Proceedings of the International Conference
Nanomaterials: Applications and Properties
Vol. **2** No 4, 04NABM02(4pp) (2013)

Preparation and Medico-Biological Tests Nanocolloids Radiopharmaceuticals on the Based of Modified DTPA Labeled Technetium-99m

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(Received 16 May 2013; published online 30 August 2013)

Technetium-99m recovery process in the presence of stannous Sn (II) with the aim to determine it is necessary and sufficient quantity, that would provide a complete "recovery" of 99mTc in the reaction mixture has been investigated. The studies were identified conditions for receiving radioactive nanopreparations based on a modified molecule DTPA. The authors has been shown that the direct interaction of 99mTc eluate solution with a solution of a mixture of SnCl2 $DTPA_{mod}$ and radiochemical impurity content unreduced 99mTc (VII) in the preparation labeled with nanocolloids is 2,7% and the yield nanocolloids with the size of 100 nm is about 80%. The medical-biological testing of the agents 99mTc (IV) - DTPAmod on test animals for the determination of the functional suitability for the scintigraphic imaging of lymph nodes has been carried out.

Keywords: Technetium-99m, Modified molecule DTPA, Molybdenum, Nanocolloid labeled technetium-99m.

PACS numbers: 47.57.J –, 62.23. –c

1. INTRODUCTION

In the world practice nanocolloid preparations labeled with a short-lived radionuclide technetium-99m (^{99m}Tc) are widely used for diagnostic tests in oncology, cardiology, for the detection of inflammatory diseases of the musculoskeletal system, cirrhosis, hepatitis and other diseases.

The use of radioactive nanocolloids in oncology is based on the possibility of the rapid and effective identification of "sentinel" lymph nodes (SLN), which are the first lymph nodes where the lymph from a malignant tumor flows. These nodes, by filtering the afferent lymph, become a "trap" for malignant cells, so their biopsy is an objective diagnostic criterion for distributing a malignant process. The optimal effective method of identifying areas of localization of SLN is scintigraphy or radiometry with using technetium-99m-labeled nanocolloids [1].

As a rule, nanocolloid preparations are made on the basis of compounds forming stable hydrosols. The decisive factor for the success is not so much their chemical composition as the size of the nanoparticles. It is known that the optimum particle size for lymphoscintigraphy is 20-100 nm. Such particles are derived from tissues at a rate which does not allow them to penetrate into the bloodstream. On the other hand, particles with sizes less than 20 nm can easily pass into the bloodstream, which prevents the imaging of lymph nodes [2].

Most of the known nanocolloids radiopharmaceuticals is a simple inorganic complexes with ^{99m}Tc rhenium sulfide and antimony produced by fairly complex technology. Presupposition for the chelator DTPA radiolabelling is the ability of the molecule itself as DTPA or derivatives thereof, to form sufficiently stable complexes with various metals, including compounds with technetium-99m. This determined the goal of our work.

2. METHODS OF SAMPLE PRODUCTION AND ANALYSIS

The determination of the radiochemical purity of obtained nanocolloidal preparations were carried out by the thin - layer chromatography. The scanning of the chromatograms was carried out on the facility"Gamma-Scan-01A." On the computer screen was received the information about the location of activity peaks of the labeled and free (unreacted) ^{99m}Tc compounds.

Determining the amount of technetium-99m-labeled nanocolloidal particles was carried out by the methods based on measuring the activity of the suspension before and after filtration through filters with desired pore sizes: 100 nm. For this purpose, three samples were chosen volume of 5 ml of the original solutions and filtrates for the subsequent measurement of their activity, as well as samples - for chromatograms for evaluating the content of impurities in the filtrates of unreacted ^{99m}Tc (VII) in the tested product. Calculations of the yield of products with different particle sizes were determined using the following formulas:

$$C_{100} = \frac{A_{ucx} - A_{100}}{A_{ucx}}$$

where $A_{ис}$ - the activity of the original suspension before the filtration, A_{100} - the activity measured after the filtration through the filter of 100 nm.

For the original of the production of the preparation ^{99m}Tc (eluate) was used the chromatographic generator "^{99m}Tc-GT-TOM" produced by the Institute of Physics and Technologies in Tomsk Polytechnic University.

3. RESULTS AND DISCUSSION

Obtained in the first stage of research Nanocolloids based on chemically modified DTPA molecules exhibit a certain similarity with a protein molecule - the presence of an amide (peptide) links, the existence in the zwitter-ionic form. Simple chemical transformation yielded a number of derivatives of DTPA-modified hydrophobic groups. Molecule itself DTPA, as well as its metal complexes, hydrophilic and are therefore not able to form colloidal particles. Adding hydrophobic fragments yielded water-insoluble complexes of DTPA-modified. At its core are amphiphilic compounds exist as zwitterions - ions are both peers once the cation-and anion-active surfactants.

On the molecule's solubility and its ability to form colloidal particles of a defined size primarily affect its geometry features. Furthermore, the stability of colloidal systems affected by the ratio between the amount of hydrophilic and hydrophobic groups (hydrophilic-lipophilic balance, HLB). Computer simulations carried out with these data allowed to calculate the size of the molecules and their resulting ionic radius in order to give them the desired properties. It was found that the addition of a molecule of DTPA two long straight-chain aliphatic chains with an aromatic group that performed the role of a "bridge" between the molecule DTPA and aliphatic chains (C21 - C22), has given the ability of the modified DTPA form colloidal solutions at physiological pH values. Compounds with shorter chains have shown a great deal poorer.

To create a desired solution nanocolloid particle size (100 nm), it was necessary to select an organic solvent or inorganic, acidic or alkaline. The solvents were tested following solutions: saline - solution 0.9% NaCl, 5% solution of $NaHCO_3$, HClconts., dehydrated ethyl alcohol. To test the solubility of the modified selected substance in a individual bottle were added 5 mg of sample $DTPA_{mod}$, and then each of the vials prepared administered solvents. The solubility of the substance was determined visually by using a dedicated set representing a light booth with an optical device. The results are shown in Table 1.

Table 1 – $DTPA_{mod}$ dissolution in different solvents

Solvent	Process of dissolution
0,9 % solution NaCl	It does not dissolve even under heating to 80^{0}C
HClconcent.	It does not dissolve even under heating to 80^{0}C
10 % solution Na_2CO_3	Dissolves when heated, to form opalescent solution
Dehydrated alcohol	Soluble without heating, to form a clear solution

The studies are shown, that the acid solutions $DTPA_{mod}$ not dissolve in anhydrous alcohol $DTPA_{mod}$ completely dissolved and does not form colloidal solutions. In alkaline solutions $DTPA_{mod}$ forms hazy opalescent colloidal type. On this basis, as the solvent for preparing solution nanocolloid $DTPA_{mod}$ was chosen 5% solution of $NaHCO_3$.

The next step study was the determination of the average particle size of the resulting colloidal solution. The essence of the method consists in determining the particle size by the optical density of the colloidal systems in different parts of the visible spectrum (λ = 400 - 800 nm) using a spectrophotometer Unico 2802 (S). In the original colloidal solution was added 5% $NaHCO_3$ solution and the solutions were prepared with different concentrations by $DTPA_{mod}$: 0.333 mg/ml (solution number 1), 0.167 mg/ml (solution number 2), 0.083 mg /ml (solution number 3). Prepared in four 10x10 mm cuvettes were injected 3 ml of solution number 1, 2 and 3, and in one (reference solution) - 3 ml of a 5% solution of $NaHCO_3$. Optical transmission study $DTPA_{mod}$ gels were measured at wavelengths λ from 400 to 800 nm. The error in determining the optical density D% sample solutions was < 1%. The resulting optical density values are shown in Table 2.

Table 2 – The values of optical density of the solutions with different concentrations nanocolloids

Длина волны, λ	lg λ	D	lg D	D	lg D	D	lg D
		Раствор № 1		Раствор № 2		Раствор № 3	
400	2,602	0,786	-0,105	0,265	-0,577	0,099	-1,004
450	2,653	0,611	-0,214	0,2	-0,699	0,074	-1,131
500	2,699	0,495	-0,305	0,159	-0,799	0,059	-1,229
550	2,740	0,411	-0,386	0,131	-0,883	0,048	-1,319
600	2,778	0,349	-0,457	0,11	-0,959	0,041	-1,387
650	2,813	0,301	-0,521	0,093	-1,032	0,035	-1,456
700	2,845	0,263	-0,580	0,081	-1,092	0,031	-1,509
750	2,875	0,233	-0,633	0,072	-1,143	0,027	-1,569
800	2,903	0,208	-0,682	0,064	-1,194	0,025	-1,602

Based on the data we plotted lgD = f (lg λ). Product according possible to determine the slope of X, which, accordingly, was 1.91, 2.03 and 1.98. According to the methodology used, the range of values for x = 0.2 - 3.8 average r (nm) is calculated using the formula:

$$R = -32{,}662X+157{,}92 \quad (1)$$

As a result, it was found that the radius of the colloidal particles in the test solution number 1, 2 and 3 are 96, 91 and 93 nm. Hence the average particle radius was 93 nm, and the diameter of -187 nm. From this it follows that under the chosen conditions nanocolloid solution preparation, the concentration of active ingredient ($DTPA_{mod}$) and the degree of dilution does not affect the average particle size.

In order to reduce the particle size has been tried nanocolloids combination of two methods: heating and sonication. For this purpose, in two bottle introduced on 5 ml nanocolloids $DTPA_{mod}$ a concentration 0.222 mg/ml. The bottle were placed in an ultrasonic bath filled with hot water (t ≈ 70 ° C): the first bottle 10 min. And a second bottle for 40 min. Then spent determining the optical density of the solutions for the above described method. Based on the data obtained and built according lgD = f (lg λ). The slope of the dependencies were respectively 2.56 and 2.88. Subsequent calculations on the formula (1) showed that the radius of the particles in solution of the first and second vials respectively decreased to 74 and 60 nm, and diameters - 148 and 120 nm.

Since the technique used to evaluate only allowed the mean particle radius, to verify that the solution particles with a diameter less than 100 nm, the solution of the first bottle was further passed through a filter with a pore size of 100 nm, followed by measuring the optical density of the filtrate, and determining the radius of the particles. The calculated value of the mean radius was about 44 nm. From that it was concluded that for obtaining solutions with predetermined nanocolloids value particle size (less than 100 nm) to carry out their filtration through a filter with a pore size of 100 nm.For radiolabeling of ^{99m}Tc in nanocolloid substance has been proposed a method of direct mixing of solutions containing $DTPA_{mod}$ with a solution of sodium pertehnetatat obtained from chromatographic generator «^{99m}Tc-GT-TOM." But as shown by our study, in the absence of a reducing agent to a modified interaction ^{99m}Tc DTPA is not happening. Regardless of the choice of a mobile phase a single peak is observed in the chromatograms of $R_f = 0,9$, which corresponds to the ions of ^{99m} Tc-pertechnetate.

As is known ^{99m}Tc present in the original eluate to with the highest degree of oxidation of (VII), does not have a high reactivity, however, in a low oxidation is sufficiently reactive. For restoring ^{99m}Tc (VII), present in the original eluate ^{99m}Tc, tin chloride dihydrate (II) ($SnCl_2 \cdot 2H_2O$) was used.Therefore, preliminary studies were carried out to establish the necessary and enough amount of Sn (II), which provides the complete recovery of ^{99m}Tc (VII) in the eluate from the ^{99}Mo/^{99m}Tc-generator The results are shown in Table 3.

Table 3 – Changing the content of 99mTc (VII) in the eluate from the ^{99}Mo/^{99m}Tc-generator depending on the concentration of tin (II)

Concentration (Sn(II)), mg/ml	Tc(VII),%
0.1400	0.0
0.1050	0.0
0.0700	0.7
0.0350	3.0
0.0175	7.0
0.00875	10.0

The table shows that the optimum amount of Sn (II) in the reaction mixture provides the content ^{99m}Tc (VII) of less than 10% corresponds to a value in the range of from 0.00875 to 0.0175 mg / ml.

Subsequent radiolabeling ^{99m}Tc nanokolloidnuyu substance carried in two ways: by direct mixing solutions containing $DTPA_{mod}$, $SnCl_2$ and ^{99m}Tc, and by dissolving the dry residue obtained after evaporation of solutions with $DTPA_{mod}$ and $SnCl_2$, ^{99m} Tc in the eluate. The mixture was then heated in a water bath (70-80 ° C) for 30 min. After cooling to room temperature in an ultrasonic bath, filtration of the products obtained through filters with a pore diameter of 100 nm. Then, samples were taken for chromatography. The results of these studies are shown in Table 4.

From the presented table shows that the most interesting result is the direct interaction of ^{99m}Tc eluate solution with a solution of a mixture of sodium chloride and $DTPA_{mod}$ tin containing a certain amount of Sn. The output labeled nanocolloids based on the modified molecules $DTPA_{mod}$ with particle size less than 100 nm was 80% with a total content of radiochemical impurities unreduced ^{99m}Tc (VII) in the resulting formulation 2.7% (Fig. 1). This is a fairly good indicator, closely matching the regulatory requirements for these drugs.

When interacting with ^{99m}Tc eluate dry residues derived from alcohol mixtures containing $DTPA_{mod}$ and $SnCl_2$, radiochemical impurity contents unreduced ^{99m}Tc (VII) obtained in preparation nanocolloid was about 6% and the yield nanocolloids labeled with a size less than 100 nm was 74%.

Table 4 – The results studies

Ingredients of mixture	Activity, imp	Contents ^{99m}Tc(VII), %	Yield %
[$DTPA_{mod}$ +Sn(II)] $_{solution}$ +^{99m}Tc	550 965	3,5	
After heating	484 552	3,0	
Filtrate 100	387 642	2,7	80
[$DTPA_{mod}$ +Sn(II)]$_{dry}$.+^{99m}Tc	545 128	7,0	
After heating	476 179	5,8	
Filtrate 100	352 372	4,8	74

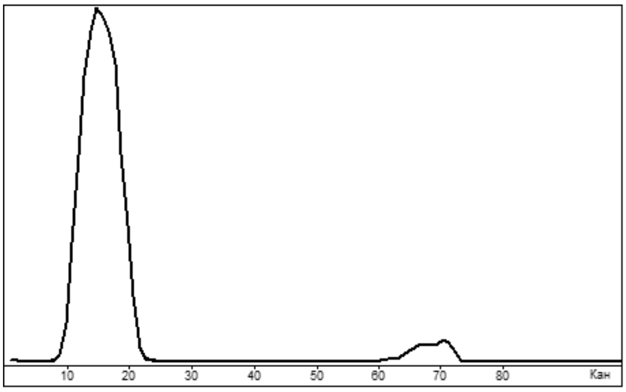

Fig. 1 – Radiochromatogramma ^{99m}Tc elution with a solution of a mixture of $DTPA_{mod}$ and $SnCl_2$

Medico-biological tests of the colloidal drug on the basis of modified molecules $DTPA_{mod}$ labeled with 99mTc were carried out at the Research Institute of Oncology, SB RAMS Tomsk city, on the white line of male rats "Wistar" with the mass of 300-350. The animal's body scintigram obtained at various time intervals are shown in Figure 2.

On the scintigrams of 60 and 120 minutes is a clearly visible sentinel lymph node, located between the bladder and the place of injection. The level of accumulation of the drug in the lymph node was 3.67% of the total introduced activity, which is enough for its safe visualization. This result exceeds to the standard requirements for such drugs (0.5 - 1.7%) and proves the functional suitability of our synthesized labeled with technetium-99m, nanocolloid on the basis of modified molecules $DTPA_{mod}$.

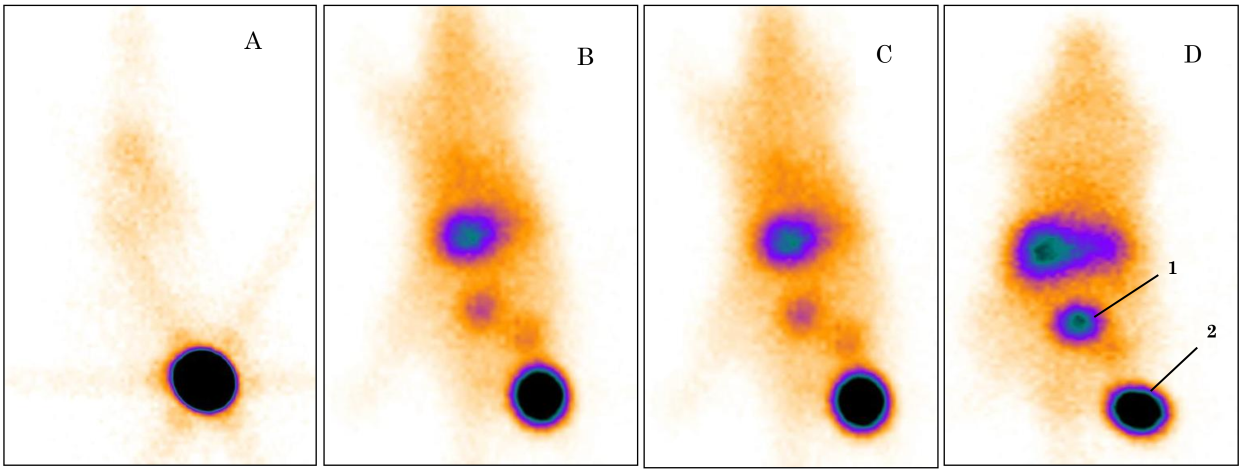

Fig. 2 – The drug distribution in the body of rat at the introduction of a nanocolloid [$DTPA_{mod}$ и $SnCl_2$]: A) Immediately after injection, B) 30 minutes after injection, C) 60 minutes after injection, D) 120 minutes after injection. The numbers is indicated: 1 - lymph node, and 2 - Location of the drug

4. CONCLUSION

1. The research process of restoration of ^{99m}Tc in the presence of stannous Sn (II) have been studied. As a result, it has been found that the optimum concentration of the reducing agent, Sn (II) in the radiopharmaceutical must be in the range from 0.00875 to 0.0175 mg / ml.

2. The conditions for obtaining radioactive nano-preparations on the basis of the modified molecule DTPA. It is shown that the direct interaction of ^{99m}Tc eluate solution with a solution of a mixture of $SnCl_2$ $DTPA_{mod}$ and radiochemical impurity content unreduced ^{99m}Tc (VII) in the preparation labeled with Nanocolloids is 2.7% and the yield nanocolloids with the size of 100 nm is about 80%.

3. Medico-biological tests of labeled ^{99m}Tc on the molecules $DTPA_{mod}$ have been carried out on rats in order to study their distribution in the organism of the experimental animals and determine the functional suitability for the scintigraphic imaging of lymph nodes. The level of accumulation of the drug in the lymph node is 3.67% of the total introduced activity, which is enough for its safe imaging.

This work has been carried out in the frame of state program «Sciense» № 3.1192.2011.

RERERENCES

1. P. Reimann, *Phys. Rep.* 361, 57 (2002).
2. S. Maza et al., *QJ Nuc.Med* 30/5, 651 (2003).
3. C.B. Sampson, *Textbook of Radiopharmacy theory and Practice* (London, United Kingdom: Gordon and Breach; 1994).