The Study of Interaction of Modified Fatty Acid with ^{99m}Tc in Alcoholic Media

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Abstract. The paper presents the results of laboratory research aimed at the development of methods of synthesis of new radiodiagnostic agents based on modified fatty acid labelled with technetium-99m intended for scintigraphic evaluation of myocardial metabolism. In particular, the interaction of substance with ^{99m}Tc in alcoholic media and the use of ethanol as solvent in the synthesis of the radiopharmaceutical were studied.

INTRODUCTION

The results of laboratory research aimed at developing methods for the synthesis of a new radiopharmaceutical (RF) based on modified fatty acid labelled with technetium-99m. This agent is intended for studying the metabolism of cardiac muscle and this will allow differentiating viable heart tissue and scar tissue. An important task of cardiology is the diagnosis of viable myocardium, since its presence in an ischemic area is a direct indication for invasive correction of coronary insufficiency [1, 2]. The new radiopharmaceutical is designed for use with a radio-labeled ^{99m}Tc. This gives the best potential performance for scintigraphic images by comparison with the known analogs based on ^{123}I. The production and introduction into clinical practice of this radiopharmaceutical will ensure improvement in the quality of medical care and the employment for specialists in the field of radiochemistry and nuclear medicine.

MATERIALS AND METHODS

As a substance for radiolabelling fatty acid 15- (4-carboxymethyl (2-carboxymethyl (2-carboxymethyl (4- (14-carboxytetradecyl) phenylcarbamoylmethyl) aminoethyl) aminoethyl)-aminomethylcarboxamidophenyl)-pentadecanoic acid (PDA-DTPA) derivative was synthesized (Fig. 1).

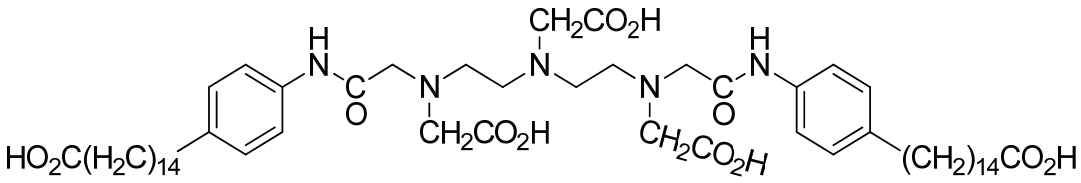

FIGURE 1. Chemical formula of 15- (4-carboxymethyl (2-carboxymethyl (2-carboxymethyl (4- (14-carboxytetradecyl) phenylcarbamoylmethyl) aminoethyl) aminoethyl)-aminomethylcarboxamidophenyl)-pentadecanoic acid (PDA-DTPA)

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The main prerequisite for its use was as follows. PDA-DTPA molecule includes two main structural fragments ensuring successful functioning of the new radiopharmaceutical. The first is that phenylpentadecanoic acid fragments should provide bioavailability of the radiopharmaceutical to myocardium. The second key fragment is substituted diethylenetriaminepentaacetic acid (DTPA) which serves for chelate binding of ^{99m}Tc [3–6].

At the first stages of the research, ethanol was used as a solvent substance. The main positive aspect of its use as a solvent is that it dissolves not only PDA-DTPA, but the reducing agent $SnCl_2 \cdot 2H_2O$ as well. The following method was chosen to perform labelling. 1 mg PDA-DTPA weighed portion was dissolved by heating in 1.0 ml 96% ethanol. Then 1.0 ml ^{99m}Tc eluate of 0.319 GBq activity, 50 mcl Sn (II) solution at concentration of 7 mg/ml and 50 mcl ascorbic acid solution (hereinafter AA) as a stabilizing additive at concentration of 10 mg/ml were successively introduced into the vial. After stirring the mixture for 2 min, the pH was measured. Subsequently, 5 mcl volume samples were selected to determine radiochemical purity (RCP) via thin layer chromatography [7–9]. The following substances have been identified as mobile phases:

– Chloroform : ethanol : ammonia (conc) (Et) = 5 : 5 : 1 (system No. 1) chromatography time 40 min,

– Acetone was selected as a mobile phase for relatively small and well moving ^{99m}Tc (VII) pertechnetate ions, the distribution of which in the chromatogram runs fast enough (about 10 min).

Total concentration of the reactants in the mixture prepared was as follows: PDA-DTPA—0.5 mg/ml, Sn (II)—0.175 mg/ml, AA—0.25 mg/ml. Radiometer RIS-A1 was used to measure the total activity of the vial.

After incubating the mixture for 15 min, repeated sampling for chromatography in acetone (Ac) and ethanol (Et) was carried out. Then the mixture was heated on a steam bath for 30 minutes at temperature close to 100°C. Re-sampling for chromatography in the same media was made from the solution after cooling in an ultrasonic bath.

EXPERIMENTAL RESULTS AND DISCUSSION

After cooling the radiopharmaceutical prepared as described above, the formation of a colloid was observed. To assess its activity and size the resulting mixture was conducted through a filtering nozzle with pore size of 200 nm, followed by measuring the filtrate activity and chromatography sampling. The results of all measurements are presented in Table 1.

These data indicate that within 15 min after mixing the reactants, ^{99m}Tc (VII) impurity concentration in the resulting product is 1.4% (Fig. 2), while ^{99m}Tc (IV) concentration exceeds 62% (Fig. 3). After heating this figure has grown even more with simultaneous formation of a radioactive colloid possibly a PDA-DTPA colloid, which was filtered afterwards. ^{99m}Tc-PDA-DTPA-labelled product concentration was 59.6% in the resulting filtrate, but its radiochemical yield out of the total amount of added ^{99m}Tc does not exceed 0.54%, which is insufficient.

Note: The preparation of Sn (II) and ascorbic acid solutions.

a) A weighed portion of 70 mg $SnCl_2 \cdot 2H_2O$ was dissolved in 200 mcl of 1 M HCl solution followed by adjusting the solution volume to 10 ml with distilled water. $SnCl_2 \cdot 2H_2O$ concentration in the solution is 7 mg/ml.

b) A 100 mg weighed portion of ascorbic acid was dissolved in 10 ml of water—the concentration is 10 mg/ml.

To reduce colloiding, the cause of which can be Sn (II) restoration during its hydrolysis forming SnO2 or PDA-DTPA sedimentation in aqueous medium with a pH of 6, two possibilities of labelling reaction were investigated: the reaction of dry, recrystallized in an alcohol PDA-DTPA and Sn (II) with ^{99m}Tc eluate solution and the reaction of interaction of PDA-DTPA with the product of eluate and Sn (II) interaction. The procedure of these experiments is shown below.

TABLE 1. Filtrate radioactivity measurement results

Sample	Activity, GBq	Peak 1, %	Peak 2, %	Labelled complex yield, %
After stirring $pH_{initial} = 6.5$	0.319	Ac 96.4	3.4	–
In 15 min	0.257	Ac 97.8 Et 62.6	1.4 37.3	36.0
After 30 min heating	0.257	Ac 97.3 Et 78.7	1.6 9.4	19.7
200 nm filtrate	0.0029	Ac 82.0 Et 22.1	18.3 77.73	59.6

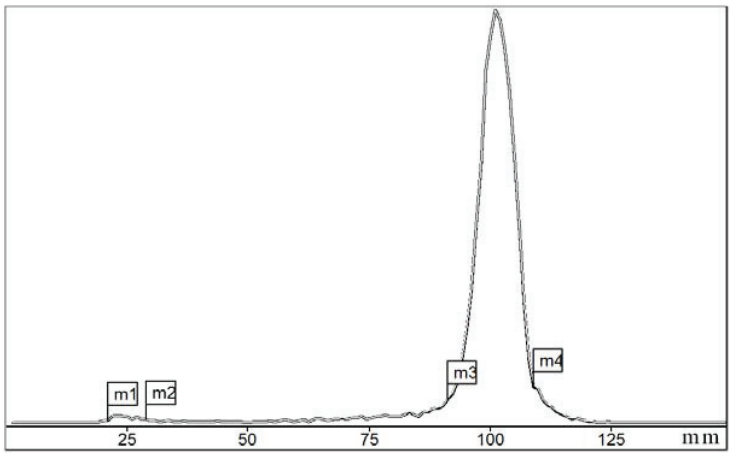

FIGURE 2. Chromatogram, 15 min after mixing in acetone

A 1 mg PDA-DTPA weighed portion was placed in a separate vial and was dissolved in 0.30 ml of 96% alcohol. The resulting clear solution (pH = 6.0) was evaporated to dryness. Then, 10 mcl alcohol solution containing 35 mg/ml Sn (II) was introduced in the same vial and was also evaporated to dryness. Subsequently, 2.0 ml ^{99m}Tc eluate and 50 mcl ascorbic acid solution at a concentration of 10 mg/ml were added to the resulting product. After stirring for 2 min, samples were taken for chromatography in AC.

The solution was heated on a steam bath for 30 min (t = 100°C) and after cooling the activity of the vial was measured and 5 mcl samples were taken for chromatography in acetone—Ac and Et mixture. Sn (II) concentration in the mixture was 0.175 mg/ml, and AA concentration—0.250 mg/ml. The resulting mixture passed through a 200 nm filter, the filtrate activity was measured, and again samples for the chromatography in the same media were taken. The results are presented in Table 2.

1 mg PDA DTPA weighed portion was placed in a vial, dissolved in 0.30 ml of 96% alcohol and evaporated to dryness. Then 2.0 ml ^{99m}Tc eluate and 50-mcl AA solution (10 mg/ml) were introduced into the second vial. After stirring for 2 min, the samples were taken for chromatography in AC. The resulting mixture was introduced into the first vial, mixed; pH was measured and samples for chromatography were taken. Then the solution was heated on a steam bath for 30 min (t = 100°C). The formation of a colloid was observed after cooling in an ultrasonic bath for 20 min. We measured the activity of the vial and took samples for chromatography in Ac, and Et mixture. The resulting mixture passed through a 200 nm filter and again the samples were taken for the chromatography in the same media. The radioactivity of the samples has decreased. The results are shown in Table.

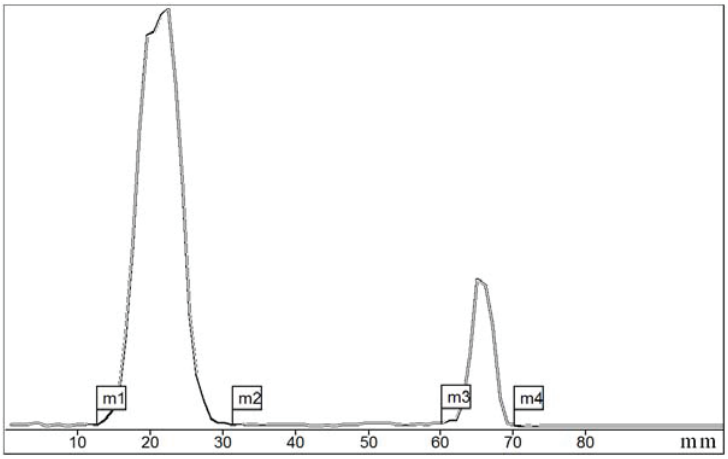

FIGURE 3. Chromatogram, 15 min after mixing in system No. 1

TABLE 2. Filtrate radioactivity measurement results

Sample	Activity, GBq	Peak 1, %	Peak 2, %	Labelled complex yield, %
After stirring $pH_{initial}$ = 5.5–6	0.514	Ac 28.9	69.3	
After 30 min heating	0.467	Ac 48.7 Et 25.9	48.8 66.8	25.3
200 nm filtrate	0.203	Ac 22.3 Et 1.0	75.3 97.3	23.7

TABLE 3. Filtrate radioactivity measurement results

Sample	Activity, GBq	Peak 1, %	Peak 2, %	Labelled complex yield, %
After stirring pH 5.5–6	0.419	Ac 95.4	3.9	
After 30 min heating	0.377	Ac 99.3 Et 92.4	0.4 1.9	7.2
200 nm filtrate pH 5.5–6	0.008	Ac 0 Et 0	0 0	0

Table 2 permits concluding that complete restoration of ^{99m}Tc (VII) does not occur in the interaction between ^{99m}Tc eluate and dry initial reactants. After heating the mixture, labelled complex yield is 25%. Approximately the same amount of the restored ^{99m}Tc (IV) colloid is formed which is then filtered off after passing the mixture through a 200 nm filter. Compared to the results of Table 1, the yield of labelled product in the filtrate increased to 23.7% versus 0.54%.

CONCLUSION

On the based results shown in Table 3 we can conclude that in the pre-reduction of ^{99m}Tc eluate until its interaction with the dry PDA-DTPA almost complete restoration of ^{99m}Tc (VII) is observed. On the contrary, the formation of PDA-DTPA-^{99m}Tc labelled product in the form of solution does not occur, since the entire substance PDA -DTPA at pH 5.5–6 precipitates completely and it is filtered off on a 220 nm filter.

The experiments show that receiving PDA-DTPA-^{99m}Tc in ethanol and eluate—0.9% NaCl solution, having a pH of ~6, the desired product yield is insufficient. This forms a large amount of radiochemical impurities in the form of a restored ^{99m}Tc (IV) colloid. The next stage of our research will be the study of PDA-DTPA behavior in aqueous media and its effect on the solubility of the pH of the medium.

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The study reported in this article was conducted according to accepted ethical guidelines involving research in humans and/or animals and was approved by an appropriate institution or national research organization.

The study is compliant with the ethical standards as currently outlined in the Declaration of Helsinki.

All individual participants discussed in this study, or for whom any identifying information or image has been presented, have freely given their informed written consent for such information and/or image to be included in the published article.

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