperature variation that are easily noticed.

In addition to the commonly used elution mode of separation (a narrow plug produced at the beginning of the capillary), a frontal analysis is

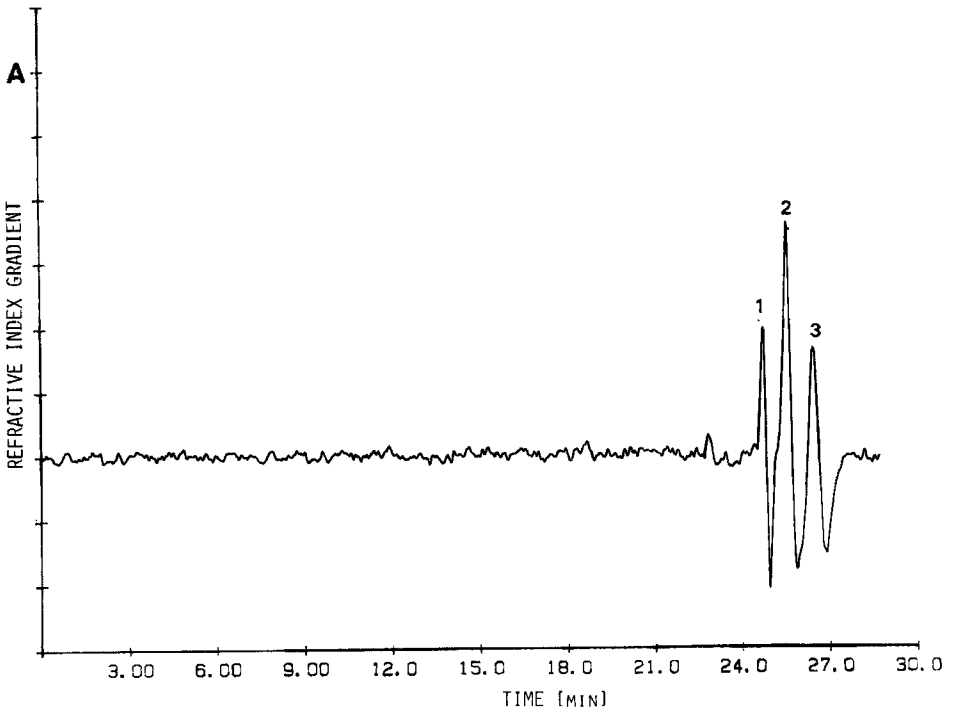

Figure 6

(a) Elution electropherogram of the three underivatized amino acid mixture (about 5 mM for all): glycine (1), L-alanine (2), and L-asparagine (3). Sample volume: about 10 nL, fused silica capillary 50 μm x 50 cm, 0.0125 M phosphate buffer pH=7, voltage = 10 kV.

(b) Integral of the electropherogram from Figure 6a.

(continued)

frequently applied (15). In this approach the sample mixture is introduced continuously for a relatively long period of time. Now, the separation between "fronts" of various components occurs. A final result resembles an integral of an elution chromatogram (Figure 2c). The frontal mode of separation has advantages in quantitation of asymetric peaks, in trace

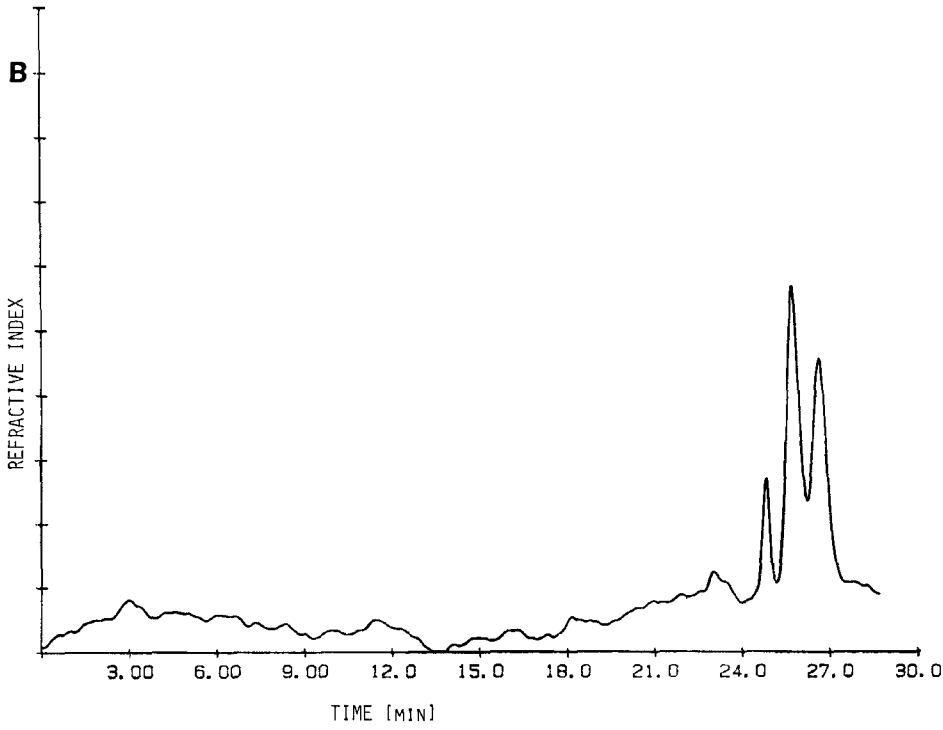

Figure 6 (continued)

analysis and in determination of physicochemical constants (16). Significant limitation of this method is the large volume of sample required. However, in capillary systems frontal analysis utilizes only small fraction of the sample required in elution packed column technology. For example, in the capillary zone electrophoresis method using 50 μm capillaries, the sample volume is only a few μL. The frontal analysis has an additional advantage in relation to capillary methods which require "sharp injection." It is much easier to produce a "sharp" front than a "sharp" and narrow plug (17).

In severely tailing cases (peptides separations (17)), the correct quantitative information in frontal analysis can be obtained only when a

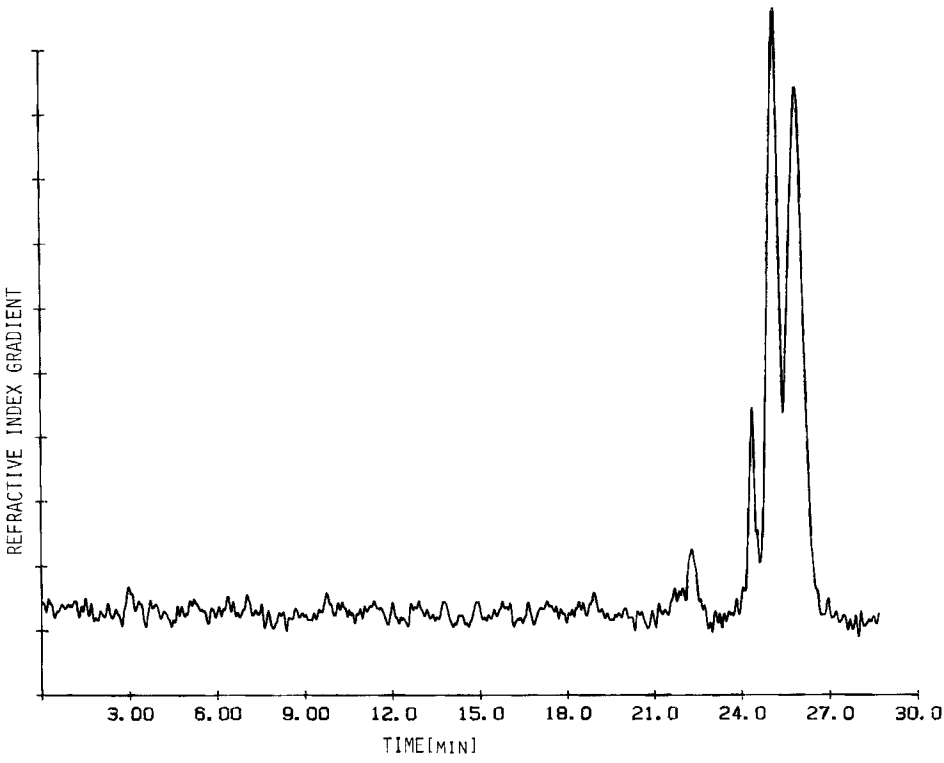

Figure 7

Frontal electropherogram of the three amino acid mixtures. Same conditions as in Figure 6. Only the first part is shown.

differenciating detector is used (18). This method shows the sample components as peaks, more familiar to chromatographers than integrals. To date, a differential response was obtained as a difference between signals from two identical detectors placed close to one another. This approach cannot be accepted in capillary methods due to requirements put on the detection volume which have been discussed before. The concentration gradient detector discussed in this paper is an ideal choice for this application since it produces output which is a derivative of that expected

by a concentration method. Figure 7 shows a typical frontal electropherogram detected by the Schlieren optics sensor.

Conclusion

The concentration gradient sensor based on Schlieren Optics can be successfully applied to detect sample components separated by high efficiency capillary methods. This technique has good sensitivity considering its universal response. An additional improvement in sensitivity is expected for future more efficient methods. The simple optical arrangement of this method allows robust and inexpensive design of the detector. This sensor can be built entirely from sillicon components which enable an integration of the device. Finally, the selective absorption mode of concentration gradient sensor recently introduced can be applied to detect the sample components which have not been resolved in the separation process (12). This method can also characterize the sample by its absorptivity coefficient at a given wavelength, since both absorption and concentration information can be provided simultaneously.

Acknowledgement

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