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UV DETECTION AT 206 nm IN ISOTACHOPHORESIS

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SUMMARY

The theoretical and practical possibilities and limitations of UV detection at 206 nm in isotachophoresis are discussed with respect to the operational systems, capillary material, UV light source and detection.

INTRODUCTION

For UV detection in isotachophoresis, a high-frequency (HF) plasma source is usually applied[1,2] and mercury or iodine is mostly used, which means that detection is possible at discrete wavelengths only. The 254 nm line of mercury is most commonly chosen. For protein work the 280 nm line of iodine is a practical choice. A UV-sensitive photodiode in conjunction with a filter is then used as a detector. The 206 nm line of the iodine source is a useful additional possibility, provided that the capillary material and operational systems used meet certain requirements.

EXPERIMENTAL

UV light source

Laboratory-made HF oscillation plasma sources[2] were used. Either mercury or iodine was chosen. The wavelengths that can be used in the UV range of interest are listed in Table I.

Detector

A UV-sensitive photodiode (Type 330-02, Hamamatsu, Japan) was used in conjunction with commercially available UV filters (LKB, Bromma, Sweden) with

TABLE I

USABLE WAVELENGTHS

Wavelength (nm)	*Light source*
206	Iodine
254	Mercury
280	Iodine
340	Iodine

0021-9673/85/$03.30

a band width of *ca.* 25 nm. The UV light was conducted towards and from the slit by means of quartz rods.

Capillary

The capillary material was PTFE, purchased to a special specification from Habia (Breda, The Netherlands). The inner diameter was 0.2 mm and the thickness of the wall was approximately 0.075 mm.

Operational systems

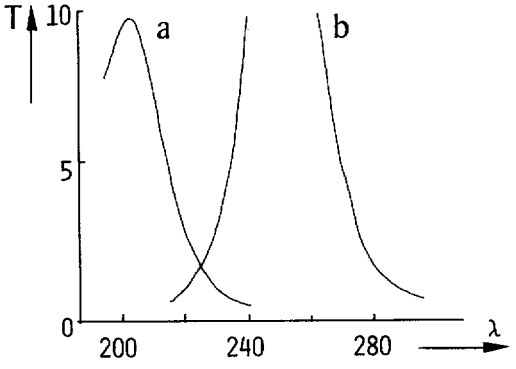
The operational systems were prepared from analytical-reagent grade chemicals, purchased from either Merck (Darmstadt, F.R.G.) or Sigma (St. Louis, MO, U.S.A.).

RESULTS AND DISCUSSION

Requirements for 206 nm detection

The most important limitation to the use of the 206 nm line of iodine is the relatively low signal finally obtained, which is caused by the fact that the 206 nm line is relatively weak, the loss in air is considerable, there is a significant loss in the capillary wall, isotachophoretic buffers can show absorption, the filter transmission is lower than usual and the sensitivity of the photodiode is low.

The loss of signal in air can be minimized by placing the quartz rods as close as possible to the capillary wall. Also, the UV source and filter should be mounted against the quartz rods. The capillary wall is one of the most important sources of loss of UV light at 206 nm. The thickness of the wall should be minimal without affecting the rigidity of the capillary. A thickness of *ca.* 0.075 mm will give satisfactory results with the material mentioned, but significant batch-to-batch variations may be observed. The transmission of the 206 nm filter used was less than that of an ordinary 254 nm filter, both as combined interference and cut-off filters (see Fig. 1). The band width, measured at 50% of maximum transmission, was *ca.* 25 nm for both filters. The decrease in sensitivity of the photodiode is shown in Fig. 2.



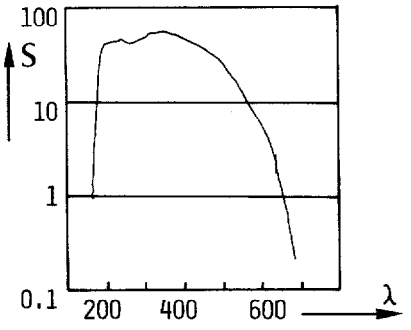


Fig. 1. Transmission of commercially available UV filters (LKB) for (a) 206 and (b) 254 nm. Both are combined interference and cut-off filters.

Fig. 2. Relative spectral sensitivity (*S*) of the Type R330-02 vacuum photodiode from Hamamatsu.

The absorption of the buffers is considerably increased at wavelengths below 240 nm, as shown in Fig. 3. These spectra were measured in a 10 mm cuvette. Only the very small pathlength used in capillary isotachophoresis makes it possible to use some of the buffers at 206 nm. The path length in the separation compartment, as used by us, is approximately 0.2 mm. It is obvious that in capillaries with larger inner diameter, such as those commercially available, buffer absorption will increase proportionally. Table II lists some of the commonly used leading buffers with their absorptions at 206 nm relative to water in a 0.2 mm I.D. capillary.

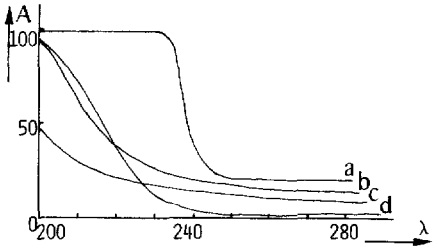


Fig. 3. Absorption (A) of some leading buffers used in isotachophoresis as measured in a 10 mm cuvette, (a) Cl^-–His; (b) Cl^-–Tris; (c) HCl; (d) K^+–acetate, all 0.01 M.

Table II shows that only the commonly used histidine buffer (pH 6) has an unacceptable absorption. In this instance the absorption, due to the counter ion, also shows a considerable pH dependence. An application of this effect is the determination of the pH in non-UV-absorbing zones or to mark the boundaries of these zones. A good alternative for a pH 6 leading buffer is hydroxylamine, which has a comparable pK value and a low absorption at 206 nm. In all the buffers mentioned so far the 206 nm absorption is due mainly to the counter ion and not to the leading ion. Leading ion or terminator ion absorption will, not, of course, limit the applicability of 206 nm detection (ref. 2, p. 335). Additives to increase the sharpness of the boundaries are frequently needed, especially at high pH, high fields strengths and low leading electrolyte concentrations. Hydroxyethylcellulose (HEC) or hydroxypropylmethylcellulose (HPMC) are commonly used. The effect of these non-ionic additives on the 206 nm absorption was not investigated separately. Mowiol and HEC, however, do not interfere, as is shown by the low absorption of the pH 2.9 buffer,

TABLE II

UV ABSORPTION AT 206 nm OF SOME ISOTACHOPHORETIC BUFFERS, MEASURED IN A 0.2 mm I.D. PTFE CAPILLARY, RELATIVE TO WATER

No.	*Leading ion*	*Counter ion*	*pH*	*Absorption at 206 nm (%)*
1	0.01 Cl^-	Histidine$^+$	6.0	85
2	0.01 Cl^-	Tris$^+$	8.2	6
3	0.01 Cl^-	ACA$^+$	4.5	9
4	0.01 Cl^-	ALA$^+$	2.9	3
5	0.01 Cl^-	Ammediol$^+$	8.9	16
6	0.01 K^+	Acetate$^-$	5.0	8

mentioned in Table II. Both additives were used here at a concentration of 0.05 and 0.2%, respectively.

Possible applications

One of the most promising applications of UV detection at 206 nm in isotachophoresis is the specific detection of sample constituents with peptide bonds. The sensitivity of UV detection of these compounds is considerably increased. Fig. 4 shows the detection of a dipeptide derivative, analysed as a cation, at both 206 and 254 nm.

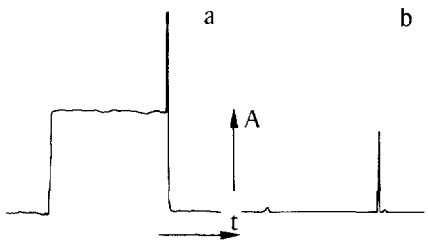


Fig. 4. Analysis in operational system No. 6 (Table II) of a cationic dipeptide derivative, glycylglycine methyl ester, detected at (a) 206 and (b) 254 nm. Terminator, H^+.

This will be especially useful if a high-resolution universal detector is not available. The single peptide bond in this monovalent ion already results in 20% absorption. Experiments with oligopeptides containing 10–20 amino acids have indicated that the absorption at 206 nm increases with increasing number of peptide bonds. When analysing peptides and proteins, there can be a considerable contribution to the 206 nm absorption from a number of amino acid groups also.

Fig. 5 shows the analysis of a severely contaminated histidylhistidine sample at 206 and 254 nm, analysed as a cation. Human serum albumin was analysed as an anion at pH 8.2 with Tris as a counter ion.

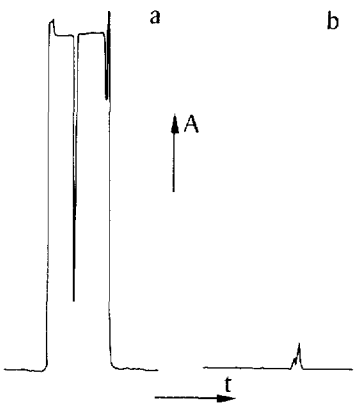


Fig. 5. Analysis in operational system No. 6 (Table II) of a contaminated histidylhistidine sample detected at (a) 206 and (b) 254 nm. Terminator, H^+.

Fig. 6 shows the detector response at 206, 254 and 280 nm. A greater signal increase is attained by switching from 254 to 206 nm rather than from 254 to 280 nm in this instance. Additional impurities and absorption of the terminator are observed at 206 nm. The latter is caused by the terminating ion or by the counter ion in the terminator zone, where the counter ion concentration and the pH are usually higher than in the leading zone.

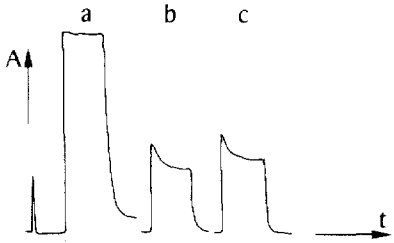


Fig. 6. Human serum albumin, analysed in operational system No. 2 (Table II) and detected at (a) 206, (b) 254 and (c) 280 nm. Terminator, HEPES.

When analysing proteins by isotachophoresis, non-UV-absorbing spacers are usually applied to separate the individual (UV-absorbing) zones and spikes[1,3]. A choice can be made between a discrete set of spacers at certain mobilities or continuous series of spacers, such as ampholines. The use of commercially available ampholines, however, is not satisfactory at 206 nm, as can be seen from the high absorption at that wavelength (see Fig. 7).

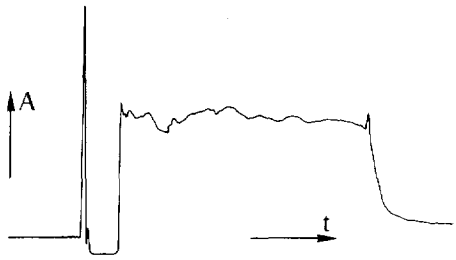


Fig. 7. A mixture of ampholines, p*I* 7–9, analysed in operational system No. 2 (Table II) and detected at 206 nm. Terminator, HEPES.

In conclusion, we can say that UV detection at 206 nm in isotachophoresis is possible if the capillary material and operational systems used meet certain standards. The increased response for the detection of peptides and proteins has been demonstrated. The specificity for peptide bond detection at this wavelength will yield new prospects for identification, especially when dual wavelength detection is available[4].

REFERENCES

1 L. Arlinger and R. Routs, *Sci. Tools*, 17 (1970) 21.
2 F. M. Everaerts, J. L. Beckers and Th. P. E. M. Verheggen, *Isotachophoresis; Theory, Instrumentation and Applications*, Elsevier, Amsterdam, Oxford, New York, 1976.
3 A. Vestermark, *Cons. Electrophoresis, Thesis*, University of Stockholm, 1966.
4 Th. P. E. M. Verheggen, J. C. Reijenga, F. M. Everaerts, *3rd International Symposium on Isotachophoresis, Goslar, F.R.G., 1982.*