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RAPID-SCANNING SPECTROPHOTOMETRY AS A NEW DETECTION SYSTEM IN CHROMATOGRAPHY

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SUMMARY

Rapid-scanning spectrophotometry, applied as a new detection system in gas or liquid chromatography, enables absorption spectra of the components at the exit of the column to be displayed continuously and instantaneously, and chromatographic curves to be recorded simultaneously at several fixed wavelengths. This procedure permits the identification of the separated compounds during the actual process of separation. The advantages of the additional recording of ratios of absorbances at two wavelengths are demonstrated for the case of partial separation. The method is exemplified by the separation of mixtures of fluorene, carbazole and acenaphthene.

INTRODUCTION

Numerous physico-chemical methods have been applied to the detection of the separated components at the exit of a chromatographic column. Their usefulness varies with the dependence of the measured signal on the concentration of the substance to be determined, and with the sensitivity, accuracy and scope of the method. Detectors based on the measurement of absorbance at a given wavelength, isolated with filters or with a monochromator, have an over-all high sensitivity, usually reaching 10^{-2} absorbance units on the full scale; with respect to particular substances, this sensitivity varies with the spectrum. The techniques used in this detection and the associated problems have been discussed by Veening[1].

Rapid-scanning spectrophotometry[2], if applied as a detection system, offers new possibilities for the simultaneous identification of the spectra and for the quantitative determination of the analyzed substances. This method of detection permits the instantaneous display and evaluation of the spectra of the components and, owing to the multi-channel character of the method, the simultaneous recording of several chromatographic curves.

The method can be used in any type of chromatography; we applied it at first to column liquid chromatography.

DETECTION METHOD

The rapid-scanning spectrophotometer (Spectromonitor) is used as the detection unit and operates on the following principle[2,3].

The spectrum is periodically scanned, at up to 100 spectra per second, by a deflection generator connected to the electronically controlled monochromator (Fig. 1), which consists of a cathode-ray tube (CRT), the phosphor screen of which is situated in the focal plane of the monochromator. The luminous spot on the screen of the CRT is a movable light source of high luminance.

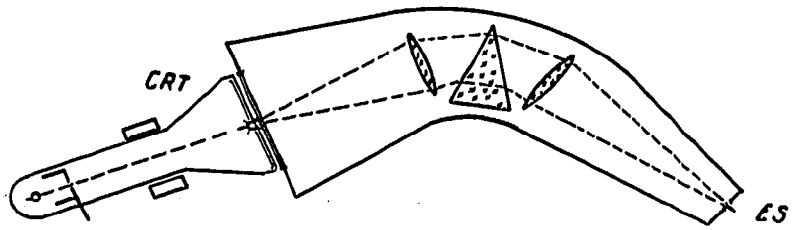

Fig. 1. Scheme of the simplest electronically controlled monochromator. CRT, cathode-ray tube with a luminous spot on its phosphor screen as the movable light source. In the exit slit, ES, of the monochromator is focused light of wavelength determined by the instantaneous position of the luminous spot on the screen.

The luminous spot can easily be shifted accross the screen by magnetic deflection of the electron beam; to each position of the luminous spot along its path corresponds a particular wavelength of the light focused on the exit slit of the electronically controlled monochromator. The deflection of the electron beam controls the scanning of the spectrum, whereas the intensity of the light source is easily changed by controlling the beam current with the cathode potential of the CRT. The monochromator used was a prism monochromator in earlier experiments and a grating monochromator in later work.

The Spectromonitor operates in a double-beam system with two matched photomultipliers. The spectra are displayed in a rectangular co-ordinate system, transmittance *versus* wavelength, on a television-type image tube.

The spectral range for the CRTs with a newly developed phosphors and quartz front face is 230–750 nm, the spectral resolution is $\pm$ 2 nm and the photometric error is within $\pm$ 1 % transmittance.

This spectrophotometer can be equipped with a multi-channel recorder in order to record simultaneously several curves of the transmittance (T), absorbance (A) or of some functions of these variables.

RATIO RECORDING METHOD

Sometimes the measurement of the ratio of absorbances for two different wavelengths may supply additional information on the complexity of a single chromatographic peak.

The ratio for two substances (1 and 2) partially separated on the column can be represented as

$$\frac{A(\lambda_1,V)}{A(\lambda_2,V)} = \frac{A'(\lambda_1)\,g'(V) + A''(\lambda_1)\,g''(V)}{A'(\lambda_2)\,g'(V) + A''(\lambda_2)\,g''(V)}$$

where g represents a Gaussian-type function, *e.g.*,

$$g' = \exp\left[-\frac{(V - V')^2}{2\sigma'^2}\right]$$

and $A'(\lambda_1)$ and $A'(\lambda_2)$ are absorbances for substance 1 at its maximal concentration in a volume V'. For simplicity, we assume that $\sigma' = \sigma''$ and approximate the ratio through a Maclaurin expansion:

$$\frac{A(\lambda_1,V)}{A(\lambda_2,V)} = C_1 + C_2\,(V'' - V')\,[A'(\lambda_1)\,A''(\lambda_2) - A'(\lambda_2)\,A''(\lambda_1)] \cdot V + \ldots$$

where C_1 and C_2 are constants.

The absorbance ratio as a function of the eluate volume, V, is constant only for two substances with identical spectra, or at least with the same absorbances for the two wavelengths,

$$A'(\lambda_1)\,A''(\lambda_2) - A'(\lambda_2)\,A''(\lambda_1) = 0.$$

The chromatographic curve $A(\lambda_i, V)$ in this case will have no Gaussian character.

In the case when $V'' - V' = 0$, constancy of the absorbance ratio corresponds either to a single substance or to more than one substance within a chromatographic peak that have not been separated by the process.

In other cases, for two investigated substances the ratio of absorbances will be a linear function of V as long as both approximations hold: that of the first two members of the Maclaurin series and that of equality of the σ parameters.

The ratio $A(\lambda_1, V)/A(\lambda_2, V)$ gives only qualitative information on the system, but this information is usually not obtained with single-channel detectors.

MATERIALS AND PROCEDURES

All reagents and solvents were of analytical-reagent grade and their physical constants agreed with those given in the literature. 4-Methylpyridine (4-MePy) contained less than 0.3% of 3-MePy.

Chromatographic experiments were carried out at 20° using the elution technique.

The column, 3 cm long and 0.5 cm I.D., was filled with 0.3 g of clathrate phase, $[Ni(4\text{-MePy})_4(NCS)_2]\cdot 0.7(4\text{-MePy})$, as described earlier[4].

The composition and structure of the sorbent depends on the mobile phase remaining in equilibrium with it. The mobile phase consisted of a solution of NH_4SCN and 4-MePy in methanol–water or ethanol–water mixtures.

RESULTS

The separation of mixtures of fluorene, acenaphthene andcarbazole on a clath-

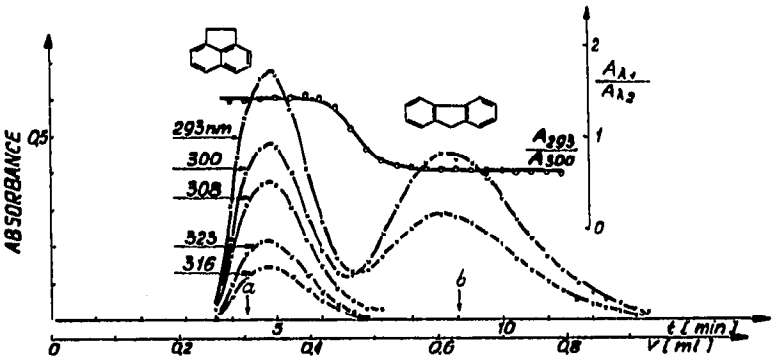

Fig. 2. Chromatographic elution curves of a mixture of fluorene and acenaphthene recorded by the use of the rapid-scanning spectrophotometer. ×—×, Curves measured as absorbances at constant wavelength); ○—○, ratio $A_{293\ nm}/A_{300\ nm}$. The column was filled with 300 mg of the clathrate $[Ni(4\text{-}MePy)_4(NCS)_2]\cdot 0.7(4\text{-}MePy)$. The mobile phase was 0.4 *M* NH_4SCN plus 0.43 *M* 4-MePy in a 60% (v/v) solution of methanol in water. The meaning of the vertical arrows *a* and *b* is explained in Fig. 4.

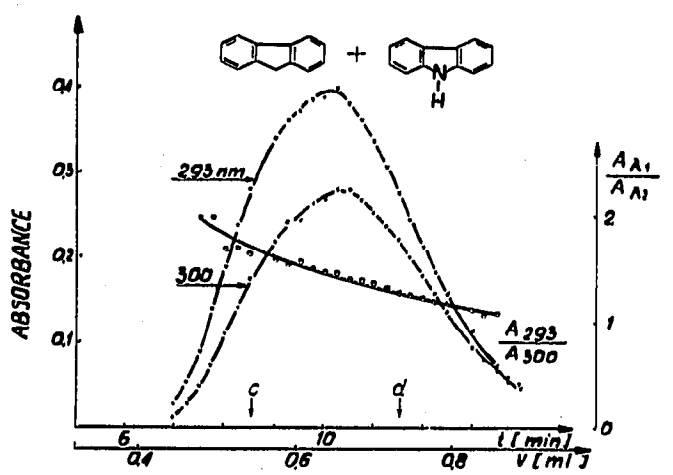

Fig. 3. Chromatographic elution curves of a mixture of fluorene and carbazole recorded with the rapid-scanning spectrophotometer. The column and conditions were the same as in Fig. 2 for the mixture of fluorene and acenaphthene. The meaning of the vertical arrows *c* and *d* is explained in Fig. 4.

rate column[5] has been carried out as an example of the new spectrophotometric method of detection in liquid chromatography.

The separation of a mixture of fluorene and acenaphthene on the clathrate column is shown in Fig. 2.

The changes in absorbance recorded at several wavelengths as well as the ratio $A_{293\ nm}/A_{300\ nm}$ prove a good separation of both components.

With a mixture of fluorene and carbazole (Fig. 3), the chromatographic curves measured as absorbances at several wavelengths show only a single peak. The ratio of absorbances is not constant, which indicates the incomplete separation of the two components. This is an example of a system in which the separation could not be detected by recording the usual chromatographic curves, but the changes in the ratio

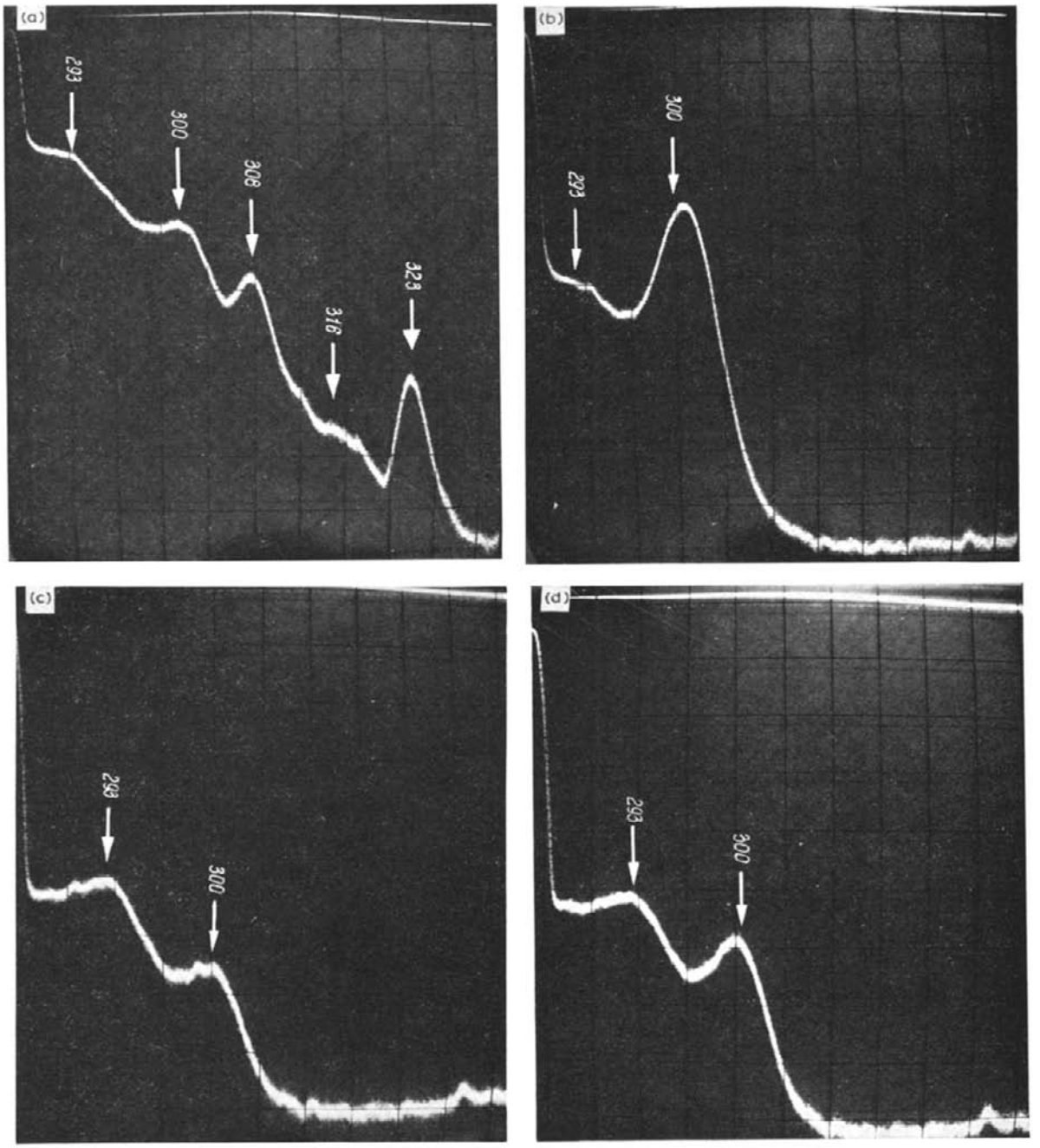

Fig. 4. Spectral transmittance curves in the near UV region, as photographed from the Spectromonitor screen, at the moments indicated by the vertical arrows *a* and *b* in Fig. 2 and *c* and *d* in Fig. 3. 0% transmittance at the top and 100% at the bottom of the screen.

of the absorbances at two wavelengths clearly demonstrate the presence of the two components within the single chromatographic peak.

The method of rapid-scanning spectrophotometry, with its continuous display of the spectra, applied to chromatographic detection (see Fig. 4), together with the ratio recording, permits the instantaneous identification of the separated fractions, as well as the detection of components that are usually described as unresolved.

This special spectro-chromatographic equipment is now being applied to different chromatographic separation procedures.

SCOPE AND LIMITATIONS OF THE METHOD

Rapid-scanning spectrophotometry is applicable to most chromatographic techniques, including gas chromatography. The scope of the method is limited to classes of compounds that absorb in the UV and visible regions, which excludes, for example, alkanes and alkenes. All aromatic compounds, most heterocyclic, carbonyl compounds, etc., are suitable for this method of detection.

Another limitation lies in the sensitivity and accuracy of the method, which also depend on the spectra.

For a typical molar absorptivity of $\varepsilon_{max.} \approx 2 \times 10^4\ cm^{-1}\ M^{-1}$ and an optical path length in the detection cell of 0.1 cm, the absorption measurable at $A \approx 0.1$ corresponds to a concentration $C \approx 5 \times 10^{-5}\ M$ in a volume of 50 μl, *i.e.*, to $2 \cdot 10^{-9}$ mole of such a component as the practical limit of detectability in the present equipment, at a signal-to-noise ratio of 10:1.

REFERENCES

1 H. Veening, *J. Chem. Educ.*, 47 (1970) A549, A675 and A749.
2 Z. R. Grabowski and J. Koszewski, *Acta IMEKO 1967*, Vol. 3, Hungarian Scientific Society for Measurements and Automation, Budapest, 1968, p. 125.
3 Z. R. Grabowski, J. Koszewski and J. Jasny, *Chem. Anal. (Warsaw)*, 17 (1972) 717.
4 W. Kemula, D. Sybilska and H. Kwiecińska, *Rocz. Chem.*, 39 (1965) 1101.
5 W. Kemula and D. Sybilska, *Nature (London)*, 185 (1960) 237.