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Capillary electrophoretic and thin-layer chromatographic characterization of rhenium complexation with 1-hydroxyethylidenediphosphonic acid

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Abstract

1-Hydroxyethylidenediphosphonic acid (HEDP) labeled by short-lived radionuclides with the nuclear properties suitable for the therapeutical purposes (^{186}Re, ^{188}Re, ^{166}Ho) is similar to the other phosphonates widely applied in the radiopharmaceutical field for the treatment of palliative bone metastases. One of the important steps for the preparation of compounds of radiopharmaceutical interest is the quality control comprehending the radiochemical and chemical purity determination. Chromatographic methods as TLC and HPLC are mostly used for this purpose. Our experiments were focused on the application of capillary electrophoresis with UV detection to the study of rhenium complexation with HEDP. The influence of pH, concentration of the ligand and the reaction time were determined. Taking in account our previous results, the Re:$SnCl_2$ molar ratio 1:500 (for 0.1 m*M* Re) was applied to reduce perrhenate to lower oxidation states which enables the Re–HEDP complexation. Different background electrolytes were tested. The mixture of 40 m*M* Na_2HPO_4 with 15 m*M* HEDP adjusted to pH 8 was selected as the most suitable system because it enabled the separation of different forms of Re–HEDP complexes. The results obtained in this study were compared to those obtained by thin-layer chromatography with radiometric detection.

Keywords: Rhenium; Metal complexes; Hydroxyethylidenediphosphonic acids; Phosphonic acids

1. Introduction

Rhenium isotopes ^{186}Re and ^{188}Re are commonly applied as labeling agents in nuclear medicine. Both isotopes are suitable for therapeutic use by means of β-irradiation. The maximum range of ^{186}Re (E_β = 1.07 MeV) in tissues is about 5 mm which means that it is convenient for the application to the small tumors. On the other hand, ^{188}Re (E_β = 2.12 MeV) reaches higher range (10–11 mm) in tissues and it is more appropriate for tumors of large masses [1,2]. The selection of isotope is also governed by technical aspects of their production and by their half-life [3–5].

The preparation of complex compounds labeled with ^{186}Re and ^{188}Re is more complicated than in case of complexes of monovalent ions (e.g. holmium, yttrium) because of the necessity to lower the oxidation state of rhenium by reduction using $SnCl_2$ for successful complex formation. Derivatives of phosphonic acid e.g. methylenediphosphonic acid

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(MDP) and hydroxyethylidenediphosphonic acid (HEDP) belong to the group of the most common ligands for rhenium labeling. While the complexes of rhenium analogue technetium with diphosphonate ligands are widely used for the imaging and diagnosis of bone disease and most especially metastatic bone cancer, the complexes of radioactive rhenium (^{186}Re, ^{188}Re) with 1,1-hydroxyethylidenediphosphonic acid have been shown to be the effective palliatives for the treatment of the intense pain associated with this disease [6,7].

One of the important steps for the preparation of compounds of radiopharmaceutical interest is the quality control comprehending the radiochemical and chemical purity determination. Radiochemical purity (radiochemical yield of complexation) is usually performed by the thin-layer chromatography with radiometric detection [8,9].

Despite extensive studies of the synthesis and the composition of rhenium diphosphonates, very little is actually known about the formulation and structure of these medically important agents, largely because these substances are not sufficiently stable to allow analytical HPLC separations [10], nor have been able to grow suitable single crystals for an X-ray structure determination. The EXAFS spectroscopy (extended X-ray absorption fine structure) was successfully applied for structural characterization of model rhenium diphosphonate complexes [11] synthesized via reduction of perrhenate by different excess of stannous chloride.

Capillary electrophoresis (CE) with UV and proton-induced X-ray emission (PIXE) detection was recently carried out to study the kinetics of decomposition of Re(III)–HEDP complex [12] and to study the perrhenate reduction mechanism [13].

The studies described in this paper are focused on the application of CE in the field of the characterization of rhenium complexation with HEDP. Different background electrolytes were tested for this purpose. The influence of pH, concentration of the ligand and the reaction time were determined. Taking in account our previous results [13], the Re:$SnCl_2$ molar ratio 1:500 (for 0.1 m*M* Re) was applied to reduce perrhenate to lower oxidation states which enable the Re–HEDP complexation. The results obtained in this study were compared to those obtained by thin-layer chromatography (TLC) with radiometric detection.

2. Experimental

2.1. Chemicals

Chemicals used in these studies were from the following sources: $KReO_4$, HEDP (both Fluka, Buchs, Switzerland); $SnCl_2$, boric acid, acetic acid, phosphoric acid, Na_2HPO_4, HCl and NaOH (all Lachema, Brno, Czech Republic). All chemicals were of analytical reagent grade.

^{186}Re for the experiments of TLC with radiometric detection was prepared by irradiation of 10.5 mg of potassium perrhenate in the reactor LVR-15 (Nuclear Research Institute, Řež, Czech Republic) with a capacity of 9 MW for 82.5 h. The stock solution acquired by dissolving of irradiated perrhenate in 1 ml of distilled water was used for labeling experiments.

The samples for the determination of the influence of pH (range 2–10) on the Re–HEDP complexation were prepared by mixing of calculated volumes of 5 m*M* $KReO_4$, 0.5 *M* $SnCl_2$ and 0.25 *M* HEDP stock solutions to get the Re:$SnCl_2$:HEDP ratio 1:500:500 for the final rhenium concentration 0.1 m*M*. The Re:$SnCl_2$ ratio was selected on the basis of the results of the perrhenate reduction study [13]. After pH adjustment by 1 *M* NaOH the sample volume was made up to 5 ml by Milli-Q water. Reaction mixtures were incubated for 60 min at room temperature followed by filtration through the 0.45-μm filtration disc (Millex HV, Millipore, Prague, Czech Republic). Samples obtained in this way were directly injected for CE analysis.

In the case of the studies of the influence of HEDP concentration and the kinetics of decomplexation of rhenium complexes two representative pH values were selected—pH 2 and 5. The samples containing HEDP in the varying concentration were prepared similar way as the samples for the influence of pH. The concentrations of Re, $SnCl_2$ and HEDP in final solution were 0.1, 50 and 0–100 m*M*, respectively. The kinetics of the complexation and decomplexation of rhenium and HEDP was measured in the sample containing Re, $SnCl_2$ and HEDP in the ratio of 1:500:500. The yield of the complexation was measured after 0–6 h and, subsequently, after 1–5 days.

The samples for the TLC experiments were pre-

pared as described above except the stock solution of $K^{186}ReO_4$ was applied for the preparation.

All experiments were done in triplicate. The RSD values of data measured by both CE and TLC did not exceed 5%.

2.2. Capillary electrophoresis

Electrophoretic experiments were performed in an untreated fused-silica capillary [48 cm long (39 cm to the detector)×75 μm I.D.] purchased from Composite Metal Services (The Chase Hallow, Worcester, UK) mounted in the CAPEL 105 capillary electrophoresis system (Lumex, St. Petersburg, Russia). Detection was done by UV detector set to 214 nm. Before analysis the capillary was conditioned by sequentially washing with water (5 min), 0.1 *M* HCl (5 min), water (5 min), 1.0 *M* NaOH (5 min), water (5 min) and the run buffer (3 min). Between individual runs the capillary was rinsed by run buffer for 5 min. The samples were injected into the capillary hydrodynamically (1.5 kPa/30 s) by setting the electrophoresis apparatus to the requested value and the analyses were run at reverse polarity, −10 kV, at 20 °C for 20–30 min.

A 40 m*M* Britton–Robinson buffer solution (an equimolar mixture of boric, acetic and phosphoric acids, pH 2–10) and a set of 40 m*M* phosphate buffers of different pH values (6–8.5) and various concentration of HEDP (0–25 m*M*) were tested to find the most suitable run electrolyte for the Re–HEDP analysis. The former was previously successfully applied in the study of perrhenate reduction [13].

2.3. TLC with radiometric detection

The combination of the TLC on the silica-gel based system ITLC-SG Gelman/acetone and paper chromatography on Whatman No.1/0.9% saline was applied for the determination of the radiochemical yield of complexation of rhenium with HEDP. The former chromatographic system allowed to separate free perrhenate ($R_F = 1$) from Re–HEDP complex and reduced rhenium ReO_2 ($R_F = 0$). The latter one separated reduced ReO_2 ($R_F = 0$) from free perrhenate and Re–HEDP complex ($R_F = 0.6$–0.9) [9]. The detection was performed by the radio-TLC scanner (Mini-Scan TLC Radiochromatography System, Bioscan, Washington, DC, USA) with a scintillation detector BFC 3600 controlled by TLC software LAURA VERSION 1.5.

3. Results and discussion

3.1. Buffer selection

First of the run electrolytes tested for the Re–HEDP analysis, 40 m*M* Britton–Robinson buffer solution, was chosen for its excellent buffering capacity over a wide interval of pH values (pH 2–10) and for its previous successful application in the perrhenate reduction study [13]. Nevertheless, as demonstrated in Fig. 1, rhenium complexation with HEDP causes just the additional decrease of the perrhenate peak area (lowered already as the result of perrhenate reduction) and no peak, which would belong to rhenium complexes, appears. Similar re-

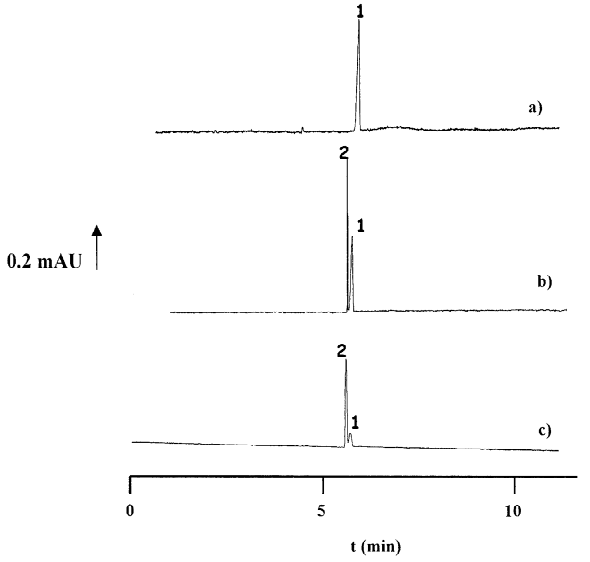

Fig. 1. Electropherograms of rhenium compounds in Britton–Robinson buffer 1=ReO_4^-, 2=unknown; (a) 0.1 m*M* $KReO_4$; (b) perrhenate reduction (0.1 m*M* $KReO_4$+50 m*M* $SnCl_2$, pH 2.5, reaction time 1 h); (c) rhenium complexation with HEDP (0.1 m*M* $KReO_4$+50 m*M* $SnCl_2$+50 m*M* HEDP, pH 2.5, reaction time 1 h). Experimental conditions: BGE 40 m*M* Britton–Robinson buffer, pH 2, injection 30 s/1.5 kPa, −10 kV, 214 nm.

sults were obtained for the whole pH range studied. Thus, this run electrolyte is, from the point of view of rhenium complexation, convenient for the determination of the total yield of reduction and complexation with HEDP because it is not feasible to quantify the percentage of Re–HEDP in the reaction mixture from the decrease of the perrhenate peak area. Due to these facts, 40 m*M* Britton–Robinson buffer solutions at pH 2–10 were applied for the study of the influence of pH.

Testing of other buffers showed more-or-less comparable electropherograms where rhenium complexation was possible to observe only via the decrease of the perrhenate peak area and no peak of rhenium complexes appeared. One of the reasons for this behavior could be, similarly to the problems with the HPLC separation of Re–HEDP [10], the insufficient stability of rhenium complexes during the electrophoretic separation. Thus the run buffers with the addition of HEDP for the stabilization of rhenium complexes were tested. The interaction of rhenium with HEDP from the run buffer should be negligible in this case due to the presence of a high excess of HEDP over rhenium in the reaction mixtures, necessary to get the optimum complexation yield, as will be discussed below. A 40 m*M* Na_2HPO_4 buffer at pH 8 was selected as a background electrolyte after preliminary experiments and the optimum concentration of HEDP in the buffer was determined as demonstrated in Fig. 2. When 15–25 m*M* HEDP is added in the run buffer, three well-defined peaks corresponding to Re–HEDP complex compounds appear [probably a mixture of Re(III), Re(IV) and Re(V)]. These results support the theory about the complex character of rhenium–HEDP compounds in the radiopharmaceutical preparation.

Considering the results discussed above, a run buffer containing 40 m*M* Na_2HPO_4 + 15 m*M* HEDP, pH 8, helps the sufficient stabilization of Re–HEDP compounds during the electrophoretic separation. Thus, it was applied for the study of the influence of HEDP concentration on the yield of rhenium complexation and for the study of the kinetics of complexation and decomplexation. A quantification of results was made from the change of the perrhenate peak area because the intensities of the peaks of Re–HEDP complexes were low in comparison with perrhenate and the linear relation between the rhenium concentration and the areas of Re–HEDP complexes was not proved.

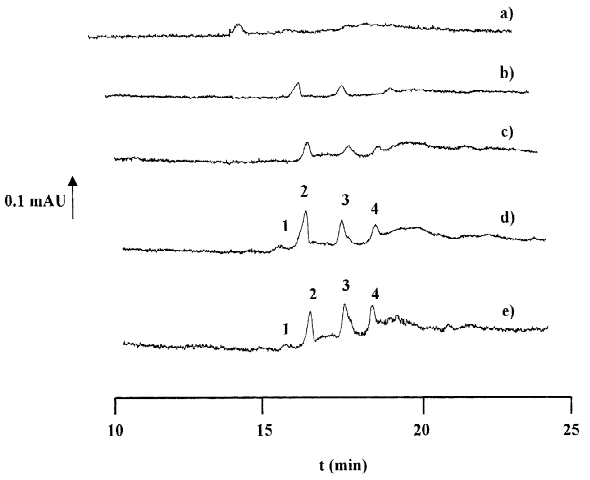

Fig. 2. Electropherograms of Re–HEDP separation; influence of HEDP concentration in run buffer; 1 = ReO_4^-; 2–4 = Re–HEDP complex compounds; electrolytes: 40 m*M* Na_2HPO_4, pH 8+ (a) 0 m*M* HEDP; (b) 5 m*M* HEDP; (c) 10 m*M* HEDP; (d) 15 m*M* HEDP; (e) 25 m*M* HEDP. Samples: 0.1 m*M* $KReO_4$ + 50 m*M* $SnCl_2$ + 50 m*M* HEDP, pH 5.5, reaction time 1 h. Analysis: injection 30 s/1.5 kPa, −10 kV, 214 nm.

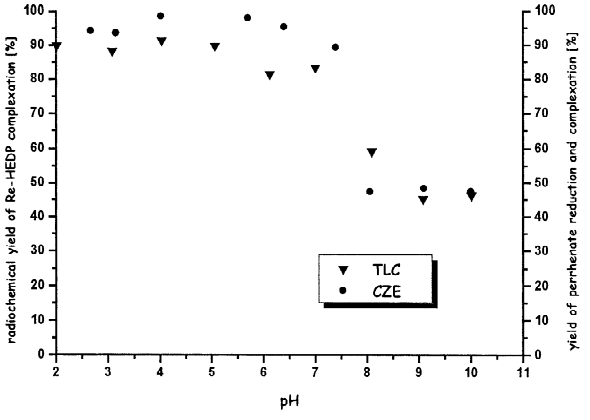

Fig. 3. Influence of pH on the Re–HEDP complexation; radiochemical yield of Re–HEDP complexation measured by TLC; total yield of perrhenate reduction and complexation determined by CZE. CZE: samples, 0.1 m*M* $KReO_4$ + 50 m*M* $SnCl_2$ + 50 m*M* HEDP, pH 2–10, reaction time 1 h. BGE: 40 m*M* Britton–Robinson buffer, pH 2–10, injection 30 s/1.5 kPa, −10 kV, 214 nm. TLC: ITLC-SG/acetone; Whatman No.1/0.9% NaCl; for samples and other experimental conditions see Ref. [9].

3.2. Influence of pH

The influence of pH on the yield of rhenium complexation with HEDP is illustrated in Fig. 3. The dependencies measured both by CE and by TLC with radiometric detection [9] have similar character. The data obtained by CE reach slightly higher values in comparison with TLC data because they include not only the complexation yield but also the yield of perrhenate reduction. Nevertheless, these results show that it was proved by both capillary zone electrophoresis (CZE) and TLC methods that rhenium–HEDP complexes are stable over a wide range of pH values (2–7) where the maximum complexation yield (>80%) and total reduction and complexation yield (>90%) are reached.

3.3. Influence of ligand concentration

Data describing the influence of HEDP concentration are shown in Fig. 4 for two selected pH values (pH 2 and 5). While the radiochemical yield of Re–HEDP complexation measured by TLC with radiometric detection is almost insignificant for Re:HEDP ratio up to 1:250 and it reaches its maximum (75–85%) at Re:HEDP ratio from 1:500 to 1:1000, the total yield of reduction and complexation measured by capillary electrophoresis ranges from 70 to 85% (up to Re:HEDP = 1:100) to 85–95% for Re:HEDP ratio higher than 1:100. This difference is caused by the fact that the data obtained by CE include the influence of both reduction and complexation of rhenium. It can be seen from these results, that when the Re:HEDP ratio is lower than 1:500 the complexation of reduced rhenium with HEDP is minimal and the colloid ReO_2 is formed. Data are comparable for both pH values studied. Thus the Re:HEDP ratio 1:500 was chosen as the optimum for the other experiments.

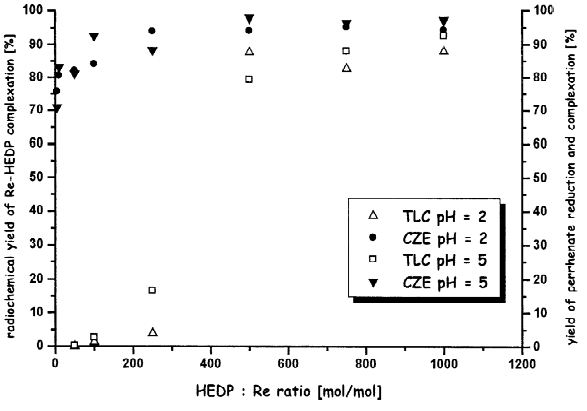

Fig. 4. Influence of HEDP concentration on the Re–HEDP complexation; radiochemical yield of Re–HEDP complexation measured by TLC; total yield of perrhenate reduction and complexation determined by CZE. Samples: Re concentration = 0.1 m*M*, $SnCl_2$ concentration = 50 m*M*, HEDP concentration = 5–100 m*M*, pH 2.0–2.4 and 5.0–5.4, reaction time 1 h. CZE: BGE 40 m*M* Na_2HPO_4 + 15 m*M* HEDP, pH 8; injection 30 s/1.5 kPa, −10 kV. TLC: ITLC-SG/acetone; Whatman No.1/0.9% NaCl; for other experimental conditions see Ref. [9].

3.4. Kinetic studies of Re–HEDP

The time dependence of rhenium complexation for pH 2 and 5 is shown in Fig. 5a (t = 1–6 h) and 5b (1–5 days). Both capillary electrophoresis and TLC with radiometric detection confirmed that 1 h is a sufficient time to get a complexation yield of around 80% and higher and thus 1 h reaction time is convenient for the preparation of Re–HEDP radiopharmaceuticals. While the complexes prepared at pH 2 are stable for 5 days (their complexation yield reaches more than 80%), there can be observed a decrease of the complexation yield after the second day of contact of reactants when samples prepared at pH 5. Nevertheless, the stability of Re–HEDP complexes is sufficient for radiopharmaceutical purposes because from the radiopharmaceutical point of view a stability of the order of hours is required. Results obtained by both analytical methods used are comparable; differences are caused by the inclusion of rhenium reduction to the yield determined by CE. The data measured at pH 5 after third day of contact of reactants showed that the decomplexation of Re–HEDP is faster than the reoxidation of reduced rhenium.

3.5. Character of Re–HEDP complex compounds

The identification of individual peaks in elec-

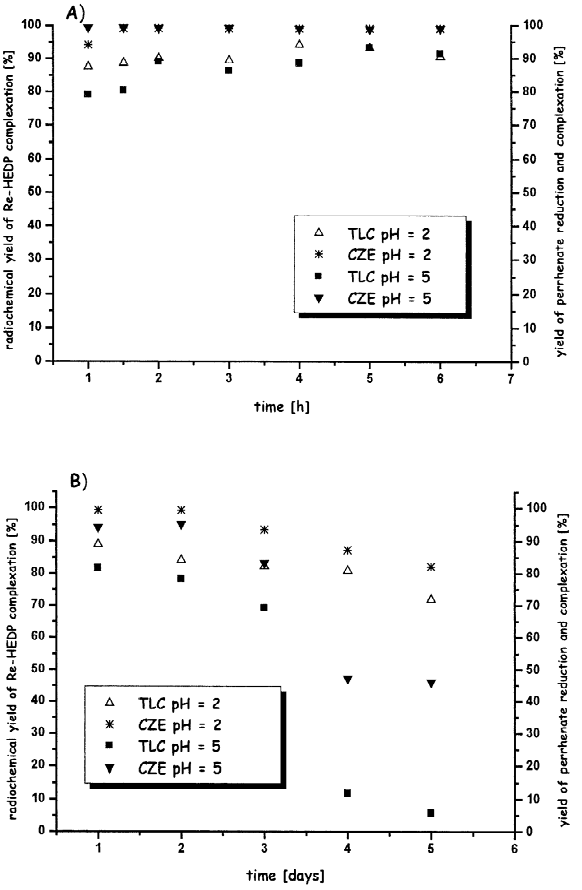

Fig. 5. Kinetics of the Re–HEDP complexation and decomplexation; radiochemical yield of Re–HEDP complexation measured by TLC; total yield of perrhenate reduction and complexation determined by CZE. Samples: Re concentration = 0.1 m*M*, $SnCl_2$ concentration = 50 m*M*, HEDP concentration = 50 m*M*, pH 2.0–2.4 and 5.0–5.4, reaction time (A) 0–6 h, (B) 1–5 days. CZE: BGE −40 m*M* Na_2HPO_4 + 15 m*M* HEDP, pH 8; injection 30 s/1.5 kPa, −10 kV, 214 nm. TLC: ITLC-SG/acetone; Whatman No.1/0.9% NaCl; for other experimental conditions see Ref. [9].

tropherograms is illustrated in Fig. 6. When free perrhenate is injected, a single sharp peak appears as is shown in Fig. 6a. After the reduction by stannous chloride the perrhenate peak area decreases (Fig. 6b) and three peaks corresponding to Re–HEDP compounds appear after HEDP addition to the system (Fig. 6c). Their identification was supported by Fig. 6d where higher concentration of perrhenate was added in the reaction mixture and it caused an increase of Re–HEDP peak areas. Fig. 6e shows the

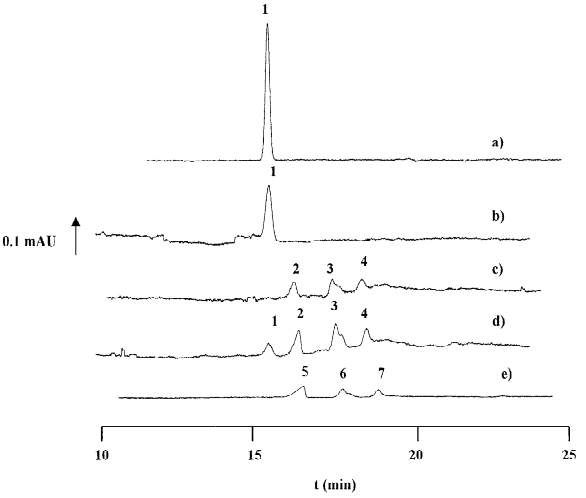

Fig. 6. Electropherograms of Re–HEDP analysis in phosphate buffer; 1 = ReO_4^-; 2–4 = Re–HEDP complex compounds; 5–7 = Sn–HEDP compounds (a) 0.1 m*M* $KReO_4$; (b) 0.1 m*M* $KReO_4$ + 50 m*M* $SnCl_2$, pH 5.5, 1 h; (c) 0.1 m*M* $KReO_4$ + 50 m*M* $SnCl_2$ + 50 m*M* HEDP, pH 5.5, 1 h; (d) 0.7 m*M* $KReO_4$ + 50 m*M* $SnCl_2$ + 50 m*M* HEDP, pH 5.5, 1 h; (e) 50 m*M* $SnCl_2$ + 50 m*M* HEDP, pH 5.5, 1 h. BGE: 40 m*M* Na_2HPO_4 + 15 m*M* HEDP, pH 8; injection 30 s/1.5 kPa, −10 kV, 214 nm.

reaction mixture containing stannous chloride and HEDP where three peaks of Sn–HEDP complexes with the same migration times as Re–HEDP complexes appeared. This situation indicates the complex character of rhenium–HEDP compounds prepared by the procedure identical with the technique used for radiopharmaceutical preparation. The reaction mixture contains at least three different Re–HEDP complex compounds [obviously a mixture of Re(III), Re(IV) and Re(V)] and the results discussed above indicated the possible incorporation of Sn into the structure of rhenium complexes. This assumption is in the agreement with information published in the literature [11].

4. Conclusions

CE proved its applicability in the field of Re–HEDP complexation studies. Both CE and TLC with radiometric detection confirmed the optimum pH interval for the Re–HEDP complexes preparation (pH 2–7), composition of the reaction mixture

(Re:HEDP = 1:500 at least) and the time stability of Re–HEDP (2–5 days after the contact of reactants). The results obtained by CZE and TLC with radiometric detection are comparable; the differences are caused by the inclusion of rhenium reduction to the yield determined by CE.

The CE experiments demonstrated that in the reaction mixture at least three different forms of rhenium complexes with HEDP are formed. The results indicated the possible incorporation of Sn from stannous chloride into the structure of rhenium complexes. These observations can be applied in the field of the chemical and radiochemical purity control of radiopharmaceuticals on the basis of phosphonates labeled by ^{186}Re and ^{188}Re.

Acknowledgements

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