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Development of a technology for obtaining a substance based on the ligand potassium-sodium salt of 1-hydroethylenediphosphonic acid with divalent tin, for the preparation diagnostic kit of Generator technetium-99m

Rikhsiev A.Z., Usarov Z.O., Abdukayumov A.M.,Zikirov M.

Enterprise «Radiopreparat» at the INP AS Republic Uzbekistan, Tashkent

ABSTRACT: The optimal ratios of the main substance potassium-sodium salt of 1-hydroethylenediphosphonic acid and the reducing agent divalent tin (Sn^{2+}) were studied in the preparation of the HEDP(Sn) substance by obtaining the HEDP^{99m}Tc radiopharmaceutical used in medicine to detect bone metastases.
The influence of the concentration of the reducing agent Sn^{2+} and the ligand complexing agent on the efficiency of the formation of the HEDP^{99m}Tc complex in solutions in the range of 0.1-0.42 mg/ml and 1.0-4.0 mg/ml, respectively, and pH 4.0-6.0 was studied. The efficiency of formation of a stable complex ^{99m}Tc with potassium-sodium salt of 1-hydroethylenediphosphonic acid HEDP^{99m}Tc containing: Sn2+ 0.3 mg/ml and HEDP 2.5 mg/ml and solution pH 5.0 20 min after the addition of radioactive ^{99m}Tc was determined, which was more 99.5%. The stability of the substance HEDP(Sn) during storage from the date of preparation to 12 months was determined by obtaining the radiopharmaceutical HEDP^{99m}Tc, which was 12 months.

KEYWORDS: radiopharmaceutical; substance; radionuclide; technetium-99m.

I. INTRODUCTION

The problem of timely and accurate diagnosis has been and remains one of the main problems of clinical medicine. To solve it, such a symbiosis of natural and exact sciences is used, such as radionuclide diagnostics or diagnostic nuclear medicine, as this clinical discipline is commonly called in the world. Modern nuclear medicine is characterized by a variety of diagnostic and therapeutic methods that objectively provide the doctor with unique opportunities, the use of which often has no other alternative. In nuclear medicine, one of the most requested diagnostics is the diagnosis of skeletal diseases. It should be noted that metastatic lesions of the skeleton are detected in more than 60% of patients with breast, lung, prostate, and colon cancer. For the diagnosis of diseases, the most sensitive and special method is scintigraphy with osteotropic radiopharmaceuticals [1-3]. The method is based on the introduction of a radiopharmaceutical drug tropic to the bone tissue into the patient's body, and its subsequent registration during distribution and accumulation in the skeleton. Among these compounds, bisphosphonates occupy a place, used in the detection and treatment of any skeletal disease. After the discovery of polyphosphate labeled with Tc-99m for bone imaging in 1971, G. Subramanian at all, developed and obtained several compounds labeled with Tc-99m pyrophosphate and bisphosphonate for bone imaging [4-8]. These drugs, created on the basis of inorganic pyrophosphates, are derivatives of phosphonic acids, which differ in their chemical structure - the replacement of the oxygen atom in the pyrophosphate molecule with a carbon atom - P-C-P. In addition, there are two radicals in the structure of the side chains of bisphosphonates, one of which facilitates the chemical binding of bisphosphonates to hydroxyapatite, and the other determines the biological effect of drugs on bone cells. Bisphosphonates have a high affinity for bone hydroxyapatite crystals, which makes it possible to obtain the greatest number of signals from the area of the human body under study.
This work is devoted to the study of the formation of the complex (HEDP^{99m}Tc) potassium-sodium salt of 1-hydroethylenediphosphonic acid (HEDP) with the radionuclide technetium-99m, selecting the optimal ratios of the main

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substance potassium-sodium salt of 1- hydroethylenediphosphonic acid and the reducing agent divalent tin (Sn^{2+}) in solution with different pH. Also, the study of the kinetics of the formation of the complex (HEDP^{99m}Tc) potassium-sodium salt of 1-hydroethylenediphosphonic acid (HEDP) with the radionuclide technetium-99m and the shelf life of the HEDP(Sn) substance.

II. METHODS, MATERIALS, REAGENTS AND EQUIPMENT

When preparing the substance of the complex potassium-sodium salt of 1-hydroethylenediphosphonic acid (HEDP) with divalent tin (Sn^{2+}), the following chemical reagents were used:
- potassium-sodium salt of 1-hydroethylenediphosphonic acid (HEDP), purity not less than 99%, Alfa Aesar company;
- tin dichloride, purity not less than 99%, Sigma Aldrich;

In experimental studies, the method of radiotracers was used with using the radioactive nuclide $Na^{99m}TcO_4$ obtained from the technetium-99m generator manufactured by the State Enterprise "Radiopreparat".
Radiometric measurements of the samples were carried out in a gamma ray spectrometer with semiconductor Ge(Li), detector and Aspect software using a spectrometric device SU-03P No. 0037-06. The preparation of experimental batches of the substance HEDP with divalent tin (Sn^{2+}) was carried out in a reaction devices (figure 1). The substance solution poured into vials with a capacity of 10 cm^3, 1 ml each , were lyophilized in an freeze-dryer Epsilon 2-16D.
Qualitative and quantitative characteristics of the main component potassium-sodium salt of 1-hydroethylenediphosphonic acid (HEDP) and excipients were determined by the spectrophotometric method on an LKB Biochrom Ultrespec II spectrophotometer (Sweden). To determine the percentage of the complexed HEDP^{99m}Tc, free pertechnetate ions $^{99m}TcO_4^-$, and hydrolyzed reduced technetium $^{99m}TcO_2$ (HRT), 3MM chromatographic paper (Whatman) and a chromatographic plate with a thin layer of silica gel (Art. 5553, Merck) were used.

III. METHOD OF OBTAINING A SUBSTANCE BASED ON THE HEDP LIGAND AND THE REDUCING AGENT SN^{2+}

The preparation of experimental batches of the HEDP(Sn) substance was carried out in the reaction devices in the following sequence (fig.1.)- the reaction three-necked flask (1) was installed on a magnetic stirrer (9) in a laminar box, and then a 200 ml ligand solution containing HEDP, ascorbic acid was introduced through a separating funnel (2) to supply chemical ingredients for synthesis. We turned on the magnetic stirrer for constant stirring of the reaction vessel solution and immediately turned on the supply of purified argon at a rate of 5×10^{-6} m^3/s, which is fed from the auxiliary vessel (7) into the ligand solution, using an inert gas supply tube (3), within 10 minutes. The tube (4), which removes argon from the reaction flask, must be in the open position. After 10 minutes, without stopping the supply of argon to the reaction flask and while stirring the solution of the reaction flask, a hydrochloric acid solution containing stannous chloride ($SnCI_2$) was quickly introduced through a separating funnel to supply chemical ingredients. In this case, the pH value of the solution medium should be pH=5.0±0.5, which is controlled by a pH meter (10). After entering the solution with tin dichloride, the supply of argon was continued for 10 minutes at the same rate. After completion of the synthesis, the supply of argon to the reaction flask was stopped by closing the valves in the argon outlet tube (4), the valve of the argon inlet tube (8) and the valve of the vessel for supplying chemical ingredients for synthesis (2). After these process through tubes for pumping out the solution of the substance (5), equipped with a membrane filter (0.22 μm) (6), the solution of the complex compound was pumped out of the reaction flask into the dispensing device, then dispensed into vials with a capacity of 10 cm^3, 1 ml each and lyophilized by freezing on freeze-dryer Epsilon 2-16D for 24 hours. After completion of drying, immediately inside the chamber of the freeze-dryer Epsilon 2-16D, they were filled with purified argon gas, then the vials were removed from the freeze-dryer and hermetically sealed with rubber stoppers and crimped with aluminum caps.

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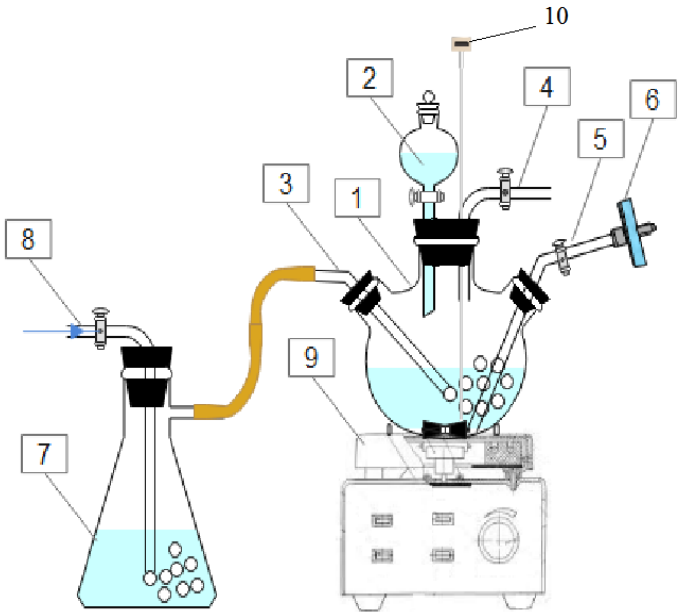

Figure 1. Device for the synthesis of a substance - a complex compound based on the HEDP ligand with Sn^{2+}

IV. METHOD OF OBTAINING THE HEDP^{99M}TC COMPLEX

To obtain the HEDP^{99m}Tc complex in the HEDP(Sn) substance, 5 ml of sodium pertechnetate ($Na^{99m}TcO_4$) solution obtained from the technetium-99m generator was added. After complete dissolution, the HEDP(Sn) substance was kepted for 5 minutes and the effectiveness of the formation of the HEDP^{99m}Tc complex was checked, the amount of the formed HEDP^{99m}Tc complex, unrelated to the $^{99m}TcO_4^-$ and hydrolyzed $^{99m}TcO_2$(HRT) was determined by paper and thin-layer chromatography.

V. CHROMATOGRAPHIC METHODS FOR DETERMINING THE FORMATION OF THE HEDP^{99M}TC COMPLEX

a) On chromatographic paper 3MM (Whatman), size 15 x 200 mm, marked the start line at a distance of 15 mm from one of the edges. 0.001 - 0.005 ml of the drug solution was applied to the start line of the strip. After drying the stain on the chromatographic paper, chromatography was performed by the ascending method until the marked front line was reached with the solvent (approximately 40 minutes), using an isotonic 0.9% sodium chloride solution for injection as a solvent. In the specified chromatography mode, the Rf value of hydrolyzed $^{99m}TcO_2$ (HRT) is 0.0 ± 0.05, and for the HEDP^{99m}Tc complex and $^{99m}TcO_4^-$ pertechnetate ions is 1.0. From the resulting chromatogram, the count rate was measured from the area containing the HRT and from the entire chromatogram by the radiometric method.
b) On a strip of a chromatographic plate with a thin layer of silica gel (Art. 5553, Merck) measuring 15x100 mm, a start line was marked at a distance of 15 mm from one of the edges. 0.001-0.005 ml of the drug solution was applied to the start line of the strip. After drying the stain on the chromatographic paper, chromatography was performed by the ascending method until the marked front line was reached with the acetone solvent (approximately 20 minutes). In the indicated chromatography mode, the Rf values of $^{99m}TcO_4^-$, are 0.95 ± 0.05 and 0.0 for hydrolyzed $^{99m}TcO_2$ (HRT) and HEDP^{99m}Tc. From the obtained chromatogram, the count rate was measured from the area containing free pertechnetate ions, and from the entire chromatogram by the radiometric method. Radiochemical impurities in the drug solution were calculated by the formula:

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$RCI = (\sum HRT + \sum^{99m}TcO_4^- / \sum A_{ch})*100\%$,

where, RCI - Radiochemical impurities in the drug solution; ∑ HRT - is the sum of the activity count rate of hydrolyzed reduced technetium-99m; $\sum^{99m}TcO_4^-$ -is the sum of the activity count rate of free pertechnetate ions; $\sum A_{ch}$. - is the sum of the activity count rate for the entire chromatogram.

VI. DETERMINATION OF THE STABILITY OF THE SUBSTANCE HEDP(SN)

The method for determining the stability of a HEDP(Sn) substance is as follows: to study the stability of the HEDP(Sn) substance, previously prepared substances with a shelf life of 3, 6, 9 and 12 months are selected, then these substances are labeled with the 99mTc radionuclide to obtain the HEDP^{99m}Tc complex. The criterion for the stability of a HEDP(Sn) substance is the preservation of its quality, i.e. appearance, quantitative content of basic substances and efficiency of labeling with radionuclide ^{99m}Tc.

VII. RESULTS AND DISCUSSION

When studying the effect of the concentration of HEDP, Sn^{2+} and pH on the efficiency of the formation of the HEDP^{99m}Tc complex in solutions, it varied within 1.0-4.0 mg/ml, 0.1-0.42 mg/ml and 4.0-6.0 respectively. With the minimum amount of Sn^{2+} (0.1 mg/ml) involved in the reduction reaction of 99mTcO4-, complexation of ^{99m}Tc with HEDP content of 1.0 mg/ml and solution pH 5.0, the maximum level of bound 99mTc was ≤ 83.7%. A high level of ^{99m}Tc binding to HEDP ≤ 99.5% was observed in substances with a quantitative content: Sn^{2+} 0.3 mg/ml, with HEDP 2.5 mg/ml and solution pH 5.0. The results obtained are shown in table1.

Table 1. Efficiency of formation of the HEDP^{99m}Tc complex depending on the concentration of HEDP, Sn^{2+} and the pH of the complex solution (in percent), 20 minutes after adding $^{99m}TcO4^-$ to the substance.

HEDP mg/ml	Content of Sn^{2+}, mg/ml								pH, solution complex
	0,1	0,2	0,24	0,3	0,36	0,38	0,4	0,42	
1,0	82,6	83,1	85,6	86,9	86,1	84,2	82,1	81,7	4,0
	83,7	**85,7**	**87,1**	**88,2**	**87,6**	**84,9**	**83,7**	**81,9**	5,0
	82,9	82,6	85,2	86,3	85,7	83,3	81,9	80,5	6,0
1,4	84,6	85,1	88,6	90,9	88,7	87,2	86,1	84,7	4,0
	85,7	**87,7**	**89,1**	**92,2**	**91,6**	**90,5**	**86,7**	**84,9**	5,0
	84,5	84,9	88,2	90,8	88,3	87,1	85,9	84,2	6,0
1,8	88,1	89,4	95,4	96,1	93,7	92,2	89,0	86,7	4,0
	89,7	**92,7**	**96,1**	**96,9**	**94,3**	**93,5**	**89,3**	**86,9**	5,0
	88,0	89,2	95,2	95,8	88,3	87,1	85,9	84,2	6,0
2,0	92,9	95,9	97,6	98,1	96,9	95,0	91,3	90,5	4,0
	95,1	**97,2**	**98,9**	**99,1**	**98,6**	**97,0**	**92,1**	**90,5**	5,0
	92,8	95,5	97,2	98,0	96,2	93,1	90,9	90,2	6,0
2,5	93,1	96,4	97,9	98,6	97,7	95,2	91,5	90,7	4,0
	95,7	**97,7**	**99,1**	**99,5**	**99,1**	**98,5**	**92,3**	**90,9**	5,0
	92,8	96,5	97,2	98,5	97,1	95,1	90,9	89,2	6,0
3,0	92,7	95,6	97,4	98,0	96,7	95,0	91,1	90,2	4,0
	95,1	**97,1**	**97,5**	**99,0**	**97,0**	**95,0**	**91,9**	**90,3**	5,0
	92,6	95,5	97,2	98,0	96,1	92,9	90,8	90,0	6,0
4,0	91,8	94,9	95,8	97,0	96,1	93,0	89,0	85,2	4,0
	92,7	**95,6**	**97,4**	**98,0**	**96,7**	**95,0**	**91,1**	**90,2**	5,0
	92,6	95,5	97,2	98,0	96,1	92,9	90,8	90,0	6,0
	Efficiency of formation of the HEDP^{99m}Tc complex, %								

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From the results of table 1, it can be seen that a batch of a substance with a quantitative content of potassium-sodium salt of 1-hydroethylenediphosphonic acid in the range of 2.0-3.0 mg/ml, Sn^{2+} 0.3 mg/ml, and a solution pH of 5.0, forms a fairly stable complex with 99mTc with an efficiency of ≤ 99.5%.

Increasing the concentration of Sn^{2+} to 0.38 mg/ml in the reaction mixtures had no significant effect on the nature of the formation of ^{99m}Tc complexes with potassium-sodium salt of 1-hydroethylenediphosphonic acid. But a further increase in the concentration of Sn^{2+} in the reaction mixtures to 0.42 mg/ml, the formation of the ^{99m}Tc complex with HEDP begins to decrease, and this is apparently due to the fact that an excess amount of Sn^{2+} leads to the reduction of $^{99m}TcO_4^-$ ions in pertechnetate to a lower oxidation state of technetium.

When studying the rate of formation of the HEDP^{99m}Tc complex, the concentration of Sn^{2+} in the reaction mixture was 0.3 mg/ml, HEDP 2.5 mg/ml, and the pH of the solution was 5.0. The results obtained are shown in table 2.

Table 2 - Kinetics of formation of the HEDP^{99m}Tc complex at different time intervals

MDP, mg/ml	Sn^{2+}, mg/ml	Time, (minutes, hour)						
		5min	10 min	15 min	20 min	1 h	2,5 h	5 h
2,5	**0,3**	**96,7±0,4**	**98,4±0,4**	**99,3±0,4**	**99,5±0,4**	**99,2±0,4**	**98,6±0,4**	**97,1±0,4**
		Efficiency of formation of the HEDP^{99m}Tc complex, %						

As can be seen from the results in table 2, at a concentration ratio of potassium-sodium salt of 1-hydroethylenediphosphonic acid to tin of 2.5:0.3 in the reaction mixtures, the HEDP^{99m}Tc complex was formed almost instantly and their amount remained at a high level for 5 hours. A high level of ^{99m}Tc bound to the potassium-sodium salt of 1-hydroethylenediphosphonic acid (99.5±0.4%) was observed after 20 min and remained at this level until almost 1 h. After 1 h, the complex begins to hydrolyze and by 5 h the condition remains 97.1%.

To study the stability of the HEDP(Sn) substance, previously prepared substances with a shelf life of 3, 6, 9 and 12 months with a content of HEDP and Sn^{2+} 2.5:0.3 mg/ml were selected with radionuclide 99mTc labeling followed by the formation of the HEDP^{99m}Tc complex and tested quantitative and qualitative characteristics of the complex. The results obtained are shown in table 3.

Table 3. Stability studies of HEDP(Sn) substance

The name of indicators	Data on the day of production	Time after production 3 months	Time after production 6 months	Time after production 9 months	Time after production 12 months
Description	white lyophilisate	white lyophilisate	white lyophilisate	white lyophilisate	white lyophilisate
Content Sn^{2+}, mg/ml	0,33	0,32	0,31	0,32	0,3
Content HEDP, mg/ml	2,55	2,52	2,54	2,51	2,52
Efficiency of complex formation HEDP^{99m}Tc, in %	99,5±0,4	99,3±0,4	99,4±0,4	99,2±0,4	99,3±0,4

From the given data in table 3. shows that the quality of the substance during storage of 12 months differs slightly from the quality of the data on the day of production. Apparently, this is due to the presence of an inert argon gas inside the vial with the substance, which prevents the decomposition of the substance and the oxidation of Sn^{2+}, thereby ensuring quality for 12 months and a shelf life of 12 months can be assigned to the substance.

VIII. CONCLUSION

Thus, in the course of the work, the optimal ratios of the main substance potassium-sodium salt of 1-hydroethylenediphosphonic acid and the reducing agent (divalent tin) were determined in the complex formation reaction of HEDP^{99m}Tc, which was 2,5 mg/ml and 0.3 mg/ml, respectively, as well as the optimal conditions for labeling the potassium-sodium salt of 1-hydroethylenediphosphonic acid with the radionuclide technetium-99m. The stability of the

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complex potassium-sodium salt of 1-hydroethylenediphosphonic acid with the radionuclide technetium-99m was determined and the preliminary shelf life of the HEDP(Sn) substance was determined, which is 12 months.

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