On the separation of $^{99m}TcO_4^-$, ^{99m}Tc-DTPA and ^{99m}Tc-citrate as marker species for the determination of Tc chemical forms in plant material using capillary zone electrophoresis

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ABSTRACT

The present paper addresses the potential use of capillary zone electrophoresis (CZE) as an analytical tool in 99-technetium speciation studies. In order to optimise sampling, storage and analytical procedures, the three marker compounds $^{99m}TcO_4^-$, ^{99m}Tc-DTPA and ^{99m}Tc–citrate were synthesised and used in test-measurements with CZE. The results underline the superior separation power of the CZE technique, and indicate good CZE performance for the stable $^{99m}TcO_4^-$ and ^{99m}Tc–DTPA compounds. The data suggest that CZE may be used without problems for various Tc-compounds of intermediate mobilities. The specific data of ^{99m}Tc–citrate suggest that with this marker compound a threshold lability is reached for the use of CZE in plant Tc–speciation studies. This result means that CZE cannot be used in analyses of Tc–compounds which are less stable than Tc-citrate. Future CZE work will comprise the synthesis and use of Tc-markers of intermediate mobilities and stabilities; furthermore, effects of marker matrices and the plant matrix on CZE performance will be investigated.

INTRODUCTION

Technetium-99 ($^{99}_{43}$Tc, $E_\beta = 292$ keV) is generally recognised as an important waste product of nuclear power generation, as a result of its high fission yield (6%) and its radioactive half-life of 2.1×10^5 yr (Kotegov *et al.*, 1968; Till, 1986; Yoshihara, 1996). Moreover, compared with other fission products, both the environmental mobility and the bioavailability of Tc are high (Wildung *et al.*, 1979; Sparkes and Long, 1988; Shaw and Bell, 1994), which both result in rapid movement of Tc in the food chain.

The measurement of Tc in the environment is problematic and time-consuming, due to a combination of low environmental concentrations, difficulties in the complete separation from contaminants, and laborious analytical procedures (Holm, 1993; Nevissi *et al.*, 1994). To overcome these problems, both the high accumulation and the high selectivity of Tc-uptake by plants (Shaw and Bell, 1994) might serve as a basis for the detection of Tc in soils/soil-waters.

In order to indicate, or even quantify, Tc in soils indirectly with the use of (sensor) plants, the large variations (up to five orders of magnitude) in reported soil-to-plant transfer factors (IUR, 1989) have to be well understood. In this context, the chemical forms of Tc (Tc-species) in both soils and (different parts of) plants are very important, because Tc (which is a group 7 transition metal) can exist in various oxidation states and, per oxidation state, may occur in various chemical forms, all depending on environmental conditions (Stalmans, 1986; Van Loon, 1986).

Pertechnetate ($Tc^{VII}O_4^-$) is reported as the most important Tc-species in the environment under oxidising conditions. TcO_4^- is also thought to be a very important Tc-species in both the uptake of Tc by the roots and the transport to the shoots (Cataldo *et al.*, 1986; Van Loon, 1986). However, to our knowledge only a single experiment has been performed to investigate the Tc-species present in plant roots (Lembrechts, 1986): the results suggested that in spite of the application of Tc as TcO_4^-, only 58% of total Tc in the roots occurred as pertechnetate. These data indicate that, although earlier papers suggest TcO_4^- as the most important Tc-species in the xylem sap (Cataldo *et al.*, 1978; Roucoux, 1980), other Tc-species, which are probably synthesised in the roots, may also be transported into the leaves via the xylem vessels. Here, the question arises of which Tc-species are predominantly responsible for the strong accumulation of Tc in plant leaves (Wildung *et al.*, 1977; Cataldo *et al.*, 1978; Lembrechts, 1988; Van Loon, 1986; IUR, 1989).

Re-examination of the data on xylem TcO_4^- should that uncertainties persist with respect to the unequivocal determination of the Tc species: the methods used were not tested for Tc-species other than TcO_4^-, and, furthermore, no tests were performed to check the possible re-oxidation of reduced Tc-species during sampling and storage. Here, for xylem sap, and, more generally speaking, for plant material, the presence of various organic acids and amino acids (White *et al.*, 1981; Sauter and Van Cleve, 1992; Senden *et al.*, 1992) should be regarded as potential complexing ligands for Tc^{IV} and/or Tc^{V} (Laurie, 1987; Pedrosa de Jesus, 1987). Harms *et al.* (1996a) noted various effects of longer-term storage on Tc chemical forms, and oxidising conditions during sampling and/or sample preparation may yield re-established TcO_4^- forms due to oxidation of initially present reduced Tc.

As may be clear from the above, a discussion on the possible Tc chemical forms in plant materials, and a discussion on the effects of methods selected for sampling, sample analysis, and sample handling should be preceded by the development of an analytical procedure which permits the clear recognition of various Tc-species. Here, the problem arises of how to check the procedure as such: any separation procedure may bring about changes in the initially introduced chemical species (Steinebach, 1993).

As is necessary for all separation techniques, we chose marker species ($^{99m}TcO_4^-$, ^{99m}Tc–DTPA and ^{99m}Tc–citrate) to indicate the threshold lability for complex-survival of the separation procedure as such. For this purpose, we selected $^{99m}TcO_4^-$, because it is the product of re-oxidation of reduced Tc-species. ^{99m}Tc-DTPA was chosen because of both its kinetic stability and its application in an earlier and comparable study (Harms *et al.*, 1996a). The third marker species, ^{99m}Tc–citrate, was used because: *(1)* Tc–citrate is a well-known, relative unstable Tc-species used in ligand-exchange labelling procedures of radiopharmaceuticals; *(2)* citrate is a well-known metal complexing ligand; and *(3)* citrate is present in plant materials and in xylem sap in relative high concentrations and has been reported as a component in metal-translocating complexes (Tiffin, 1972; White *et al.*, 1981; Volkert *et al.*, 1982; DePamphilis *et al.*, 1983; Özker *et al.*, 1988; El-Kolaly and El-Wetery, 1990; Özker *et al.*, 1992).

The present paper addresses the separation of these markers for the analysis of Tc chemical species in plant materials. Capillary zone electrophoresis (CZE) was selected as the separation method to investigate, because CZE gives the opportunity to repress strongly column (*i.e.* capillary wall) interactions with relatively labile Tc-complexes, and CZE shows superior separation characteristics compared to, *e.g.*, HPLC techniques (Altria *et al.*, 1990).

In order to use Tc-concentrations which approximate environmental (plant) conditions, we applied ^{99m}Tc rather than the environmentally relevant ^{99}Tc radio-isotope: ^{99m}Tc is a short-living, γ-emitting Tc-isotope (half-life 6.02 h, $E_\gamma = 141$ keV) of high specific radioactivity, which makes that CZE could be performed with on-line detection of Tc, using a NaI γ-detector.

EXPERIMENTAL

Chemicals

$^{99m}TcO_4^-$ was eluted from a ^{99}Mo–^{99m}Tc generator (Mallinckrodt Nuclear, The Netherlands) with 15 mmol L^{-1} NaCl. All reagents were of analytical grade. Stock solutions of 2.0 mmol L^{-1} $SnCl_2$ were daily prepared in 100 mL of 10 mmol L^{-1} HCl that had been purged with N_2 for 30 minutes.

Labelling procedures

The labelling procedures used are common in nuclear medicine, thus only slight modifications were used as compared to others (Volkert *et al.*, 1982; Münze *et al.*, 1983; Chilton and Thrall, 1990; Özker *et al.*, 1988, 1992).

^{99m}Tc-diethylenetriaminepentacetic acid (DTPA) was synthesised at room temperature by the addition of 0.1 mL of the $SnCl_2$ stock solution to 2 mL of an aqueous solution containing 1.7 mL $^{99m}TcO_4^-$ eluate (10 nmol L^{-1}), 0.1 mL 2-morpholinoethanesulfonic acid (MES)-buffer (0.3 mol L^{-1}) pH 5.2 and 0.1 mL DTPA calcium trisodium (0.2 mol L^{-1}) pH 5.2. The ^{99m}Tc-complex was allowed to stand for 0.5–2 h.

^{99m}Tc–citrate was synthesised in the same way as ^{99m}Tc–DTPA was, only 0.1 mL DTPA calcium trisodium (0.2 mol L^{-1}) was replaced by 0.1 mL disodium citrate (0.2 mol L^{-1}).

$^{99m}TcO_4^-$ for injection was added to the same matrix as ^{99m}Tc-citrate, only the $SnCl_2$-solution was absent.

Analytical procedures

Paper chromatography

Paper chromatography was performed for the quality control of the labelling of $^{99m}TcO_4^-$, ^{99m}Tc-DTPA, and ^{99m}Tc-citrate as described earlier (Colombetti *et al.*, 1976). From this method, the percentages of $^{99m}TcO_4^-$, reduced-hydrolysed ^{99m}Tc, and complexed ^{99m}Tc were calculated.

Capillary zone electrophoresis

CZE was performed with the experimental system schematically outlined in Figure 1. A Prince device (Lauer Labs B.V., Emmen, The Netherlands) permitted hydrodynamic sample introduction by application of pressure at the injection end of the capillary. This device regulated the temperature at 26°C of the first 32 cm of the capillary. The high voltage supply was a reversible polarity 0–30 kV unit (model HCN 140–35 000, F.u.G. Electronik GmbH, Rosenheim, Germany). The 75 μm × 61.5 cm fused silica capillaries were obtained from

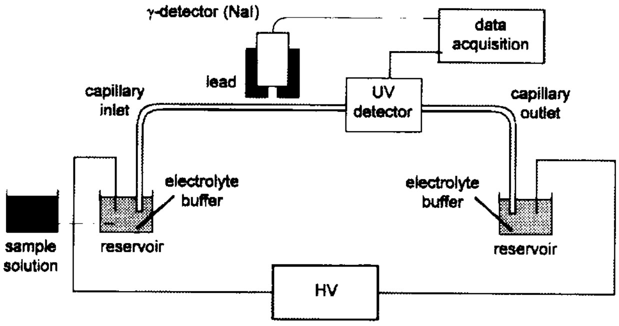

Figure 1 *The experimental system used for radiopharmaceutical analysis. HV = high-voltage supply.*

(Chrompack Nederland B.V. (Bergen op Zoom, The Netherlands). Polyacrylamide coated fused silica capillaries were home-made as described earlier (Van der Schans *et al.*, 1995). The samples were introduced by hydrostatic pressure (3 s at 50 mbar, which corresponds to an average sample size of 15.6 nL and an average injection plug length of 3.54 mm).

Detection systems

A lead-collimated NaI-detector (slit width 0.5 cm) was used on line for radioactivity counting at 38 cm from the inlet. An UV/vis-detector (model PU 4225, ATI Unicam, Cambridge, UK) was used at 50.5 cm from the inlet, to perform direct UV-detection for mesityl oxide (electroendosmotic flow (EOF)-measurement) and indirect UV-detection for the mobility of free citrate at 230 and 254 nm, respectively. For the latter approach, potassium chromate (5 mmol L^{-1}) was added to a 25 mmol L^{-1} MES-buffer. This solution was adjusted at pH 5.2 using NaOH. Subsequently, 10 mmol L^{-1} NaCl minus the NaOH concentration was added to the running buffer.

RESULTS AND DISCUSSION

^{99m}Tc-marker synthesis

Both ^{99m}Tc-DTPA and ^{99m}Tc-citrate were synthesised in high yields (96 ± 2 %, and 99 ± 2 %, respectively; n=4) within 1 hour, as determined with paper chromatography (Colombetti *et al.*, 1976). Throughout the synthesis procedures, a MES-buffer was elected as it could fulfil the requirements of an appropriate pK_a, low UV-absorption, low mobility, and an inertness for both $^{99m}TcO_4^-$ and reduced ^{99m}Tc. The latter MES-property was confirmed by a recovery of 101 ± 2 % (n=4) in paper chromatography of reduced, hydrolysed Tc. Also the purity of $^{99m}TcO_4^-$ was verified (99 ± 1 %, n=4).

Operating conditions

General

A general optimisation of the operating conditions of the CZE system was accomplished by restriction of the Joule heating. The Joule heating of the system is generated by the passage of electric current, depends on the power, and is determined by the capillary dimensions, conductivity of the buffer and the applied voltage. In addition to the suppression of heating effects by the CZE cooling system, the selected capillary had a rather large inner diameter (i.d.) of 75 μm, to compromise between Joule heating and signal-to-noise ratio. A capillary of 100 μm (i.d.) resulted in a much higher Joule heating, while an internal diameter of 50 μm resulted in a much lower ^{99m}Tc-signal.

^{99m}Tc-samples

CZE was performed using 25 mmol L^{-1} MES-buffer and 15 mmol L^{-1} NaCl/NaOH. Higher ion strengths in solutions resulted in excessive Joule heating of the CZE-system. To avoid asymmetric peak shapes in CZE, ^{99m}Tc-samples were prepared of comparable ion strengths (see Figure 2, for an example of a typical $^{99m}TcO_4^-$ signal). This means that the elution of $^{99m}TcO_4^-$ from the ^{99}Mo–^{99m}Tc generator was performed at 10-fold diluted NaCl; *i.e.* 15 mM NaCl rather than 150 mM NaCl. Further dilution resulted in a strong decrease in the $Na^{99m}TcO_4$ yield (results not shown).

In order to obtain an indirect UV-signal for the free citrate, 5 mmol L^{-1} potassium chromate was added to the electrolyte. In this case, the NaCl/NaOH concentration was lowered from 15 mmol L^{-1} to 10 mmol L^{-1}.

CZE wall modification

To reduce wall interactions with ^{99m}Tc, a permanent capillary wall modification was carried out by silylation, followed by deactivation using polyacrylamide as the neutral functional group (Van der Schans *et al.*, 1995). The modification was checked by measurement of the absence of any EOF, which was confirmed from the anodic to the cathodic side after addition of mesityl oxide to the injection plug. No UV-signal was observed within 90 minutes at 230 nm. The potential UV-response of mesityl oxide was verified by an additionally applied hydrostatic pressure (100 mbar), which resulted in a clear UV-signal (results not shown).

Ohm diagram

The reproducibility of compound mobilities strongly depends on the stability of temperature during CZE. An Ohm diagram was set up (voltage *vs* current), which showed excessive heat generation at higher voltages, in turn indicated by a disproportional (*i.e.* non-linear) increase in current with voltage (Figure 3). Therefore, CZE was operated at –10 kV in all determinations.

Determination of recoveries

In order to determine recoveries of ^{99m}Tc-components in CZE, the detector efficiency had to be established, both with respect to geometry and to the compound velocity. The geometric detection efficiency was determined as 12% by measurement of the ^{99m}Tc-signal, from a reference $^{99m}TcO_4^-$-solution that completely filled a capillary column. The detection efficiency as function of compound velocity was determined experimentally, using $^{99m}TcO_4^-$ in CZE under various applied voltages (Figure 4). The latter efficiency is not reported by the pioneers of ^{99m}Tc-analysis in CZE, which may be the

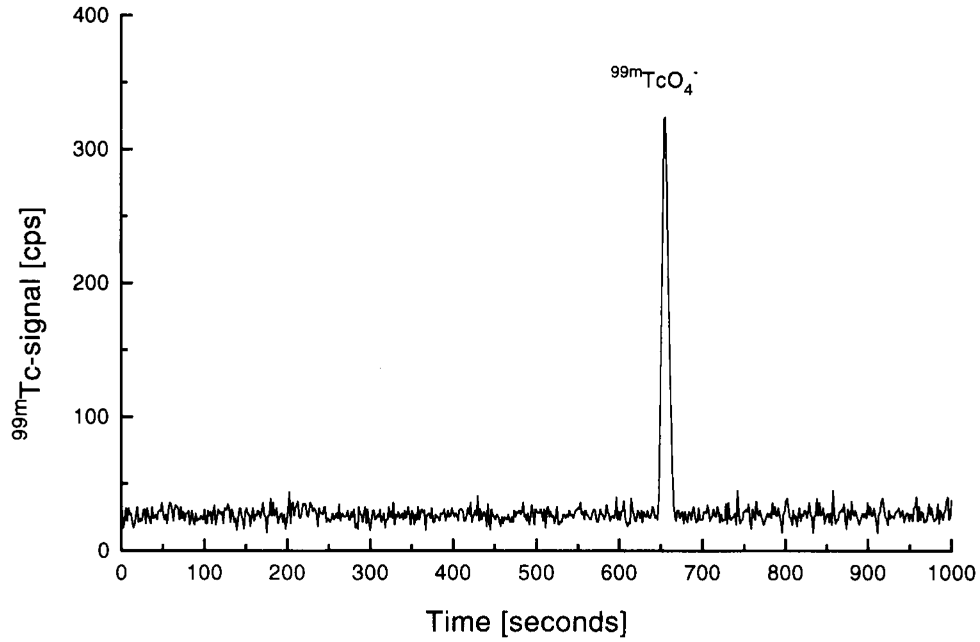

Figure 2 *Electropherogram of $^{99m}TcO_4^-$. Operating conditions: –10 kV across a 75 μm × 61.5 polyacrylamide coated capillary filled with 25 mmol L^{-1} MES, 15 mmol L^{-1} NaCl/NaOH, pH 5.2. Sample introduction 3 s at 50 mbar.*

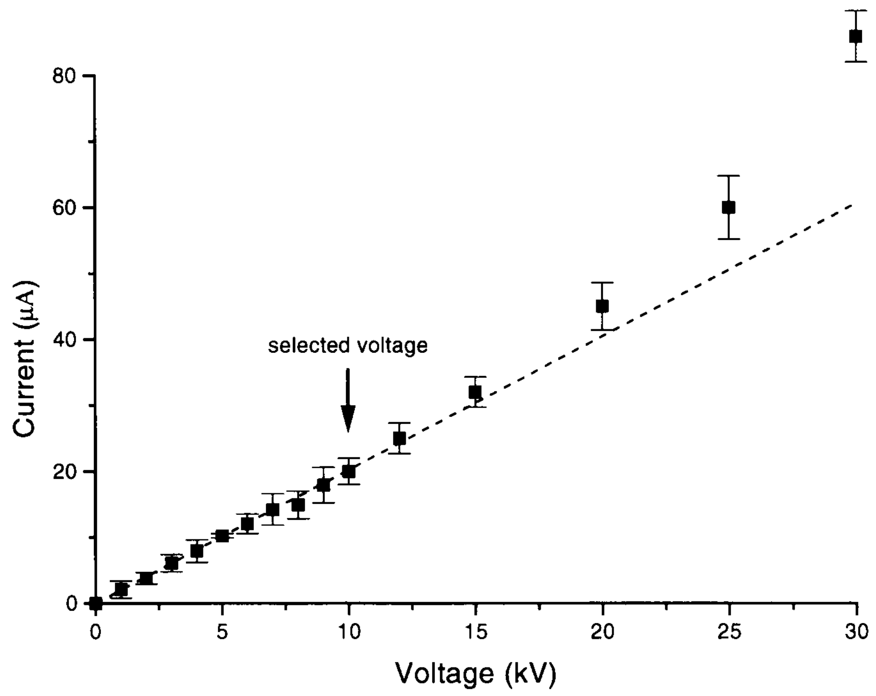

Figure 3 *An Ohm's law plot (current vs. voltage) to monitor Joule heating. Operating conditions: 75 μm × 61.5 polyacrylamide coated capillary filled with 25 mmol L^{-1} MES, 15 mmol L^{-1} NaCl/NaOH, pH 5.2. Sample introduction 3 s at 50 mbar.*

reason for the absence of ^{99m}Tc-recovery data in the paper by Altria *et al.* (1990). The line drawn in Figure 4, is generated by fitting the function $y = a \times z^{-1}$, with y = detector response and z = compound velocity. The data fitted well, as indicated by a χ^2 of 1.2.

Determination of mobilities and column efficiency

The separation power and efficiency of CZE may be expressed in terms of the compound-specific parameters, *i.e.* effective mobility μ_{eff} and the height equivalent to a theoretical plate (HETP). The latter parameter permits comparison between the performance of CZE with other separation techniques and gives a direct indication of the technique potentials in Tc-compound separations.

The effective mobility can be given as:

$$\mu_{eff} = \mu_a + \mu_{EOF}$$

where μ_a is the apparent mobility in the presence of an EOF, and μ_{EOF} is the velocity of a neutral marker which

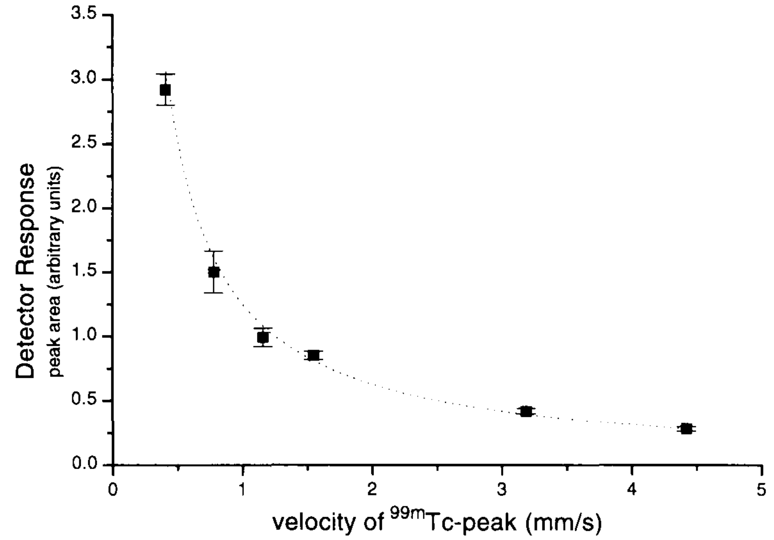

Figure 4 *The peak area of the detector response as a function of the velocity of the $^{99m}TcO_4^-$ peak. Operating conditions: 75 μm × 61.5 polyacrylamide coated capillary filled with 25 mmol L^{-1} MES, 15 mmol L^{-1} NaCl/NaOH, pH 5.2. Sample introduction 3 s at 50 mbar.*

moves at a velocity equal to the EOF. (Kuhn and Hoffsjetter-Kuhn, 1993). Using mesityl oxide as a neutral marker, the polyacrylamide coated capillary could be shown as exhibiting a zero EOF velocity, thus $\mu_{eff} \equiv \mu_a$ under the conditions applied.

Therefore, μ_{eff} values for ^{99m}TcO$_4^-$, ^{99m}Tc–DTPA, ^{99m}Tc–citrate, and of the free citrate ligand were calculated as:

$$\mu_{eff} = \mu_a = (L_d \times L_0) / (t_m \times V),$$

with L_d as the length of the column between inlet and detector, L_0 as the total column length, t_m as the migration time, and with V as the applied voltage.

The HETP values for the various compounds were calculated as HETP = L_0/N, with N as the number of theoretical plates, in turn measured as $N \equiv 5.54\ (t_m/w_{1/2})^2$, with $w_{1/2}$ as the peak width at half maximal height.

Data of μ_{eff} and HETP are shown for both CZE and SE–HPLC in Table 1, suggesting superior CZE characteristics.

The analysis of ^{99m}TcO$_4^-$, ^{99m}Tc–DTPA, and ^{99m}Tc–citrate

Figures 2, 5 and 6a show the ^{99m}TcO$_4^-$, ^{99m}Tc-DTPA and ^{99m}Tc-citrate signals obtained in the polyacrylamide capillary, using the above recommended operating conditions. Figure 2 presents the sharp peak for ^{99m}TcO$_4^-$ after 660 seconds. The data were in agreement with the anionic character of pertechnetate, in the sense that the mobility was from the cathodic to the anodic side. The low HETP-value for this peak (Table 1) indicates the separation power of CZE. Although this value is only half that of SE–HPLC, the HETP-values are relatively high compared to ordinary CZE, primarily due to the high injection volumes necessary to obtain adequate activity signals for ^{99m}Tc. Table 1 presents the recoveries obtained in CZE: for pertechnetate this recovery is near 100%, indicating the absence of any losses due to chemical reactions.

The electropherogram of ^{99m}Tc–DTPA (Figure 5) is in agreement with an even better HETP-value if compared to SE–HPLC (Table 1). As for pertechnetate, the ^{99m}Tc–DTPA data agree with the idea of a negatively charged Tc-complex (Harms *et al.*, 1996a). Again, recoveries were obtained as near 100 % (Table 1), suggesting the stability of the ^{99m}Tc–DTPA complex.

Figure 6a gives the results for the relatively labile ^{99m}Tc–citrate, corresponding to a negatively charged ^{99m}Tc-complex. In contrast to the sharp peaks for ^{99m}TcO$_4^-$ and ^{99m}Tc-DTPA, a strong tailing was observed in the electropherogram of ^{99m}Tc–citrate, which proba-

Table 1 *The recoveries, mobilities and HETP-values of both the ^{99m}Tc–marker species, and the free citrate in CZE with the standard deviations (n=4).*

	^{99m}TcO$_4^-$	^{99m}Tc–DTPA	^{99m}Tc–citrate	Free citrate
Recovery (%)	98 ± 7	96 ± 5	69 ± 17	ND
Mobility (10^{-4} cm^2 s^{-1} V–1)	3.5 ± 0.2	1.4 ± 0.1	2.9 ± 0.3	4.1 (0.03)
HETP CZE (mm)	0.0235 ± 0.013	0.0169 ± 0.008	ND	ND
HETP SE-HPLC (mm)	0.025[a]	0.042[a]	NA	NA

ND = not detectable
NA = not available
[a]Personal comment by A.V. Harms (Harms *et al.*, 1996).

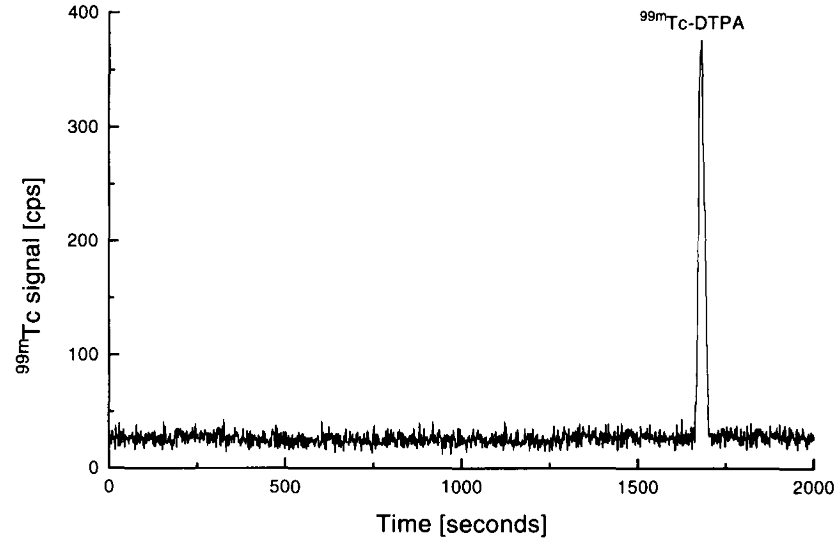

Figure 5 *Electropherogram of* ^{99m}*Tc–DTPA. Operating conditions: –10 kV across a 75 μm × 61.5 polyacrylamide coated capillary filled with 25 mmol L*$^{-1}$ *MES, 15 mmol L*$^{-1}$ *NaCl/NaOH, pH 5.2. Sample introduction 3 s at 50 mbar.*

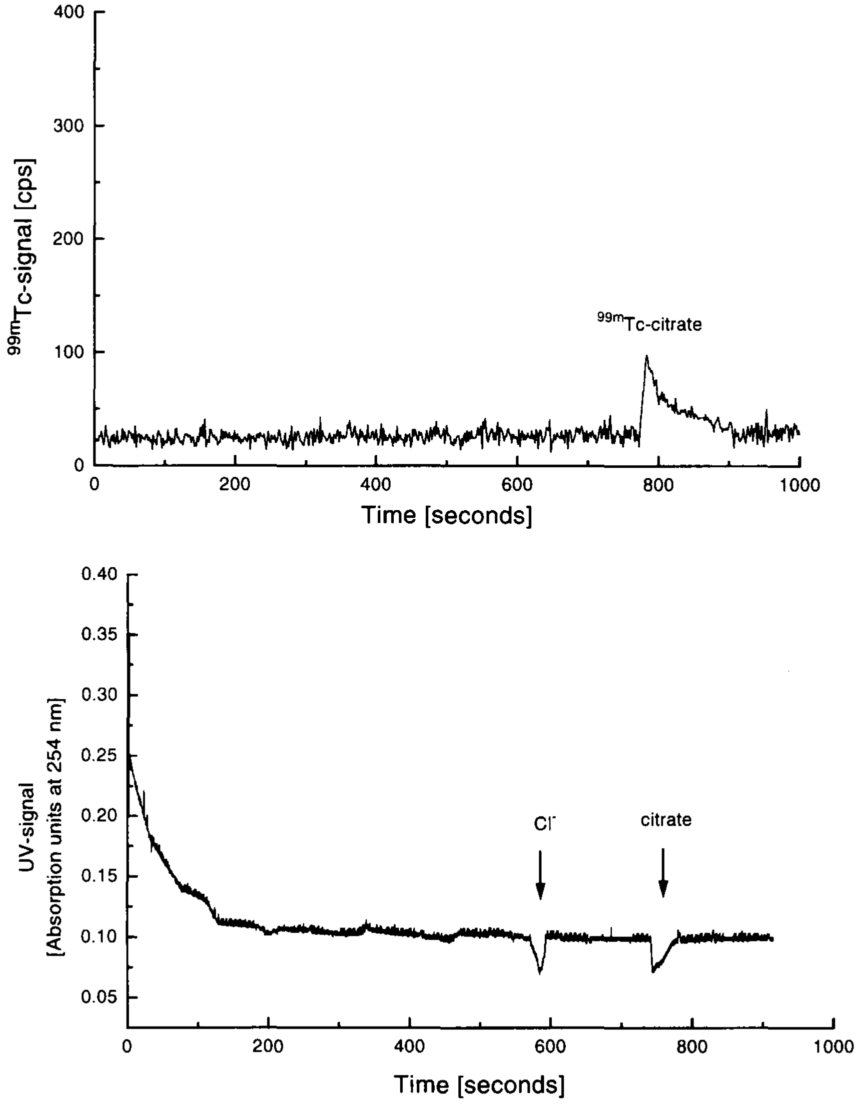

Figure 6 *Electropherograms of both* ^{99m}*Tc-citrate (a) and free citrate (b) using the gamma-detector and the indirect UV approach, respectively. Operating conditions: –10 kV across a 75 μm × 61.5 polyacrylamide coated capillary filled with 25 mmol L*$^{-1}$ *MES, 10 mmol L*$^{-1}$ *NaCl/NaOH and 5 mmol L*$^{-1}$ *potassium chromate, pH 5.2. Sample introduction 3 s at 50 mbar.*

bly indicates a progressive disproportionation of the Tc^V–complex in $^{99m}Tc^{VII}O_4^-$ and relatively high-molecular Tc^{IV}–citrate (Münze *et al.*, 1983). The latter is also confirmed by both the 67% recoveries of ^{99m}Tc-citrate (Table 1) and the slightly lower mobility of Tc^{IV}–citrate compared to Tc^Vcitrate in gel electrophoresis (Münze *et al.*, 1983). The progressive disproportionation of Tc^V–citrate may be related to the different mobility of the free citrate ligand (Figure 6b and Table 1).

Although the recovery and peak efficiency for the relatively labile ^{99m}Tc–citrate are lower than for $^{99m}TcO_4^-$ and ^{99m}Tc–DTPA, the data in Table 1 clearly show that the three markers can be separated. The differences in mobility are relatively high, also permitting a distinguishment of ^{99m}Tc-complexes with intermediate migration times. The specific data of ^{99m}Tc–citrate suggest that with this marker a threshold lability is reached for the use of CZE in separation procedures in plant Tc-speciation studies.

Future CZE work on Tc-speciation

The present study indicates the good performance and separation power of CZE for various Tc-compounds. With Tc–citrate, a threshold lability is reached for the applications of the CZE technique in Tc speciation studies.

Future work will comprise the synthesis and tests on Tc-compounds of intermediate mobilities and stabilities, all meant to expand the calibration of the CZE-system, which is regarded as necessary for a further optimization of procedures for analysis, sampling and storage of (plant) Tc-compounds.

In this context, CZE will be also investigated for the effects of marker matrices and of plant matrix material on the separation and recoveries of Tc-compounds.

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