PROCEEDINGS OF THE INTERNATIONAL CONFERENCE NANOMATERIALS: APPLICATIONS AND PROPERTIES
Vol. **1** No 1, 01NNBM01(4pp) (2012)

Production and Medical-Biological Tests of Nanocolloids on the Basis of Aluminum Oxide Labeled by Technetium-99m

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(Received 29 May 2012; published online 15 August 2012)

The authors have studied regularities of adsorption of ^{99m}Tc (VII) on the activated gamma-oxide, Al_2O_3. It has been shown that the sorption capacity of the oxide on the radionuclide depends on its acid treatment. 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 ^{99m}Tc in the reaction mixture has been investigated. The adsorption process of the restored ^{99m}Ts on the nanoscale powder of gamma-aluminum oxide has been studied, and the method of obtaining nanocolloids ^{99m}Tc (IV)-Al_2O_3 has been developed. The medical-biological testing of the agents ^{99m}Tc (IV) - Al_2O_3 on test animals for the determination of the functional suitability for the scintigraphic imaging of lymph nodes has been carried out.

Keywords: Technetium-99m, Aluminum Oxide, Active Centers, Molybdenum, Adsorption, Nanocolloid Labeled Technetium-99m

PACS number: 82.70.Dd

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 nanokolloid radiopharmaceuticals represent simple inorganic complexes of ^{99m}Tc with rhenium sulfide and antimony, obtained by a complicated technology. However, we have carried out preliminary studies which showed that stable colloidal compounds could be obtained in simpler way - by means of adsorption of the reduced ^{99m}Tc on the alumina gamma [3]. The original premise for the use of alumina as a "carrier" label of ^{99m}Tc is its relatively low toxicity, combined with good adsorption properties, availability and low cost. However, the research on obtaining ^{99m}Tc labeled nanocolloids on the basis of gamma-Al_2O_3 oxide has been conducted by anybody to date. This has determined the purpose of our work.

2. METHODS OF SAMPLE PRODUCTION AND ANALYSIS

As the object of these studies was used the nanopowder with low-temperature (cubic) modification of the gamma-oxide Al_2O_3. The specific surface area of the was 320 m^2/g. According to electron microscopy, the particles were irregular in shape and had a non-smooth surface. Their average length was in the range of 8-10 nm with the diameter of 2 nm. For the experiments, the original suspension of aluminum oxide was prepared by diluting the sample of about 5 mg of gamma-Al_2O_3 oxide nanopowder with the 7-10 nm particles diameter in 10 ml of water. To prevent the partial loss of oxide in the sediment a further processing of the suspension in an ultrasonic bath was carried out, after which the activation of the surface of the gamma - oxide 0.05 M HCl was brought up to pH = 2. The adsorption process was carried out under static conditions by mixing 2 ml of the suspension with 2 ml of the eluate followed by injecting Sn (II) with the rate of 0.0175 mg/ml.

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: 220, 100 and 50 nm. For this purpose, three samples were chosen with the at 3 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:

$$C_{220} = \frac{A_{\text{èñ}} - A_1}{A_{\text{èñ}}};\quad C_{100} = \frac{A_1 - A_2}{A_1};\quad C_{50} = \frac{A_2 - A_3}{A_2}$$

where $A_{\text{ис}}$ – the activity of the original suspension be-

fore the filtration, A_1 – the activity measured after the filtration through the filter of 220 nm, A_2 – the activity after the filtration through 100 nm, A_3 – the activity after the filtration through 50 nm.

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.

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

Before the adsorption of various anions on the oxide Al_2O_3 its acid activation is previously carried out in order to create stable adsorption centers on the surface. In this context, at the first phase were found optimal conditions for acid treatment, which would provide the maximum amount of adsorption of the radionuclide.

Then the adsorption characteristics of aluminum oxide for different amounts of absorbed hydrochloric acid was studied. The change of the sorption capacity of aluminum oxide depending upon the amount of absorbed acid are shown in Fig. 1.

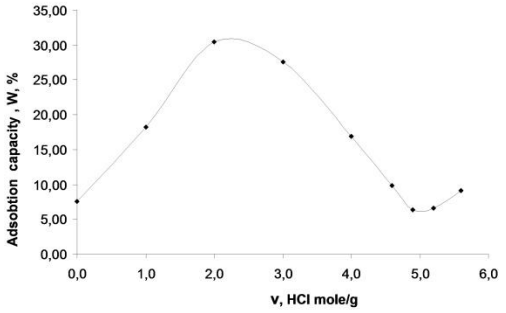

Table 1 – Appointment of special paragraph styles

Fig. 1 – The change of sorption capacity of aluminum oxide depending upon the amount of absorbed acid.

Abstract is an essential part of the article metadata. One should always remember that the main purpose of the abstract is to attract the target reader. Abstract passed to the abstract database, where the automatic search widely used.

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From this relation we see that the maximum adsorption is more than 30 % of the introduced radionuclide activity, being observed in the oxide with the absorbed mass of $2 \cdot 10^{-4}$ mol/g.

However, using the data obtained, it follows that ^{99m}Tc, which is present in the original eluate to with the highest degree of oxidation of (VII), does not have a high sorption capacity. Therefore, we have carried out a study on the adsorption of reduced technetium-99m, which is known to be chemically more active in the lower oxidation state. 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 99Mo/99mTc-generator. The results are shown in

Table 1 – Changing the content of ^{99m}Tc (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), %
0.14	0
0.105	0
0.07	0.7
0.035	3
0.0175	7
0.00875	10

They imply thatot the optimal amount of Sn (II) in the reaction mixture, which ensures the content of ^{99m}Tc (VII) at less then 10 %, corresponds a value ranging from 0.00875 to 0.0175 mg/ml.

In order to avoid the hydrolysis of tin, which leads to its oxidation and reduction of it restoring properties, in the future, for preparing a nanocolloid solution, instead of the solution of tin, we are planning to try its medical counterpart - the lyophilizate.

For the investigation, the initial aluminum oxide nanocolloids was prepared by dissolving about 5 mg of gamma-Al_2O_3 oxide nanopowder with the particle diameter of 7-10 nm in 10 ml of water. As a part of the oxide was precipitating, in addition to the process the suspension was treated in an ultrasonic bath until there was no more visible residue. For the subsequent activation of the gamma-oxide surface was carried out its acid treatment, by adding of 0.05 M HCl to pH = 2 in the bottle. For evaluating the radiochemical yield of fractions with a given diameter of the particles the filtration of the product through filters with a pore diameter of 220, 100 and 50 nm was performed.

The experiment was conducted using the following program. A prepared solution of aluminum oxide nanocolloids with 2 ml volume, were added 2 ml of prereduced technetium-99m (the introduced in the radiopharmaceuticals (RPC) concentration of tin (II) was C_{Sn} = 0.0175 mg / ml). After mixing, the mixture was handled in an ultrasonic bath for 10 min. The subsequent filtration of the product through the filter of 220 nm showed that all of ^{99m}Tc-labeled colloid (IV) – Al_2O_3 had the a size of more than 220 nm. The content of the impurity of unreduced ^{99m}Tc (VII) in the filtrate increased from 5 % (original mix) to 56 % due to the fact that ^{99m}Tc (VII) adsorbs poorly on the oxide and freely passes through the filter.

We have preliminary studied the effect of temperature on the magnitude of nanocolloids and the radiochemical purity of the drug which has shown that the optimum temperature regime of the introducing a radiolabel is in

the range from 70 to 80° C at 30° min heating.

The first attempt to influence the size of the particles formed in the reaction mixture, was holding a "competitive" interaction reaction of ^{99m}Tc (VII) simultaneously with aluminum oxide and the reducing agent Sn (II). To initiate the reaction was used heating the mixture, as well as the introducing the additives of ascorbic acid and gelatin into the reaction mixture.

Then in the first bottle was added 10 ml of freshly prepared solution of Sn (II) with the concentration of C_{Sn} = 0.0175 mg/ml.

In the second bottle, at first was add 100 μkl of ascorbic acid concentration of 10 mg/ml, followed by 10 ml of Sn (II) with the same concentration.

The third bottle was sequentially added 100 μkl of ascorbic acid, 10 ml of solution of tin (II) with the same concentration, and after that 100 ml of 10 % solution of gelatin.

Then all the bottles were heated in a water bath (70-80° C) for 30 min. After cooling to the room temperature in an ultrasonic bath was carried out the filtration of products obtained through filters with the pore diameters of 220, 100 and 50 nm. The results of these studies are shown in Table 2.

Table 2.

Ingredients of mixture	Activity, imp.	Contents ^{99m}Tc(VII), %	Radiopharmaceuticals, %	Yield %
Al_2O_3+^{99m}Tc+Sn(II)	549 963	10	88	
filtrate 200	484 907	15	85	82
filtrate 100	355 591	8	85	62
filtrate 50	62 917	54	42	5,9
Al_2O_3+^{99m}Tc+АК+Sn(II)	424 128	8	92	
After heating	370 179	1	99	
filtrate 200	245 972	3	97	66
filtrate 100	79 030	4	96	19
Al_2O_3+^{99m}Tc+АК+Sn(II)+ gelatin	383 569	22	78	
After heating	383 569	7	93	
filtrate 200	309 761	9	91	77
filtrate 100	288 059	7	93	76

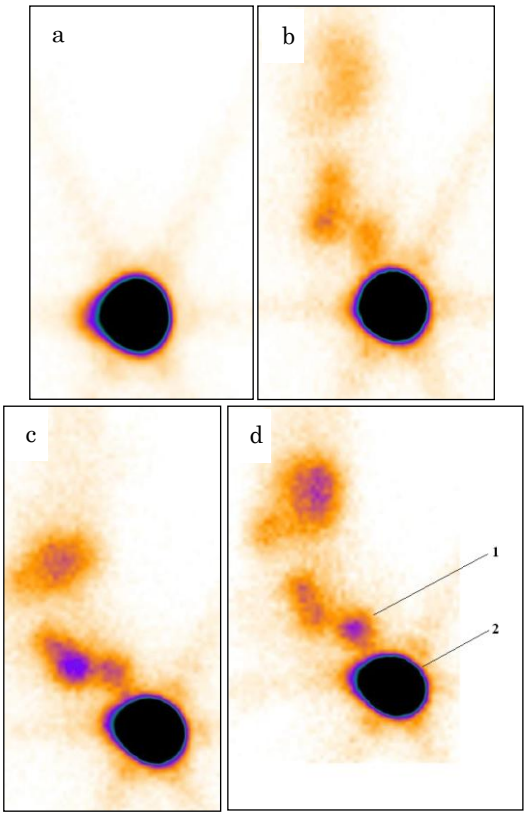

Fig. 2 – The drug distribution in the body of rat at the introduction of a suspension [Al_2O_3 + ^{99m}Tc + AK + Sn (II) + Gelatin]: a) Immediately after injection, b) 30 minutes after injection, c) 60 minutes after injection, d) 120 minutes after injection. The numbers is indicated: 1 – lymph node, and 2 – Location of the drug

From the table it follows that in the absence of chemical additives, the yield of labeled nanocolloids of the size less than 100 nm is 62 % for the overall radiochemical purity of the product 85 %, while the output of ≤ 50 nm colloid - less than 6 %.

The introduction of ascorbic acid to the reaction mixture has resulted in an increase in the radiochemical purity of the filtrate of 100 nm up to 96 %.

Medico-biological tests of the colloidal drug on the basis of oxide- alumina Al_2O_3 labeled with ^{99m}Tc 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 Fig. 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 1.63 % of the total introduced activity, which is enough for its safe visualization. This result corresponds closely 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 gamma-oxide alumina.

4. CONCLUSION

1. The regularities of the adsorption of ^{99m}Tc (VII) on activated oxide, gamma-$_{Al2O3}$ have been studied. It has been shown that the sorption capacity of the oxide on the radionuclide depends on its acid treatment. It has been established that the maximum adsorption of 99mTc on the oxide is observed at the absorbed amount of acid $2 \cdot 10^{-4}$ mol/g.

2. 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 RPC must be in the range from 0.00875 to 0.0175 mg/ml.

3. For the first time the adsorption process of the reconstructed ^{99m}Ts on the nanoscale powder of gamma-aluminum oxide has been studied.

4. Medico-biological tests of labeled ^{99m}Ts on oxide Al_2O_3 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 1.5 % 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».

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