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ON-COLUMN RADIOMETRIC DETECTOR FOR CAPILLARY ISOTACHOPHORESIS AND ITS USE IN THE ANALYSIS OF ^{14}C-LABELLED CONSTITUENTS

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SUMMARY

A radiometric detector suitable for capillary isotachophoresis (ITP) is described. This detector, based on solid scintillation counting, consists of three subunits, *viz.*, an on-column detection module, measurement electronics and a microcomputer system. A key part of the detector, a small volume detection cell, has a sensing part made of a plastic scintillator. Counting efficiencies of 10–15% were typical for these cells of effective volumes of 70 or 210 nl (0.3 mm I.D.) for ^{14}C-labelled constituents in a coincidence mode of measurement. By using the cell of effective volume of 210 nl, *e.g.*, a detection limit of *ca.* 16 Bq was achieved for [U-^{14}C]acetate under typical ITP working conditions. It is shown by practical examples (cytidine 5′-tri- and diphosphates) that the detector can be very useful in the purity control of ^{14}C-labelled chemicals by ITP. A practical utility of the detector in monitoring a biotransformation of ^{14}C-labelled Cytostasane (a cytostatic drug) by ITP indicates its potential, *e.g.*, in pharmaceutical research. From the ITP analysis of an [U-^{14}C]protein hydrolysate it can be deduced that the radiometric detector in conjunction with suitable spacing constituents is convenient also for analytical work with complex mixtures of radiolabelled constituents.

INTRODUCTION

The separating abilities associated with an inherent concentrating power as well as typical submicroanalytical features make capillary isotachophoresis (ITP) an

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attractive alternative in the analysis of radioactive and/or radiolabelled ionogenic compounds. To exploit these analytical potentials of ITP, off-line techniques were proposed by Arlinger[1–4]. This approach to radiometric detection was shown to be useful also in analytical applications characterized by complex sample matrices[5–7].

However, when a wider analytical use is considered these off-line combinations are less convenient for the following reasons: (i) increased labour requirements; (ii) a time delay between the separation and detection; (iii) a possible loss of resolution during the isolation step; (iv) uncertainties in the recoveries of the analytes, *e.g.*, when they are isolated from the trapping strips[1–7]; (v) risk of contamination due to extensive handling of radioactive material. These disadvantages stimulated feasibility studies devoted to the developments of on-column radiometric detection in ITP[8–10]. Despite sceptism (see, *e.g.*, refs. 3 and 4), it was clearly shown that this detection mode is applicable in ITP for a wide range of radionuclides. For example, with the exception of ^{3}H (very weak β emitter), it is suitable to the detection of β radiation emitted from nuclides currently employed in biosciences[8–10].

The aim of this work is to describe an on-column radiometric detector for ITP developed recently in our laboratory. As is illustrated practically, based on solid scintillation counting, this detector is very convenient for the analysis of ^{14}C-labelled constituents present in various matrices.

EXPERIMENTAL

Instrumentation

A CS isotachophoretic analyser (ÚRVJT, Spišská Nová Ves, Czechoslovakia) was used. It was assembled in the column-coupling configuration of the separation unit[11,12] by using modules as delivered by the supplier. An on-column radiometric detection module developed in this work was assembled into the analytical stage of the separation unit (see Figs. 1 and 2). The measurement electronics of the detector comprised commercially available subunits for the radioactivity measurement (JANA, ÚRVJT), see Fig. 2. Data acquisition from the radiometric detector was performed with a PMD 85 microcomputer (Tesla, Bratislava, Czechoslovakia). This microcomputer system served also for post-analysis treatment of data and for printing of the results (see below).

For preparative ITP experiments a discontinuous fractionation technique[12,13] was employed in the column-coupling configuration of the separation unit[14].

Chemicals

Chemicals used for the preparation of the leading and terminating electrolytes were obtained from Sigma (St. Louis, MO, U.S.A.), Serva (Heidelberg, F.R.G.), Reanal (Budapest, Hungary) and Lachema (Brno, Czechoslovakia). They were purified by conventional methods[15]. Water supplied by a two-stage laboratory demineralization unit (Rodem-1; OPP, Tišnov, Czechloslovakia) and further purified on a mixed-bed ion exchanger (Amberlite MB-1; BDH, Poole, U.K.) was used for the preparation of the solutions. Hydroxyethylcellulose 4000 (Serva) was used as an anticonvective additive to the leading electrolytes. Its stock solutions were purified on a mixed-bed ion exchanger (Amberlite MB-1).

Carbon-14-labelled chemicals were obtained from the Institute for Research,

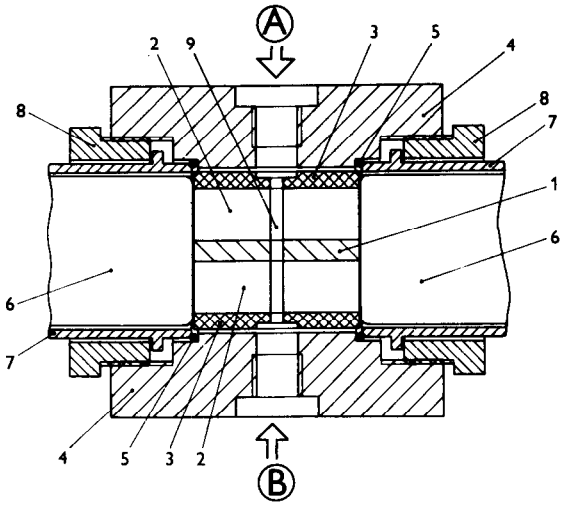

Fig. 1. On-column part of the radiometric detector. 1 = Sensing layer made of plastic scintillator; 2 = transparent plastic material compatible with the scintillator; 3 = black layer made of plastic material preventing stray light effects from the side of the column (A) and from the side of the counter-electrode compartment (B); 4 = duralumin housing of the on-column module; 5 = O-rings for light-tight connections of the photomultipliers (6) to the detection cell; 7 = metal housing for the photomultipliers; 8 = screws fixing photomultipliers; 9 = capillary channel in the detection cell.

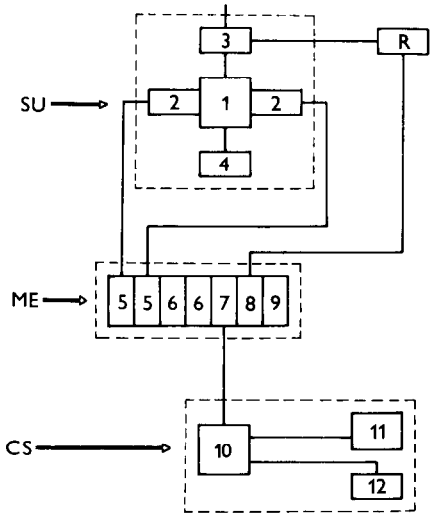

Fig. 2. Block diagram of the on-column radiometric detector for ITP. SU = On-column part of the detector (see Fig. 1); ME = measurement electronics; CS = microcomputer system; R = line recorders; 1 = duralumin housing for the detection cell; 2 = photomultipliers; 3 = conductivity detector; 4 = refilling block with the counter-electrode compartment for the analytical column; 5 = power supplies for photomultipliers; 6 = amplifiers; 7 = coincidence modul; 8 = ratemeter; 9 = system power supply; 10 = microcomputer; 11 = monitor; 12 = printer.

Production and Applications of Radioisotopes (Prague, Czechoslovakia). Reaction mixtures from a biotransformation study of ^{14}C-labelled 5-bis(2-chloroethyl)amino-1-methylbenzimidazolyl-2-butanoic acid (Cytostazane) were obtained from Dr. V. Ščasnár (Institute of Pharmacology, Biomedical Centre of Slovak Academy of Science, Bratislava, Czechoslovakia).

RESULTS AND DISCUSSION

Description of the detector

The detector developed in this work consists of three subunits, *viz.*, (1) on-column detection module, (2) measurement electronics, (3) microcomputer system (see Figs. 1 and 2). In its design we paid special attention to the detection module since it is a key subunit when the overall analytical performance of the detector is considered. Fig. 1 shows that it consists of a light-tight assembly made of metal (4) in which an exchangeable detection cell (1–3) is placed between a pair of photomultipliers (6). The sensing part of the detection cell (1) is made of a plastic scintillator (SPB 31; Tesla, Přemyšlení, Czechoslovakia). A capillary channel in the cell (9) has a diameter identical to that of the I.D. of the analytical column (0.3 mm). In spite of the fact that very different values for the average range of ^{14}C β radiation in aqueous solutions can be found in the literature (see, *e.g.*, refs. 16 and 17), such a diameter of the capillary channel in the sensing part is rather large to achieve an high counting efficiency in the radioactivity measurement (see below). However, we preferred compatibility of the on-column module with current ITP columns on the account of lower counting efficiency.

To achieve an high resolving power of the detector it is desirable to use a very thin scintillating layer in the detection cell ($\approx$ 0.1 mm). While such a thickness of the sensing part is technologically feasible, from a theoretical treatment of our type of detector it can be concluded that the thickness should not be less than 1 mm as otherwise a considerable decrease in the precision of the radioactivity measurement is unavoidable[10]. This means that our type of detection cell to some extent compromises the resolving power of the detector relative to that achieved by the separation process. To keep the precision of the radioactivity measurement at an optimum, we used cells having a thickness of the sensing layer of 3 mm (effective cell volume 210 nl) while problems due to the decreased resolving power were solved by using (when required) discrete spacing constituents (see below).

A block diagram of the detector is given in Fig. 2. In the design of the measuring subunit (ME in Fig. 2) we preferred a coincidence mode of the radioactivity measurement to minimize detector noise. This subunit is also provided with a ratemeter module and thus enables recording of an analogue signal from the detector by a line recorder. This possibility is convenient for a quick test of the detector in troubleshooting. The signal acquired by the computer system, however, is more convenient for the evaluation of the ITP analysis. The computer is coupled to a coincidence module of the measuring subunit provided with a circuit converting the registered counts into TTL pulses. The low capacity of the computer memory restricts the abilities of the system in real time processing of the acquired data. Therefore, during an experiment only the data acquisition is possible, while data handling has to be performed subsequently.

An algorithm describing the data acquisition is available elsewhere[10]. The minimum counting time (fixed by the hardware) was 1 s. Software, written in Assembly language, enables in post-analysis mode the following operations with the acquired data:

(1) graphical plotting of the data on the system display
(2) selection of the counting time in the interval 1–60 s
(3) selection of a start time for display of the data
(4) selection of a scale on the axis of the registered counts for a given time interval
(5) calculation of the sum of the registered counts for a chosen time interval in absolute or relative mode
(6) printing of a hardcopy of the display

Some performance characteristics of the detector

The counting efficiencies of the detection cells developed in this work were evaluated with [U-^{14}C]acetate. A commercially available preparation used for this purpose was purified in our laboratory by preparative ITP (see Experimental). This purification step was necessary in order to avoid systematic errors due to the presence of other ^{14}C-labelled constituent(s) in the preparation. ITP was performed in operational system 1 (Table I). The nett number of counts as registered on passage of the acetate zone through the detection cell (divided by the mean residence time of the labelled ions in the cell) was related to the nett number of counts obtained for the same amount of purified acetate and for the same time interval by liquid scintillation counting (LSC). When an 100% counting efficiency is assumed for the measurements by LSC the counting efficiencies for our detection cells were in the range 10–15%. Such values are considerable lower in comparison to those reported for ^{14}C-labelled compounds in packed cells designed for liquid chromatography (see, *e.g.*, refs. 17–19).

TABLE I

OPERATIONAL SYSTEMS

His = Histidine; MES = morpholinoethanesulphonic acid; Glu = glutamic acid; HEC = hydroxyethylcellulose; BALA = β-alanine; CAPR = caproic acid; Tris = tris(hydroxymethyl)aminomethane; Gly = glycine; HOAc = acetic acid.

Parameter	*System No.*			
	1	*2*	*3*	*4*
Solvent	Water	Water–methanol	Water	Water
Proportion	–	80:20	–	–
Leading ion	Cl^-	Cl^-	Cl^-	Cl^-
Concentration (m*M*)	10	10	10	10
Counter ion	His	BALA	Tris	MES
pH_L	6.0	3.85	8.0	6.1
Additive to the leading electrolyte	HEC	HEC	HEC	HEC
Concentration (%, w/v)	0.2	0.2	0.2	0.2
Terminating ion	MES (Glu)	CAPR –	Gly –	HOAc –
Concentration (m*M*)	5	5	5	5

A less favourable ratio of the diameter of the capillary channel in the detection cell to the average range of ^{14}C β radiation in aqueous solutions (see above) is the main but probably not the only explanation for these differences (see, *e.g.*, ref. 20). On the other hand, packed detection cells have some serious disadvantages for use in ITP:

(i) packed cells having effective volumes of several tens of nl are difficult to manufacture,

(ii) less favourable surface/volume ratio, typical for the packed cells, can lead to problems due to adsorption phenomena[19],

(iii) particulate material is also less favourable when a suppression of the electroosmotic flow is desirable,

(iv) interactions of the separands with the particulate material may have undesirable influences on their effective mobilities during the detection,

(v) assembly of a packed cell into the ITP separation compartment increases its hydrodynamic resistance, thus making a rapid refilling of the compartment between experiments with viscous solutions of the leading electrolytes practically impossible.

These facts suggest that, despite its inherently lower counting efficiency for ^{14}C-radiolabelled constituents, our capillary type of detection cell has some favourable features from the point of view of ITP. In addition, a positive impact of the concentrating power of ITP on the precision of the radioactivity measurement (see below) makes it applicable also for typical trace analysis problems.

By using a general equation for the precision of the radioactivity measurement in flowing systems (see, *e.g.*, refs. 20 and 21), for our type of on-column detector in the ITP steady-state we obtained[10]

$$\delta_x = \frac{v_{iso}}{SOl_d\bar{c}_{x,x}l_x} + \frac{v_{iso}R_{c,o}(l_d + l_x)}{S^2O^2l_d^2\bar{c}_{x,x}^2l_x^2} + \frac{R_{c,o}(l_d + l_x)^2}{t_0S^2O^2l_d^2\bar{c}_{x,x}^2l_x^2} \quad (1)$$

where δ_x is the relative standard deviation of the measurement, v_{iso} is the steady-state migration velocity, $R_{c,o}$ is the counting rate for the background measurement (determined in the time interval t_0), l_d is the thickness of the scintillating layer in the detection cell in the direction along the capillary tube having the cross-section O, $\bar{c}_{x,x}$ is the concentration of the constituent X in its zone or in the interzonal boundary layer (when its amount is not sufficient to create its own zone) while l_x is the length of the zone X or the thickness of the interzonal boundary layer, respectively, and S is the sensitivity of the detection which includes both the decay constant of the radionuclide and the counting efficiency of the detector.

From eqn. 1 it is clear that the precision of the radioactivity measurement by the detector is related to the factors describing the ITP working conditions (v_{iso}, $\bar{c}_{x,x}$, l_x) as well as to those which characterize the detector and the radionuclide employed (l_d, S, O, $R_{c,o}$, t_0). Since the steady-state data in ITP can be calculated[22,23], eqn. 1 is useful to estimate their importance in the on-column radiometric detection. Thus, when the precision in the radioactivity measurement at the steady-state concentration of the analyte is compared to that corresponding to its concentration in the sample (under otherwise identical conditions), it is possible to quantify the rôle of the concentration adaptation of the analyte on the precision of the radioactivity measurement. The plots in Fig. 3, calculated for a concentration range of more than four decades, show that in some instances (low concentrations of the analytes in the samples) this adaptation

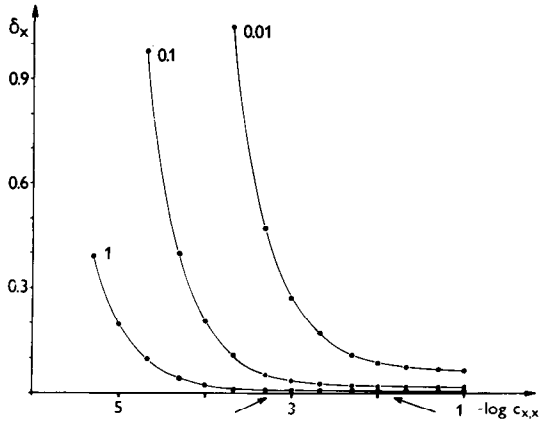

Fig. 3. Calculated dependence of the relative standard deviation of the radioactivity measurement (δ_x) on the concentration of the ^{14}C-labelled constituent present in the zone migrating isotachophoretically. Plots for relative abundances 0.01, 0.1 and 1 of the labelled form in the zone were obtained for the same injected amount of the constituent (21.2 nmol) migrating through the on-column detector at v_{iso} = 0.5 mm/s. The total concentration of the constituent was the variable and the length of its zone was calculated with respect to the constant injected amount. For the rest of the parameters the following values were used in the calculations (for the meaning of the symbols see the text); l_d = 3 mm; t_0 = 500 s; $R_{c,o}$ = 4.5 counts/s; O = 0.071 mm^2. A counting efficiency of 10% and a specific activity of 2.31 kBq/nmol (at 100% isotopic abundance) were used for the calculation of S. The arrows indicate the range of steady-state concentrations of the analytes in their zones under typical working conditions in ITP.

process is of key importance in the on-column radiometric detection in ITP. These plots also show that, in general, an high concentration of the leading ion is desirable when this detection is employed. This is true, especially, for analytes accompanied in the samples by their unlabelled analogues (dilution of the labelled form). However, the solubilities of the separands under the chosen ITP conditions as well as thermal effects determine the highest concentration of the leading ion in practical analysis.

The isotachopherograms in Fig. 4 were obtained from the on-column radiometric detector for [U-^{14}C]acetate at various concentrations of the leading anion. Since the rest of the parameters influencing the precision of the radiometric measurement (see eqn. 1) were kept constant, the practical importance of the concentrating power of the ITP separation process in the on-column detection is clearly illustrated. Here, the experiment corresponding to the isotachopherogram B can be taken as a reference since the volume of the acetate zone was similar to the injection volume, *i.e.*, neither dilution nor concentration occurred in this instance. On the other hand, in experiment A (Fig. 4), acetate was diluted during the separation to half of its sample concentration, while in C it was concentrated *ca.* 5 times. As expected from eqn. 1, lower precisions were typical for experiments with lower concentrations of the leading anion (longer zones of the analyte) for the same injected amount. However, a further increase in its concentration (D in Fig. 4) did not improve the precision as in C the acetate zone was shorter than the thickness of the sensing part of the detection cell.

An experimental estimation of the detection limit was carried out with the purified [U-^{14}C]acetate (see above) in system 1 (Table I). The measurements were

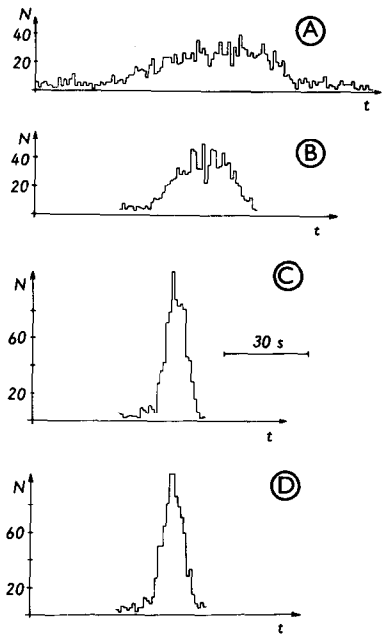

Fig. 4. Isotachopherograms from the analysis of [U-^{14}C]acetate at various steady-state concentrations. The compositions of the operational systems were as for system 1 (Table I) except that the concentrations of the leading anions were: 1 (A), 2 (B), 10 (C) and 20 m*M* (D). The driving currents during the detection were 2.5, 5.0, 25 and 50 μA for A, B, C and D, respectively. Volumes of 1 μl [U-^{14}C]acetate (purified as described in the text) were injected. *N*, *t* = increasing number of counts and time, respectively.

carried out with the detection cell having a thickness of 3 mm of the scintillating sensing layer. This cell was chosen for this work because from a theoretical treatment of the detection limit in the on-column radiometric detection[10] it is apparent that such a thickness of the sensing layer is an optimum when a compromise between a low detection limit and an high resolving power has to be achieved. The value of the signal-to-noise ratio of 2 (corresponding to $\delta_x = 0.5$) was taken as the detection limit and in our particular case it was *ca.* 7 pmol (16 Bq). This experimentally obtained value was in a reasonable agreement with the one predicted with the aid of eqn. 1 (9 pmol, 21 Bq).

The sensing part of the detection cell employed is *ca.* 30 times longer relative to a reasonable estimate of the thickness of the interzonal boundary layer[24]. This is an obvious disadvantage of the cell from the point of view of the overall resolving power of the detection. As already mentioned, the use of suitable spacing constituents can alleviate this disadvantage. With respect to an high selectivity of detection, the choice of suitable spacing constituents is less restrictive than, for example, in UV photometric detection.

From eqn. 1 it can easily be deduced that an high precision in the radioactivity measurement requires a low migration velocity. Such a requirement is associated with a decreased sharpness of the zone boundaries (see, *e.g.*, refs. 22 and 24) and, therefore, it is hardly acceptable for high resolution detectors. On the other hand, from previous

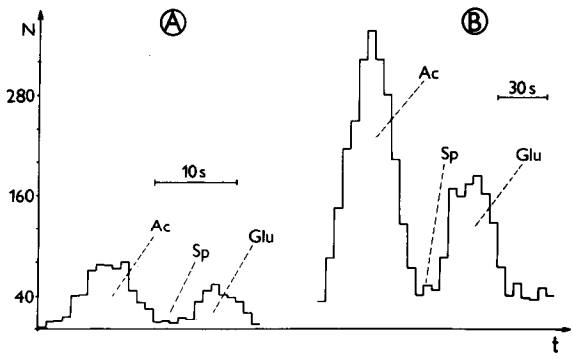

Fig. 5. Influence of the migration velocity on the number of registered counts. Volumes of 1 μl of the sample (prepared by mixing commercially obtained preparations of [U-^{14}C]acetate (Ac) and [U-^{14}C]glutamate (Glu) in the ratio 1:1) were injected in both A and B together with 1.5 nmol of β-bromopropionate (Sp). The driving currents during the separations were identical except during the detection they were 50 (A) and 10 μA (B).

discussion it is apparent that for on-column radiometric detection this is to a certain extent a reasonable way to increase the precision in the radioactivity measurement. This possibility is illustrated by the isotachopherograms given in Fig. 5. In these experiments the same amounts of a sample containing [U-^{14}C]acetate, [U-^{14}C]-glutamate and β-bromopropionate (spacing constituent) were separated under identical working conditions, except that the driving currents during the detection were 50 μA in A and 10 μA in B. To illustrate the difference in the registered counts the isotachopherograms were plotted at "chart speeds" having the same ratio as the driving currents during the detection.

ITP analysis of ^{14}C-labelled nucleotides

At present, the application of radiolabelled compounds is widespread, especially in various branches of bioscience. Of these compounds the ones labelled with ^{14}C are mostly used. Therefore, in spite of the fact that the detector described above is suitable also for the detection of other β emitters, in this work we paid attention to its use in the analysis of ionogenic compounds carrying this label.

^{14}C-labelled nucleotides represent an important group of compounds employed in biochemical and biological research. For obvious reasons, purity control of the preparatives employed for these purposes is essential, especially when impurities or naturally occurring compounds disturb the reaction(s) under investigation. The isotachopherograms given in Fig. 6 were obtained from the analysis of commercially available cytidine 5'-triphosphate (CTP) labelled with ^{14}C (ref. 25). A freshly delivered preparation (37 MBq/ml) was used in this instance. The actual content of the labelled CTP was 91.2% as determined by high-performance liquid chromatography (HPLC) in the laboratory of the supplier. From the ITP analysis it was apparent that besides CDP and CMP (cytidine 5'-di- and monophosphates, respectively) it contained also other ^{14}C-labelled impurities of which the one marked with u (Fig. 6) represented 13.8% of the registered counts. When we assumed that only the labelled constituents migrating between the leading and terminating anions were present in the sample, the

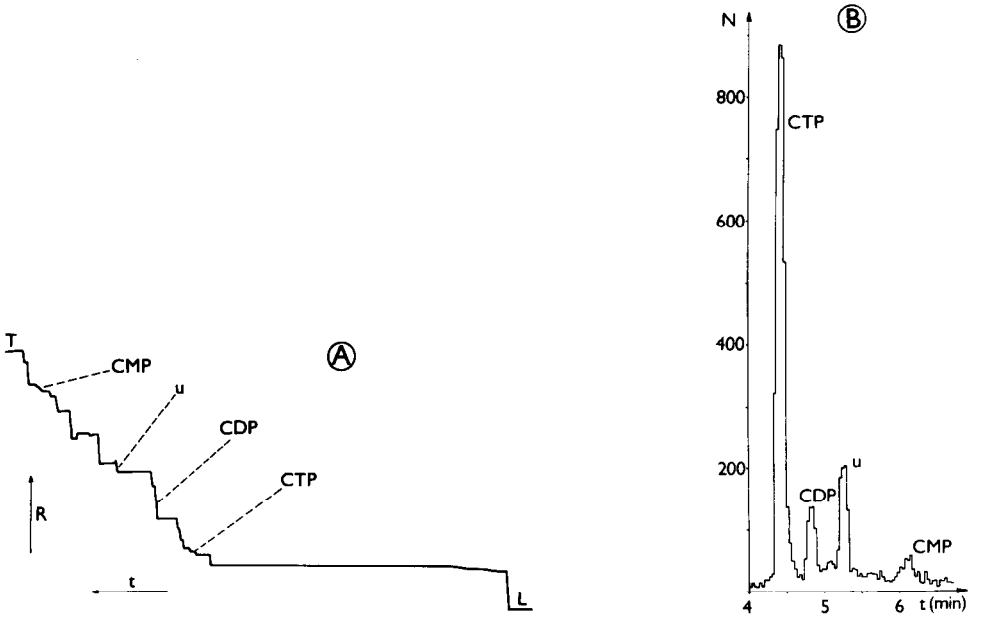

Fig. 6. Isotachopherograms from the analysis of [U-^{14}C]CTP. The separation was carried out in system 2 (Table I) and 1 μl of CTP was injected together with a mixture of spacing constituents, a (Table II). Records from the conductivity (A) and radiometric (B) detectors were registered at 45 μA. The driving current in the preseparation column was 200 μA. N, R, t = increasing number of counts, resistance and time, respectively.

actual content of CTP (expressed via relative distribution of the registered counts) was 63.9%. This value indicates a large discrepancy between ITP and HPLC. The difference was large also when CDP and CMP present in the sample were ascribed to the decomposition of CTP since its production (78.3% of the registered counts). A reasonable agreement between HPLC (91.2%) and ITP (92.0%) was obtained only when the constituent was considered as originating from CTP, *e.g.*, via a radiolytic degradation or as unresolved from it by HPLC.

In the analysis of ^{14}C-labelled CDP stored for a longer time (immediately after the expiration time), several labelled constituents were detected under similar conditions to those employed in the analysis of CTP (see Fig. 7). Of these only CDP and CMP were identified by spiking the sample with the unlabelled nucleotides. To save the preparative for further experimental work, we employed preparative ITP to isolate the labelled CDP. The isolation was carried out in the operational system 2 (Table I) by using the column-coupling configuration of the instrument adapted for the preparative work (see Experimental). An isotachopherogram from the radiometric detector shown in Fig. 7B was obtained in the analytical control of the preparative experiment. In this instance a small aliquot of the isolated fraction was taken for the analysis. This experiment performed with a delay of 20 h after the isolation also shows a degree of hydrolytic conversion of CDP kept in the trapped solution (pH $\approx$ 4.0). Nevertheless, these experiments clearly indicate an high purification efficiency of the preparative ITP in this instance. We feel that this approach to the purification of

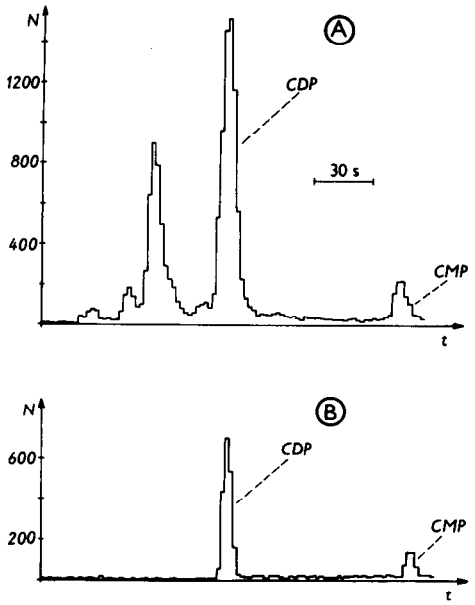

Fig. 7. Purification of [U-^{14}C]CDP by preparative capillary ITP. The working conditions were as in Fig. 6. In A, 5 μl of the CDP preparative were injected and in B the injection volume was 2 μl of the same preparation purified as described in the text.

radiolabelled ionogenic compounds is of general applicability, especially for less stable compounds currently employed in biological and biochemical research. Its advantages are obvious: (i) pure and well defined material can be prepared immediately before the experiment; (ii) the capillary type of separation unit enables purification in a reasonable time (*ca.* 25 min in our instance); (iii) in some instances, hundreds of μg of target compounds can be obtained in one experiment; (iv) with the exception of the columns, the same instrumentation can be used for both the preparative and analytical experiments.

ITP profiling of biotransformation products of ^{14}C-labelled cytostasane

Analysis of radiolabelled transformation products is a current task, *e.g.*, pharmacological evaluation of drugs. Since the biotransformation products are often ionogenic in nature, ITP is a method of choice in this research area. In this work we briefly investigated its capabilities in the analysis of samples obtained from a biotransformation study of ^{14}C-labelled cytostasane. Previous work with this drug in which HPLC was employed to monitor the course of biotransformation did not give satisfactory results in spite of the fact that at least one product was detected[26]. Since no more preliminary data were available we performed cationic and anionic ITP profiling of the labelled products present in the incubation mixture.

ITP profiles as obtained by the radiometric detector in the operational systems 3 and 4 (Table I) are given in Fig. 8. The cationic profiles were obtained in a system in which the effective mobility of H^+ is very low (for an estimation see ref. 27). Only

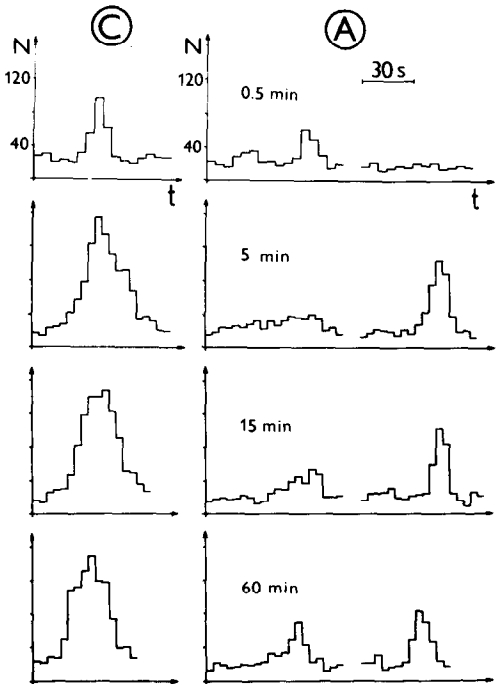

Fig. 8. Cationic (C) and anionic (A) ITP profiles of radiolabelled biotransformation products of ^{14}C-labelled Cytostasane. The ^{14}C-labelled drug was incubated with hepatocytes ($4 \cdot 10^6$ cells/ml) in Krebs-Henseleit buffer solution at pH 7.4 (the hepatocytes were obtained by a collagenase perfusion of rat liver). Volumes of 1 ml of the incubation mixtures taken at 0.5, 5, 15 and 60 min were centrifued (3000 rpm) and the supernatants were stored at −25°C until the ITP measurements. Volumes of 10 μl of the supernatants were taken for ITP. The driving current during the detection in the cationic profiling was 20 μA and in the anionic profiling was 40 μA. The zone of the carbonate spacing the radiolabelled constituents in the anionic profiling (*ca.* 120 s) was omitted from the isotachopherograms. For other details see the text.

under such extreme conditions could we achieve the cationic migration of the labelled constituent(s). From the response of the detector, the presence of only one constituent (migrating with an effective mobility similar to that of H^+) is visible. This, however, cannot be accepted as conclusive since the number of radiolabelled cationically migrating constituents must be determined in experiments in which the degree of chemical homogeneity of this zone is investigated, *e.g.*, the use of suitable spacing constituents, preparative experiments followed by analysis under various working conditions, etc.

The anionic profiles were evaluated in the operational systems 1–3 (Table I). Radiolabelled constituents migrated only at $pH_L = 8.0$ (system 3). Under these conditions two zones were detected by the radiometric detector (Fig. 8). The sum of the registered counts for these zones for a given incubation time was very close to half of the registered counts in the corresponding cationic profiles. At the same time, the migration velocity in the cationic system was approximately half of the value in the anionic system. When we consider the influence of the migration velocity on the number of registered counts (see Fig. 5 and accompanying explanation), it is seen that

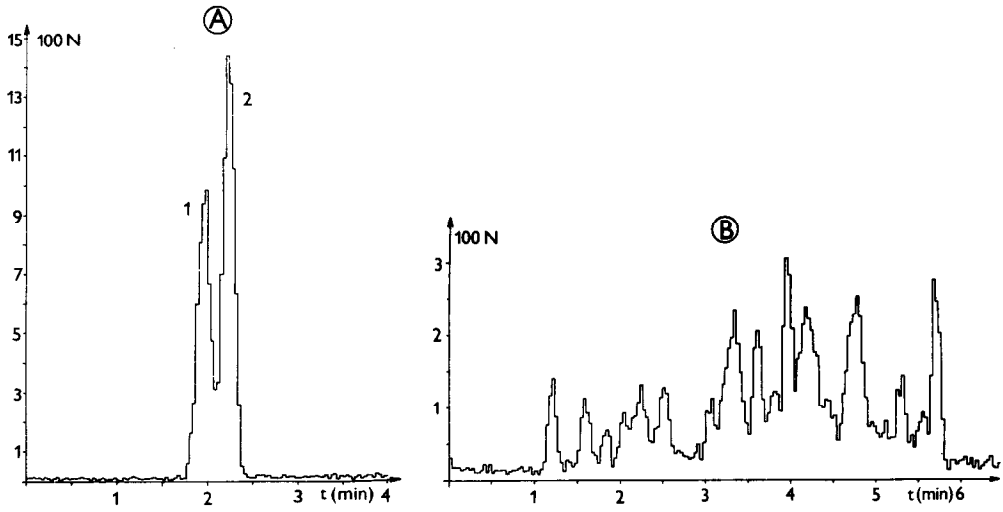

Fig. 9. Isotachopherograms from the analyses of an [U-^{14}C]protein hydrolysate. Volumes of 50 μl of the sample isolated as described in the text were injected without (A) and with (B) the spacing constituents, b (Table II). These volumes corresponded to 2.5 μl of the original sample having a specific activity of 40 MBq/ml. The driving currents were 250 and 50 μA in the preseparation and analytical columns, respectively.

the same amounts of the radiolabelled analytes were extracted in both profiling systems. This result suggests that in the cationic system there are two unresolved radiolabelled constituents formed by the biotransformation process. In addition, from their migration behaviours we can deduce that they have typical zwitterionic properties.

These preliminary experiments imply that ITP with the on-column radiometric detector may be a very useful analytical tool in biotransformation studies, *e.g.*, in combination with other separation and identification methods.

Detection of ^{14}C-labelled constituents present in a protein hydrolysate

ITP is a method suitable for the analysis of complex mixtures of ionogenic compounds. In this respect the column-coupling configuration of the separation unit[11,12] has many practical advantages. To illustrate these possibilities in the analysis of complex mixtures of radioactive or radiolabelled ionogenic constituents we carried out experiments with an [U-^{14}C]protein hydrolysate.

In the direct analysis of the hydrolysate (ref. 25, p. 33) we observed a trailing zone along the terminator (operational system 1). As this zone might be misinterpreted as a memory effect of the detection cell, we employed in these experiments a fraction trapped by preparative ITP (see Experimental). This fraction included the constituents migrating in the mobility interval determined by the leading and terminating ions of the operational system 1 (Table I).

In the analysis of the fraction two zones were resolved by the radiometric detector (Fig. 9A). To confirm their purities the fraction was analyzed under identical conditions with a mixture of spacers (b, Table II). An isotachopherogram obtained

TABLE II

COMPOSITIONS OF THE MIXTURES OF SPACING CONSTITUENTS

Mixture	*Constituents*[a]
a	Formate, trichloroacetate, lactate, succinate, N-acetylleucine, glutarate, glutamate, monomethyl succinate, acetate, monoethyl succinate, mono *n*-propyl succinate, propionate, butyrate, monomethyl adipate
b	Tartronate, malonate, citrate, succinate, glutarate, adipate, N-acetylglutamate, glycolate, acetate, dichloroacetate, trichloroacetate, β-bromopropionate, butyrate, aspartate, valerate, N-hydroxyethyliminodiacetate, glutamate, enantate, glucuronate, α-aminoadipate, α-aminopimelate, pantothenate

[a] The orders of the constituents agree with their migration orders at pH_L = 3.85 (a) and 6.0 (b).

from such an analysis (Fig. 9B) clearly shows that this fraction of the protein hydrolysate which should ideally contain only amino acids migrating anionically at pH 6.0 with effective mobilities higher than that of MES (aspartic and glutamic) was a complex mixture of radiolabelled constituents. Some of these constituents migrated between the spacers and some of them were spread along their zones. Reasons for the presence of such a number of radiolabelled constituents in the analyzed fraction were not investigated in this work. However, it can be explained via autoradiolytic and biochemical processes as well as via the presence of some peptidic fragments. Nevertheless, the isotachopherograms from the analysis of this sample clearly indicate that the on-column radiometric detector in conjunction with the column-coupling configuration of the separation unit is a promising analytical alternative also for complex mixtures of radiolabelled ionogenic compounds.

CONCLUSIONS

From this work it is clear that the described on-column radiometric detector is a very convenient solution to the detection of ^{14}C-labelled constituents under typical ITP working conditions. Its inherently lower counting efficiency for this radionuclide (in comparison to the packed detection cells used in liquid chromatography) is at least compensated by the concentrating power of the ITP separation process. In the detection of ^{14}C we can expect a further improvement of the counting efficiency by decreasing the diameter of the capillary channel in the detection cell. With the exception of ^{3}H (undetectable), however, the detector provides considerable higher counting efficiencies for other currently employed β radiation emitting nuclides (^{32}P, ^{35}S, ^{99m}Tc, etc.).

Problems associated with the lower resolving power of the detector (when compared to current high resolution detectors in ITP) can be solved via the use of spacing constituents. However, in this approach the resolution of some constituents with very close physico-chemical properties can be tedious. In this respect, a further improvement of the counting efficiency of the detection in the suggested way will automatically provide a possibility to increase the resolution of the detection via a decrease in the thickness of the sensing part of the detection cell without sacrificing the precision in the radioactivity measurement.

In this work we found preparative ITP to be a very suitable method for purification of radiolabelled chemicals. It is obvious that its use can be extended to isolations of the radiolabelled constituents from reaction mixtures, *e.g.*, in experiments aimed at the identification of radiolabelled products.

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