

SYNTHESIS OF AU (III) POLYACRYLATES AND STUDY OF THEIR TUMORICIDAL ACTIVITY

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Aurumacryl is an incomplete metal salt of poly(acrylic acid) that exhibits hemostatic activity and inhibits the growth of transplantable carcinomas *in vivo*. The samples of aurumacryl synthesized following the original technique are insufficiently soluble, which complicates the study of the mechanisms involved in their synthesis and underlying their cytotoxic effect. The aim of this work was to study the impact of the following factors on aurumacryl properties: the molecular weight of the polyacrylate polymer in a range between 2 and 1,000 kDa, the presence of a counterion H⁺ or Na⁺, the molar ratio of AuCl₄ to the polyacrylate polymer (1 : 5 and 1 : 10), the total concentration of the polyacrylate polymer during synthesis (0.1 and 3%), and the type of drying (lyophilization). By comparing the cytotoxicity of aurumacryl samples with significantly different molar ratio of gold ions to the polyacrylate polymer against human breast carcinoma cells (MCF-7), we established that the proportion of the polymer and its molecular weight in the sample do not affect the biological properties of the synthesized substance. Using UV spectroscopy, we revealed that the concentration of Au (III) ions in aurumacryl determines its cytotoxicity.

Keywords: aurumacryl, polyacrylate, chloroauric acid tetrahydrate, antitumor therapy, cytotoxicity, MCF-7

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СИНТЕЗ И ИССЛЕДОВАНИЕ ПРОТИВООПУХОЛЕВОЙ АКТИВНОСТИ ПОЛИАКРИЛАТОВ ЗОЛОТА (III)

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Аурумакрил проявляет гемостатическую активность и обладает онколитическим и онкостатическим действием в отношении перевиваемых карцином *in vivo*. Образцы аурумакрила, полученные по известной методике, недостаточно стандартизированы по растворимости, что не позволяет изучить механизмы реакции и цитостатического действия. Целью работы было исследовать влияние на свойства аурумакрила следующих параметров проведения реакции его синтеза: молекулярная масса полиакрилатного полимера (в диапазоне 2–1000 кДа), наличие в качестве противоиона H⁺ или Na⁺, соотношение иона тетрахлораурата и звена полиакрилатного полимера (1 : 5 или 1 : 10), общая концентрация полиакрилатного полимера в момент синтеза (0,1 или 3%), а также влияние лиофильного высушивания аурумакрила. Сопоставление цитотоксической активности полученных образцов аурумакрила со значительно различающимся соотношением ионов золота и полиакрилатного полимера в отношении клеток карциномы молочной железы человека MCF-7 позволило установить, что удельное содержание в препарате полиакрилатного полимера и его молекулярная масса практически не сказываются на биологических свойствах конечного продукта. Методом УФ-спектроскопии показано влияние концентрации ионов Au (III) в аурумакриле на их цитотоксическую активность.

Ключевые слова: аурумакрил, полиакрилат, золотохлористоводородная кислота, тетрахлораурат, противоопухолевое средство, цитотоксическая активность, MCF-7

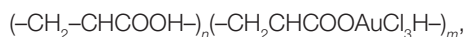
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Aurumacryl first described in [1] is an incomplete metal salt of poly(acrylic acid) (PAA) containing gold (III) ions. Based on the amount of the initial reactants consumed during aurumacryl synthesis, it was concluded that the final metal wt% in the substance would be 8.03% whereas the molecular weight of the polymer would fall within the range between 100 and 300 kDa. The chemical formula of aurumacryl is



where $n = 1,263$ and $m = 124$.

In the original study, aurumacryl was synthesized by combining aqueous PAA solutions and chloroauric acid tetrahydrate (HAuCl_4) at room temperature [1]. Briefly, HAuCl_4 was taken in the amount sufficient to produce 8.3% wt of gold in the resulting gold polyacrylate and added to a 5.7% PAA solution (the molar mass of the solute M_r was 100,000). The reaction mixture was stirred, and the solution was then left in a vacuum desiccator. The resulting substance was a dense yellow film. Its physical and chemical properties can be inferred from the absorption bands of its IR spectra characteristic of carboxyl and carboxylate groups at 1,720 and 1,570 cm^{-1} wavelengths, respectively. However, the actual content of aurumacryl components in the resulting compound, including polyacrylic acid, gold ions (III), gold ions (I), elemental gold, and other anions and cations, cannot be deduced from the presented data.

The aim of this work was to improve the technique for aurumacryl synthesis in order to obtain a chemically stable standard preparation sufficiently soluble in water and to elaborate a method for testing aurumacryl quality thereby predicting its biological activity. The substance was synthesized using commercially available PAA and sodium polyacrylate (PANa) with known molecular weights. The molar ratio between AuCl_4^- anions and PAA in the reaction mixture was 1 : 5 and 1 : 10.

Unlike our colleagues, we thoroughly desalted the PAA polymer by continuous-flow dialysis, which took a few days, and dried the solutions of the obtained polymer-gold complexes in a freeze dryer instead of leaving them to air-dry. The lyophilized aurumacryl samples were cryopreserved in liquid nitrogen and vacuumized.

To evaluate our improved method of synthesis, we tested aurumacryl for solubility and cytotoxicity against MCF-7 cells, measured the amount of gold salts in the produced samples by UV-spectroscopy, and estimated the content of gold nanoparticles in aurumacryl using transmission electron microscopy.

METHODS

Reagents

Commercially available PAA and PANa (Sigma Aldrich; USA) with different polymerization degrees (i.e., the average number of monomers per molecule) of 23; 80; 160; 1,389; 1,944; 3,472; and 13,889 were used as starting materials. The average degree of polymerization and polymer dispersion properties were specified by the manufacturer. Another component used for aurumacryl synthesis was dry chloroauric acid (Aurat; Moscow) with 48% gold content.

Preparing polyacrylic polymers for synthesis

For synthesis we chose PAA with an average molecular weight of 1,633 Da, 11,360 Da, 100,000 Da, 140,000 Da, 250,000

Da, and 1,000,000 Da (the molecular weight was specified by the manufacturer) and sodium polyacrylate (SigmaAldrich; USA) with an average molecular weight of 2,100 Da, 15,000 Da, 132,350 Da, 185,370 Da, 330,980 Da, and 1,324,000 Da.

Prior to synthesis, all PAA samples were additionally purified by flow dialysis continued for several days and thoroughly dried by freezing.

Then, weighted amounts of the polymers (50 mg) were dissolved at room temperature in 50 and 1.67 ml of water to obtain 0.1% and 3% wt polymer solutions, respectively. During the 12-h process the polymers were continuously stirred on the magnetic stirrer.

Aurumacryl synthesis

Chloroauric acid was added to the solutions of pre-deionized polymers as described below:

- 50 μL of the aqueous solutions containing 23.8 and 47.6 mg of chloroauric acid were added dropwise to 0.1% wt PAA solutions;

- 30 μL of the aqueous solutions containing 23.8 and 47.6 mg of chloroauric acid were added dropwise to 0.3% wt PAA solutions;

- 50 μL of the aqueous solution containing 18.4 and 36.7 mg of chloroauric acid were added dropwise to 0.1% wt PANa solutions;

- 30 μL of the aqueous solution containing 23.8 and 47.6 mg of chloroauric acid were added dropwise to 0.3% wt PAA solutions.

Synthesis was carried out at room temperature and took 24 h. The reaction mixture was continuously stirred on the magnetic stirrer at 60 rpm.

Low molecular weight components were separated from the obtained solutions by continuous-flow dialysis using MWCO 15,000 dialysis tubing. The solutions of gold-polymer complexes (1.7–20 ml) were poured into the tubing and dialyzed against water for 3 h at room temperature.

After that, the solutions were frozen at -70°C and lyophilized, except for a few samples that were not freeze-dried but stored at $+4^\circ\text{C}$ under aseptic conditions instead.

Determining solubility of aurumacryl samples

Solubility was determined for each of the obtained dry aurumacryl samples. Briefly, 100 μL water aliquots were added one by one to 1 mg of the sample at room temperature and its behavior was monitored for 30 min. If the sample did not dissolve, another water aliquot was added to it. The procedure was repeated until complete dissolution was achieved.

Measuring the conversion of gold ions in concentrated aurumacryl solutions

The conversion of gold ions was measured in 100 mM HCl at $\lambda = 226.5$ nm. For the measurements, we took a 10 μL aliquot of a concentrated solution obtained in the previous solubility assessment step and 10 μL of a solution that was not dried immediately after the synthesis. The aliquot was introduced into 3 ml of a 100 mM HCl solution and then its absorbance $A_{226.5}$ at 226.5 nm was measured. The conversion of gold salts was determined for each sample based on the calculated $A_{226.5}$ ($\approx[\text{HAuCl}_4]$, μM) relative to the total solids concentration (the residual moisture in the dry sample was ignored). For further toxicity experiments in MCF-7 cells, concentrations of all samples were normalized based on absorbance at $\lambda = 226.5$.

Studying the cytotoxic activity of aurumacryl samples in the MCF-7 cell line with an MTT assay

Five thousand cells were seeded onto the Costar 96-well plate containing 180 μL of the DMEM medium (Paneco; Russia) per well supplemented with 10% fetal bovine serum. The cells were cultured at 37 °C and 5% CO_2 for 24 h. Then 20 μL of the aqueous aurumacryl solution with the normalized concentration (absorbance at 226.5 nm) were added into each well and continued incubation at 37 °C and 5% CO_2 for 24 h. After that, 10 μL of the MTT reagent 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (item 57360-69-7; Sigma Aldrich; USA) were added at a final concentration of 0.5 mg/ml to the culture, and the cells were incubated for 3 h. Absorbance from the wells A_{570} at 570 nm was measured by the Multiskan Go spectrophotometer (Thermo Fisher Scientific; USA).

Measuring the proportion of gold nanoparticles in aurumacryl samples by transmission electron microscopy

A 3 μL aliquot of the aqueous aurumacryl solution containing 1–5 μg of the gold-polymer complex was applied onto the Fine 200 Copper Grid Mesh (SPI Supplies; USA) with a diameter of 3.5 mm, air-dried for 5 min and vacuumized. The analysis was carried out using the JEM-100B microscope (JEOL; Japan) at the accelerating voltage of 120 kV. The microscope was equipped with an accessory for X-ray phase-contrast imaging.

The images of the particles detected within the field of view were analyzed in ImageJ (a free software by the National Institutes of Health; USA): the square area of each dark spot was calculated allowing for the zoom factor. At least 10 randomly chosen fields of view were analyzed for each sample. Once the linear sizes of the particles and their number was identified, we calculated the proportion of the particles falling within each of 5 diameter ranges of 1–10, 11–20, 20–50, 50–100, and >100 nm in Windows Excel.

RESULTS

We synthesized 23 aurumacryl samples from PAA and sodium polyacrylate with various molecular weights at 2 different molar ratios between AuCl_4^- and PAA of 1:5 and 1:10; the initial polymer concentration in the reaction mixture was 3% or 0.1%.

A total of 10 freeze-dried samples were re-dissolved in water showing good solubility.

Chloroauric acid is known to undergo gradual hydrolysis in aqueous solutions with neutral and alkaline pH; the reaction produces hydrochloric acid, which increases the acidity of the medium [2]. At $\text{pH} < 1$ this reaction is impossible. In our experiment, the aqueous solution of chloroauric acid in 100 mM HCl had an absorbance peak at $\lambda = 226.5$ nm, the extinction coefficient ϵ being $3.5 \times 10^4 \text{ l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$. These values are consistent with the published literature [2]. For the polyacrylates synthesized in the course of our experiment the extinction coefficient was below $10^6 \text{ l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$. Gold nanoparticles had zero absorbance at $\lambda = 226.5$ nm, except for the light scatter effects. Therefore, we measured the proportion of gold ions in the synthesized substance at $\lambda = 226.5$ nm in 100 mM HCl (see *Methods*).

To test a hypothesis that the cytotoxic activity of aurumacryl is determined by the proportion of Au (III) in the substance, the solutions of the synthesized aurumacryl samples and the control sample prepared following the original technique (sample 1) were normalized to the Au (III) concentration calculated from absorbance at $\lambda = 226.5$ nm. The solutions were tested for

cytotoxicity at concentrations of 9.1 mU/ml (the IC_{50} of the control sample) and 91.0 mU/ml, which is 10 times higher than the IC_{50} of the control sample. The results were averaged based on 3 independent measurements and are presented in Table 1.

Although concentrations of Au ions expressed as $\mu\text{mol}/\text{mg}$ differed between the solutions, all normalized aurumacryl samples exhibited the same cytotoxic potency as the control aurumacryl sample prepared following the original technique. The biological activity of the normalized aurumacryl samples was identical both at concentrations equaling IC_{50} of the control sample and at those 10 times higher than IC_{50} . This proves that *in vitro* cytotoxicity of aurumacryl is determined by Au ions. The polymer component of the compound seems to work as a shield protecting Au ions from the interaction with other organic molecules that can reduce them to inactive gold nanoparticles.

We hypothesized that lower conversion of gold ions in the process of aurumacryl synthesis is due to their spontaneous reduction to elemental gold that aggregates into nanoparticles. To test this hypothesis, we studied a few synthesized aurumacryl samples by transmission electron microscopy (TEM) (Table 2).

The bigger was the molecular weight of the polymer used for aurumacryl synthesis, the higher was the proportion of gold nanoparticles sized > 10 nm, which in essence are aggregates of smaller particles under 10 nm in size. Regardless of PAA molecular weight, the proportion of aggregated nanoparticles, as compared to the proportion of unaggregated, increased dramatically when the molar ratio of Au (III) to PAA was reduced from 1 : 5 to 1 : 10. When PANa was used instead of PAA as the starting component for aurumacryl synthesis, the average size of gold nanoparticles increased and more pronounced aggregation of gold nanoparticles was observed. For example, in the samples synthesized from PANa with polymerization degrees of 3,472 and 13,889 at the 1 : 10 molar ratio between gold ions and PANa, the proportion of gold nanoparticles sized ~100 nm was 75%. In other samples synthesized from PANa, such nanoparticles were not detected. The control aurumacryl sample synthesized following the original technique contained a remarkably high proportion of gold nanoparticles with an average diameter of 20–50 nm, which exceeded the proportion of such nanoparticles in other tested samples several-fold.

To validate our hypothesis about the cytotoxicity of aurumacryl samples being dependent on the proportion of Au ions and independent of the proportion of polyacrylate, elemental gold and other components, the most active samples 3, 10 and 17 (Table 1) were scrutinized for their cytotoxic activity against MCF-7 cells. The aurumacryl solutions were normalized to ion gold concentrations based on absorbance at $\lambda = 226.5$ nm in 100 mM HCl. Aurumacryl synthesized following the original technique was used as a control sample. The results of the test are shown in Fig. 1.

The correlation between the cytotoxic activity of the sample and the Au (III) concentration was the same for samples 3 and 10, as well as for the control sample, although the samples were different in their composition and the applied method of synthesis. Sample 3 was synthesized from PAA with an average molecular weight of 1.6 kDa (low polymerization degree) and was freeze-dried immediately after synthesis. Sample 10 was synthesized from PAA with a molecular weight of 250 kDa and was stored as a solution. The molar ratio of gold ions to PAA in the reaction was 1 : 32 for the control sample and 1 : 10 for samples 3 and 10. Thus, the tested samples had different solubility and a different molar ratio of Au (III) to PAA. Table 2 suggests that the samples may have contained

different proportions of gold nanoparticles. However, after the concentrations of the tested samples were normalized to Au (III), they exhibited identical cytotoxicity against the MCF-7 cells.

The correlation between the cytotoxic activity of sample 17 synthesized from PANa with an average molecular weight of 5.75 kDa and spared from freeze-drying and the concentration of gold ions in it implies that sample 17 has some properties missing by other samples. At low concentrations in the solution (0–10 mU/ml) its cytotoxicity is higher than that of the samples synthesized from PAA whereas at higher concentrations of 25

and 90 mU/ml its cytotoxic potency is worse. It means that the interactions between chloroauric acid and PAA and between chloroauric acid and PANa produce substances in which the biological activity of Au ions is slightly different.

Aurumacryl synthesized from PANa (as opposed to PAA) can hardly be used in clinical practice because of its insufficient chemical stability during storage. Regardless of the molecular weight of the polymer, both solutions and lyophilisates have a tendency to the spontaneous reduction of Au ions that subsequently form gold nanoparticles, which causes a dramatic decline in their cytotoxicity.

Table 1. Synthesis conditions, physical, chemical and biological properties of aurumacryl samples

Sample ID	Concentration of the initial polymer during synthesis, %wt	Mr of the initial polymer, kDa	Counterion	Molar ratio between Au (III) anions and PAA or PANa in the reaction mixture, mol:mol	Max concentration of aurumacryl solution based on total solids concentration, mg/ml	A _{226.5} of the concentrated aurumacryl solution, U/ml	Concentration of Au ions, mmol/mg of solids	Proportion of surviving MCF-7 cells at aurumacryl concentration A _{226.5} expressed as ml (MTT assay)	
								9.1 mU	91.0 mU
1	5.7	100–300	H	1 : 32	2.7	0.91	12.5	5	49
2	3.0	1.6	H	1 : 10	29.4	2.40	32.8	5	51
3	3.0	1.6	H	1 : 10	28.3	3.00	41.0	5	47
4	3.0	11.5	H	1 : 10	29.4	1.00	15.5	5	21
5	3.0	100	H	1 : 10	29.4	1.53	21.0	4	45
6	3.0	100	H	1 : 10	33.3	3.00	41.0	5	46
7	3.0	140	H	1 : 5	29.4	4.80	65.5	5	42
8	3.0	140	H	1 : 10	29.4	2.00	23.6	4	51
9	3.0	140	H	1 : 10	47.0	4.30	58.7	5	44
10	3.0	250	H	1 : 10	29.4	2.00	24.2	4	60
11	3.0	250	H	1 : 10	16.7	0.96	13.2	5	54
12	0.1	250	H	1 : 10	4.2	0.77	10.5	6	43
13	3.0	1000	H	1 : 10	29.4	0.56	7.7	5	44
14	0.1	1000	H	1 : 10	5.0	0.69	9.4	6	49
15	3.0	1.6	Na	1 : 10	29.4	1.27	17.4	5	45
16	0.1	1.6	Na	1 : 10	27.3	0.39	5.4	6	41
17	3.0	5.75	Na	1 : 10	29.4	1.00	14.8	6	39
18	3.0	100	Na	1 : 10	29.4	1.00	18.4	4	39
19	0.1	100	Na	1 : 5	12.6	2.58	35.3	6	46
20	3.0	140	Na	1 : 10	29.4	1.33	18.2	11	42
21	3.0	250	Na	1 : 10	29.4	1.30	17.8	6	44
22	0.1	250	Na	1 : 10	13.3	0.37	5.2	7	41
23	3.0	1000	Na	1 : 10	29.4	0.37	5.2	11	49
24	0.1	1000	Na	1 : 10	10.0	0.54	7.5	9	38

Note: Freeze-dried samples dissolved in water are shown in grey; other samples were not lyophilized but stored as solutions instead.

Table 2. Distribution of gold nanoparticle sizes in aurumacryl samples synthesized from PAA and PANa

M _r of the polymer, kDa	Counterion	Molar ratio of Au (III) ions to PAA or PANa in the reaction mixture, mol:mol	Proportion of nanoparticles of a given diameter, %				
			1–10 nm	11–20 nm	20–50 nm	50–100 nm	>100 nm
1.6	H	1 : 5	76.0	14.0	10.0	0.0	0.0
1.6	H	1 : 10	57.5	25.0	15.0	2.5	0.0
11.5	H	1 : 10	84.0	12.0	4.0	0.0	0.0
250	H	1 : 10	42.9	48.6	8.6	2.9	0.0
1000	H	1 : 10	36.0	50.0	2.0	0.0	12.0
11.5	Na	1 : 5	82.0	8.0	6.0	4.0	0.0
140	Na	1 : 5	78.0	16.0	6.0	0.0	0.0
140	Na	1 : 10	0.0	2.5	0.0	22.5	75.0
100–300	H	1 : 0.0018	46.7	23.3	30.0	0.0	0.0

DISCUSSION

The tumoricidal effect of aurumacryl was investigated both *in vivo* and *in vitro* in the study [3]. The authors report that a single intraperitoneal injection of aurumacryl at a dose of 20 mg per 1 kg mouse weight causes 80–90% growth inhibition of solid tumors in mice, including Lewis lung carcinoma, large intestine adenocarcinoma ACATOL and Ca-755 adenocarcinoma, in comparison with the controls. In the experiments *in vitro* the aurumacryl concentration of 1 mg/ml kills ~70% of the MCF-7 cells (human breast carcinoma) by triggering both necrosis and apoptosis [3]. The mechanism of the selective tumoricidal effect of aurumacryl *in vivo* is yet to be elucidated; it is known, though, that gold ions do not selectively accumulate in the tumor following the intraperitoneal injection of aurumacryl but are evenly distributed in the body of the mouse and eliminated through excretion [3].

Over the past 3 years there have been reports that acrylic acid polymers can effectively deliver different cytotoxic and antiproliferative agents to cancer cells *in vitro* and *in vivo* (see below), meaning that polyacrylic particles of different molecular weight can selectively transport such agents to cancer cells and enhance their activity inside the cancer cell. Importantly, polyacrylates can selectively accumulate in dendritic cells and macrophages. This can improve the presentation of antigens carried by a polyacrylate agent to lymphoid cells as shown in the successful test of a polyacrylate conjugated to the peptide antigen mimetic of the HPV-16 oncoprotein 7 [4]. Polyacrylate particles immobilized inside the virus-like particles composed of the hepatitis B HBs-antigen enhanced the cytotoxic effect of doxorubicin [5]. There is evidence that peroxovanadate delivered to A495 lung carcinoma cells can effectively inhibit their growth [6]. Another study demonstrates a possibility of using theranostic polyacrylate-calcium phosphate complexes as carriers of gold nanoclusters in the therapy and diagnostic imaging of solid tumors [7].

Polyacrylic clusters can be used to deliver titanium oxide nanoparticles, ferromagnetic contrast agents, and salts and oxides of other metals to the tumor to increase its sensitivity to radiotherapy, achieve a cytotoxic effect or facilitate bioimaging based on the principles of fluorescence or magnetic resonance

[8–11]. Polyacrylate can be employed to deliver methylene blue used as a photosensitizer for contrast-enhanced radiotherapy to human breast carcinoma cells (MCF-7) [12]. All data mentioned here indirectly suggest that polyacrylates are capable of selectively targeting cancer cells *in vivo*. The underlying mechanism is unclear but the existing evidence leads us to hypothesize that PAA itself cannot exhibit cytotoxic activity against cancer cells. It is probably Au ions (III) carried by PAA that make the complex toxic for cancer cells.

This supposition can be confirmed by the findings of the study [13] of Au (III) compounds dichloro(ethylenediamine)aurum and dichloro(S-methyl-L-cysteine)aurum that exhibit pronounced cytotoxicity against human lymphoid leukemia cells. The authors of the study prove that just like cisplatin Au (III) ions can form DNA adducts in living cells causing their death.

The insufficient solubility of aurumacryl synthesized as described in [1] complicates the testing of its safety and therapeutic activity against cancer cells in biological models, as well as its use in clinical practice. The effective aurumacryl dose in the mouse model is 20 mg per 1 kg body weight. The required concentration of aurumacryl in the solution would be 1 mg/ml [3]. Given that a mouse weighs about 20 g, the volume of the injected solution would be 200 μ L per mouse, approaching the acceptable threshold: further increase in the injection volume would threaten the survival of mice regardless of the composition of the injected drug. Besides, dry samples stored at +4 $^{\circ}$ C for 6 months show a sharp decline in their solubility.

By varying the parameters of the initial polymer and the molar ratio of PAA or PANa to chloroauric acid, we were able to study the impact of these factors on the reaction yield. We revealed that PAA concentrations in the produced aurumacryl and its molecular weight had almost no effect on the biological properties of the obtained substance. In contrast, the concentration of Au (III) ions measured from the absorbance of aurumacryl solutions ($\lambda = 226.5$ nm) turned to be an accurate predictor of their *in vitro* cytotoxic activity in the cell culture.

However, the molar ratio of the polymer to AuCl_4^- anions, the molecular weight of the polymer and the absolute concentration of the polymer solution have a profound impact on the gold ion concentration in the synthesized sample and

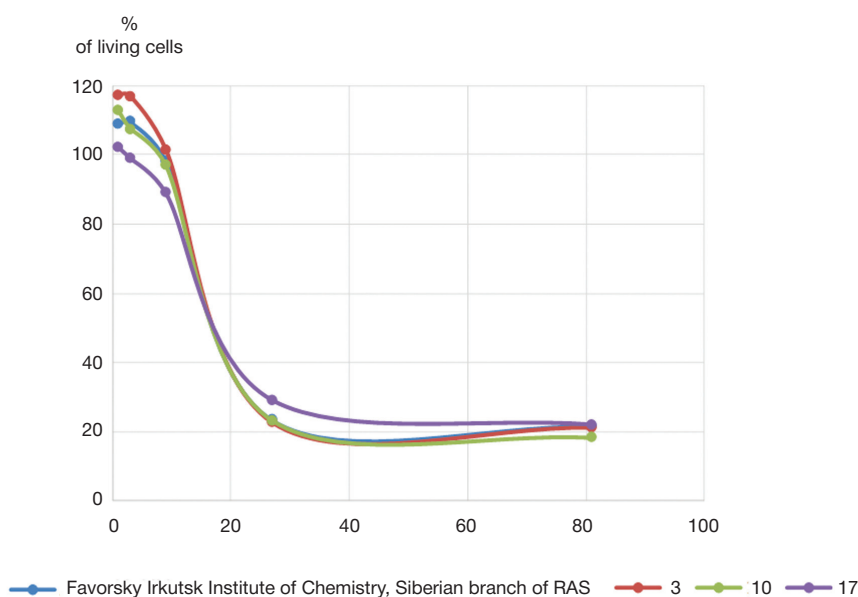


Fig. 1. The graph shows a correlation between the Au (III) concentration in the tested aurumacryl samples and their cytotoxicity against human breast carcinoma cells (MCF-7). Concentrations at $A_{226.5}$ expressed as mU are plotted on the X axis. The proportion of the surviving cells measured by the MTT assay is plotted on the Y axis. The curves were constructed for samples 3, 10 and 17 synthesized in the course of this work and for the control sample synthesized at Irkutsk Institute of Chemistry (see Table 1).

the solubility of the latter. In our experiment, the solubility of the sample was the highest when aurumacryl was synthesized from the polymers with polymerization degrees of 1,389 and 1,944. The use of the polymers with higher molecular weight resulted in a slightly worse solubility of the aurumacryl sample if the initial polymer had the polymerization degree of 3,472, and caused a dramatic drop in the solubility of the sample if the degree of polymerization was 13,889. The use of the low molecular weight polymers with polymerization degrees of 23, 80 and 160 enhanced the solubility of the synthesized substance in some cases, but because the produced substance tended to increasingly form nanoparticles, the reproducibility of the synthesis technique remained poor. The polymer with a polymerization degree of 1,389 was slightly less beneficial for the solubility of the sample than the polymer with a polymerization degree of 1,944.

The 1 : 5 molar ratio of chloroauric acid to the polymer with an average molecular weight of 140 kDa ensured a slightly better reaction yield than the 1 : 10 ratio. The concentrated solution of the polyacrylate polymer (3%) significantly increased the gold ion conversion in comparison with the diluted solution (0.1%). The presence of Na^+ as a counterion for the carboxyl group of the polyacrylate polymer reduced the gold ion conversion by 10%. Besides, the presence of Na^+ in the sample negatively affected the chemical stability of the samples during storage. The samples synthesized from PANa showed the signs of steady spontaneous reduction of Au (III) ions to metal nanoparticles regardless of the PANa molecular weight when stored at +4 °C for 5–30 days. Thus, pre-deionization of the polymer aimed at eliminating the cations of alkali metals is required to ensure the suitability of the polyacrylate polymer for aurumacryl synthesis.

Importantly, we revealed that the interaction between AuCl_4^- anions and the polyacrylate polymer can cause reduction of gold atoms to elemental gold aggregating into nanoparticles. This reaction results in the low gold conversion. We were able to come to this conclusion using transmission electron spectroscopy, which facilitates observation of electron-opaque particles securely attached to the polymer. The presence of gold nanoparticles of 1–100 nm in diameter in aurumacryl samples does not affect the cytotoxic activity of aurumacryl against MCF-7 cells at the studied range of concentrations: it only indirectly suggests that the proportion of bioactive gold ions decreases in such samples.

Based on the correlation between the concentration of gold ions measured from the absorbance at 226.5 nm and the cytotoxic activity of the obtained aurumacryl samples, we developed a method for assessing the quality of the synthesized aurumacryl. We applied this method to demonstrate that regardless of the synthesis technique and the molar ratio of Au ions to the polyacrylate polymer, the cytotoxicity of aurumacryl against the MCF-7 cell line was proportional to its $A_{226.5}$. It means that aurumacryl quality assessment should include measurements of the absorbance of the saturated solution in the UV spectra at $\lambda = 226.5$ nm. To avoid the impact of hydrolysis on the measurements, the analysis should be performed in the acidic media (such as 100 mM of HCl). Using this principle, we were able to assess the reaction yield and optimize the conditions of the reaction.

The fact that the concentration of gold ions in the sample is a predictor of aurumacryl toxicity against MCF-7 cells regardless of the concentration of the polyacrylate polymer in it, leads us to conclude that the polyacrylate polymer does not have a critical role in transporting gold ions into cancer cells or at least does not limit the efficacy of gold permeating the cell membranes *in vitro*. Still, it is possible that in *in vivo* experiments the role of the polyacrylate polymer in delivering the gold particles to the tumor is quite important. In order to investigate the properties of polyacrylate polymer carriers in biological systems, aurumacryl cytotoxicity needs to be studied first against other cell lines and primary cell cultures *in vitro*. Their nonspecific toxicity and tumoricidal effects should also be compared *in vivo*.

Unlike our colleagues who resorted to air-drying and used a vacuum exicator, we freeze-dried the obtained aurumacryl samples immediately after synthesis. This type of drying significantly improves the solubility of aurumacryl under physiological conditions up to 30 mg/ml of solids and ensures chemical stability necessary for long-term storage.

By optimizing the synthesis technique, we were able to obtain an aurumacryl solution with $A_{226.5} = 4.8$ (equivalent to the optical path length of 1 cm), which is 5.3 times higher than the best result demonstrated by the aurumacryl samples synthesized according to the original technique ($A_{226.5} = 0.9$). The cytotoxicity of our samples increases proportional to $A_{226.5}$. The use of concentrated solutions will facilitate the testing of aurumacryl biological activity *in vivo* and *in vitro* and promote the application of the compound as an tumoricidal agent in the future.

We believe that aurumacryl applications in medicine should not be limited to adjuvant chemotherapy of carcinomas. TEM findings suggest that aurumacryl is capable of forming gold nanoparticles when interacting with reductants. Considering the big atomic radius of gold, such particles can serve as radiosensitizers to enhance the effect of ionizing radiation on tumor cells [14]. This approach is underpinned by the affinity of aurumacryl to cancer cells discovered in this work and in [3], leading us to expect that gold nanoparticles will tend to selectively accumulate in malignant cells instead of healthy tissue.

CONCLUSIONS

By comparing the cytotoxicity of aurumacryl samples with significantly different molar ratio of gold ions to the polyacrylate polymers, we established that the proportion of the polymer and its molecular weight in the sample do not affect the biological properties of the synthesized substance. On the contrary, the concentration of Au (III) ions measured from the absorbance of aurumacryl solutions at $\lambda = 226.5$ nm is an accurate predictor of aurumacryl cytotoxicity against the MCF-7 cells *in vitro*. To synthesize the sample with the highest Au (III) conversion and the best solubility in water, free PAA should be thoroughly deionized prior to the synthesis. The recommended molecular weight of PAA is 140 kDa; its recommended concentration is 3%. The molar ratio of chloroauric acid to PAA should be 1 : 5. It is important to freeze-dry the solution immediately after synthesis.

We hope that our findings will be useful for planning a preclinical trial of aurumacryl.

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