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Pharmaceutical Development of the Therapeutic Radiopharmaceutical Based on B-Emitting Samarium-153 in Thermally Responsive Carrier for Brachytherapy of Tumors of Various Locations

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Abstract

The present paper discusses the development of radiopharmaceuticals for brachytherapy based on thermally responsive copolymers with chelated beta-emitting radionuclide ^{153}Sm. The preparation is made of two components, each loads individual function. The first component, being a dilute solution of copolymer chains of N- isopropylacrylamide and allylamine which are esterificated by the chelating agent – diethylenetriaminepentaacetic acid and radioactive labeled ^{153}Sm, is extracted due to column fractionation of reaction mixture by acetate buffer and is used as a solvent to prepare the second component solution – a thermally responsive homopolymer of N- isopropylacrylamide with average molecular weight W ≤ 100 kDa. Semi diluted at room temperature solution of non-entangled chains of polymer- gelifier experiences the sol-gel transition at heating in a living organism and turns into dense physical gel in which copolymer macromolecule-carriers of a radionuclide are immobilized. To prevent reptation disentanglement of active copolymer chains and their exceeding of physical gel bounds, the gelifier concentration C_{pg} shall be subject to the relation $6 \leq C_{pg}[\eta]_{pg} \leq 8$, where $[\eta]_{pg}$ is inherent viscosity of polymer-gelifier.

Keywords: Radiopharmaceutical; Radionuclide; Thermally Responsive Polymer; Samarium-153; N-Isopropylacrylamide; Allylamine; Diethylenetriaminepentaacetic Acid; Polyvinylpyrrolidone; Samarium Chloride

Introduction

In medical practice of malignant tumors treatment brachytherapy is actively used, and it is based on injection of radioactive sources directly into a tumor. New methods are based on suppression of macromolecular radioisotope carries mobility near the preparation injection site due to their binding in a polymer gel. Preference is given to (co)polymers in aqueous solution of which there is the reversible sol-gel transition caused by properties changes in a rather narrow interval of a medium factors [1]. Aqueous solution of thermally responsive (co)polymer homogenous and low-viscous at room temperature, after the transition becomes sufficient firm gel containing radionuclide atoms (RN) and is in fact a localized radiation source for brachytherapy.

The present paper sets the task to develop a radio pharmaceutical, based on component-wise division of self-collapsing radio pharmaceutical functions. The solver is that, being in the aqueous solution copolymer macromolecule-carriers of radionuclide serve as a therapeutic agent affecting a tumor; and thermally responsive chains of a homopolymer-gelifier function as a compactor of polymeric phase of the preparation. The task performance supposes to perform the synthesis of macromolecules of copolymer N-isopropylacrylamide (NIPA) and allylamine (AAm) and further polymer-similar transformations in linker amino groups – that is the reactions of esterification by the chelating agent of diethylenetriaminepentaacetic acid dianhydride (DADTPA) and labeling of $^{153}Sm^{3+}$.The stability constant of complex compound ^{153}Sm-DTPA is 20–21 [2]. Compulsory stage of splitting an active co polymeric component from low-molecular compounds of radiolabelled product is supposed to be performed by the elution with acetate buffer on a chromatographic column and collection of diluted fraction of the effluent containing not less than 1 weight % of radioactive co polymeric macromolecules. The idea of radiopharmaceutical development is to use this fraction as a solvent for preparation of polymer-gelifier poly-N-isopropylacrylamide (PNIPAM) solution with sufficient average molecular

weight and required concentration; and this polymer-gelifier solution is a radiopharmaceutical itself. The role of the polymer-gelifier is to form a space fluctuation network which after phase transition turns into a dense conglomerate of macromolecules and prevents the disentanglement of immobilized chains of radionuclide copolymer-carrier. The research objective was to find the favorable ratio of hydrodynamic and molecular characteristics of the preparation components which satisfy the requirements for innovative radiopharmaceuticals used in endoradiotherapy of solid tumors.

Experimental Part

Synthesis of (co)polymers based on NIPA (Sigma-aldrich, 97%) and AAm (Merck, 99%) was performed by radical (co) polymerization in freshly distilled dioxane with the use of recrystallized azobisisobutyronitrile (AIBN) as an initiator. The reaction mixture in glass vials was vacuumed, sealed, put into a thermostat at a given temperature and held for a fixed time, and after its end the vial content was precipitated by diethyl ether (Table 1).

[NIPA], mol/l	[AAm], mol/l	[AIBN], mol/l	Solvent	T, °C	t, h	Conversion Ψ, %	[η], dl/g	Mη, kDa	Concentration NH_2-groupsin copolymer, mol.%
0.80	0.12	$3.2•10^{-4}$	1,4-dioxane 90 weight %	70	20	87	0.16	14	1.5
0.88	0.105	$3.2•10^{-4}$	1,4-dioxane 90 weight %	60	23	74	0.24	28	1.5
0.88	-	$3.2•10^{-4}$	1,4-dioxane 90 weight %	66	4	94	0.54	104	-

Table 1: Synthesis conditions and properties of received polymers

Due to the fact that AAm is a strong chain transfer agent, we failed to obtain samples of copolymer-carrier with medium-viscosity molecular weight M_η > 40kDa. MWD of copolymers are unimodal. Dry residue was re dissolved in freshly distilled tetrahydrofuran (THF) and precipitated with diethyl ether, and then we calculated the conversion degree and the copolymer composition. Inherent viscosity [η] of aqueous solutions of (co)polymer samples was measured with Ubbelohde viscosimeter at 20 °C in 0.5M solution $LiNO_3$. To calculate the medium-viscosity molecular weight of PNIPA with small amount of AAm ≤ 1.5 weight % we use the Marc-Kun-Hauvink's equation $[\eta] = 4.7•10^{-4}•M_w^{0.61}$[3].

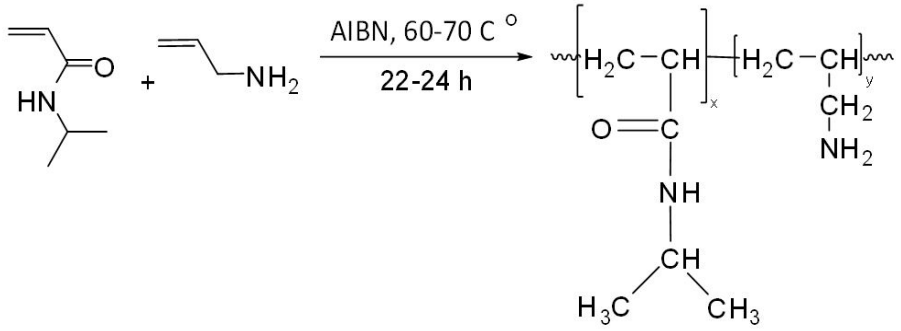


Figure 1: Reaction scheme of N- isopropylacrylamide and allylamine copolymerization

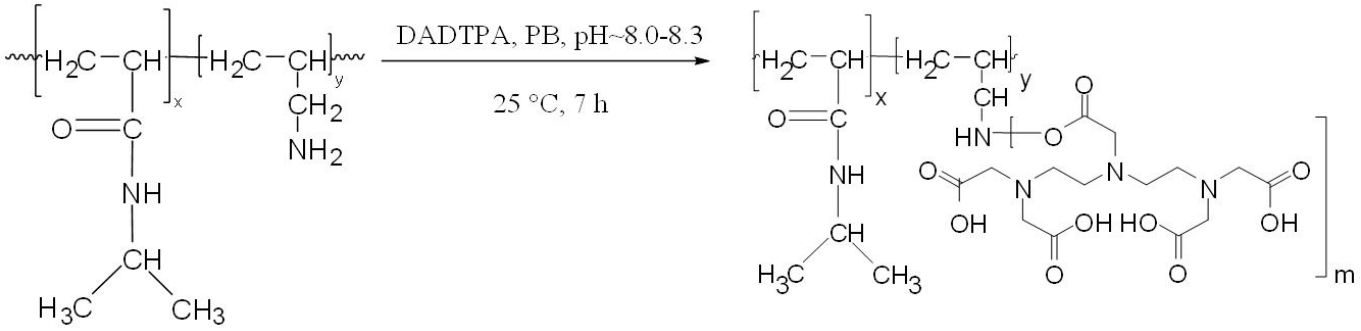


Figure 2: Scheme of esterification reaction

Synthesis of polymer-gelifier in the form of homopolymer PNIPA with average molecular weight of 80 – 100 kDa was performed in the same way as synthesis of polymer-carrier without AAM. At final stage of the extraction both polymer and copolymer undergo liophilization.

MWD of PNIPA homo- and copolymers samples was specified by gel-penetrating chromatography (GPC) at Waters chromatographer with refractive index detector equipped with three chromatographic columns of high resolution HR4, HR5 and HR6 [4]. The columns are filled with polystyrene gel. For calibration we use tight-dispersive PS-standards within the range

of MW = $5 \bullet 10^3$ - $1 \bullet 10^7$. THF served as a solvent, elution was performed at the temperature of 30 °C and speed of 0.8 ml/min, solutions concentration was 0.05–0.2 weight %. Parameters of the copolymer-carrier were calculated with GPC diagram using the calibration with PS-standards and conversion coefficient for PNIPA Q* = 1.2. Figure 3 shows the estimated data for three samples synthesized under the conditions described in Table 1.

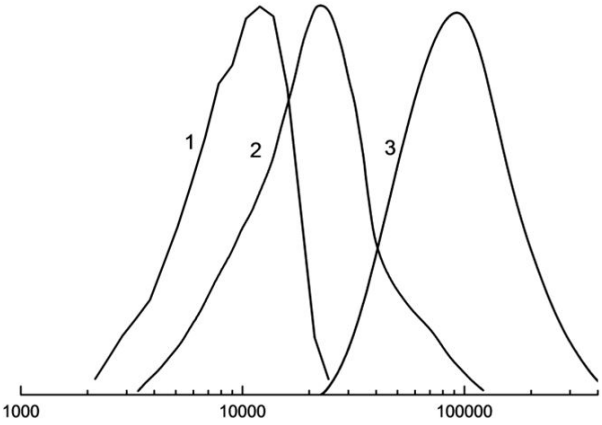


Figure 3: MWD curves: 1 – low-molecular sample of copolymer (Mw = 12 kDa, Mn= 9.4 kDa, Mw/Mn= 1.35); 2 – "most suitable" copolymer-carrier (Mw = 30 kDa, Mn= 20 kDa, Mw/Mn= 1.5); 3 – homopolymer-gelifier ([η] pg = 0.54, Mw = 102 kDa, Mn= 75 kDa, Mw/Mn= 1.36)

The values of the low critical solution temperature and phase separation temperature (Tps) of PNIPA solutions and its copolymers were estimated from the dependence of optical density of aqueous solutions on temperature with use of interval method at spectrophotometer Agilent 8453 UV-vision. The Tps range of (co)polymer samples in the present paper was 32 – 34 °C (Figure 4).

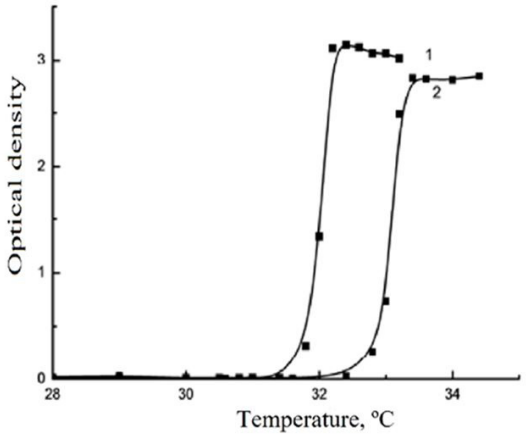


Figure 4: Curves of PNIPA cloudiness in a salt solution (1) and in water (2)

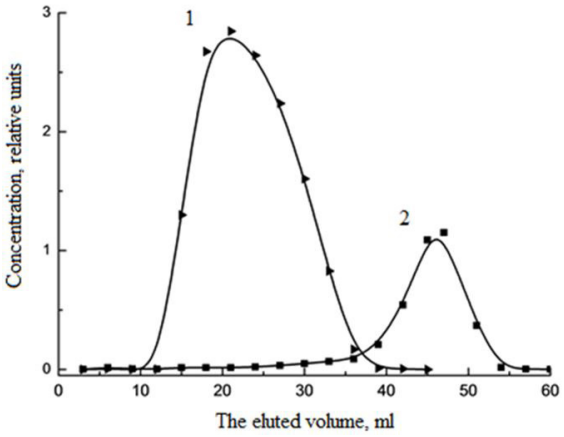


Figure 5: Curves of column fractionation of individual substances: copolymer-carrier (curve 1) and DTPA (curve 2)

Addition of chelating agents into a chain was performed by esterification of aminogen groups of DADTPA copolymer in a glass jacket vessel under continuous mixing and temperature 25 °C in 2% solution of copolymer in phosphate buffer with pH ~ 8.0 – 8.3 for 7 hours (Figure 2). Reaction products were divided on a preliminary calibrated column filled with swollen Sephadex and using water as a mobile phase (Figure 5).

The excess of not included in the chain residues of diethylenetriaminepentaacetic acid were removed from the aqueous solution by dialysis through a membrane with pore diameter correspond to MW ~ 3 kDa, and polymeric fractions after dialysis underwent the lyophilization. Esterification degree was calculated by the content of aminogen groups in copolymer before and after esterification (Figure 6). The presence of primary aminogen group in a polymer chain is indicated by the reaction with picrylsulfonic acid in a media of Sodium Tetraboarte aqueous solution, which allows calculating its content by spectrophotometric method. The method description is given in [5]. Picrylsulfonic acid easily reacts with primary aminogen groups of amino acids in an aqueous solution under pH>8 with formation of yellow (orange) adducts.

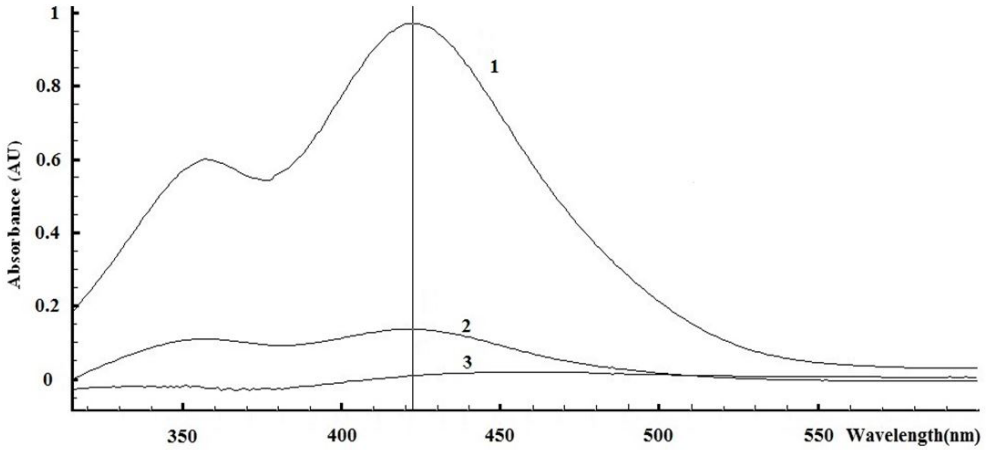


Figure 6: Optical absorption spectra in UV-spectrum of copolymer sample solution with different esterification degree: 1 – non-esterified sample, 2 – 80 %, 3 – ~100 %

Color derivatives are recorded at the wavelength of 250 and 420 nm with extinction coefficient (10 – 15). 10^3 l/mol•cm.

Radiolabelling of esterified copolymer was performed in acetate buffer (pH = 5.4) mixed with radioactive $^{153}SmCl_3$ hydrochloride solution during 1 hour at temperature 29 °C. Table 2 shows main nuclear-physical characteristics of ^{153}Sm radionuclide, taken from [6]. $^{153}SmCl_3$ radionuclide was prepared by irradiation of $^{152}SmCl_3$ target in WWR-c nuclear reactor.

Decaytime, h	Energy of β-particles, keV	Intensity of β-particles with maximum yield, %	Energy of γ-quanta, keV	Intensity, %
$T_{1/2}$= 46.7	203	35	42	57
T_e = 66.8	229	43	70	5.4
	268	21	103	28.3

Table 2: Main nuclear-physical characteristics of 153Sm radionuclide

The problem of complexation of multivalent metal ions with chelating agents was studied in several papers [7-10].

To determine radiochemical purity (RCP) of a preparation without gelifier we used thin-layer chromatography method including chromatography of radioactive fractions on Sulifol plates in aqueous solution of 1 M ammonium sulphate, and following activity measurement of plate fragments with gamma-spectrometer or the whole plate on gamma-scanner (Figure 7).

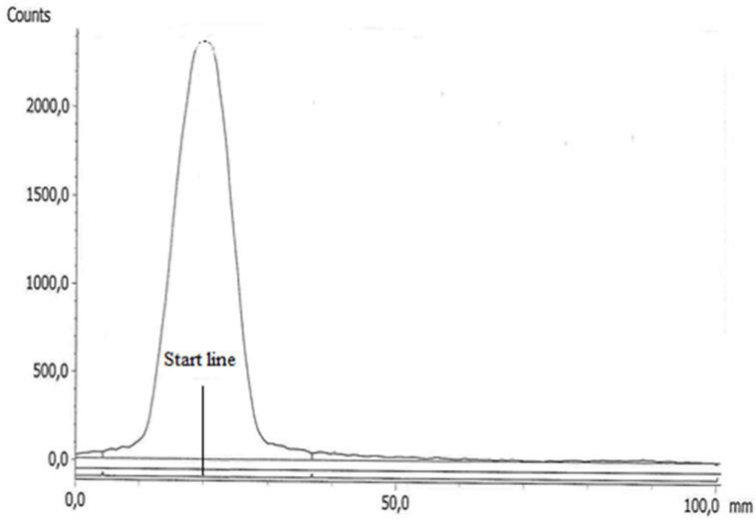


Figure 7: Chromatogram of column effluent of copolymer-carrier: fixed phase – Silufol plates, mobile phase – aqueous 1M solution of ammonium sulphate

Samples with radiochemical purity RCP ≥ 95 % were considered eligible for the further testings.

Results and Discussion

The selection of gelifier and its parameters is based on the following physical interpretation of a fluctuation quasi network which is formed in a solution by chain contacts of different macromolecules [11]. Dilute solution where a single chain moves independently from other chains is not suitable for our objectives, as after phase transition it transfers to coacervate solution of milk dispersion type. Semi dilute solutions are the solutions which area is restricted by the values of concentrations of c* coils overlapping and entanglement of ce chains not matching each other. In solutions of neutral macromolecules in the range of $5 \le c_e/c^* \le 10$ chains are not entangled, and viscosity is a subject to the power law $\eta \sim c^{5/4}$ [12]. At concentration $c_e \approx 10c^*$ entanglement increases power law parameter by threefold: $\eta \sim c^{15/4}$. It in its turn leads to unnecessarily high absolute viscosity of thermally responsive polymer solutions at room temperature that causes trouble to membrane filtration and use of standard injection needles.

Phase transition in a body activates collapse of the polymeric phase, and possibility of intra-chains contacts increases, anchoring knots become a promotion factor to hold immobilized chains which are radionuclide carrier in three-dimensional network. In this manner, to prevent reptation disentanglement of relatively short active copolymer chains and their exceeding of physical gel bounds, the physical models require that the gelifier concentration C_{pg} shall be subject to the relation $5 < C_{pg}[\eta]_{pg} < 10$. The gelifier function can be successfully performed by PNIPA with appropriate MWD parameters and concentration in solution for injections. Upper limitations on molecular weight are set to simplifyremoval of polymer coils having appropriate hydrodynamic volume from the body through kidneys.Lower limits are due to the fact that short chains of PNIPA with $MW < W_{cr} \approx 14$ kDa don not fit into three-dimensional cross-linking network.

In gel-penetrating chromatography (GPC) method an exclusion fractionation of macromolecules is performed upon their hydrodynamic volumes $f = M[\eta]$ [4]. It is postulated that under identicalprocedure conditions gel-penetrating chromatography for macromolecules of researchable polymer and PS-standards which are removable with the same retention volume, the hydrodynamic volumes of these polymers and PS-standards are equal. Q-factor is a proportionality coefficient between MW ofpolymer chain and its contour length. Within the frame of freely jointed Gaussian chain model the ratio $Q = S \bullet M_0/A$ (M_0 – molecular weight of monomer unit, S – number of units in a segment, A –segment size) is fair, so that during Q estimation there is no need to consider particular composition of polymer chain. Classic values $S_{PS} \approx 7.9$, $A_{PS} \approx 2$ nm allow determine QPS ≈ 410 нм-1, and exactly PS-standards most often calibrate columns for gel-penetrating chromatography [13]. In fact, ratio $Q^* = Q/Q_{PS}$ is a conversion coefficient while calculation average MW of researchable polymer: $Mw = Q^*M_{wPS}$, $Mn = Q^*M_{nPS}$. Putting the reported values $S_{PNIPA} = 62$, $A_{PNIPA} = 14$ nm, $S_{PVP} = 14.5$, $A_{PVP} = 3.65$ nm, calculated in accordance with data, we get estimated values $Q^* \approx 1.2$ and 1.1 for PNIPA and polyvinylprollidone (PVP) correspondingly [14,15]. The stated calculation follows that hydrodynamic volumes of macromolecule coils of PVP and PNIPA with equal MW are the same with in reference accuracy. Medical-biological researches stated that injected in body macromolecules of PVP with MW < 30 kDa are removed through kidneys within few days [16]. Macromolecules with 30 kDa< MW < 100 kDa are leaving body much more slowly – within several months. As both polymers are not bio-degraded, it can be expected that biological fractionation of PNIPA chains happens in the similar way, as there is no quantitative reported information for this process. Consequently, in relation of removal from a body PNIPA and PVP are "bioequivalent".

Macroscopic dynamic behavior of concentrated solutions or macromolecules melts is described with inter chain topological knots model – anchoring with longer but limited life time [11]. Anchoring effects are recorded only for chains MW of which exceeds the critical value W_{cr}. Empirically was found the relation $W_{cr} \approx 2W_{anch}$, where W_{anch} equals to molecular weight of chain segments between knots of anchoring network. For aqueous solution of PNIPA it is found that size of independent cooperative kinetic unit of hydrated macromolecule during the transfer from diluted phase to the phase enriched with polymer is $W_{coop} \approx 7$ kDa [15]. It is reasonable to grant that $W_{coop} \approx W_{anch}$. Then $W_{cr} \approx 14$ kDa. In respect thereof, the experiment result from the paper is representative, showing that removal of a fraction with MW < 12 – 14 kDa from PNIPA sample due to dialyses through membranes with corresponding pose size promotes formulation of a dense compact gel upon reaching the temperature of thermo tropic transition with elastic modulus typical for firm jelly $G \sim 10^3$ Pa; otherwise there is a formation of milk dispersion [17]. Thus, phase transaction in a body causes collapsing of polymeric phase which can be considered as concentrated solution and topological knots are becoming a factor promoting holding of chains – radionuclide carriers in three-dimensional network.

To illustrate the validity of the development theoretic basis, there were made series of tests for the radiopharmaceutical thermal stability based on the model measurements of kinetics of radioactivity release from thermal radiogel to normal saline. Therein molecular and hydrodynamic properties of polymeric components were being changed for their optimization. For thermal stability tests there was prepared 1 – 2 ml of polymer-gelifier solution with the concentration of C_{pg} = 10 – 15 weight %, wherein a column copolymer fraction with volume radioactivity of 50 – 100 MBq/ml was used as a solvent. The vial with test solution and cylinder for the test with the fixed volume of normal saline V_{f0} = 80 ml were incubated in thermostat at 37 °C for 15 minutes, and then the opened vial was put inside the cylinder with normal saline. After that we registered the kinetics of radionuclide release in normal saline volume, in addition, after the time t from the test beginning we measured activity at in 1 ml sample, and then calculated the activity quantity $q_t = a_t V_{ft}/A_0$ came out from thermal radio gel to normal saline considering samples volume and natural decay of 153Sm with constant $T_e \approx 67$ hours (Table 3). The trials lasted 3 – 4 days continuously. As a positive example we would like to show

the results of one trial. The radiopharmaceutical was prepared by radiolabelling of esterified lyophilized copolymer (80 mg) at the temperature 29 °C for 1 hour in a mixture of 2 ml acetate buffer (pH = 5.45) and 1 ml hydrochloride solution of $^{153}SmCl_3$ of 1.85 GBq activity. The reaction mixture was divided on a column by elution with acetate buffer, and then the volume activity of polymer fractions was measured at gamma-spectrometer. After that, PNIPA polymer-gelifier ($[\eta]_{pg}$ = 0.54 dl/g) with the concentration $C_{pg} \approx$ 12 weight % and initial activity A0 = 62 MBq/ml was dissolved in 1 milliliter of the first polymer fraction (V* = 15 – 21 ml, activity 540 MBq, radiochemical purity ≈ 97 %).

Trialtime, t, h	Activity of 1 ml – normal saline sample, a_t, kBq/ml	Proportion $q_t = a_t V_{\phi t}/A_0$, %
1	8	1
24	30	5.5
48	17	4.6
72	13	5

Table 3: Kinetics of radioactivity release from thermal radiogel to normal saline volume at 37 °C

As Table 3 shows, the relative proportion of activity released in normal saline was kept constant at a very low level $q_t \approx 5\%$. The sample is acceptable as a preparation for pre-clinical trials. It shall be noted that in the considered example the product $[\eta]_{pg} C_{pg} = 0.54 \cdot 12 \approx 6.5$ is relating to semi dilute solution $5 < C_{pg}[\eta]_{pg} < 10$, in which upper limit corresponds to chains entanglement c_e and lower limit – to concentration of diluted solution $c^* \approx 1/[\eta]_{pg}$.

In negative examples, where unacceptable high release of active copolymer chains from thermal radio gel to normal saline can be observed, the value of criterial product goes down to $C_{pg}[\eta]_{pg} < 5$. When criterial value becomes too high $C_{pg}[\eta]_{pg} > 10$, there is no opportunity to take the preparation into syringe and make injection due to high value of the radiopharmaceutical solution absolute viscosity at room temperature. Based on the analysis of favorable situations massif, it is concluded that in positive examples the condition $5 < C_{pg}[\eta]_{pg} < 10$ tapers to in equation $6 \le C_{pg}[\eta]_{pg} \le 8$ or $C_{pg}[\eta]_{pg} \approx 7 \pm 1$.

For pharmacokinetic studies used: 30 F1 mice, females with a body weight of 16-20 g, 30 C57Bl/6 mice, females with a body weight of 16-20 g. Stamm S37 (mouse sarcoma) was maintained in ascites form in ICR male mice (CD-1) with an inoculation interval of 7-9 days. A suspension of tumor cells isolated from ascites was inoculated into C57Bl / 6 hybrid mice subcutaneously on the side in an amount of 1×10^6 cells/mouse in 0.06 ml of saline.

B16 cells (mouse melanoma) were cultured in plastic bottles with a 25 cm^2 cell growth surface (Costar, USA) on DMEM environment with L-glutamine (PanEco, Russia) with 10% fetal calf serum (ETS; PanEco, Russia), moistened atmosphere at 37 °C, in an atmosphere of 5% CO_2.

Cells from 3 to 8 culture passages were used for grafting. Cells were removed from the vials with Versene's solution (PanEko, Russia), washed in DMEM environment not containing ETS, and inoculated to C57Bl/6 mice subcutaneously on the side, in an amount of 1×10^6 cells/mouse, in a volume of 0.06 ml.

7 days after tumor inoculation, a single radiopharmaceutical with an activity of 0.18–0.37 MBq in a volume of 0.1 ml was inoculated intratumoral to mice.

At various times (5 min, 1, 3, 24, 72 and 168 h) after intratumoral injection of the radiopharmaceutical, 5 animals were euthanized for each period by decapitation, samples of organs and tissues were isolated, placed in plastic tubes, weighed on an electronic scale Sartorius (Germany) and radiometry was performed using an automatic gamma counter Wizard version 2480 of PerkinElmer/ Wallac (Finland).

At the time of inoculation in a separate tube was taken a sample of radiopharmaceutical in the amount of 0.1 ml for use as a standard injected dose. In addition, 5 mice with sarcoma-S37 and melanoma B16 were placed in individual cells to collect urine. Then, at certain times urine was collected in tubes and radiometry was carried out.

According to radiometry data, specific activity per 1 g of organ or tissue was calculated for each observation period, as well as the total activity content in the organ or tissue using directly obtained results of weighing organs or tables of the average weight of the relevant organs or tissues of mice. The specific content of radioactivity in organs and tissues of mice F1 with sarcoma-S37 at different times after intratumoral injection of the radiopharmaceutical is presented in Table 4 and in Figures 8 and 9, the total content-in Table 5. 7 days after injection the activity level in tissue of sarcoma S37 was 57.2% of administrated dose, and in tissue of melanoma B16 – 42.2% of administrated dose.

	Name of organ, tissue	Time after radiopharmaceutical injection					
		5 min	1 h	3 h	24 h	72 h	168 h
1	Blood	0,89±0,35* 0,99±0,10 **	0,90±0,35 0,66±0,11	0,25±0,05 0,20±0,02	0,015±0,002 0,017±0,002	0,004±0,001 0,009±0,002	0,005±0,001 0,032±0,015
2	Thyroid	0,11±0,03 0,40±0,14	0,13±0,04 0,29±0,03	0,09±0,03 0,19±0,04	0,08±0,04 0,27±0,03	0,13±0,03 0,35±0,14	0,20±0,05 0,53±0,19

	Name of organ, tissue	Time after radiopharmaceutical injection					
		5 min	1 h	3 h	24 h	72 h	168 h
4	Liver	0,21±0,06 0,32±0,05	0,69±0,08 0,85±0,10	0,82±0,10 0,92±0,20	0,98±0,03 1,07±0,06	0,69±0,07 1,10±0,13	0,76±0,08 1,14±0,07
5	Kidneys	1,41±0,52 1,93±0,26	4,63±0,27 5,10±0,37	6,45±0,75 6,85±0,42	6,52±0,50 7,41±0,60	4,06±0,54 5,31±0,62	3,61±0,44 5,07±0,39
6	Spleen	0,09±0,03 0,13±0,02	0,13±0,01 0,18±0,01	0,10±0,02 0,12±0,02	0,09±0,01 0,09±0,01	0,033±0,002 0,090±0,010	0,063±0,013 0,060±0,020
7	Heart	0,23±0,06 0,27±0,04	0,24±0,03 0,37±0,04	0,14±0,01 0,15±0,03	0,066±0,006 0,080±0,010	0,061±0,026 0,080±0,020	0,053±0,007 0,090±0,020
8	Brain	0,015±0,005 0,030±0,006	0,018±0,002 0,025±0,005	0,008±0,001 0,012±0,001	0,008±0,001 0,011±0,004	0,005±0,001 0,012±0,002	0,007±0,003 0,016±0,006
9	Stomach	1,31±0,60 4,22±2,41	0,56±0,14 2,10±1,58	0,22±0,04 0,50±0,18	0,13±0,01 0,17±0,03	0,09±0,01 0,14±0,02	0,10±0,01 0,15±0,03
10	Intestines	0,19±0,06 0,26±0,02	0,22±0,03 0,33±0,02	0,14±0,03 0,19±0,02	0,11±0,01 0,28±0,07	0,08±0,02 0,18±0,04	0,10±0,04 0,11±0,02
11	Leather	1,16±0,53 0,19±0,04	1,34±0,37 0,46±0,10	0,38±0,03 1,15±0,68	0,49±0,26 0,13±0,02	0,33±0,08 0,11±0,02	0,11±0,01 0,11±0,02
12	Muscle	0,15±0,04 0,06±0,01	0,44±0,08 0,09±0,01	0,09±0,01 0,05±0,01	0,070±0,030 0,017±0,003	0,060±0,020 0,021±0,004	0,018±0,002 0,035±0,012
13	Hip bone	0,21±0,07 0,16±0,03	0,39±0,04 0,38±0,05	0,29±0,01 0,42±0,05	0,41±0,07 0,56±0,01	0,51±0,05 0,66±0,10	0,58±0,08 0,80±0,06
14	Tumor	186,7±8,08 211,5±27,4	185,8±30,2 175,4±18,2	108,3±11,2 115,2±40,4	122,1±16,4 145,0±13,2	129,9±9,19 68,9±7,72	56,2±6,60 23,4±7,51

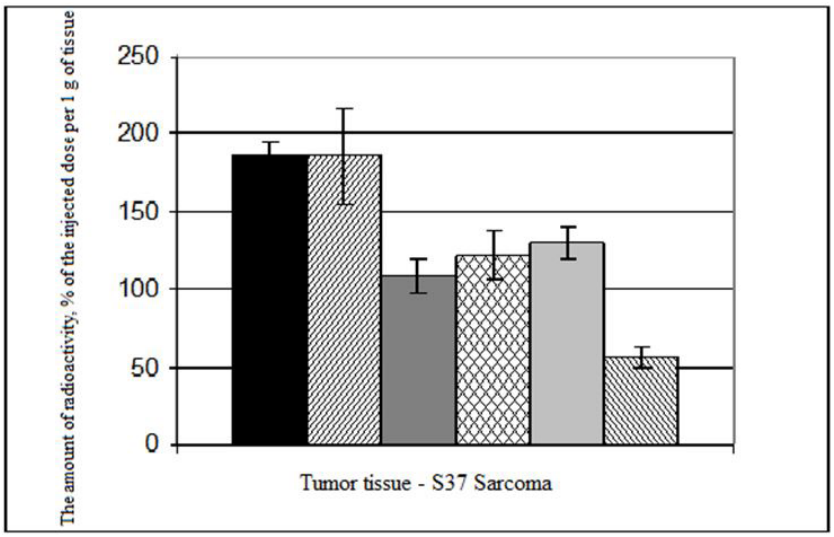
* – F1 mouse with sarcoma-37; ** – C57Bl / 6 mice with B16 melanoma

Table 4: Specific content of the activity in the organs and tissues of F1 mice with S37 sarcoma and B16 melanoma after intratumoral injection of the radiopharmaceutical (% of the injected dose per 1 g of organ or tissue)

	Name of organ, tissue	Time after radiopharmaceutical injection					
		5 min	1 h	3 h	24 h	72 h	168 h
1	Blood	1,32±0,51* 1,27±0,11**	1,28±0,49 0,80±0,08	0,037±0,06 0,26±0,03	0,021±0,003 0,021±0,002	0,007±0,002 0,013±0,003	0,007±0,001 0,045±0,020
2	Thyroid	0,005±0,001 0,015±0,004	0,005±0,001 0,010±0,001	0,004±0,001 0,007±0,002	0,003±0,002 0,010±0,001	0,006±0,002 0,014±0,005	0,008±0,002 0,021±0,006
3	Lungs	0,08±0,02 0,06±0,01	0,066±0,004 0,20±0,12	0,037±0,008 0,05±0,02	0,016±0,001 0,020±0,003	0,010±0,001 0,029±0.007	0,011±0,001 0,017±0,004
4	Liver	0,20±0,05 0,27±0,03	0,65±0,07 0,66±0,05	0,78±0,11 0,77±0,13	0,90±0,03 0,88±0,05	0,66±0,04 1,03±0,09	0,66±0,07 1,01±0,06
5	Kidneys	0,16±0,05 0,22±0,03	0,54±0,03 0,58±0,02	0,83±0,10 0,80±0,08	0,83±0,04 0,92±0,04	0,51±0,03 0,62±0,06	0,43±0,05 0,54±0,03
6	Spleen	0,022±0,005 0,013±0,002	0,024±0,003 0,017±0,001	0,026±0,005 0,012±0,003	0,027±0,003 0,008±0,001	0,013±0,001 0,011±0,002	0,026±0,004 0,010±0,003
7	Heart	0,022±0,006 0,030±0,005	0,026±0,002 0,040±0,006	0,014±0,001 0,015±0,002	0,007±0,001 0,008±0,001	0,007±0,002 0,009±0,002	0,005±0,001 0,010±0,002
8	Brain	0,005±0,001 0,009±0,002	0,005±0,001 0,009±0,002	0,003±0,001 0,004±0,001	0,003±0,001 0,004±0,001	0,002±0,001 0,004±0,001	0,002±0,001 0,005±0,002
9	Stomach	0,21±0,11 0,56±0,26	0,07±0,01 0,23±0,09	0,031±0,005 0,07±0,02	0,020±0,002 0,027±0,004	0,013±0,002 0,024±0,003	0,015±0,003 0,019±0,003
10	Intestines	0,04±0,01 0,04±0,01	0,024±0,003 0,04±0,01	0,017±0,003 0,025±0,002	0,012±0,001 0,034±0,006	0,008±0,001 0,029±0,003	0,019±0,009 0,016±0,004
11	Leather	3,19±1,06 0,47±0,06	3,72±1,14 1,02±0,21	1,02±0,09 2,81±1,21	1,28±0,61 0,30±0,05	0,92±0,18 0,29±0,03	0,27±0,02 0,30±0,04
12	Muscle	1,41±0,32 0,51±0,08	4,06±0,52 0,66±0,05	0,81±0,13 0,38±0,06	0,65±0,20 0,14±0,02	0,56±0,15 0,20±0,04	0,16±0,02 0,31±0,10
13	Hip bone	0,016±0,005 0,010±0,001	0,029±0,002 0,023±0,002	0,022±0,001 0,028±0,003	0,030±0,004 0,037±0,001	0,039±0,004 0,051±0,005	0,041±0,005 0,057±0,003
14	Tumor	85,18±3,95 88,25±2,88	67,61±2,79 66,14±4,14	61,19±2,38 60,93±3,60	61,85±1,26 56,51±2,28	57,67±2,25 43,06±1,72	57,22±0,58 42,22±5,95

* – F1 mouse with sarcoma-37; ** – C57Bl/6 mice with B16 melanoma

Table 5: The total activity content in the organs and tissues of F1 mice with S37 sarcoma after intratumoral injection of radiopharmaceutical (in % of the injected dose to the whole organ or tissue)



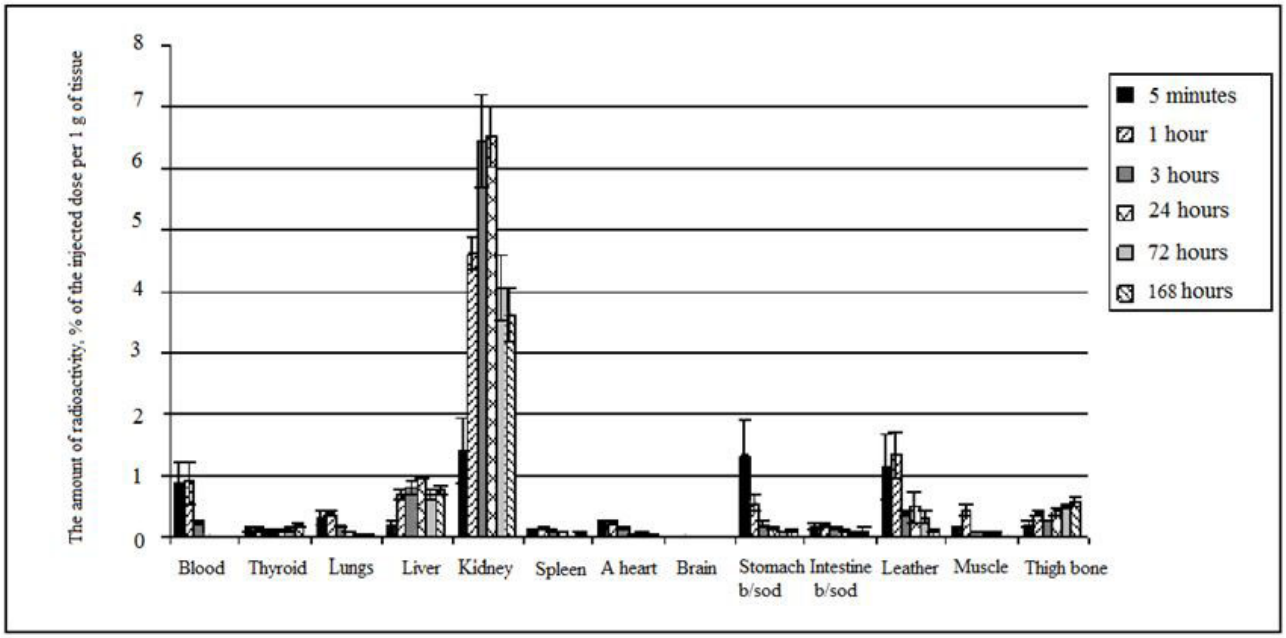
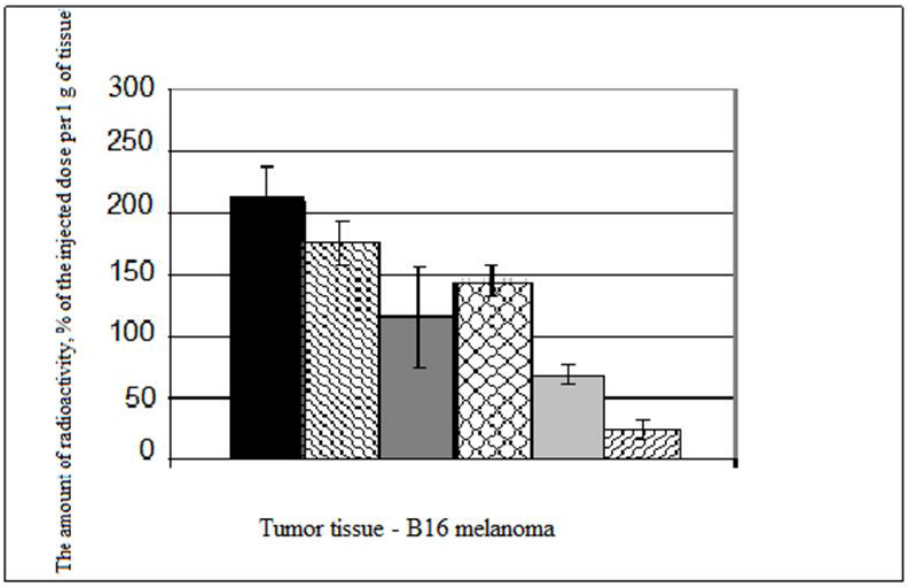


Figure 8: Specific radioactivity (in % of the injected dose per 1 g of tissue) in organs and tissues of mice F1 with sarcoma S37 at different times after intratumoralinjection of radiopharmaceutical (Top: 1-tumor tissue (sarcoma S37); Bottom: internal organs and other tissues)



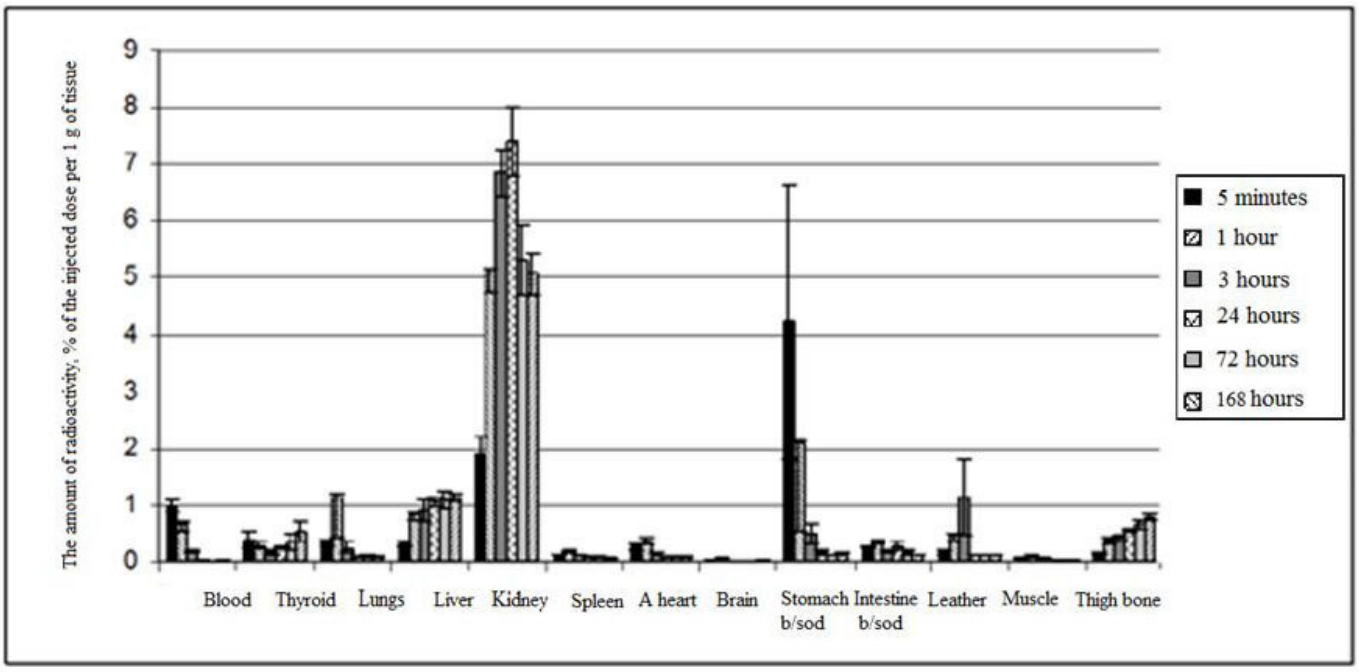


Figure 9: Specific radioactivity (in % of the injected dose per 1 g of tissue) in organs and tissues of mice C57Bl/6 with melanoma B16 at different time's intratumoralinjection of radiopharmaceutical (Top: 1-tumor tissue (melanoma B16); Bottom: internal organs and other tissues)

In addition, the retention of labeled preparation by tumor tissue of sarcoma S37 is higher than by melanoma B16 tissue. Peak value of activity in liver was 1.14%/g. Transient increase of labeled preparation concentration was observed in stomach (up to 4.22 %/g in 5 minutes), lungs (up to 1.15 %/g in 1 hour) and skin (up to 1.34 %/g in 1 hour), but after that it decreased fast. High level of activity was observed in kidneys, as the most part of the activity was removed by urinary system. Activity concentration in thyroid, spleen, heart, brain, small intestine, muscular tissue and thigh bone was lower than 1 %/g during the whole testing time. Statistically significant difference between activity levels in internal organs and tissues in mice with sarcoma S37 and melanoma B16 were little if any observed [18].

Conclusion

As a result of the performed studies, the properties complex of the proposed therapeutic radiopharmaceutical which is perspective for radionuclide endotherapy of solid tumors can be formulated as following:

– composition – homogenous aqueous solution of macromolecules of thermally responsive polymer PNIPA with WM ≤ 100 kDa and containing copolymer PNIPA chains with chelated ions of $^{153}Sm^{3+}$ radionuclide;

– the radiopharmaceutical is produced by dissolving of thermally responsive polymer-gelifier PNIPA in acetate buffer effluent released from chromatographic column in the range of hold volume of active macromolecules while dividing the products of labeling the chelately modified copolymer – carrier of ^{153}Sm radionuclide; concentration of polymer-gelifier solution C_{pg} at room temperature is selected from the range corresponding to the mode of semidelute solution $C_{pg}[\eta]_p g \approx 7\pm1$;

– expected mechanism – intratumorally injected radiopharmaceutical solution in the injection site fast achieves body temperature and due to self-collapsing of thermally responsive polymer-gelifier turns into dense chains conglomerate, that assures the lost of translational mobility of immobilized chains of copolymer-carrier with chelated radionuclides; formed in such a manner sources of local therapeutic irradiation affect cancer cells and barely impact healthy tissues;

– obtained results enable the opportunity to use this radiopharmaceutical for local therapy of tumors.

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