Kinetic study on ligand-exchange reaction between ethylenedicysteine (EC) and ^{99m}Tc-glucoheptonate (^{99m}Tc-GH)

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Abstract A detailed kinetic study of ligand-exchange reaction between ^{99m}Tc-GH and EC was carried out. The rate constants (*k*) of ligand-exchange reaction at different EC concentrations and different pH values were calculated. The *k* values ($L \cdot mol^{-1} \cdot min^{-1}$) were 3.7×10^3, 4.1×10^3, 3.9×10^3 and 3.9×10^3 at EC concentrations ($\mu mol \cdot L^{-1}$) of 559.7, 279.9, 186.6 and 55.97, respectively, while *k* values were 3.7×10^3, 3.4×10^3, 1.9×10^3 and 3.1×10^2 when pH values were 10, 9, 8 and 7, respectively, and *k* had an increase of the order of magnitude from pH 7 to pH 8. It demonstrated that *k* of the ligand exchange reaction was pH dependent and pH value of the reaction solution must be equal to or little higher than 8 in order to make the labeling yield of ^{99m}Tc-EC higher than 90%.

Keywords ^{99m}Tc-EC, Ligand exchange reaction kinetics, Rate constant

CLC numbers R817, O615

1 Introduction

^{99m}Tc-L, L-ethylenedicysteine（^{99m}Tc-EC）is a new type of renal imaging agent. It can be labeled very easily and efficiently at room temperature through direct labeling at pH 12.[1] But the need for direct labeling at pH 12 is an unfavourable factor for the practical use in patients. On the basis of the labeling experiments, we developed a ligand-exchange labeling method, which contains two kit formations: kit A is a 10 mL vial containing 1.5 mg L,L-EC and 20 mg mannitol, and kit B is a 10 mL vial containing 8 mg sodium glucoheptonate and 80 μg $SnCl_2 \cdot 2H_2O$. Labeling can be easily performed by adding ^{99m}Tc pertechnetate (2~6 mL generator elute) to kit B to form ^{99m}Tc-glucoheptonate (^{99m}Tc-GH), then ^{99m}Tc-GH is transferred to kit A to give ^{99m}Tc-EC. This simple ligand-exchange reaction is based on the fact that ^{99m}Tc-EC is more stable than ^{99m}Tc-GH. The final pH of ^{99m}Tc-EC is 8, which is in accordance with the physiological pH value of human's body. The radiolabeling yield (RLY) and radiochemical purity (RCP) of ^{99m}Tc-EC is always over 95%. In order to interpret and instruct the clinic usage of ^{99m}Tc- EC, a detailed kinetic study of ligand-exchange reaction between ^{99m}Tc-GH and EC was carried out.

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2 Experimental

2.1 Materials

L,L-EC and GH kits (RCP was over 98%) were prepared in our laboratory. Other chemical reagents were from Shanghai Chemical Co, and all were of chemical or analytical grade. Xinhua No.1 paper was commercially purchased. ^{99}Mo-^{99m}Tc generator was from China Institute of Atomic Energy (Beijing). Packard Cobra γ-counter was made in USA.

2.2 Preparation of ^{99m}Tc-EC

^{99m}Tc-EC is prepared as the following ligand-exchange reaction:

$$^{99m}\text{Tc-GH} + \text{EC} \rightarrow {}^{99m}\text{Tc-EC} + \text{GH}$$

Freshly prepared ^{99m}Tc-GH is transferred to the aqueous solution of EC at pH 8, and after being shaken, ^{99m}Tc-EC was formed.

2.3 Determination of RLY and RCP

Xinhua No.1 paper with developing system of $Me_2CO/H_2O/con.NH_3 \cdot H_2O$ = 9/3/1(*V*/*V*) was used. R_f values for $^{99m}TcO_2$, ^{99m}Tc-GH, ^{99m}Tc-EC and $^{99m}TcO_4^-$ were 0, 0, 0.5~0.6 and 1.0, respectively.

2.4 Ligand exchange reaction at different EC concentrations

According to the literature,[2] ^{99m}Tc-GH(RCP must be over 98%, 80 μL, 3.6~7.4 MBq) was added to 1mL of 0.5mol • L^{-1} phosphate buffer containing different amounts of EC(150, 75, 50 and 15 μg), then the sample was taken out at different time intervals and the RLY was determined.

2.5 Ligand-exchange reaction at different pH values

A solution of EC (30 μL, 5 g • L^{-1}) was added to 1 mL of 0.5 mol • L^{-1} phosphate buffer at different pH values (pH = 10, 9, 8, 7). After completely skaking, ^{99m}Tc-GH (RCP must be over 98%, 80 μL, 3.6~7.4 MBq) was added, then the sample was taken out at different time intervals and the RLY was determined.

2.6 Calculation of rate constant

Ligand-exchange reaction can be expressed as follows:

$$^{99m}\text{Tc-GH} + \text{EC} \overset{k}{\leftrightarrow} {}^{99m}\text{Tc-EC} + \text{GH}$$

t=0	a	b	0
t=t	a-x	b-x	x

where k is rate constant for the formation of ^{99m}Tc-EC.

Supposing the reaction is a second order reaction, then

$$d[^{99m}\text{Tc-EC}]_t/dt = k[^{99m}\text{Tc-GH}]_t[\text{EC}]_t$$
$$dx/dt = k(a-x)(b-x)$$
$$k dt = dx/[(a-x)(b-x)]$$

Integrating of each side of the above equation between t=0, x=0 and t=t, x=x gives:

$$kt = [\ln b(a-x) - \ln a(b-x)]/(a-b) \qquad (1)$$

where $a = [^{99m}\text{Tc-GH}]_0$, $b = [\text{EC}]_0$, $x = [^{99m}\text{Tc-EC}]_t$. Since $a \approx x = 10^{-9}$~10^{-8}mol • L^{-1}, $b = 10^{-5}$~10^{-4}mol • L^{-1}, $b >> a \approx x$, Eq.1 can be simplified as:

$$kt = [\ln a/(a-x)]/b$$
$$bkt = \ln [1/(1-x/a)] \qquad (2)$$

Since R_f values of ^{99m}Tc-EC and ^{99m}Tc-GH are 0.5~0.6 and 0.0 respectively, the above x/a was the count percentage of ^{99m}Tc-EC to ^{99m}Tc-GH. Its value equals the RLY of ^{99m}Tc-EC, and it can be determined by TLC. Assuming x/a equals P, Eq.2 can be written as:

$$\ln[1/(1-P)] = bkt$$

Plot ln[1/(1-P)] vs t should show a linear relationship and the rate constant k for the formation of ^{99m}Tc-EC can be calculated from the slope.

3 Results and discussion

The RLYs for ligand exchange reactions at different EC concentrations and different pH values are shown in Fig.1 and Fig.2 respectively. The exchange reaction between ^{99m}Tc-GH and EC was rapid and it reached the equilibrium 1 min after beginning. ^{99m}Tc-EC was able to put into clinical use 2 min post-preparation when the ligand-exchanging method was employed. The RLY of ^{99m}Tc-EC increased with the increase of EC concentration, but the RLY of ^{99m}Tc-EC had no evident variation for the EC concentration from 279.9 μmol • L^{-1} upwards. The RLY of ^{99m}Tc-EC increased with the increase of pH values, and exhibited a significant increase from pH=7 (60%) to pH=8 (95%). The RLY of ^{99m}Tc-EC had no evident variation when pH value was 8.0 upwards. In order to make the labeling yield of ^{99m}Tc-EC higher than 90%, pH value of the reaction solution must be equal to or higher than 8.

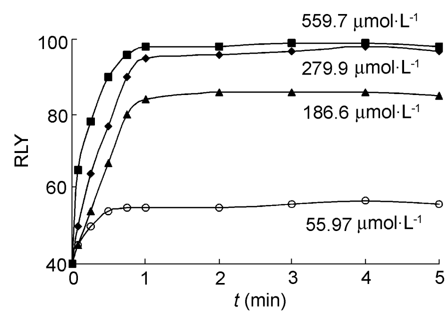

Fig.1 Ligand-exchange reaction at different EC concentrations.

Curves ln[1/(1-P)] vs t (Fig.3) at four different EC concentrations showed a better linear relationship. The slopes of every straight line were different, however the rate constant k calculated was the same (see Table 1). Since k did not change with the EC concentrations, the ligand exchange reaction was a second order reaction as expected.

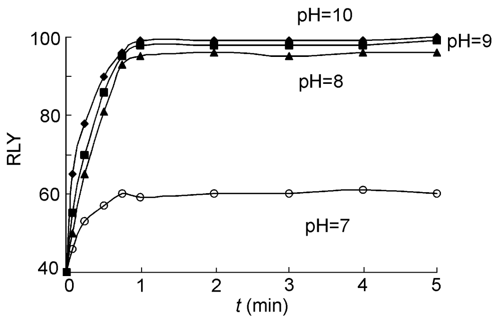

Fig.2 Ligand-exchange reactions at different pH values.

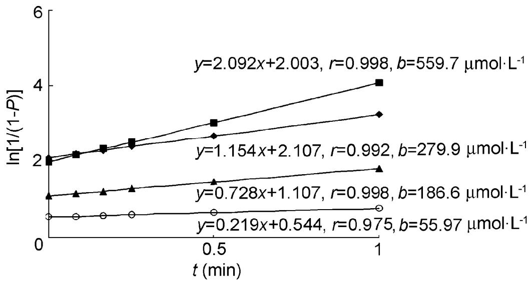

Fig.3 ln[1/(1-*P*)] vs *t* at different EC concentrations.

Table 1 Rate constants at different EC concentrations

EC concentration (μmol • L^{-1})	559.7	279.9	186.6	55.97
k (×10^3 L • mol^{-1} • min^{-1})	3.7	4.1	3.9	3.9

Curves ln[1/(1-*P*)] vs t (Fig.4) at different pH values also showed a better linear relationship. The rate constant k (see Table 2) increased with the increase of pH values. It demonstrated that k of the ligand-exchange reaction was pH dependent. k had a significant increase, from 3.1×10^2 (for pH=7) to 1.9×10^3 (for pH=8), which may be the reason why the solubility of EC increased when pH was higher than 7. This was a further evidence to validate the fact that pH value must be equal to or little higher than 8 when using ligand-exchange method to prepare ^{99m}Tc-EC.

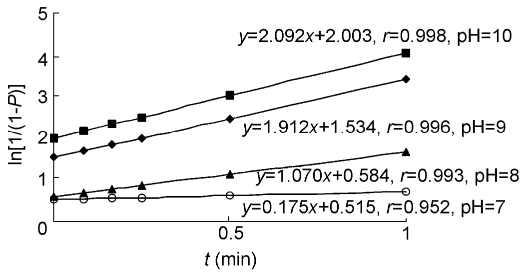

Fig.4 ln[1/(1-*P*)] vs *t* at different pH values.

Table 2 Rate constants at different pH values

pH	7	8	9	10
k (×10^3 L • mol^{-1} • min^{-1})	0.31	1.9	3.4	3.7

References

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2 Wu C Y, Kuang Q F, Fang P *et al*. Nucl Tech (in Chinese), 1993, **16**(5): 265-270