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Note

On-line radiometric detection in capillary isotachophoresis

I. Preliminary experiments

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The wide range of applications of capillary isotachophoresis (ITP) is stimulating the development of new detection techniques, especially those having the capability of improving the selectivity of detection and, consequently, the overall performance of ITP.

Detection of the zones with the aid of high-resolution techniques is advantageous in ITP[1]. Therefore, mainly different alternatives of a.c. and d.c. conductivity measurements are used as universal detection techniques (see, *e.g.*, refs. 1–5). UV photometric detection is the only selective detection technique that is currently employed in capillary ITP[1,6]. A fluorimetric detector recently developed for high-performance zone electrophoresis[7] seems to be easily adaptable for capillary ITP.

For those working with radioactive and/or radiolabelled ionic compounds, an on-line radiometric detector is desirable. To our knowledge, no successful attempt to develop an on-line radiometric detector for capillary ITP has been reported and this paper is the first to deal with this subject.

When designing an on-line radiometric detector suitable for capillary ITP we must take into consideration the random nature of the disintegration of radionuclides and, consequently, the relationship between the precision of the radioactivity determination and the number of counts registered (see, *e.g.*, refs. 8 and 9). In other words, the cell used for the detection of radionuclides in capillary ITP should provide an acceptable precision of the radioactivity determination through a high counting yield of the detection process and/or through the counting time, which is adjustable to a certain extent[8].

To optimize the counting time for a given configuration of the detection cell,

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we can employ the methods that are currently used in radiometric flow-through detectors[8] (migration velocity, length or volume of the sensing part) or we can employ specific means of capillary ITP (*e.g.*, counter-flow of the leading electrolyte). Following these requirements, we cannot neglect the need for the highest possible resolving power of the detector to exploit fully the separation capabilities of ITP.

Capillary tubes made of fluoropolymers are mostly used in instruments for capillary ITP. A finite wall thickness of the capillary tube means that some radionuclides cannot be detected using a cell with an uninterrupted capillary wall. Obviously, the type of the radiation and its energy are of major importance in this respect.

The aim of our work was to develop detection cells suitable for the detection of β-emitters as the radionuclides belonging to this category are mostly used in experiments with radiolabelled compounds in biochemical, biomedical and environmental research.

An energy of β-radiation higher than 0.7 MeV is stated to be necessary for β-particles to penetrate the wall of capillary tube made of polytetrafluoroethylene (PTFE) and the window of a G-M tube[8]. The value of 0.4 MeV is given as the lower energy limit for the β-particles to penetrate the window of a counting device[9]. Below this energy level other techniques of radioactivity detection must be employed.

EXPERIMENTAL

An instrument for ITP similar to that described by Everaerts *et al.*[1] was used. A fluorinated ethylene–propylene copolymer (FEP) capillary tube of 0.3 mm I.D. (0.15 mm wall thickness) was used. Detection was performed with a conductivity detector[1,10] and radiometric detectors (see below). Stabilized current was supplied by a unit developed by Havaši[11]. Details concerning the operating conditions are given in the legends to the figures.

RESULTS AND DISCUSSION

Radiometric detection cells for capillary ITP

Two alternatives of radioactivity detection were developed (see Fig. 1).

Detection cells with an uninterrupted capillary tube (IA and IB in Fig. 1) were

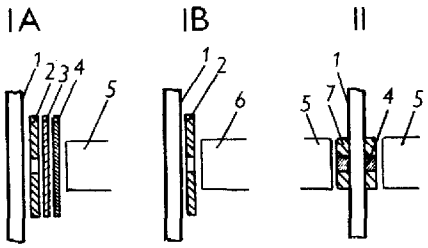


Fig. 1. Detection principles tested in the development of radiometric detection for ITP. IA, Uninterrupted FEP capillary tube with scintillating detection; IB, as in IA but a G-M tube is used for the detection of radiation; II, the solution to be evaluated is in direct contact with a solid scintillating material. 1 = Wall of the capillary tube; 2 = lead shielding with the slit; 3 = aluminium foil; 4 = scintillating foil; 5 = photomultiplier; 6 = G-M tube.

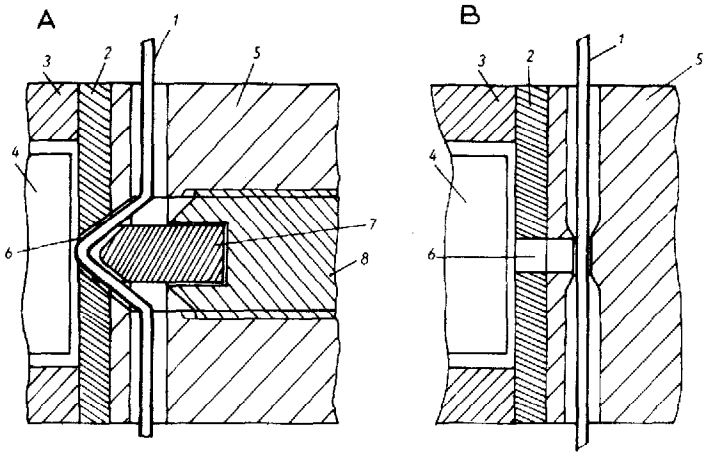


Fig. 2. Geometrical arrangements of the detection cells with an uninterrupted capillary tube. B, Straight configuration of the capillary tube; A, turned configuration of the capillary tube. 1, FEP capillary tube; 2, lead shielding; 3, metal housing for G-M tube; 4, G-M tube; 5, acrylic body of the cell; 6, split; 7, clamping piece for the placing of the capillary tube into the slit; 8, adjusting screw.

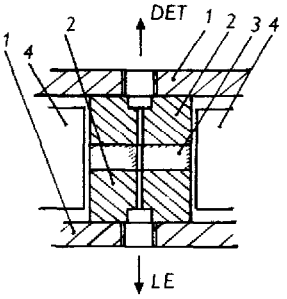
used in experiments with phosphate containing a certain amount of $^{32}PO_4^{3-}$ (E_{max} = 1.71 MeV). The proper arrangement of the sensitive part for these types of detection cells was investigated. Two extreme possibilities for the placing of the capillary tube in the cell are shown in Fig. 2. As would be expected, arrangement B (Fig. 2), resembling a photometric cell, is characterized by a low geometry factor (geometry factor = 1 for the 4π geometry measurement). Consequently, a poor signal was recorded on passage of the zone of phosphate through the cell. A considerable signal improvement was obtained when the capillary tube was turned as shown in Fig. 2A, while the slit width remained unchanged. Undoubtedly, an improvement in the geometry factor was responsible for the effect.

For already discussed reasons, low-energy β-emitters (in this work arbitrarily defined as those having E_{max} lower than 0.4–0.7 MeV) could not be detected using devices IA and IB (Fig. 1).

For this group of radionuclides mostly scintillation counting techniques are employed. To have the possibility of their on-line detection in capillary ITP (^{14}C, ^{35}S, ^{45}Ca, ^{99}Tc, for example, belong to this group) the cell shown in Fig. 3 was developed. The principles of scintillation counting were utilized in this detection cell. A short (2–3 mm) piece of a thick-walled narrow-bore tube of 0.3 mm I.D. made of a plastic scintillator (SPB-31; Tesla, Přemyšlení, Czechoslovakia) served as a sensor. Photomultipliers in coincidence were used for the radioactivity evaluation (a schematic diagram of the measuring circuitry is given in Fig. 4).

Detection limits with radiometric detection

Two characteristics describe a particular detection system in capillary ITP[1], *viz.*, a minimal detectable zone length and a minimal detectable amount of the sample material. While the former has a direct relationship with the resolving power of the detector, the latter is an absolute measure of the minimal detectable amount. For a universal detection technique these characteristics are interrelated. In general, for a



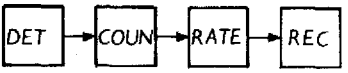


Fig. 3. Scintillating detection cell suitable for weak β-emitters. 1, Metal housing of the cell; 2, acrylic body; 3, plastic scintillator with a drilled hole of identical I.D. to the capillary tube; 4, photomultipliers. DET = Direction to the conductivity detection cell; LE = direction to the leading electrolyte compartment.

Fig. 4. Schematic diagram for the radioactivity measurement. DET = detection cell; COUN = counter (Model 200026, VEB RFT Messelektronik, Dresden, G.D.R.); RATE = ratemeter[14]; REC = line recorder.

selective detection technique such a relationship cannot be expected, as the way in which the quantitation is performed is decisive (see refs. 12 and 13).

The detection capabilities of the radiometric detectors provided with the cells described above were compared with a conductivity detector measuring in the a.c. mode[1,10]. The radiometric cell to be tested was mounted on the same capillary tube on which the conductivity cell was welded[10]. Thus, the detectors were arranged in an in-line configuration.

Isotachopherograms for the evaluation of the detection cell with an uninterrupted capillary tube (cell IA in Fig. 1) are given in Fig. 5. As can be deduced from the isotachopherograms (the radiometric signal was recorded at a chart speed equal to half of the chart speed used for the registration of the conductivity signal), the leading edge of the zone of phosphate containing part of the $^{32}PO_4^{3-}$ is registered

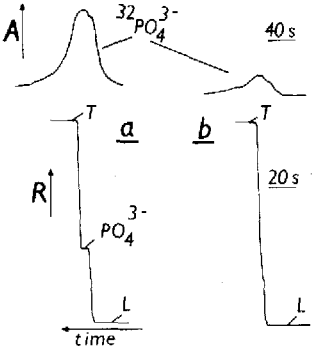


Fig. 5. Isotachopherograms for the detection of phosphate containing $^{32}PO_4^{3-}$. a = Amount injected giving responses for both the conductimetric and radiometric detectors; b = amount of phosphate below the detection limit of the conductivity detector. Leading electrolyte: 10 m*M* hydrochloric acid + histidine to pH 6.0, additive 0.2% hydroxyethylcellulose (HEC). Terminating electrolyte: 5 m*M* 2-morpholinoethanesulphonic acid (MES). Driving current: 40 μA. L = chloride; T = MES; R = increasing resistance; A = increasing counting rate (logarithmic scale).

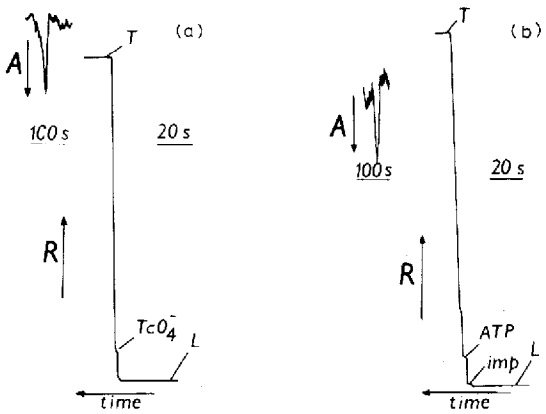


Fig. 6. Detection of weak β-emitters. a = ITP evaluation of TcO_4^-. Leading electrolyte: 10 m*M* hydrochloric acid + ε-aminocaproic acid (pH 4.8), additive 0.2% HEC. Terminating electrolyte: 5 m*M* caproic acid. A 1-μl volume of water solution of NH_4TcO_4 was injected (activity dosed, 0.69 nCi). b = ITP evaluation of ^{14}C-labelled ATP. Leading electrolyte: 10 m*M* hydrochloric acid + β-alanine (pH 3.0), additive 0.2% HEC. Terminating electrolyte: 5 m*M* caproic acid. A 2-μl volume of an aqueous solution of ATP was injected (activity dosed, 20 nCi). The driving current in both instances was 40 μA (10 μA during the activity measurement). L = Chloride; T = capronate; R = increasing resistance; A = increasing counting rate (logarithmic scale).

with a certain distortion by the radiometric detector. This distortion is partly due to the detection of β-particles before the entrance of the zone into the cell and partly due to the change of the geometry factor during the detection of the migrating zone. An analogous distortion of the trailing edge is caused by the same effects and, moreover, the high time constant of the ratemeter used[14] influences the shape of this edge in an undesirable manner.

A rough estimate of the resolving power of this arrangement of radiometric detection implies that this characteristic approaches that of a thermodetector. On the other hand, the absolute amount that can be detected and/or determined by a radiometric detector of this design is smaller than that by a conductivity detector (for a given I.D. of the capillary tube and for the operational system used).

In a similar manner, the performance of the detection cell illustrated in Fig. 3 was evaluated. Experiments with two radionuclides (^{99}Tc, ^{14}C) were carried out to test this cell. Isotachopherograms of the analysis of $^{99}TcO_4^-$ (E_{max} = 0.29 MeV) and ^{14}C-labelled ATP (E_{max} = 0.14 MeV) are given in Fig. 6. These experiments clearly show that also low-energy β-emitters can be detected by an on-line radiometric detector in capillary ITP.

CONCLUSIONS

The results imply useful possibilities for ITP in the analysis of radioactive and radiolabelled ionic compounds. The availability of an instrument suitable for the tion of several detectors, *e.g.*, conductimetric, UV photometric and radiometric, very effectively. Such a configuration of an ITP instrument seems very attractive, *e.g.*, for metabolism research where radiolabelled compounds are currently used (see, *e.g.*, ref. 16).

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