Journal of Chromatography, 332 (1985) 195–201
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 610

THE ABBÉ REFRACTOMETER AS A LIQUID CHROMATOGRAPHIC DETECTOR USING AN IMAGE SENSOR FOR BOUNDARY LOCATION

SATORU EGASHIRA
Faculty of General Education, Ehime University, Bunkyocho, Matsuyama, 790 (Japan)
(First received March 14th, 1985; revised manuscript received April 11th, 1985)

SUMMARY

A novel refractive index detector for liquid chromatography using a flow-through Abbé refractometer cell and image sensor is described. The Abbé refractometer has some advantages such as independence of the intensity of the light, a wide dynamic range and a small cell volume (less than 3.5 μl).

A linear diode array image sensor was used to determine the critical angle of refraction. As the output of the sensor is essentially discrete, interpolation to locate accurately the position of the boundary is required. The least-squares method was used in real time for this purpose. A change in the refractive index of 10^{-5} units was detected with this apparatus.

INTRODUCTION

Fresnel and deflection-type refractive index (RI) detectors are widely used in liquid chromatography and an interference type refractometer has also been reported recently[1]. This paper describes a trial of a new type of RI detector based on the Abbé refractometer.

The Abbé refractometer is a total reflection type of refractometer that gives RI by measurement of the critical angle of refraction of light through the interface between the liquid sample and a glass prism. The refractometer has the advantage of being unaffected by the intensity of the light source, giving a wide dynamic range and requiring a small-bore cell (less than 3.5 μl). However, it is difficult to determine the critical angle accurately and to convert it into an electrical signal by using analogue sensors. Accordingly, a linear diode array image sensor was used for this purpose.

PRINCIPLES

Fig. 1 shows the optical system used in the apparatus. Light from the source is scattered by a rough-surface prism and is refracted at the interface between the liquid and a smooth-surface prism. The critical angle of refraction, θ, is given by

$$\theta = \sin^{-1} (n/n_0) \tag{1}$$

0021-9673/85/$03.30

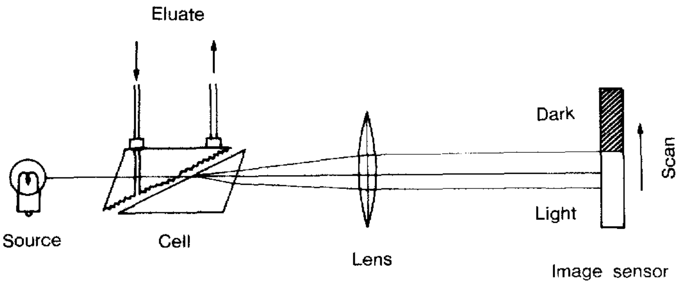

Fig. 1. Optical system.

where n and n_0 are the refractive index of the liquid and the prism, respectively.

The refracted light is transmitted into the smooth-surface prism and focused on the window of the image sensor with the aid of a lens. The critical angle is determined by locating the bondary of the light and dark areas on the window.

As the boundary location is given in terms of the segment number of the sensor, the physical resolution is limited by the dimensions and the apertures of the diode segments. Interpolation by a least-squares method was used in real time to locate the boundary accurately.

The profile of the output signal from the sensor around the boundary is shown schematically in Fig. 2. The curve of the profile should be represented by the Fresnel equation, and the boundary position of the light and dark areas should theoretically be detected as the intersection of the curve and the baseline at x_0. However, experimentally, less noisy outputs were obtained with an appropriate threshold, y'_0, giving the boundary position at x'_0. The threshold near the inflection point of the curve was found to give the minimum noise.

The linear part of the curve around its inflection point was approximated with the following first-order equation:

$$y = ax + b \tag{2}$$

where y is the height of the curve (arbitrary units) and x is the segment number of

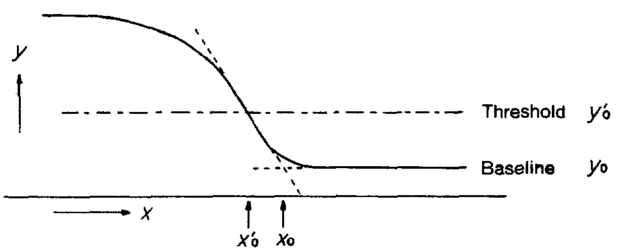

Fig. 2. Output signal of image sensor around the critical angle.

the sensor. The parameters a and b were obtained with the least-squares method as follows.

The normal equations obtained from eqn. 2 are

$$\left.\begin{aligned} A_1a + B_1b &= C_1 \\ A_2a + B_2b &= C_2 \end{aligned}\right\} \quad (3)$$

where

$$A_1 = 1/6\ N(N+1)(2N+1) \quad (4)$$

$$A_2 = B_1 = 1/2\ N(N+1) \quad (5)$$

$$B_2 = N \quad (6)$$

$$C_1 = \sum_{i=1}^{N} x_i y_i \quad (7)$$

$$C_2 = \sum_{i=1}^{N} y_i \quad (8)$$

and N is the number of samples used in the least-squares treatment.

The parameters a and b are obtained by solving eqn. 3 as

$$a = (B_2C_1 - B_1C_2)/(A_1B_2 - A_2B_1)$$

and

$$b = (A_1C_2 - A_2C_1)/(A_1B_2 - A_2B_1)$$

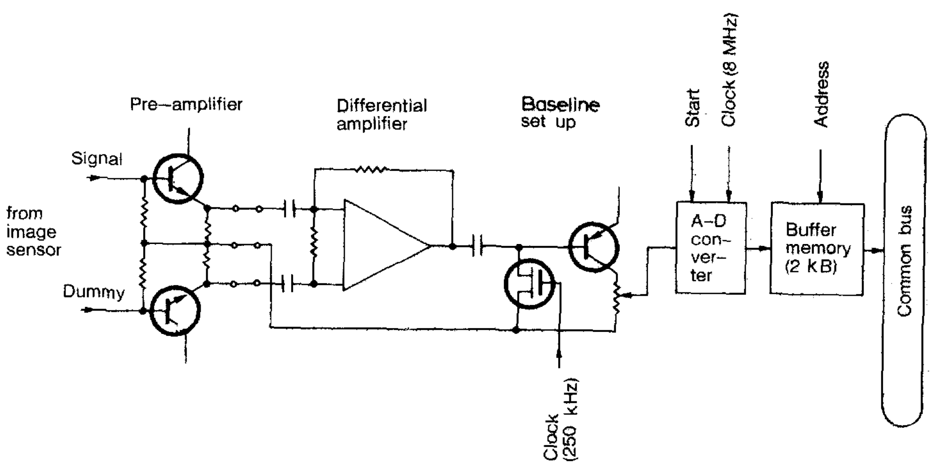

Fig. 3. Electrical circuits around the image sensor.

Applying the parameters to eqn. 2, the x coordinate (segment number) of the intersect, x_0, is given as

$$x_0 = \frac{y_0(A_1B_2 - A_2B_1) - (C_2A_1 - C_1A_2)}{C_1B_2 - C_2B_1} \tag{9}$$

where y_0 is height of the baseline. When we use the threshold instead of the baseline, x_0 and y_0 are replaced by x_0' and y_0'.

The sensitivity of diode segments vary in the range $\pm 10\%$ and dark output varies for each segments. Corrections for these variations are carried out before the least-squares treatments.

APPARATUS

The optical equipment of the Model 678 automatic urine analyser (Hitachi, Tokyo, Japan) is used in the optical part with a minor modification (Fig. 1). Eluate from the chromatographic column flows in the space between the smooth- and the rough-surface prisms.

A linear charge-coupled device image sensor (LZ-2020, 2048 segments; Sharp, Osaka, Japan) was used as the optical sensor. Electrical circuits around the image sensor are shown in Fig. 3.

The output of the sensor is amplified by a pre-amplifier and mixed with the dummy output by a differential amplifier. Then, after setting up the electrical baseline, the signal is sent to 8-bit analogue-to-digital (A–D) converter (TDC1002J, TRW LSI Products, Redondo Beach, CA, U.S.A.). The sampling interval is 0.1 sec and the

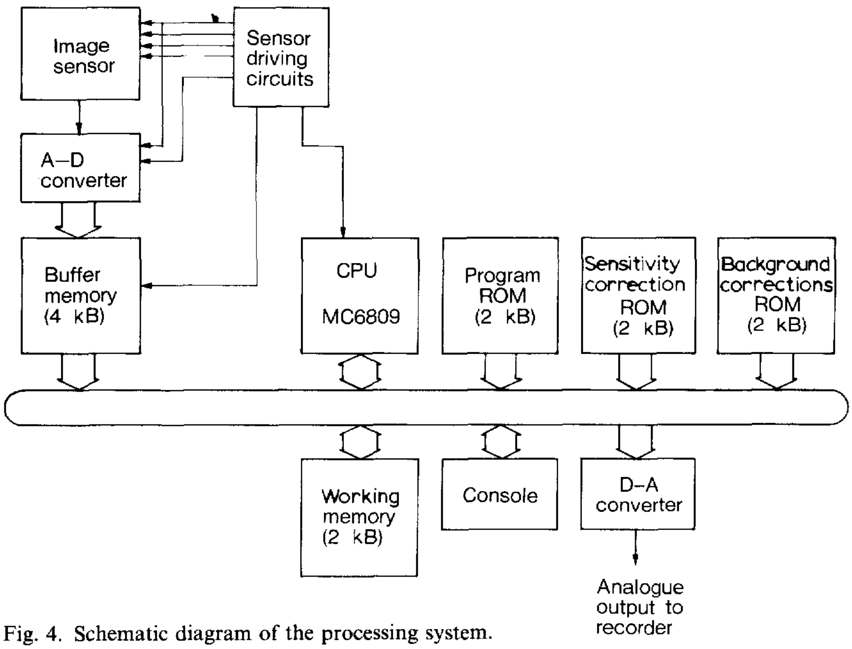

Fig. 4. Schematic diagram of the processing system.

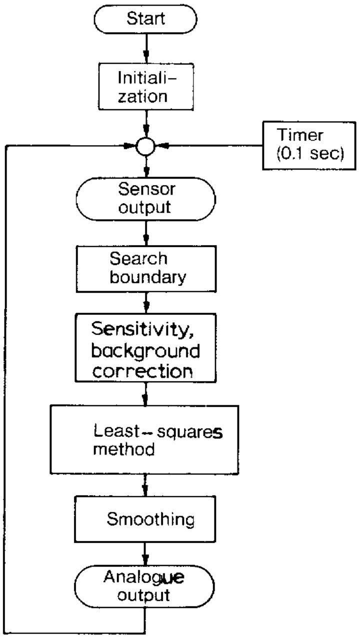

Fig. 5. Flow chart of processing.

conversion time is 1 μsec with an 8-MHz clock. The digital data are stored on a 2 kbyte (kB) buffer memory to permit accessing by the central processor.

Fig. 4 is a schematic diagram of the whole system. As the central processor (CPU), an MC6809 microprocessor (Motorola Semiconductor Products, U.S.A.) is used. The digital outputs of the A–D converter, stored on the buffer memory, are

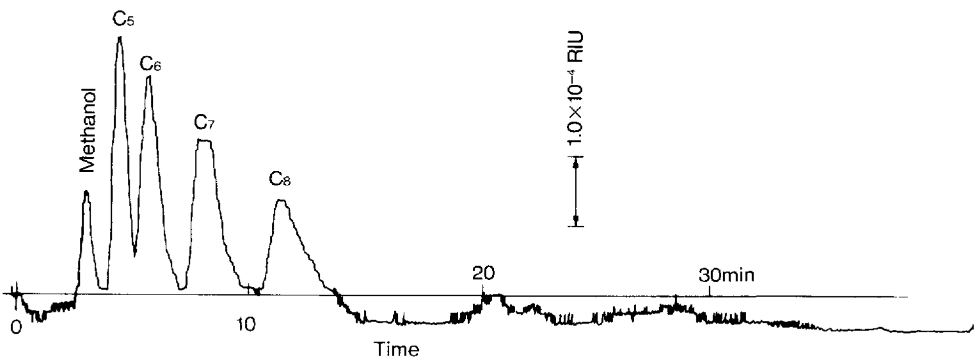

Fig. 6. Separation of cyclic ketones on a column of Develosil ODS-5 (250 × 2.6 mm I.D.) with 50% (v/v) methanol. Flow-rate: 0.5 ml/min.

read out by the CPU. The approximate position of the boundary is detected by a preliminary search. Then, after correction for the dark output and sensitivity of the sensor segments, the numerical treatment by the least-squares method is performed.

Each 2 kB of read-only memory (ROM) was provided to store the factors for the correction of the sensitivity and the dark output. Another 2 kB ROM and 2 kB random access memory were used as program and working memory, respectively, for the data processing.

The results of the numerical treatment are converted into an analogue signal again, and sent to a pen recorder to give the chromatographic elution curve. Smoothing by the moving average method is used prior to A–D conversion if necessary. A processing flow chart is shown in Fig. 5.

The sensor driving circuits supply several kinds of clock pulses for the image sensor, the CPU and the A–D converter, and address signals for buffer memory accessing.

RESULTS AND DISCUSSION

Cyclic ketones (1 mg of each) and cyclic terpenes (1 mg of each) were separated on Develosil ODS-5 (Nomura Chemical Co., Seto, Japan), as shown in Figs. 6 and 7, respectively.

Eqn. 1 represents a non-linear response of the detector. However, it was found experimentally that the system gives almost a linear output over the range n = 1.33–1.40. It took 10 msec for scanning of the image-sensor and about 200 msec for numerical processing.

One eighth of the aperture of the diode segments of the sensor could be read out by the above treatment. This corresponds to 10^{-5} refractive index units (RIU).

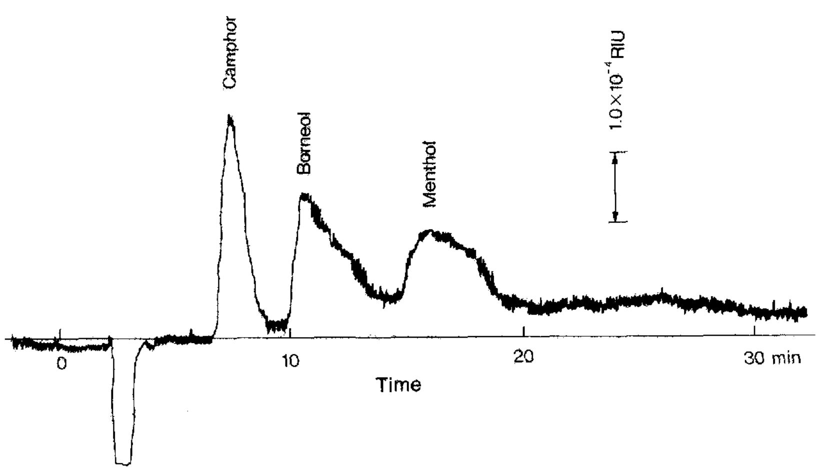

Fig. 7. Separation of cyclic terpenes. Solvent: 70% (v/v) methanol. Other conditions as in Fig. 6.

The noise level was nearly equal to the limit of read out, *i.e.*, 10^{-5} RIU. It was found experimentally that the noise level was lowest when the threshold, y'_0, was set at the inflection point of the sensor output *vs.* position (Fig. 2).

Smoothing by the moving average method also reduced the apparent noise. Thus Figs. 6 and 7 used both the moving average method and a threshold set at the inflection point.

It has been shown here that the sensitivity of the flow-through Abbé RI detector could be improved by numerical treatment. The sensitivity, determined by the 10^{-5} RIU noise level, does not show the inherent limits of the detector. The effective sensitivity of the system could be improved by reducing the noise with more sophisticated hardware or software.

ACKNOWLEDGEMENT

I thank Dr. Kyoichi Ozawa (Hitachi, Tokyo, Japan) for providing the optical assembly of the apparatus.

REFERENCE

1 M. N. Munk, in T. M. Vickrey (Editor), *Liquid Chromatography Detectors*, Marcel Dekker, New York, 1983, p. 165.