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Note

The performance of the UVM-4 UV analyser in isotachophoresis

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In UV detection a single beam of light of a given wavelength is usually employed. The selection of the wavelength is limited by the relatively small number of sufficiently intense spectral lines produced by a discharge lamp; the wavelength 254 nm is most often used[1,2]. Reijenga *et al.*[3] used dual-wavelength UV-absorption detection with two mutually perpendicular beams with wavelengths of 254 and 280 nm, and later described a device for simultaneous UV-fluorescence and UV-absorption detection[4].

It is often advantageous to be able to choose a suitable wavelength for the detection. Hanibalová[5] and Vacík and Everaerts[6] have described a modified spectrophotometer that enables measurement of the absorption spectra of individual zones or detection at a single, selected wavelength. They used the relatively large and expensive Specord UV–Vis spectrophotometer (Carl Zeiss, Jena, G.D.R.).

The use of the relatively cheap UVM-4 UV-analyzer (Development Section of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia), designed for liquid chromatographic detection, its modification and combination with a common isotachophoretic apparatus has produced a UV detector with a wavelength variable from 200 to 400 nm.

EXPERIMENTAL

The source of UV radiation is a low-voltage deuterium discharge lamp with a heated cathode ($D_2E/1$ type; Narva, Berlin, G.D.R.) emitting continuous radiation in the range 185–450 nm. A Czerny-Turner monochromator comprised a diffraction-reflection grating, 600 lines per mm, made by the holographic method in the Institute for Radiotechnology and Electronics (Czechoslovak Academy of Sciences, Prague). The effective spectral width of the radiation produced is 5 nm. The UV radiation sensor is a 1P28 Sb-Cs photomultiplier (R.C.A., Harrison, NJ, U.S.A.) the output current of which is amplified by a WSH 220 FET operational amplifier. The detector sensitivity can be varied continuously by controlling the voltage applied to the photomultiplier dynodes.

The quartz flow-through cell with a detection volume of 10 μl, originally designed for liquid chromatography, has been adapted for detection through the wall of the separating capillary. By means of a circular slit 0.4 mm wide, a detection volume of *ca.* 0.043 μl is defined on the capillary.

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RESULTS AND DISCUSSION

The operating region of the detector is characterized by the relative spectral sensitivity curve (Fig. 1). This dependence represents the spectral distribution of the output signal relative to the maximum value, when the detector space in a capillary is filled with redistilled water. The shape of this characteristic depends primarily on the spectral characteristics of the individual components of the detector, *i.e.*, the distribution of the discharge lamp energy, the transparency of the monochromator, capillary and the solvent used and the photomultiplier sensitivity.

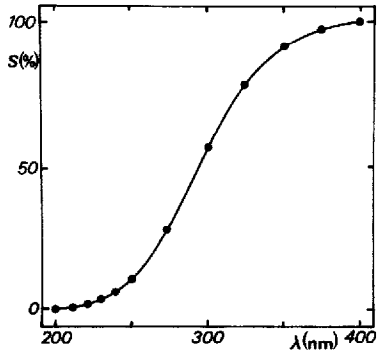
Separation capillaries made of two different materials, PTFE and FEP (a tetrafluoroethylene–hexafluoropropylene copolymer manufactured by Kablo, Vrchlabi, Czechoslovakia), were tested. With the FEP capillary, the operating range of the detector can be extended toward shorter wavelengths compared with the PTFE capillary. Fig. 2 depicts the transmittance curves for the walls of both capillaries, PTFE (0.45 mm I.D., 0.80 mm O.D.) and FEP (0.40 and 0.85 mm, respectively), measured on a Unicam SP 700 spectrophotometer.

In the detector operating range from 200 to *ca.* 250 nm, where the capillary transmittance is very small, the discharge lamp noise is pronounced and hinders exact evaluation of the detector response. Its magnitude increases from 1 to 5% of the outpout signal with decreasing wavelength.

It is possible to select a wavelength in the operating range at which the test substance exhibits strong absorption and thus the detection is sufficiently sensitive. Hence it is also possible to obtain a response for substances that absorb within the region of 200–220 nm, and which would be undetectable at 254 nm. An example of such substances is a mixture of 5 m*M* glycylglycylglycine, 5 m*M* glycylglycine and 5 m*M* glycine. This mixture was separated using operating system 1 given in Table I and Fig. 3.

The detector response at 220 nm corresponds to the n–π^* transition of the chromophore of the peptidic bond; the response at 254 nm is virtually zero.

In the detection of several substances with different absorption characteristics, an optimum wavelength can be used at which the responses of the neighbouring



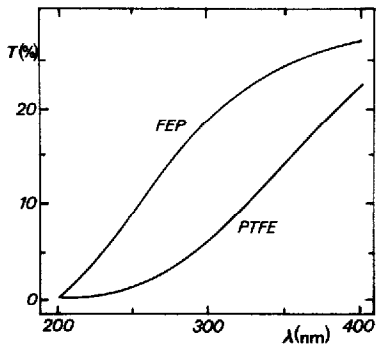


Fig. 1. Dependence of relative spectral sensitivity, S_r, on wavelength, λ, of the radiation used.

Fig. 2. Dependence of transmittance, T, of the walls of FEP and PTFE capillaries on the wavelength, λ, of the radiation used.

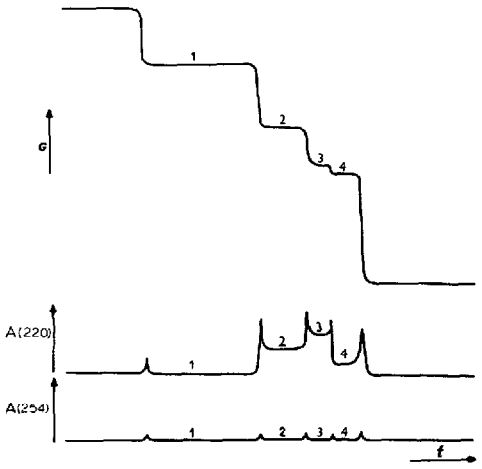


Fig. 3. Isotachopherogram of a 1-μl sample of a mixture of glycine (5 mM), glycylglycine (5 mM) and glycylglycylglycine (5 mM). G = Conductivity detector response, $A(220)$ = UV detector response at λ = 220 nm and $A(254)$ = UV detector response at λ = 254 nm. Zones: 1 = HCO_3^-; 2 = glycylglycylglycine; 3 = glycylglycine; 4 = glycine.

TABLE I

OPERATING SYSTEM 1[7]

Leading electrolyte: 0.05 M ammediol, 0.1% polyvinyl alcohol and hydrochloric acid, pH = 8.97
Terminating electrolyte: 0.01 M β-alanine and barium hydroxide, pH = 10.9
Migration current: 40 μA

zones in the isotachophorogram can well be differentiated. An example is a mixture of naphthalenedi- and -trisulphonates. The separation of a mixture of 2 μl 5 mM sodium 1,5-naphthalenedisulphonate and 10 mM sodium 1,3,6-naphthalenetrisulphonate, using operating system 2 given in Table II, is illustrated in Fig. 4. Fig. 4 demonstrates the effect of the various wavelengths used on the magnitude of the

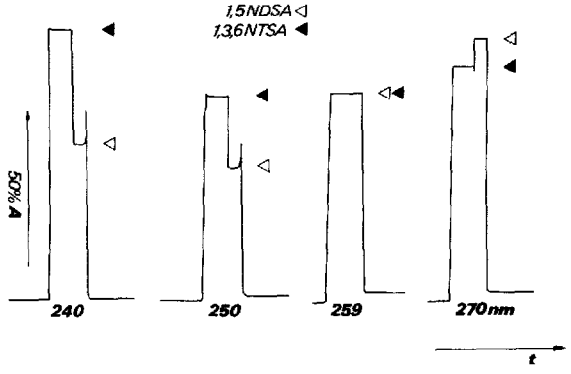


Fig. 4. UV detector response after the separation of a mixture of and naphthalenesulphonates at various wavelengths of the radiation used. △, 1,5-Naphthalenedisulphonate; ▲, 1,3,6-naphthalenetrisulphonate.

TABLE II

OPERATING SYSTEM 2[1]

Leading electrolyte: 0.01 *M* L-histidine, 0.01 *M* L-histidine monochloride
Terminating electrolyte: 0.01 *M* glutamic acid
Migration current: 100 μA

detector response. It is seen that, *e.g.*, the wavelength of 259 nm is quite unsuitable as the two substances yield the same response at this value.

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