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# Radiopharmaceutical Drug based on Aluminum Oxide

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## Abstract

**Background/Objectives:** The regularities of adsorption $^{99m}$Tc on activated nanoscale powder gamma-oxide $Al_2O3$ were investigated. **Methods/Statistical Analysis:** As object of examination nanopowder low-temperature (cubic) modification of gamma-oxide $Al_2O_3$ is used. The area of specific surface of aluminum oxide was 320 m2/g. According to submicroscopy, the particles had the improper shape and a rough surface. For obtaining of an initial drug 99mTc (eluate) in the form of a sodium pertechnetate solution, $^{99m}$Tc was produced with the chromatographic generator of "$^{99m}$Tc-GT-TOM" productions of PTI TPU. The stability of the complex was determined by measuring the RCP at room temperature (24°C) at different time points (0, 1, 2, 4 h) after preparation. **Findings:** It is shown that sorption capacity of aluminum oxide essentially depends on its acid treatment. Experimental research of process of adsorption $^{99m}$Tc in the presence of reducing agent of tin (II) is made. It's necessary and sufficient amount providing full "reducibleness" $^{99m}$Tc in a reaction mixture is spotted. For yield rising nanocolloids with particle size less than 100 nanometers and risings of radiochemical purity of drugs additives of sodium pyrophosphate, ascorbic acid and gelatine were used. Pre-award medicobiological tests of nanocolloids preparate $^{99m}$Ts-$Al_2O_3$ on the observational animals to define the functional operability for scintigraphic visualisation of lymph nodes are conducted. **Applications/Improvements:** Is of practical importance for carrying out of diagnostic researches in cardiology, oncology and other fields of medicine.

**Keywords:** Acid Treatment, Aluminum Oxide, Hypotoxicity, Labelled Radionuclides Nanocolloid Drugs, Radiopharmaceutical Drug, Reaction Mixture

## 1. Introduction

Recently, in world practice the considerable increase of interest to use labelled radionuclides nanocolloid drugs for carrying out of diagnostic researches in cardiology, oncology and other fields of medicine becomes perceptible. As the marking agent the greatest preference is donated to short-lived technetium-99m ($^{99m}$Tc) that is caused by its availability and good nuclear and physical characteristics: rather small half-life period 6,02 h and energy γ - radiations of 0,1405 MeV providing a small air dose and, at the same time, sufficient making through ability for carrying out of radiometric measuring.

Application of radioactive nanocolloids in oncology based on the possibility of rapid and effective identification Sentinel Lymph Nodes (SLN), which represent the first lymph nodes where the lymph from a malignant tumour flows off. These nodes, filtering afferent lymph, become "a trap" for malignant cells; therefore, their biopsy is objective diagnostic measure of diffusion of malignant process. An optimal method of revealing of areas of localization SLN is the scintigraphy or a radiometry with the use of labelled technetium-99m nanocolloid[1–7].

As a rule, nanocolloid drugs are made based on the compounds forming inconvertible hydrosols. It is known, for example, that optimal particle size for carrying out lymphoscintigraphy is 20-100 nanometers. Such particles are output from tissues with the rate, which does not allow them to penetrate into a blood channel. However, the particles with the size less than 20 nanometers easily

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transit in a blood channel that interferes with visualisation of lymphonoduses[8].

The biggest part from known nanocolloid radiopharmaceuticals (RP) is simple inorganic complexes $^{99m}$Tc with the rhenium and antimony sulphides, received with advanced technologies. At the same time, our pre-award researches have shown that inconvertible colloid compounds can be received easier - by carrying out of adsorption of reduced $^{99m}$Tc on gamma aluminum oxide[9]. The precondition for use of aluminum oxide as a score "carrier" of $^{99m}$Tc is it enough hypotoxicity in a combination with good adsorption properties, availability and low cost.

## 2. Materials and Methods

As object of examination nanopowder low-temperature (cubic) modification of gamma-oxide $Al_2O_3$ is used. The area of specific surface of aluminum oxide was 320 $m^2/g$. According to submicroscopy, the particles had the improper shape and a rough surface. Their average length was in limits of 8-10 nanometers at diameter - 2 nanometers (Figure 1).

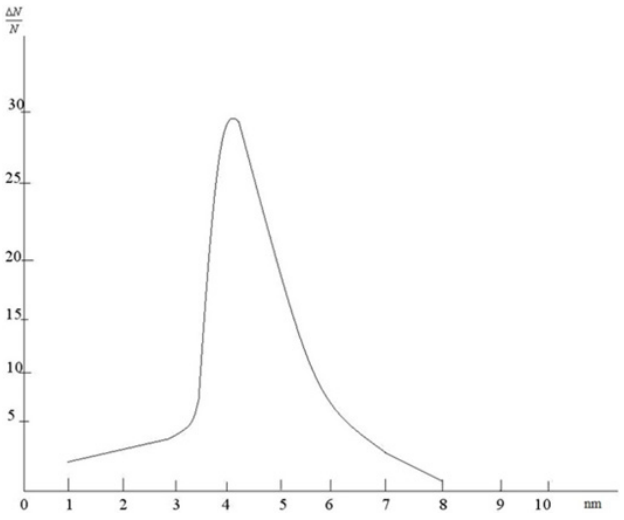


**Figure 1.** Allocation of the particles of gamma oxide $Al_2O_3$ on the conditional diameter, nanometer.

Definition of the size labelled nanocolloid particles and their radiochemical yield in suspensions were made with procedure based on measuring of activity of suspensions before and after a filtration through filters "Minisart" of the firm "Sartorius Stedim Biotech" with pore sizes: 220, 100 and 50 nanometers. With this aim from initial solutions and filtrates the hallmarks in a volume of 5 μL for the subsequent measuring of their activity were selected, also the hallmarks on chromatograms for a content estimation in filtrates of impurity of the unreacted $^{99m}$Tc (VII) were selected. The calculations of a yield with various particle sizes spotted under the formulas are given below:

$$C_{220} = \frac{A_{is} - A_1}{A_{is}} \times 100\%; C_{100} = \frac{A_1 - A_2}{A_1} \times 100\%; C_{50} = \frac{A_2 - A_3}{A_2} \times 100\%$$

Where $A_{is}$ = activity of initial suspension to a filtration; $A_1$ = the activity measured after a filtration through the filter of 220 nanometers; $A_2$ = activity after a filtration through 100 nanometers; $A_3$ = the activity measured after a filtration through 50 nanometers.

Definition of Radiochemical Purity (RCP) of received nanocolloid drugs was made with a thin-layer chromatography method. A specimen with technetium-99m in a volume of 5 μL was superimposed on a plate with a shallow layer of silica gel "Sorbfil" in the size 20×150 mm, having receded from one of the edges in 15 mm (a start line). After spot drying, a plate was put in preliminarily prepared chromatographic chamber with acetone - the height of layer at the bottom of the chamber is 1 cm. The Plate was kept during 10 mines, a time term is sufficient for the full allocation of the mobile pertechnetateions $^{99m}$Tc (VII) along the chromatograms. The information on a situation of maximums of the peaks activity labelled bond and unreacted of $^{99m}$Tc was received by their scanning with the equipment "Gamma-Skan-01A".

For obtaining an initial drug $^{99m}$Tc (eluate) in the form of a sodium pertechnetate solution, $^{99m}$Tc was produced with the chromatographic generator of "$^{99m}$Tc-GT-TOM" productions of PTI TPU. The use of low-activity $^{99m}$Tc, received of the extracting generator, for these purposes is possible[10,11].

### 2.1 Stability Studies

The stability of the complex was determined by measuring the RCP at room temperature (24°C) at different time points (0, 1, 2, 4 h) after preparation.

## 3. The Experimental Results and Their Discussion

As a rule, before carrying out of adsorption of those or other anions on oxide $Al_2O_3$ its lead-acid activation preliminary made for making on a surface the inconvertible centers

of adsorption. Thereupon, at the first stage, optimum requirements of lead-acid processing of the aluminum oxide were found, providing the maximal quantity $^{99m}$Tc of adsorptions.

In Figure 2 dependence of change of sorptive capacity of aluminum oxide on $^{99m}$Tc depending on quantity of the captured acid is presented. It is shown follows that the maximum of adsorption of a radionuclide - more than 30% from injected activity, is observed on the aluminum oxide handled HCl in number of $2 \cdot 10^{-4}$ mole/g.

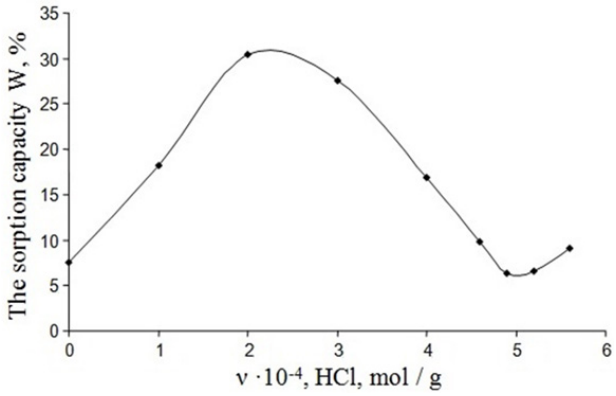


**Figure 2.** Change of sorptive capacity of aluminum oxide on $^{99m}$Tc depending on quantity of the hydrochloric acid absorbed by aluminum oxide.

At the same time, from the received data follows that $^{99m}$Tc, presenting at initial eluate with extremely oxidizing (+7), does not possess high sorption ability. Therefore, the research of adsorption on oxide $Al_2O_3$ of the reduced technetium-99m, which, as it is known, in lower oxidation states is chemically more active, were conducted. For restoration of $^{99m}$Tc (VII) in initial eluate of $^{99m}$Tc tin chloride (II) dihydrate ($SnCl_2 \cdot 2H_2O$) was used. Given the fact, that a parallel formation of large size colloid (more 220 nanometers) can be at hydrolysis $SnCl_2 \cdot 2H_2O$, pre-award definition of minimum quantity Sn (II) providing a complete reduction of $^{99m}$Tc (VII) in eluate from the generator to $^{99m}$Tc (IV) was necessary to spend. With this aim the solutions with the various content of tin chloride (II) were prepared within the change of its concentration from 0,01 to 0,14 mg/ml. Then, equal volumes of eluate of $^{99m}$Tc were entered into the prepared vials.

After, from each vial the hallmarks were selected and superimposed on the plates for chromatography. As the mobile phase, methyl-ethyl ketone was used. Radiochemical purity (RCP) of an initial RP, and also the drugs, containing Sn (II) of 0,14 and 0,0175 mg/ml, are presented in Table 1.

**Table 1.** Radiochemical purity the drugs

| Sample | RCP (%) |
|---|---|
| Initial radiopharmaceuticals | 99,9 |
| Radiopharmaceuticals + 0,14 mg/ml of Sn (II) | 99,5 |
| Radiopharmaceuticals + 0,0175 mg/ml of Sn (II) | 93,7 |

The obtained dependence of change of the content of 99mTc (VII) in a drug from entered quantity of Sn (II) (Figure 3).

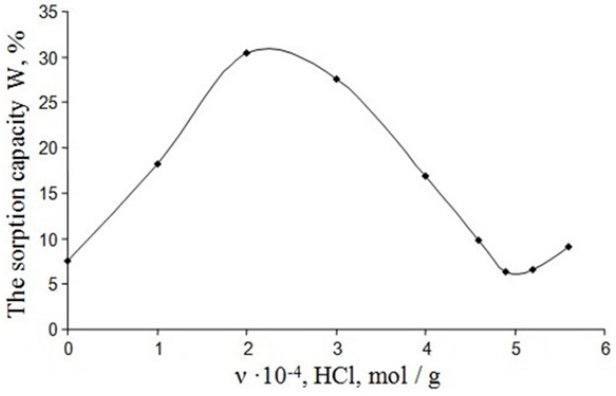


**Figure 3.** Content change of $^{99m}$Tc (VII) in a drug from entered quantity of Sn (II).

From dependence of Figure 3 it follows that for almost complete reduced of $^{99m}$Tc in the given volume of RP, concentration of Sn (II) should be not less than 0,0175 mg/ml. Introduction in RP of larger quantity of reducing agent is not expediently in order to avoid the formation of no-purpose colloid.

For carrying out of experiments, initial suspension of aluminum oxide was plotted by shot deluting nanopowder $Al_2O_3$ in mass ~5 mg in 10 ml of water. For prevention of partial fallout of aluminum oxide to a deposit additional suspension processing in an ultrasonic bath with the subsequent activation of a surface of gamma-oxide $Al_2O_3$ of 0,05 M HCl to value pH = 4-5 was made.

Adsorption process in static conditions by mixing of 2 ml of suspension with 2 ml of eluate was made with the subsequent introduction of Sn (II) to achieve the concentration equal to 0,0175 mg/ml. Also the additives of ascorbic acid (0,25 mg/ml), of sodium pyrophosphate (4,4 mg/ml) and of gelatine (2,5 mg/ml) were used. Then the received yields are filtrated through the filters with various diameter of pores to define a radiochemical yield of fractions labelled $^{99m}$Tc nanocolloids with the sizes 50,

100 and 220 nanometers. In parallel with it, a definition of radiochemical purity of the drugs was made. Effects of examinations concerning the fractions with 50-100 nanometers are presented in Table 2.

**Table 2.** Effects of adsorption of $^{99m}$Tc on gamma oxide $Al_2O_3$

| Sample | RCP (%) | Yield (%) |
|---|---|---|
| $Al_2O_3$+$^{99m}$Tc+Sn (II) | 92 | 62 |
| $Al_2O_3$+$^{99m}$Tc+ ascorbic acid +Sn (II) | 96 | 19 |
| $Al_2O_3$+$^{99m}$Tc+ ascorbic acid + sodium pyrophosphate +Sn (II) | 98 | 34 |
| $Al_2O_3$+$^{99m}$Tc+ ascorbic acid +Sn (II) + plasma | 98 | 42 |
| $Al_2O_3$+$^{99m}$Tc+ ascorbic acid +Sn (II) + gelatin | 96 | 76 |

At the subsequent hypodermic introduction to the experimental animals (rats of "Vistar"-line) drugs No. 1-3, it was determined that the drugs remain in a point of an injection within 1 h without appreciable accumulation of $^{99m}$Tc in a blood of animals that is an evidence of strong bracing of a radionuclide on a surface of nanocolloid. Along with this positive moment, also it is found out that in sentry lymph nodes the drug accumulation is not observed. So, it is suggested that the possible cause of such effect is upsizing of nanocolloid because of its coagulation at interaction with a blood. For the experimental checkout of this suggestion, human blood plasma was entered into the prepared drug (a sample No. 4) and its influence on particle sizes and radiochemical purity of separate nano-size fractions is explored.

As appears from the data presented to Table 2, contrary to the guesses the coagulating of nanocolloid at plasma addition does not occur. Moreover, the yield of fraction with particle size of 100 nanometers is increased in comparison with experiment without plasma addition. So, the nanocolloid, in spite of the fact that it has demanded particle sizes (less than 100 nanometers), move on lymphatic system with insufficient rate. Therefore, for its "transportation" to the composition of the drug a gelatine was entered which, is the biopolymer with a considerable quantity of meshes for placement in them the molecules of immobilised substance. After gelatine introduction (No. 5) the vials with a drug were heated on a water bath (70-80 °C) within 30 minutes. The chromatogram of an admixture of [$Al_2O_3$ + $^{99m}$Tc + ascorbic acid + Sn (II) + Gelatine] (Figure 4).

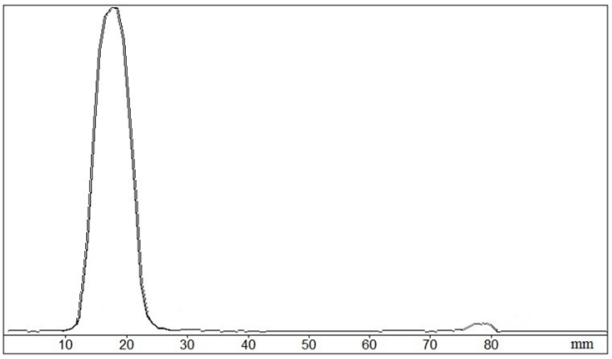


**Figure 4.** The chromatogram of an admixture of [$Al_2O_3$ + $^{99m}$Tc + ascorbic acid + Sn (II) + Gelatine] after heating to 70-80 ˚C for 30 minutes.

From the given chromatogram it follows that the drug has high radiochemical purity at level of 96%. It is good enough index as RCP of similar commercial drugs of $^{99m}$Tc, made on a basis of lyophilisate by their direct mixing with eluate from $^{99}$Mo/$^{99m}$Tc-generator, usually is in limits from 88,2% (Pentatech, $^{99m}$Tc) to 95% (Technefit, $^{99m}$Tc).

## 3.1 Stability of the Complexes

The RCP of the product (Figure 5.) was nearly constant (<96 %) over the observed period of 4 h, suggesting that the complexes possessed a great stability in the reaction mixture at room temperature.

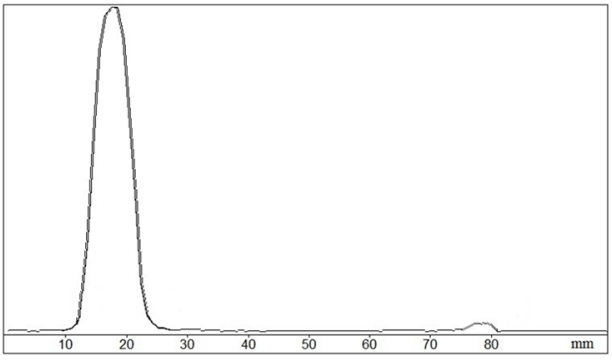


**Figure 5.** The radio chemical purity curve of [$Al_2O_3$+$^{99m}$Tc+ascorbic acid+Sn (II)+Gelatine].

Medicobiological tests of the drug containing gelatine were carried out on the base of Tomsk Cancer Research Institute of the Russian Academy of Medical Science. The content and participation of the animals in the experiment carried out according to the rules, which were

accepted by the European convention for the protection of vertebrate animals used for experimental and other scientific purposes[12].

Scintigrams of a body of the animal, received in various time terms (Figure 6).

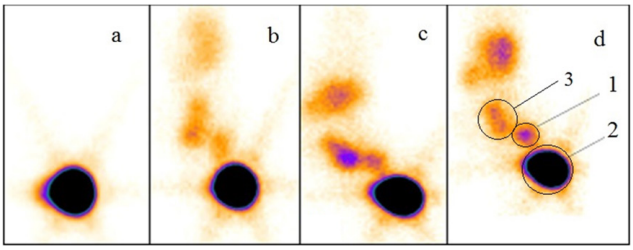


**Figure 6.** Drug allocation in an organism of a rat at suspension introduction of [$Al_2O_3$ + $^{99m}$Tc + ascorbic acid + Sn (II) + Gelatin]: (**a**) Right after drug introductions, (**b**) In 30 minutes after drug introduction, (**c**) In 60 minutes after drug introduction (**d**) In 120 minutes after introduction. And: 1 - the Lymph node, 2 - the Place drug of introduction, 3 - bladder.

On the scintigrams, corresponding to 60 and 120 min the sentinel lymph node located between a bladder and a place of introduction of a drug is distinctly determined. Thus, level of accumulation of a drug in a lymph node is 1,63% from general injected activity that is enough for its trusty visualisation. The received result corresponds closely to the standard requirements to similar drugs (0,5 - 1,7 %) and proves the functional operability of synthesized labelled by technetium-99m of nanocolloid on the basis of aluminum gamma oxide.

## 4. Conclusion

As a result of the conducted researches labelled of technetium-99m nanocolloid drugs on the basis of gamma oxide $Al_2O_3$ with high radiochemical purity and a radiochemical yield of the particles with a size to 100 nanometers more than 75% were obtained.

The patterns of adsorption of $^{99m}$Tc (VII) on labialized gamma oxide $Al_2O_3$ were explored. It is shown that sorptive capacity of aluminum oxide on a radionuclide depends on its lead-acid processing. It is shown that the peak of adsorption of $^{99m}$Tc on aluminum oxide is observed at the quantity of acid captured by aluminum oxide makes $2 \cdot 10^{-4}$ mole.

It is shown that for a complete reduced of $^{99m}$Tc in the given volume of RP a concentration of Sn (II) should be not less than 0,0175 mg/ml. Introduction in RP higher quantity of Sn (II) is not expedient, as it can lead to parallel formation of largely-size colloid. The estimate of a yield of the particles with the sizes less than 100 nanometers was made.

Researching of patterns of allocation of nanocolloid RP in an organism of the experimental animals and their functional operability for scintigraphy visualisation of lymph nodes shows that level of accumulation of a drug in a lymph node is enough for its reliable visualisation.

## 5. Acknowledgment

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