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Short communication

Capillary electrophoretic analysis of ethylene dicysteine, a precursor of the radiopharmaceutical ^{99m}Tc ethylene dicysteine

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Abstract

L,L-Ethylene dicysteine (EC) is a ligand used in nuclear medicine for the preparation of ^{99m}Tc-L,L-EC, a tracer agent for renal function studies. A capillary zone electrophoretic method is described which allows us to monitor the purity of ethylene dicysteine, for which no other suitable method has been reported to date. A fused-silica capillary (L=70 cm, l=62 cm, 75 μm I.D.) was used with 10 m*M* sodium tetraborate, pH 10.0, as the background electrolyte. The voltage applied was 20 kV and the temperature was 25°C. Benzyl alcohol served as the internal standard. The limit of detection was 0.07% and the limit of quantitation was 0.15%. ©1997 Elsevier Science B.V.

Keywords: Ethylene dicysteine

1. Introduction

The complex of ^{99m}Tc with L,L-ethylene dicysteine (^{99m}Tc-L,L-EC) has recently been developed as a useful and practical tracer agent for renal function studies [1,2]. Ethylene dicysteine (EC) is synthesized via ring opening of thiaproline (or L-thiazolidine-4-carboxylic acid) and subsequent dimerization of the intermediate radical in Na/NH_3 [3] (Fig. 1). At present, there is no suitable method for monitoring the chemical purity of the synthesized compound. However, reliable analysis of the precursor of EC is strictly required prior to the use of the compound for preparation of the radiopharmaceutical.

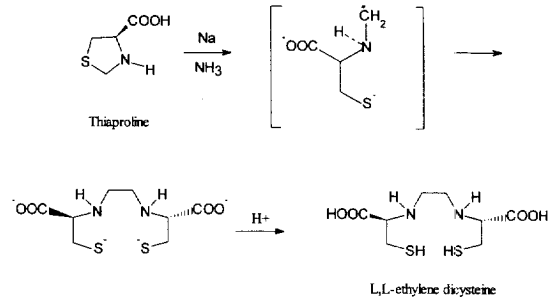

Fig. 1. Scheme for the synthesis of L,L-ethylene dicysteine.

Apart from the starting material, thiaproline, EC might contain side products from synthesis, such as N-methyl-L-cysteine and L-cysteine.

Thin-layer or liquid chromatography have been unsuccessful to date due to the high polarity of the compound. EC can actually be considered as a bis-

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amino acid with six functional groups (two labile thiols, two carboxylates and two amines). It has a very low solubility at pH values below 9.

In this paper, we investigated the usefulness of capillary electrophoresis (CE) for the analysis of this very polar, acidic substance. Since a reference standard of EC does not exist, no assay of the compound was attempted. The study was thus restricted to purity control, and it focused on the determination of electrophoretic mobilities for the several potential impurities and determination of the limits of detection and quantitation. Furthermore, the stability of EC in aqueous medium was monitored as a function of time.

In daily radiopharmaceutical practice, EC is used in the form of a kit containing EC monohydrochloride and stannous chloride. Therefore, the influence of stannous ions was also investigated.

2. Experimental

CE was performed on Spectraphoresis 500 equipment (Thermo Separation Products, Fremont, CA, USA), coupled to a 3396 series II integrator (Hewlett-Packard, Avondale, PA, USA). The UV absorption detector was set at 235 nm and injection was done hydrodynamically for 1 s. Fused-silica capillaries were from Polymicro Technologies (Phoenix, AZ, USA). Measurement of pH values was carried out using a Consort pH meter (Turnhout, Belgium) with calibration buffers prepared according to the European Pharmacopoeia [4]. The pH of running buffers was adjusted using 0.1 *M* NaOH before making up to volume. Throughout the study, all samples were dissolved in running buffer.

All reagents and commercially available reference substances were of analytical grade (Merck, Darmstadt, Germany or Acros Chimica, Geel, Belgium). L,L-Ethylene dicysteine and N-methyl-L-cysteine were synthesized as described [3]. Labelling kits for the preparation of ^{99m}Tc-EC contained the lyophilized residue of 0.5 mg ethylene dicysteine monohydrochloride and 100 μg of $SnCl_2.H_2O$ in 1 ml of 170 m*M* phosphate buffer, pH 12. Throughout the study, Milli-Q^{50} water was used (Millipore, Milford, MA, USA). All solutions were filtered through 0.2-μm nylon filters (Euroscientific, Lint, Belgium).

3. Results and discussion

On the basis of preliminary experiments with different types of background electrolytes, a capillary zone electrophoretic method was developed that allows us to distinguish EC from the above-mentioned potential contaminants. S-Methyl-L-cysteine was included in this series for comparison. A fused-silica capillary (L=70 cm, l=62 cm, 75 μm I.D.) was used with 10 m*M* sodium tetraborate, pH 10.0, as the background electrolyte. The voltage applied was 20 kV and the temperature was 25°C. The sample was dissolved in benzyl alcohol stock solution [0.3% (v/v) benzyl alcohol in background electrolyte]. Benzyl alcohol served as a neutral marker. It was chosen because of its solubility in water and UV absorption characteristics. Even at pH 10.0, it can serve as a neutral compound because it has the same migration time as acetone. It was preferred over acetone because it is not volatile and could thus serve as an internal standard at the same time. The average current observed was 32 μA. A typical electropherogram of EC is depicted in Fig. 2. Electrophoretic mobilities (in cm^2/V min) for the different substances were: EC, −0.025; thiaproline, −0.020; L-cysteine, −0.020; N-methyl-L-cysteine, −0.018 and S-methyl-L-cysteine, −0.019 (broad), −0.026 (broad).

Although thiaproline and L-cysteine cannot be distinguished from each other, this does not jeopardize the ability of the method to monitor the purity

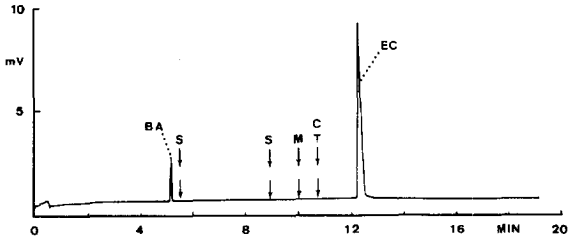


Fig. 2. Electropherogram of ethylene dicysteine dissolved in benzyl alcohol stock solution. Capillary: fused-silica, L=70 cm, l=62 cm, 75 μm I.D.; background electrolyte: 10 m*M* sodium tetraborate, pH 10.0; voltage: 20 kV; temperature: 25°C. Injection: hydrodynamically, for 1 s; detection: UV at 235 nm. BA=benzyl alcohol, EC=ethylene dicysteine, T=thiaproline, C=L-cysteine, M=N-methyl-L-cysteine and S=S-methyl-L-cysteine.

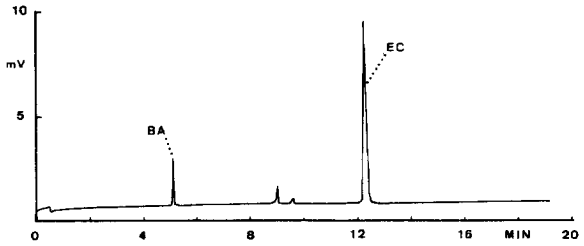


Fig. 3. Electropherogram of the sample used in Fig. 2 after 2 h and 40 min. For analytical conditions see Fig. 2.

of EC. Moreover, the presence of cysteine in EC is not very likely since it cannot be formed during the synthesis. Cysteine could be present only when the starting material thiaproline, prepared by reaction of cysteine with formaldehyde, is not pure. Electrophoretic mobilities of EC were shown to be repeatable. The limit of detection was 0.07% for EC, defined as the percentage of the original concentration (5 mg/ml) which yielded a signal-to-noise ratio of 3. The limit of quantitation was 0.15% in which case the relative standard deviation (R.S.D.) equalled 23% ($n=5$). These percentages were calculated using a sample concentration of 5 mg EC/ml, which is close to the solubility limit of EC in the background electrolyte. Using this solution, the repeatability of the method was evaluated [R.S.D.= 1.2% for the corrected peak area of EC ($n=3$)]. Fortunately, repeatability was not affected as in the case of some aminopolycarboxylic acids [5]. The latter ligands have a high affinity for metal ions and if traces of metal ions from the buffer adsorb onto the capillary wall, the ligand can also get adsorbed, causing poor repeatability.

The stability of EC in an aqueous buffer medium, pH 10.0, was monitored and was found to be poor. It is therefore advisable to analyse freshly prepared solutions. Fig. 3 shows the formation of decomposition products of unknown structure in the solution of EC, which was used to generate Fig. 2, analysed after a storage time of 2 h 40 min.

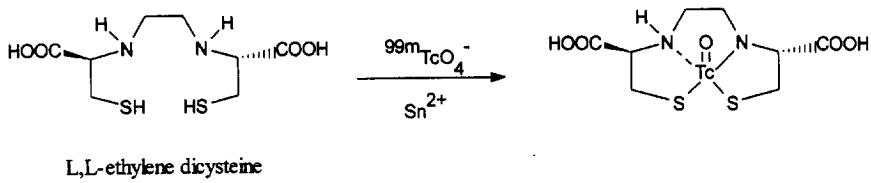
The developed method has been used to analyse samples of different batches of EC. The electropherogram of some samples showed an artefact, which was not observed in all the electropherograms of these samples. The phenomenon consisted of a peak with a surface that was in constant proportion (approx. 5%) to the main peak and with a migration time that was slightly lower than that of N-methyl-L-cysteine. However, the presence of this peak did not affect the corrected area of EC. So far, we have not been able to explain its appearance.

At present, EC is used in nuclear medicine in the form of a kit formulation with the above-mentioned composition. Stannous ions are required in these kits to reduce the heptavalent Tc in $^{99m}TcO_4^-$, which is available from a generator, to the pentavalent oxotechnetium state, which can be complexed by EC.

Previous studies have shown that the complexation of reduced technetium by EC only occurs rapidly (<1 min) and efficiently (>99%) at pH values of at least 11.0. Therefore, a phosphate buffer, pH 12, is used for the preparation of the labelling kits. ^{99m}Tc-L,L-EC (see Fig. 4) is prepared by the addition of 2 to 5 ml of generator eluate containing up to 3700 MBq of $^{99m}TcO_4^-$ in saline to the labelling vial, followed after 30 s by 0.25 ml of 0.02 *M* phosphate buffer, pH 5.

In view of the instability of EC in alkaline medium, the time between addition of the generator eluate and the acidic phosphate buffer during preparation of the radiopharmaceutical should be kept short.

In this study, we also attempted to analyse EC



L,L-ethylene dicysteine

Fig. 4. Radioactive labelling of L,L-ethylene dicysteine with $^{99m}TcO_4^-$.

after its incorporation into a labelling kit containing EC, $SnCl_2$ and phosphate buffer. The electropherograms obtained with such kits, after reconstitution with water, gave a complex picture consisting of broad and split peaks, which was probably due to complexation of stannous ions by EC. CE analysis of a solution of EC in 170 m*M* phosphate buffer, pH 12, did not show broad peaks, but still revealed a splitting of the EC peak. It was supposed that this was due to the buffering capacity of the phosphate buffer, which was higher than that of the background electrolyte. Both buffers are used in the zone of best buffering capacity (pK_a values for phosphate, in the kit, and borate, in the background electrolyte, are 12.4 and 9.2, respectively), and the phosphate buffer concentration was considerably higher (170 m*M* versus 10 m*M*). That the effect is indeed due to inadequate buffering was shown by electrophoresis of a solution of EC in 100 m*M* phosphate buffer, pH 10.0 (same pH as the background electrolyte), and a solution of EC in 0.4 m*M* phosphate buffer, pH 12.0 (same pH as the original kit buffer, but less concentrated), both containing no $SnCl_2$. No peak splitting was observed.

The reported capillary electrophoretic method offers an efficient means of determining the purity of synthesized ethylene dicysteine.

Acknowledgments

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