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DRUG SYNTHESIS METHODS AND MANUFACTURING TECHNOLOGY

LABORATORY SYNTHESIS OF Actinium-225 COMPLEXES WITH SOMATOSTATIN PEPTIDE ANALOGS FOR RADIONUCLIDIC THERAPY OF NEUROENDOCRINE TUMORS

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Somatostatin receptors overexpressed by malignant neuroendocrine neoplasms are a well-known target for radioligand peptide-receptor radionuclide therapy with metal complexes of peptide molecules, e.g., somatostatin analogs and trivalent cations of metal radionuclides. The aim of the present work was experimental production of a potential pharmaceutical composition based on metal complexes of somatostatin analogs DOTA-TATE and DOTA-TOC with the radionuclide ²²⁵Ac. Optimal conditions for the radiochemical synthesis of ²²⁵Ac complexes with peptides DOTA-TATE and DOTA-TOC with radiochemical yield 95% were established as peptide concentration 1 mg/ml with addition of ascorbate buffer and heating of the reaction mixture at 95°C for 30 min. The results will be in demand in the future for development and technology transfer of the production of radiopharmaceutical drugs to the formulating pharmacy for their clinical use.

Keywords: neuroendocrine tumors; radiopharmaceutical; somatostatin analogs; DOTA-TATE; DOTA-TOC; actinium-225; peptide receptor radionuclide therapy.

The use of radiopharmaceuticals (RPs) based on complexes of various therapeutic metal radionuclides with somatostatin peptide analogs to perform radioligand peptide-receptor radionuclide therapy (PRRT) has a long history and is described in more detail in a review [1]. RPs with metal complexes of agonists of somatostatin receptor 2

DOTA-TATE and 2 and 5 DOTA-TOC with the radionuclides Lu-177 and Ac-225 are the most well-known and widely used in many countries for therapy of inoperable malignant neuroendocrine tumors in various locations (Fig. 1).

DOTA-TATE (CAS 177943-88-3) is a synthetic octapeptide and somatostatin analog with a modified octreotide structure in which the phenylalanine in the 3-position is substituted by tyrosine and contains threonine in the 8-position. The DOTA chelate is bound to phenylalanine in the 1-position.

DOTA-TOC (CAS 204318-14-9) is a synthetic octapeptide and somatostatin analog with a modified octreotide structure in which the phenylalanine in the 3-position is substituted by tyrosine. The DOTA chelate is bound to phenylalanine in the 1-position.

Actinium-225 (²²⁵Ac, Ac-225) is a highly promising alpha emitter for application in nuclear medicine. The trivalent

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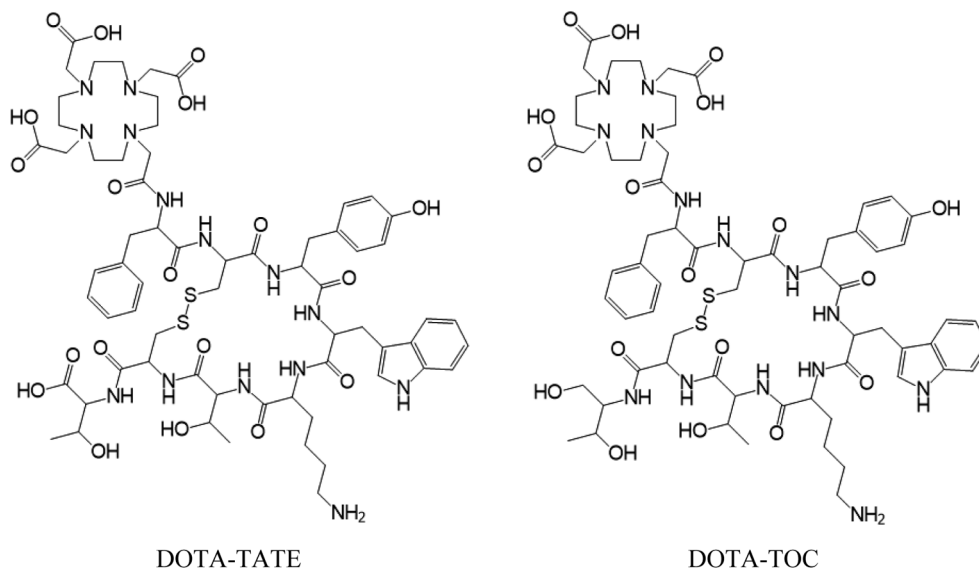


Fig. 1. Structural formulas of DOTA-TATE and DOTA-TOC.

cation persists in HCl or HNO₃ solution and can form stable coordination compounds with various cyclic chelates, particularly DOTA. Ac-225 has a half-life of ~10 d and belongs to the neptunium series ($4n + 1$). It decays to essentially stable Bi-209 and emits four α -particles (^{225}Ac , ^{221}Fr , ^{217}At , ^{213}Po) and two β -particles (^{213}Bi , ^{209}Pb) with characteristic γ -rays at 212 keV (^{221}Fr) and 440 keV (^{213}Bi). It is produced (primarily carrier-free) by isolation from a mixture with Th-229.

The first RP registered for radioligand PRRT of neuroendocrine tumors (NETs) was Lutathera (Novartis AG), which includes ^{177}Lu -DOTA-TATE as the active ingredient and excipients. It is a sterile slightly yellowish aqueous solution of activity 7.4 GBq and specific activity 370 MBq/mL containing excipients HOAc (0.48 mg/mL), NaOAc (0.66 mg/mL), gentisic acid (0.63 mg/mL), NaOH (0.65 mg/mL), ascorbic acid (2.8 mg/mL), DTPA (diethylenetriaminepentaacetic acid, 0.05 mg/mL), and NaCl (6.85 mg/mL). The total volume of the solution for injection is 20 mL with a pH in the range 4.5 – 6 [2]. This composition is protected by patent RU 2,789,366 C2 “Stable concentrated solutions of radionuclide complexes,” which comprises the metal complex ^{177}Lu -DOTA-TATE and the analogous composition of the metal complex ^{177}Lu -DOTA-TOC [3].

The next development stage of PRRT of malignant NETs was the organization and clinical trials of RPs based on the metal complexes ^{225}Ac -DOTA-TATE and ^{225}Ac -DOTA-TOC, with biodistributions like those of ^{177}Lu -DOTA-TATE and ^{177}Lu -DOTA-TOC. The use of an α -emitter as the active ingredient in an RP gives a considerably greater cytotoxic effect on tumor cells, although metal complexes with α -emitters also have potentially greater side effects, which requires further study.

The American pharmaceutical company RayzeBio Inc. (acquired by Bristol Myers Squibb in 2024) developed an RP based on the metal complex of Ac-225 and DOTA-TATE (completed phase 2 clinical trials). The portfolio of RayzeBio Inc. includes a US patent and a Patent Cooperation Treaty (PCT) application for an RP based on metal complexes of the agonists ^{225}Ac -DOTA-TATE and ^{225}Ac -DOTA-TOC and the antagonist ^{225}Ac -DOTA-JR-11 for radioligand PRRT of malignant NETs [4]. Compositions containing ^{225}Ac -DOTA-TATE, ^{225}Ac -DOTA-TOC, and ^{225}Ac -DOTA-JR-11, which remain stable up to 120 h, were disclosed in the invention. In this instance, like in the patent on the compositions ^{177}Lu -DOTA-TATE or ^{177}Lu -DOTA-TOC, the maximum resistance of the pharmaceutical composition to radiolysis is achieved by using various antioxidants.

The patent imposes limitations on the ability to design a generic of this commercial RP in Russia. However, the preparation of an RP based on metal complexes ^{225}Ac -DOTA-TATE or ^{225}Ac -DOTA-TOC of analogous composition in a nuclear pharmacy according to physician's prescriptions for individual patients is allowable according to part 5, art. 1359 of the Civil Code of the Russian Federation. The development of the laboratory technology to produce potential RPs based on metal complexes ^{225}Ac -DOTA-TATE or ^{225}Ac -DOTA-TOC for testing and further technology transfer to the RP formulating pharmacy at A. Tsyb MRRC Branch of NMRC, Ministry of Health of Russia, became a promising and required task.

The aim of the present work was to prepare a potential pharmaceutical composition based on metal complexes of the somatostatin analogs DOTA-TATE and DOTA-TOC and the radionuclide Ac-225.

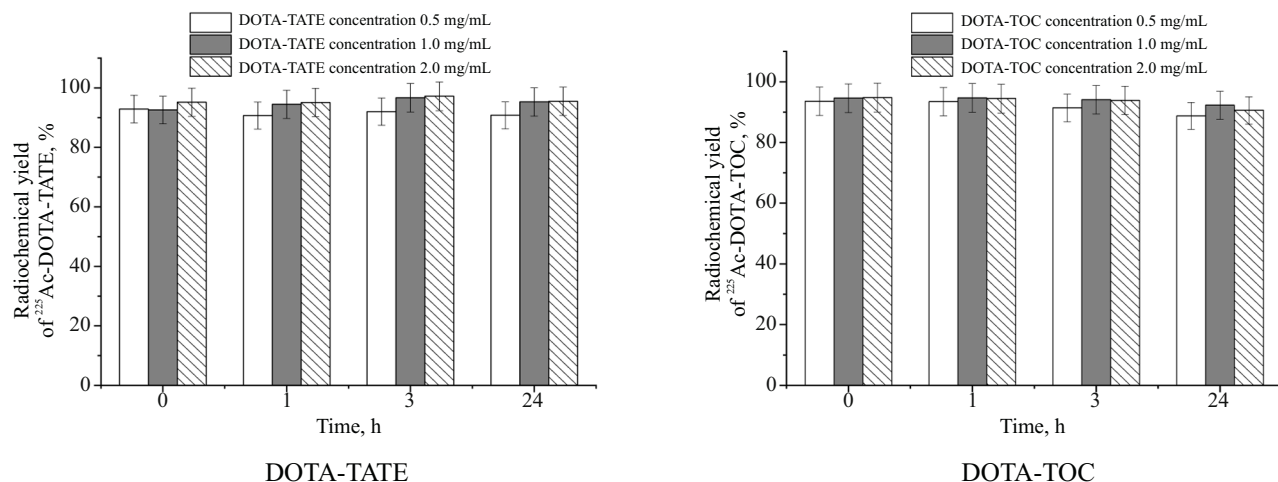


Fig. 2. Dependence of labeling reactions on concentrations of DOTA-TATE and DOTA-TOC peptides in the reaction mixtures and RCP of metal complexes at various times after the reactions.

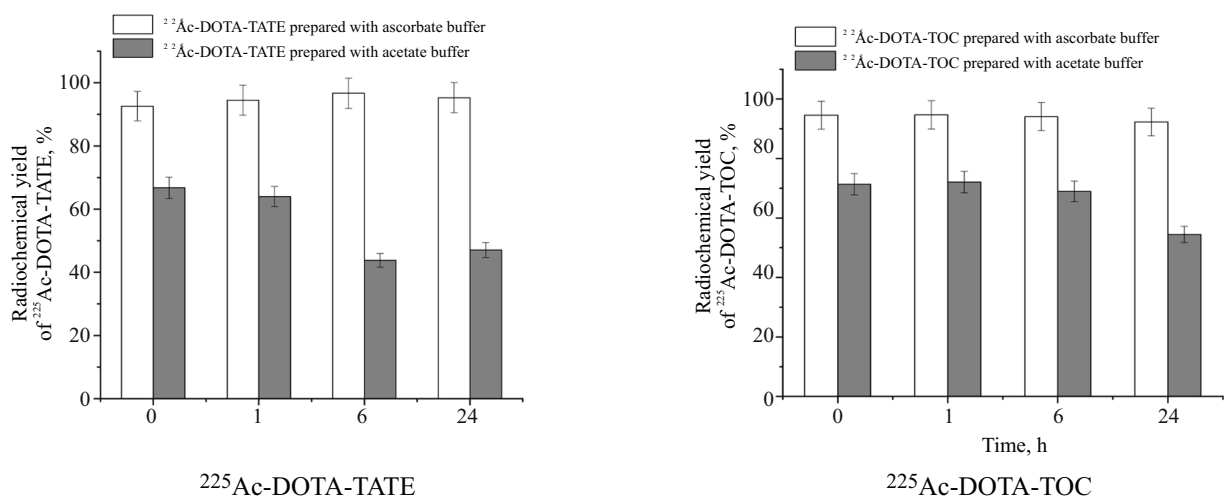


Fig. 3. RCP of metal complexes ²²⁵Ac-DOTA-TATE and ²²⁵Ac-DOTA-TOC prepared by adding ascorbate or acetate buffer solution at various times after the reactions. DOTA-TATE and DOTA-TOC peptide concentration 1 mg/mL.

EXPERIMENTAL PART

Ac-225 was obtained from AO GNTs RF FEI (Obninsk, Russia) as the chloride ²²⁵AcCl₃, dry solid after solvent removal. It was dissolved in HCl (0.1 M) before the radiochemical synthesis.

The chemical precursors DOTA-TATE and DOTA-TOC (MM 1435.62 and 1421.64, respectively) as lyophilizates of purity ≥98% were produced by AO Pharm-Sintez (Moscow, Russia).

The excipients were ascorbic acid (Sigma-Aldrich, USA), NaOH (Sigma-Aldrich, USA), NaOAc (neoFroxx, Germany), DTPA (Merck, Germany), high-purity MeCN for HPLC type 0 (Kriohim, Russia), sodium citrate dihydrate, ammonium acetate (Component-Reaktiv, Russia), Silica gel

60 TLC plates on aluminum backing (Merck, Germany), and Whatman-1 TLC paper (Cytiva, USA).

Peptides DOTA-TATE and DOTA-TOC were dissolved in deionized H₂O at concentrations 0.5, 1.0, and 2.0 mg/mL. For this, H₂O (200, 100, or 50 μL, respectively) was added to vials containing DOTA-TATE or DOTA-TOC (100 μg). The mixtures were stirred until the solids were completely dissolved. The solutions were transferred to 10-mL vials. Then, the dissolved peptides were treated with sodium ascorbate buffer (pH 5.8 – 5.9) or acetate buffer solution (pH 4.9 – 5.0) and stirred. Next, the HCl solution of Ac-225 was added. Each reaction vial was closed with a rubber stopper and sealed with an aluminum cap. The vials with the prepared reaction mixtures were placed in an NF-10A heating block (OOO NTTs Amplituda, Russia), held at 50 – 95°C for

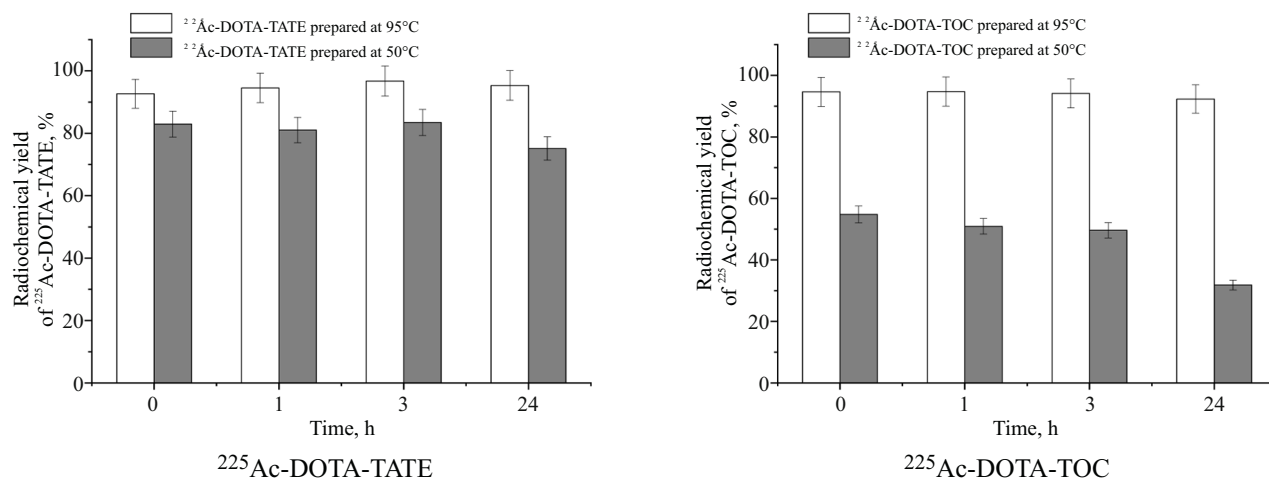


Fig. 4. RCP of metal complexes $^{225}\text{Ac-DOTA-TATE}$ and $^{225}\text{Ac-DOTA-TOC}$ prepared with heating at 95°C or 50°C for 30 min at various times after labeling reactions. Concentration of DOTA-TATE and DOTA-TOC peptides 1 mg/mL.

30 min, cooled to room temperature, and treated with sodium ascorbate solution for stabilization and DTPA solution (4 mg/mL). The mixtures were stirred and filtered through a 0.22- μm cartridge filter.

Quality control of the obtained metal complexes involved visual monitoring of the obtained solution, measurement of the pH using indicator paper with a narrow pH range (Johnson, Great Britain), and TLC to determine the radiochemical purity (RCP) of the metal complexes immediately after the synthesis and after 1, 3, and 24 h.

The RCP of the $^{225}\text{Ac-DOTA-TATE}$ and $^{225}\text{Ac-DOTA-TOC}$ metal complexes was determined by TLC on Silica gel 60 TLC strips on aluminum backing (Merck, Germany) using aqueous sodium citrate solution (0.05 M) as eluent. Free ionic ^{225}Ac in this system migrated to the solvent front (R_f 0.9 – 1.0). The metal complexes and colloidal species remained at the chromatogram origin (R_f 0.0 – 0.1). In parallel, the same samples were analyzed using MeCN– H_2O in a 1:1 ratio. The $^{225}\text{Ac-DOTA-TATE}$ and $^{225}\text{Ac-DOTA-TOC}$

complexes migrated to the solvent front (R_f 0.9 – 1.0), while free cationic ^{225}Ac remained at the origin (R_f 0.0 – 0.1). An alternative TLC method was based on the use of Whatman 1 paper strips (Cytiva, USA) as the stationary phase. The strips were held for 20 min after the separation and drying for the activity of daughter radionuclides to reach equilibrium and scanned on a TLC scanner with a Scan-RAM γ -detector (LabLogic, Great Britain, USA).

Results were statistically processed using the Origin 2019b program to calculate the arithmetic mean (M) and standard deviation (σ).

RESULTS AND DISCUSSION

The laboratory synthesis of metal complexes $^{225}\text{Ac-DOTA-TATE}$ and $^{225}\text{Ac-DOTA-TOC}$ allowed us to determine the optimum concentrations of the ligands DOTA-TATE and DOTA-TOC and the excipients in the solution containing the radionuclide in the necessary chemical

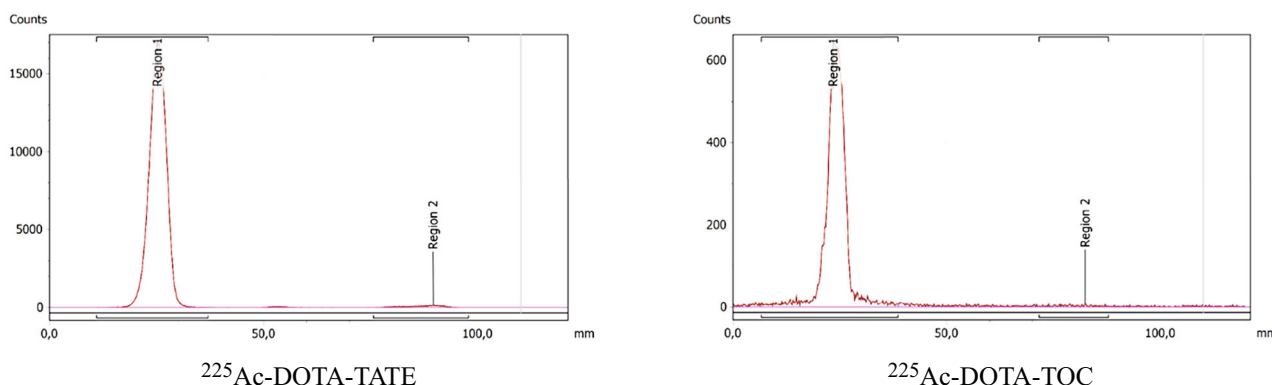


Fig. 5. TLC radiochromatograms of metal complexes $^{225}\text{Ac-DOTA-TATE}$ and $^{225}\text{Ac-DOTA-TOC}$ on Silica gel 60 TLC strips; eluent, 0.05 M aqueous sodium citrate.

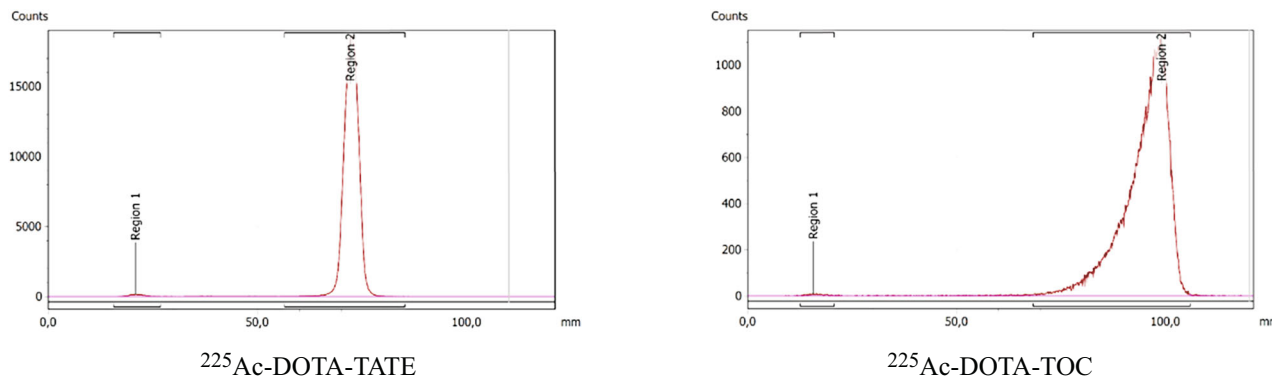


Fig. 6. TLC radiochromatograms of metal complexes ^{225}Ac -DOTA-TATE and ^{225}Ac -DOTA-TOC on Silica gel 60 TLC strips; MeCN–H₂O, 1:1.

form to give the maximum product yield. The excipients were used in the radiochemical synthesis of the RP to give it the required physicochemical properties. Also, intense radiolysis causing a reduction in the RCP of the finished drug began in aqueous solutions of the radionuclides decaying with emission of α -particles [5, 6]. Radioprotectors (stabilizers) should be added to diminish the extent of radiolysis and preserve a high RCP in the RP. Ascorbic acid is one of the most effective stabilizers [7].

The composition of the finished RP dosage form was chosen experimentally using aqueous solutions of DOTA-TATE and DOTA-TOC and ascorbate or acetate buffer solution at 50 and 95°C. The synthesized metal complexes ^{225}Ac -DOTA-TATE and ^{225}Ac -DOTA-TOC were transparent slightly yellowish solutions with pH 4.9–5.7 and without mechanical inclusions.

The percent binding of ^{225}Ac in the complex was >90% for all used peptide concentrations in the reaction mixture (Fig. 2). The most effective binding of DOTA-TATE and DOTA-TOC to ^{225}Ac occurred with a peptide concentration of 1–2 mg/mL using ascorbate buffer and heating the reaction mixture at 95°C (Fig. 2). The RCP of ^{225}Ac -DOTA-TATE (90.8–97.2%) was higher than that of ^{225}Ac -DOTA-TOC (88.8–94.7%). The RCP decreased with time for all metal complexes.

A study of the influence of the reaction mixture acidity on the binding kinetics of ^{225}Ac with DOTA-TOC demonstrated that the percent binding (up to 94.6%) was highest if ascorbate buffer solution (pH 5.8–5.9) was used rather than acetate buffer solution (pH 4.9–5.0). The maximum binding of ^{225}Ac with DOTA-TATE (92% during 24 h) was also achieved using ascorbate buffer solution (pH 5.8–5.9). The binding of ^{225}Ac with DOTA-TATE in acetate buffer was 43.8–66.8% efficient (Fig. 3).

The chelate DOTA bound to various peptides is most reactive at elevated temperatures [8, 9]. Therefore, DOTA-TATE and DOTA-TOC peptides were labeled with ^{225}Ac at elevated temperatures. The reaction of ^{225}Ac and DOTA-TOC at a reaction temperature of 95°C gave

92.3–94.6% binding of ^{225}Ac with the peptide. The binding of ^{225}Ac decreased to 54.8 and 45.9%, respectively, if the reaction temperature was lowered to 50°C (Fig. 4).

The maximum percent binding of ^{225}Ac with DOTA-TATE (92.6–96.7%) was also observed at a reaction temperature of 95°C. It did not exceed 83.4% for ^{225}Ac -DOTA-TATE at 50°C (Fig. 4).

Thus, the optimum conditions for preparing metal complexes ^{225}Ac -DOTA-TATE and ^{225}Ac -DOTA-TOC in high radiochemical yield (>95%) were found to be DOTA-TATE or DOTA-TOC concentration 1 mg/mL with ascorbate buffer solution and heating of the reaction mixture to 95°C for 30 min (Figs. 5 and 6).

The method for preparing the Ac-225 complexes did not differ in principle with that for preparing Lu-177 complexes because these trivalent metal cations could form stable coordination bonds to the cyclic chelates, including the DOTA chelate in the peptide analogs of somatostatin. Their synthesis and use have been described in detail in the literature [10–12]. However, the quality control method of the product (radiochemical impurities) with the finished metal complexes ^{225}Ac -DOTA-TATE or ^{225}Ac -DOTA-TOC in the RPs differed in principle from that for RPs based on the β - and γ -emitting radionuclide ^{177}Lu . Ac-225 decays with the emission of α -particles. Its decay is not accompanied by γ -emission. The γ -emission comes from the decay of its daughter radionuclides francium-221 ($T_{1/2} = 4.8$ min) and bismuth-213 ($T_{1/2} = 46.5$ min). The resulting ^{221}Fr , an alkali metal, could not form a metal complex because it does not form coordination bonds. This excluded quality control of the RPs of metal complexes ^{225}Ac -DOTA-TATE or ^{225}Ac -DOTA-TOC by HPLC with a common and rather reliable γ -radiometric detector. A rather rare and complicated radiometric detector with a liquid scintillator added to the eluent after eluting from the column had to be used. Such detectors are manufactured by a few companies in the world, although their use to detect metal complexes of α -emitting ^{225}Ac has not been reported in the literature. TLC with scan-

ning of the plates with detection of emitted α -particles is technically very complicated. It is practically impossible to implement. TLC monitoring can be used in a complicated version of indirect detection of γ -emitting daughter radionuclides formed after separation at the site of the ^{225}Ac complexes. TLC strips were held for 30–40 min after the chromatographic separation to accumulate daughter radionuclides and reach equilibrium. Then, they could be scanned with a solid-state γ -detector. The most accurate data were obtained if the plates were scanned after prolonged (5–6 h) storage. However, this analysis time is not suitable for timely monitoring of the RCP.

The most accurate results for indirect determination of the location of these Ac-225 species was monitoring of the radioactivity distribution after cutting the plates into fragments and measuring them in a γ -spectrometer using the characteristic line of Fr-221 ($E = 212$ keV) or the characteristic line of Bi-213 ($E = 440$ keV) [13].

The development of the optimum methods for preparation and quality control of RPs based on metal complexes of the α -emitter Ac-225, i.e., ^{225}Ac -DOTA-TATE or ^{225}Ac -DOTA-TOC, were promising and in demand for elaboration and technology transfer of the preparation of these RPs in the nuclear pharmacy at A. Tsyb MRRC, Branch of NMRRRC, Ministry of Health of Russia, to study their clinical application for further routine use in practical radionuclide therapy of oncological diseases.

Conflict of interest

We declare no conflict of interest.

Financing

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Contributions of authors

SAI conceived the planned the research; TPR and SAD synthesized the metal complexes; MBV, EDS, AAO, and ANShch performed the quality control of the metal complexes; VKT and OPV analyzed the data; VKT, OPV, SAD, and MBV prepared the manuscript.

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