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FORMATION AND STABILITY OF GOLD NANOPARTICLES IN COLLOIDS PREPARED BY CITRATE METHOD

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Gold nanoparticles (Au NPs) have found a variety of applications in different areas, particularly in biomedical practices. The activity of Au NPs strongly depends on the size and association of particles in colloid, that in turn are greatly affected by experimental parameters of the reaction. The obtaining of Au NPs even via classical procedure of citrate method can be a challenge.

In the present work we applied different experimental approaches to affect the process of Au NPs formation in the presence of sodium citrate. Au NPs were obtained using different experimental procedures and varying the ratio of reagents, their concentrations, temperature of reaction, duration of heating, the order of introduction of reagents into the reaction mixture, pH, and so on. Comparative analyses of UV-vis spectra with DLS data by number, volume and intensity basis allowed to trace the changes in Au NPs colloid, find optimal experimental conditions and predict prolonged stability of colloids. Applying size-dependent Hamaker constant to DLVO theory explains experimental results.

The formation of Au NPs strongly depends on the ratio of the functional groups of the molecule involved simultaneously in the reduction of metal ions, the binding to the surface of Au NPs and the formation of a charge for stabilization due to electrostatic repulsion. The change in the ratio of components is not enough to get a different size of Au NPs. Big concentration of the reagents mostly affects the aggregation process and colloid aging. Temperature is a critical activation factor, that should be about 100 °C, but prolonged heating causes collision induced aggregation. The initial stage of particles growth (the mechanism) can be affected with the change of pH of the system due to formation of deprotonated carboxyl groups and gold hydroxocomplexes.

Keywords*: gold nanoparticles, colloids, sodium citrate*

INTRODUCTION

Gold nanoparticles (Au NPs) have found variety of applications in different areas, particularly in medical practice for drug delivery, biomolecular imaging, diagnostics and therapy of various diseases. The therapeutic and possible toxic effects of nanosized gold are one of the main topics in modern nanoscience.

One of the most well-known methods of obtaining colloidal solutions of gold is the synthesis of gold nanoparticles in the presence of sodium citrate. It is also called the Turkevich method, although in publication of J. Turkevich in 1951 [1] the method for preparing a "standard" solution is given as a modification of the citrate method previously described by the authors in [2].

The citrate method involves the reduction of the gold precursor with sodium citrate in an aqueous solution heated to boiling. The reaction mechanism in such a system is usually described by a model of nucleation and growth of particles [1, 3]. The color change of the reaction mixture during the reaction from colorless to red through blue was described.

The mechanism of particle formation in such a system was considered in a number of papers. It includes several stages, in particular the formation of small clusters, which then form ensembles of larger particles. For example, the authors of [4] describe polycrystalline particles that subsequently aggregate.

Based on a detailed study of each stage of the process, a refined idea on the existence of an intermediate state between the initial nanoclusters and the final nanoparticles, namely network of gold nanowires bound through amorphous gold, which are subsequently fragmented to form spherical particles till

7.5 nm, was presented in [5]. The size and shape of the formed particles depend on many factors, such as reagent concentrations, their ratio, temperature, pH and other experimental conditions.

The dependence of the process of Au NPs formation on the pH value of the medium was shown in [6]: the nucleation followed by a diffusion-controlled net growth occurred when pH value was higher than 6.5, while at pH = 6.5 nucleation, random attachment (or aggregation), and intraparticle ripening (or smoothing) took place.

The authors of [7], based on data of small-angle X-ray scattering (SAXS) and X-ray absorption near edge spectroscopy (XANES) in combination with electron microscopy and absorption spectra, suggested the following mechanism of formation of Au NPs. In the first stage, about 20 % of the available gold forms nuclei with a size of 2 nm, and then their aggregation to particles with a size of 4 nm occurs (second stage). According to the authors, the increase in particle size is accompanied by a slow reduction process, and the growth of particles is due to coalescence [8] or Ostwald ripening [9]. On the third step (25–50 min) the growth of particles up to 5.2 nm occurred, that was associated with diffusion processes [10, 11]. In this case, the limiting stage is again the slow reduction of the precursor. During the final fourth stage, there is a rapid decrease in the amount of Au(III) and an increase in the Au NPs size to 7.6 nm. A possible reason for this rapid transformation is the autocatalytic reduction of Au (III) on the surface of formed Au NPs of 5 nm, that is transitional to the bulk properties [12]. The temperature and concentration are the critical parameters for obtaining NPs of different sizes.

Size of Au NPs and their association in colloid determine the activity in biomedical applications. In its turn, the morphology and stability of Au NPs are greatly affected by experimental approaches and parameters of the reaction. As it was shown, even classical procedure of obtaining of Au NPs via citrate method is still open to a variety of questions.

While accurate size of nanoparticles can be measured in electron microscope, dynamic light scattering (DLS) method is suitable for the investigation of hydrodynamic diameter and the whole size distribution of nanoparticles in Au NPs colloid. Comparative analyses of UV-vis spectra with DLS data by number, volume and intensity basis allow us to trace the changes in Au NPs colloids.

In the present work we applied different experimental approaches to affect the process of gold nanoparticles formation via chemical reduction of Au (III) in the presence of sodium citrate. Au NPs were obtained using different experimental procedures and varying the ratio of reagents, their concentrations, temperature of reaction, duration of heating, the order of introduction of reagents into the reaction mixture, pH and so on. Optimal conditions were found to adjust the size of Au NPs and produce colloids with prolonged stability.

EXPERIMENTAL

***Chemicals**:* tetrachloroauric acid ($HAuCl_4$, Merck, Germany); sodium citrate (NaCit, $Na_3C_6H_9O_9$, ACROS, Germany); sodium borohydride ($NaBH_4$, Fluka, Germany); sodium hydroxide (NaOH, Merck, Germany); DI water (H_2O).

Synthesis Au NPs. Colloidal solutions of Au NPs were obtained by the method of chemical reduction of tetrachloroauric acid ($HAuCl_4$) in the presence of sodium citrate ($Na_3C_6H_5O_7$, NaCitr). The reaction was carried out in aqueous solution (distilled water) heated to boiling point. The concentration of gold in colloidal solutions for different series of experiments varied from 1×10^{-4} to 4.5×10^{-4} M. The molar ratio of NaCitr : $HAuCl_4$ ranged from 0.5 to 10.

The effect was studied of reaction temperature, boiling time, cooling of the reaction mixture and the order of introduction of reagents on the characteristics of the formed gold nanoparticles. Additional reagents were also used to achieve this goal: sodium hydroxide (NaOH), sodium borohydride ($NaBH_4$).

Optical properties. The absorption spectra of the Au NPs colloids were recorded in a 1 cm quartz cell using a spectrophotometer Lambda 35 (Perkin-Elmer, Waltham, MA, USA).

Size distribution. Distribution of Au NPs size was assessed by a dynamic light scattering (DLS) method using laser correlation spectrometer Zeta Sizer Nano S (Malvern Panalytical Ltd, Malvern, UK) equipped with a correlator (multi-computing correlator type 7032 CE). A helium-neon laser LGN–111 was used with the output power of 25 mW and wavelength of 633 nm to irradiate the suspension. The registration and statistical processing of the scattered laser light at 173° from the suspension were performed three times during 120 s at 25 °C. The resulting autocorrelation function was treated with standard computer programs PCS–Size mode v.1.61.

RESULTS

This work aimed to develop conditions for the synthesis of gold nanoparticles with controlled size using the citrate method and with variation of such parameters like the ratio of reagents, their concentrations, temperature of reaction, duration of heating, the order of introduction of reagents into the reaction mixture, pH and so on. The starting point of the research was the conditions of classical citrate synthesis, where the metal concentration was about $3 \cdot 10^{-4}$ M (Table 1).

Table 1. Experimental conditions of chemical synthesis of gold nanoparticles by the citrate method

Initial concentration, mol/l		**Ratio**	**T °C**	**Size of Au NPs, nm**	**Duration, min**	**Reference**
$HAuCl_4$	**$Na_3C_6H_5O_7$**	**Au:NaCit**				
$3.75 \cdot 10^{-4}$	$2.5 \cdot 10^{-3}$	1:6.6(6)	75	~6	30–40	[7]
$2.5 \cdot 10^{-4}$	$2.5 \cdot 10^{-3}$	1:10	75	7.6	70–90	[7]
$2.5 \cdot 10^{-4}$	$2.5 \cdot 10^{-3}$	1:10	85	~8	35–40	[7]
$2.54 \cdot 10^{-4}$	$2 \cdot 10^{-3}$	1:(6.7)7.8	100	20 (TEM)		[3]
$2.54 \cdot 10^{-4}$	$2 \cdot 10^{-3}$	1:(6.7)7.8	100	20 (TEM)	5–45 (color)	[1]

The key method of the study is DLS, that is frequently used but often underestimated. The Au NPs size distribution by DLS in this study was analyzed as follows: 1) all three bases of the data were taken into account, namely the distribution by number, volume and intensity; 2) the particle size graphs were plotted not in logarithmic scale, but in linear one; 3) the averaging was not applied for the data registered from the suspension for 3–5 times.

EFFECT OF THE RATIO AND CONCENTRATIONS OF REAGENTS

The ideas behind this part of experiment were that (1) increasing the amount of reducing agent would accelerate the chemical reduction reaction, thereby causing the formation of smaller particles, and (2) the higher the concentration of reagents leads to the greater probability of interaction between the components and as a consequence to the faster reaction and smaller size of particles. Both ideas were not confirmed, the expected linear dependence of size on excess of the reductant (content) was not observed.

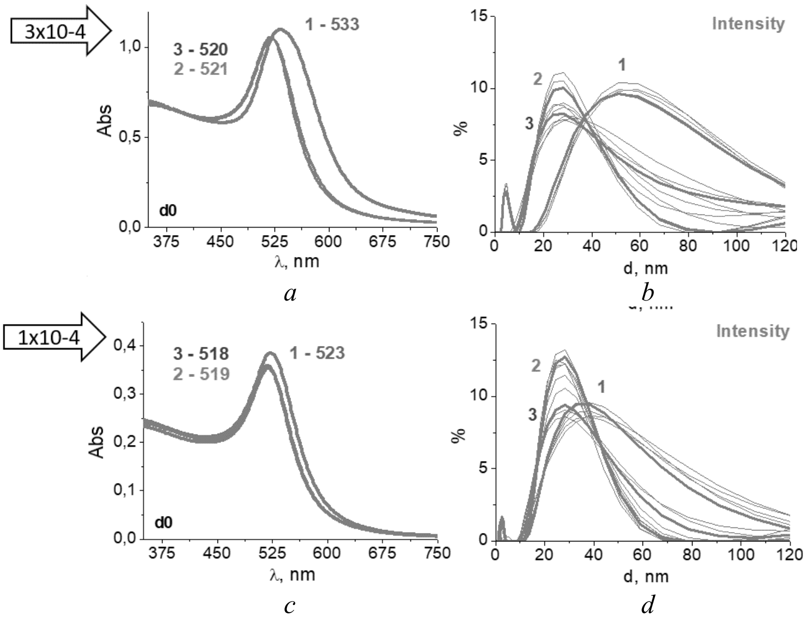
Usually, the size of Au NPs is associated with the position of the maximum of localized surface plasmon resonance (LSPR) band of gold in absorption spectra, while its width and symmetry contain information about aggregation processes. We find the correlation between LSPR band position and its shape with DLS data obtained by intensity basis (Fig. 1).

When using an equimolar amount of reagents (NaCitr : $HAuCl_4$ = 1:1), the LSPR band was the most shifted to the long-wavelength region. For colloids obtained at $C_{Au} = 3 \times 10^{-4}$ M the maximum was located at $\lambda_{max} = 533$ nm, compared to other series, $\lambda_{max} = 521$ nm for “2:1” and 520 nm for “3:1” (Fig. 1 *a*). The same tendency was for colloids with $C_{Au} = 1 \times 10^{-4}$ M: $\lambda_{max} = 523$ nm for “1:1”, compared to $\lambda_{max} = 519$ nm for “2:1” and 518 nm for “3:1” (Fig. 1 *c*). Colloids with “1:1” were the least stable, precipitating in time, which was also reflected in the color of solution.

The data from DLS intensity basis reflect the best correlation with the position of the LSPR band maximum in the absorption spectra. The average size of Au NPs formed in samples with NaCitr: $HAuCl_4$ = 2:1 and 3:1 was about 25 nm for both systems and concentrations. For “1:1” colloids particles were the largest among studied: about 50 nm for $C_{Au} = 3 \times 10^{-4}$ M (Fig. 1 *b*) and 40 nm for $C_{Au} = 1 \times 10^{-4}$ M (Fig. 1 *d*).

The low-intensity peak in the range up to 5 nm for the smallest ratio of reagents (curves *1*) probably indicates the presence of small particles (or clusters), which stay in solution when there are not enough citrate molecules for both processes of reduction of ions and stabilization of particles. They evidence an incomplete process of establishing the equilibrium in colloid. Such effect is connected with the ratio of reagents, and doesn't depend on their concentration.

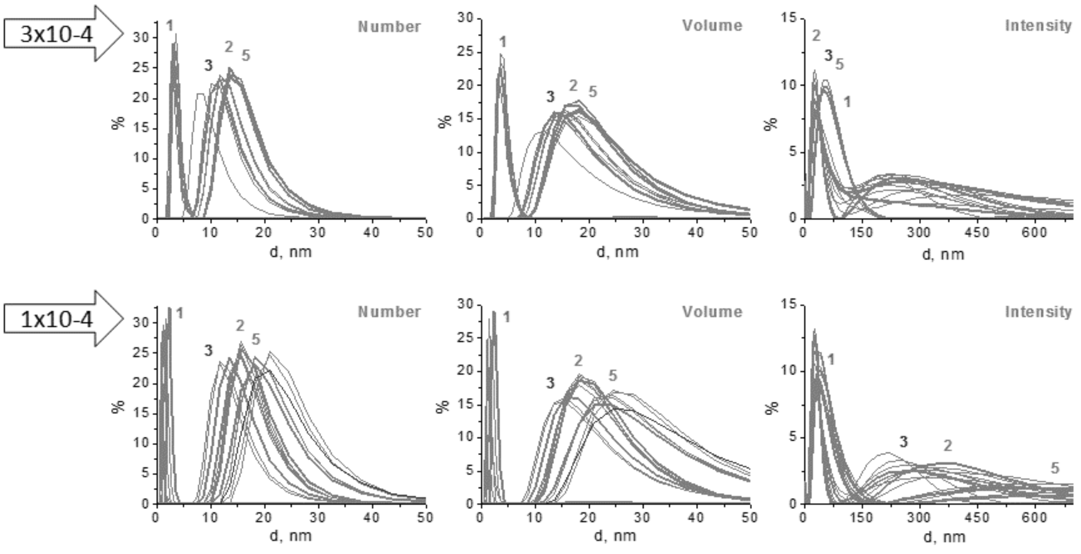
The expected effect of decreasing the size with an increase of reducer content was only for the ratios NaCitr : $HAuCl_4$ = 2:1 and 3:1. When using "3:1" system the particles were smaller according to number and intensity bases, but this difference was not a radical change in size (Fig. 2 and Table 2). A further increase in the ratio to 5:1 caused the opposite effect - an increase in size, as well as intensity of signal from the fraction of aggregated particles (Fig. 2).



a *b*

c *d*

Fig. 1. Absorption spectra (*a*, *c*) and size distribution of Au NPs by DRS intensity basis (*b*, *d*). Upper graphs reflect the distribution in samples with the gold content of 3×10^{-4} M (*a*, *b*), the lower – to 1×10^{-4} M (*c*, *d*). The numbers correspond to colloids obtained with molar ratios of NaCitr : $HAuCl_4$ = 1:1 (*1*), 2:1 (*2*) and 3:1 (*3*)



a *b* *c*

Fig. 2. The size distribution of Au NPs by DLS method by number (*a*), volume (*b*) and intensity (*c*) bases. Upper graphs reflect the distribution in samples with the gold content of 3×10^{-4} M, the lower – to 1×10^{-4} M. The numbers correspond to colloids obtained with molar ratios of NaCitr : $HAuCl_4$ = 1:1 (*1*), 2:1 (*2*), 3:1 (*3*) and 5:1 (*5*)

Table 2. The size of Au NPs according DLS data

C_{Au}, M	Ratio NaCitr:Au	Basis		
		Number	Volume	Intensity
3×10^{-4}	1:1	3–4 (85 %)	3–5 (86 %)	3–9 (14 %) / 20–200 (86%)
	2:1	12–21 (88 %)	12–28 (87 %)	16–51 (67 %)
	3:1	9–16 (85 %)	10–24 (83 %)	16–51 (62 %)
1×10^{-4}	1:1	1–2 (95 %)	1–2 (97 %)	1–3 (5 %) / 10–200 (90 %)
	2:1	12–24 (95 %)	14–33 (90 %)	16–44 (72 %)
	3:1	10–24 (96 %)	12–28 (84 %)	16–59 (68 %)

Thus, with increasing the concentration of reagents three times from 1×10^{-4} to 3×10^{-4}M for used ratios of reagents (from 1:1 to 5:1) the same nonlinear tendency in changes of the size of formed Au NPs remained. However, the effect of concentrations turned out in an increase in the number of aggregated particles.

The concentration of reagents affected redistribution of the height of the spectral signal of two fractions of Au NPs in DLS intensity basis, as shown for the "2:1" system in Fig. 3. Increasing the gold content from 1×10^{-4} to 4×10^{-4} M caused the more intensive formation of aggregates, and its percentage rised from 20 to 60 %, while for individual Au NPs decreased from 90 to 60 %. The more intensive formation of aggregates occurred because of diffusion interaction, due to a bigger probability of collisions of already formed NPs.

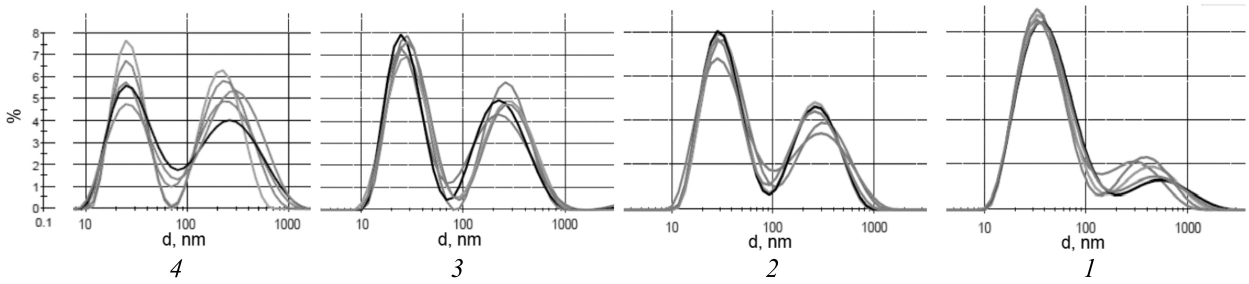


Fig. 3. The size distribution of Au NPs by DLS method intensity basis for the system with molar ratio of NaCitr : $HAuCl_4$ = 2:1. Numbers indicate the concentration of gold: 1×10^{-4} M (*1*), 2×10^{-4} M (*2*), 3×10^{-4} M (*3*), 4×10^{-4} M (*4*)

Considering the influence of aggregates on the aging processes of colloidal systems, it is advisable to carry out the synthesis with a lower content of reagents, namely at $C_{Au} = 1\times10^{-4}$ M.

EFFECT OF THE TEMPERATURE OF REACTION AND DURATION OF HEATING

The reduction of Au^{3+} ions with sodium citrate usually proceeds in a solution heated to boiling. Temperature is a critical factor for this reaction.

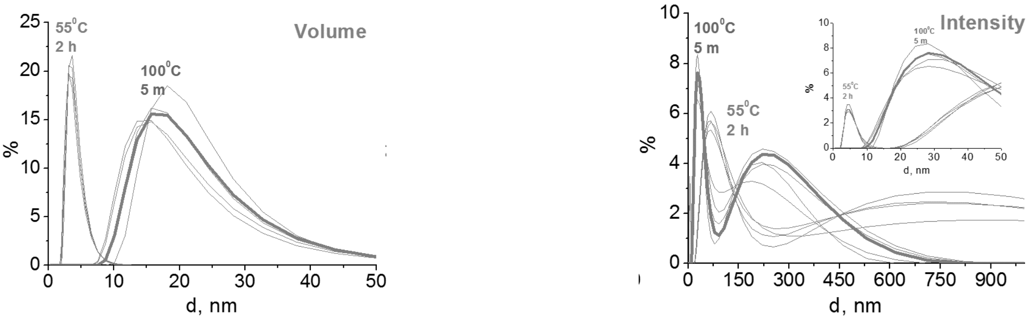
Using different temperatures of reaction for the same system, namely 55 and 100 °C, we observed a clear difference in DLS data.

In boiling solution (for 5 min) Au NPs were formed with the average size of 15–20 nm according volume basis, and in the intensity basis data two fractions of individual Au NPs and big aggregates can be distinguished. When the temperature of reaction is decreased to 55 °C, even 2 hours is not enough to reach classical red color of colloid. The DLS distribution by volume contains the signal of small particles up to 5 nm, while intensity basis shows three peaks – up to 10 nm, around 80 nm and 300–1200 nm. The colloid stays blue and precipitate in time (Fig. 4).

Thus, the low temperature is not enough to activate a rapid reaction of Au^{3+} ions reduction, that causes total aggregation of Au NPs.

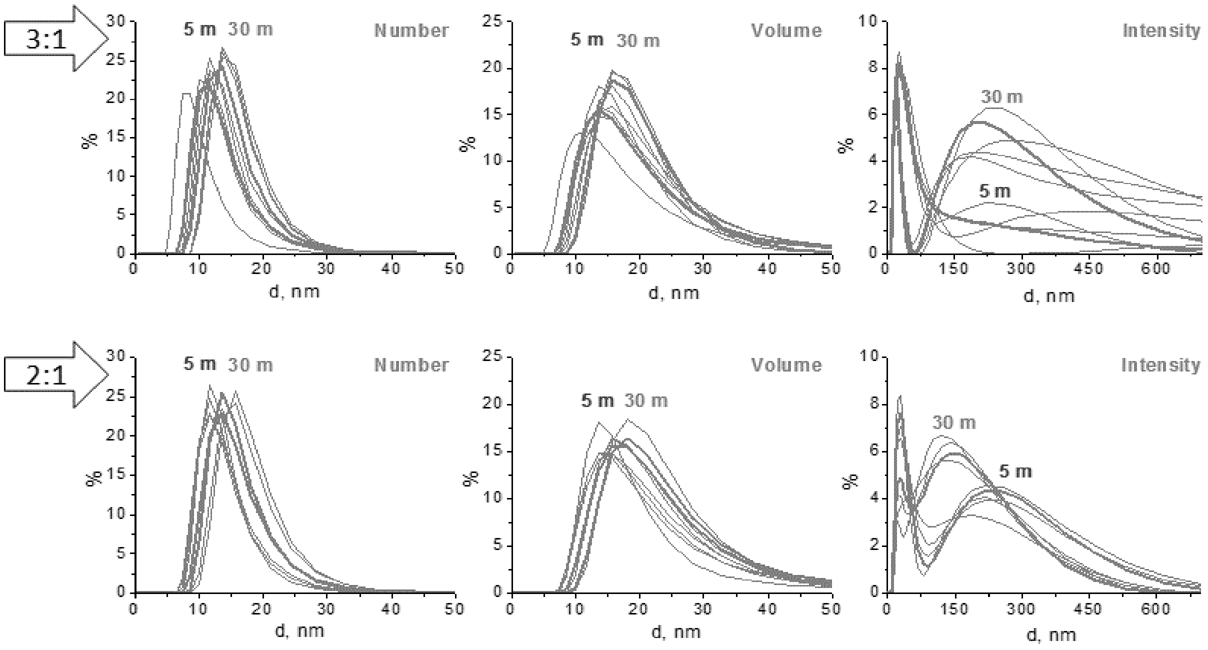
The ideas used behind duration of heating were the next: the longer the boiling is, the greater the probability of Au NPs collisions and, as a consequence, their growth are, and the larger the particles are. This idea worked out, but resulted in different way.

The DLS distributions by number and volume bases for Au NPs formed during different time of heating show almost the same size of particles, but intensity basis evidenced the formation of bigger quantity of aggregates when boiling was prolonged (Fig. 5). In particular, longer heating contributed to an increase in the probability of collisions of already formed Au NPs, rather than in the process of their formation, and thus the fraction of aggregated particles appeared.



a *b*

Fig. 4. The size distribution by DLS method by volume (*a*) and intensity (*b*) basis of Au NPs obtained at 55 and 100 °C with NaCitr : $HAuCl_4$ = 2:1. Inset shows the size range from 0 to 50 nm



a *b* *c*

Fig. 5. The size distribution of Au NPs by DLS method by number (*a*), volume (*b*) and intensity (*c*) bases. Upper graphs reflect the distribution in samples with NaCitr : $HAuCl_4$ = 3:1, the lower – to 2:1. The time of heating is indicated as 5 and 30 min ($C_{Au} = 1\times10^{-4}$M)

Therefore, to avoid the formation of aggregates that cause the aging of colloid the reaction mixture should be heated till boiling and the heating time should be reduced, namely remain 5 min, after the color of the solution changes from blue to red inherent to nanosized spherical Au NPs.

EFFECT OF THE HIGH pH VALUE OF THE MEDIUM AND THE ORDER OF INTRODUCTION OF REAGENTS INTO THE REACTION MIXTURE

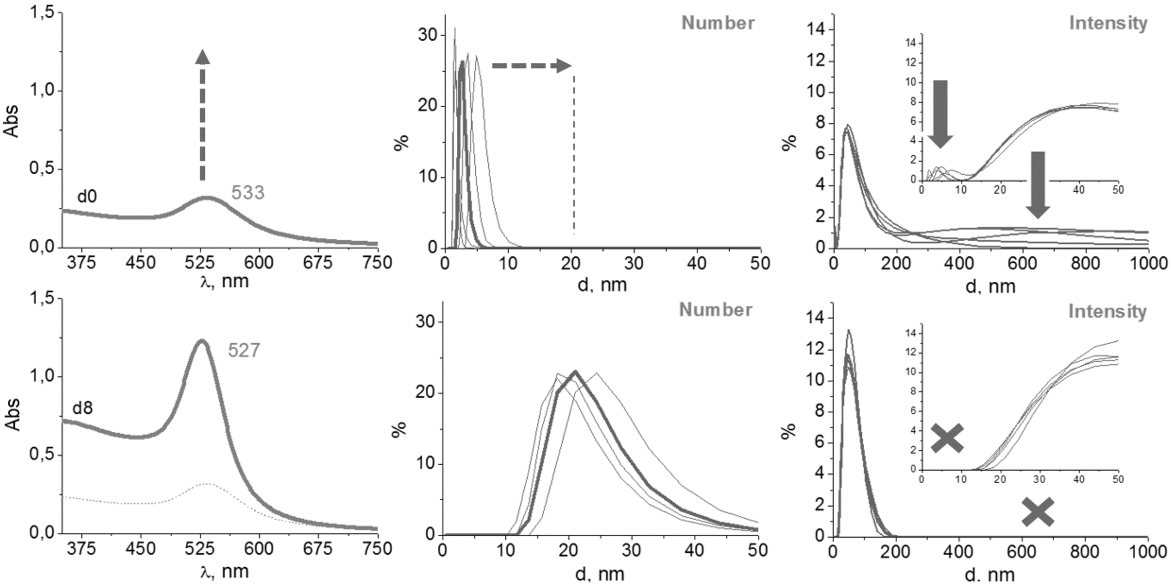
Sodium hydroxide was used to reach high pH value of initial solution on the basis of that

(1) hydroxide anions create an additional negative charge around the NPs in the form of counterions of the double electric layer on their surface, and thus increase the stability of the particles, (2) in the presence of hydroxide anions carboxyl groups of citrate are deprotonated and form complex metal salts that slows down the process of Au^{3+} ion reduction.

Alkali significantly affects the formation of Au NPs. This process can take more than a week after prolonged boiling and storing at room temperature. The LSPR band of gold in the absorption spectra grows slowly, and its maximum shifts to the short-wavelength region from 533 to 527 nm (Fig. 6 *a*).

The next day after the synthesis in the size distributions there are signal of particles less than 5 nm. When repeated measurement after a week, we observed NPs with an average size of about 20 nm (Fig. 6 *b*).

At the same time, in the size distribution by intensity of scattering, the signals of small particles up to 5 nm and large aggregates of 400–1000 nm disappear (Fig. 6 *c*). This effect occurs due to Ostwald ripening of particles in the colloidal system.



a *b* *c*

Fig. 6. Absorption spectra (*a*) and size distribution of Au NPs by DLS number (*b*) and intensity (*c*) bases. Upper graphs reflect the properties of samples with the gold content of 3×10^{-4} M after the synthesis, the lower – after a week of storage

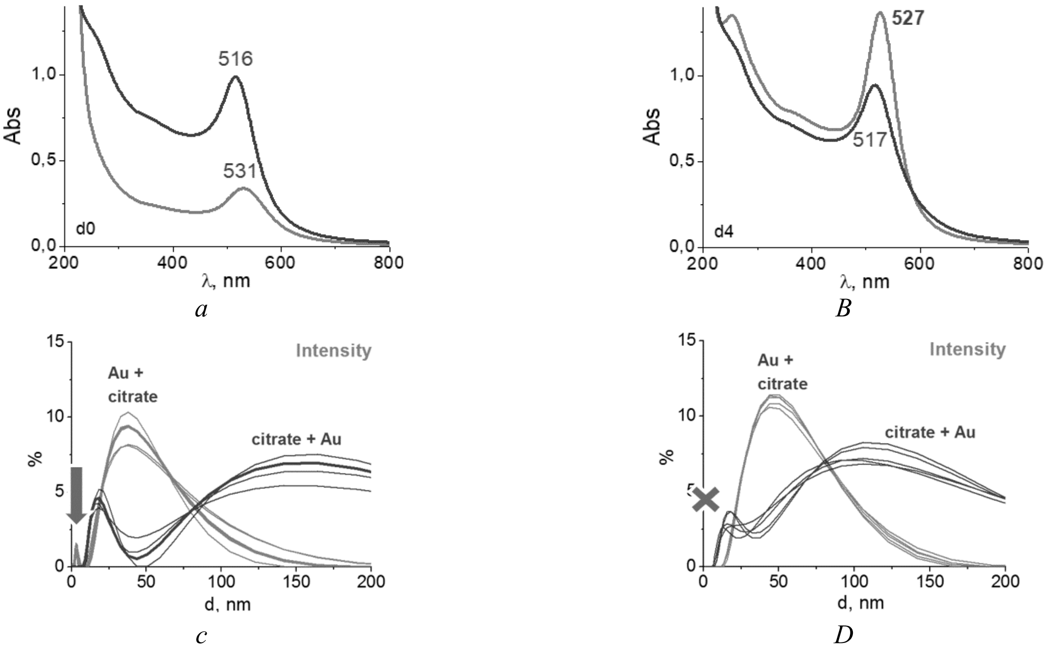
Therefore, when using alkali, the process is quite long, but it results in colloidal systems stable in time, that was confirmed also for the bigger ratios of reagents, namely for NaCitr: $HAuCl_4$ = 3:1 and 5:1 (equilibrium is established within 4–10 days).

The order of introduction of reagents into the reaction mixture with high pH value affects the rate of particle formation because hydroxide anions are the factor of deprotonation of the carboxyl groups of citrate, as well as they are the components of the hydroxocomplexes of gold.

The system with the ratio NaCitr : $HAuCl_4$ = 3:1 was studied focusing on the order of introduction of reagents. When sodium citrate was injected to boiling alkaline solution containing gold $[(HAuCl_4 + OH^-)_{boiling} + NaCitr]$, the formation of Au NPs was much slower, and the particles themselves were larger. The LSPR band of gold in the absorption spectra grows slowly during the week (Fig. 7 *a*, *b*). The average size of Au NPs according to the distribution by intensity was about 40 nm (Fig. 7 *c*, *d*).

At the same time while storing the signal of “small” NPs up to 5 nm disappears. Hydroxo-anions interact with the complex gold anion causing the ligand exchange in the inner sphere $[Au(OH)_xCl_{4-x}]^-$, a stronger bond with the metal cation is formed, and as a result the reaction is slower. Therefore, the larger particles grow.

When tetrachlorauric acid was added to the sodium citrate in deprotonated form [(NaCitr + $OH^{-}_{boiling}$ + $HAuCl_4$] (inverse reagent sequence), the reaction was much faster. However, despite the individual Au NPs with the size about 20 nm, the stability of such system was worse compare to previous one, as a big fraction of large aggregates was formed. For NaCitr : $HAuCl_4$ = 5:1 the similar trend was observed.



a *B* *c* *D*

Fig. 7. Absorption spectra (*a*, *b*) and size distribution of Au NPs by DLS intensity basis (*c*, *d*) for the system with NaCitr: $HAuCl_4$ = 3:1 and gold content of 3×10^{-4} M after the synthesis (*a*, *c*) and after a week of storage (*b*, *d*)

MECHANISM OF PARTICLE FORMATION

According to the data available in the literature, the mechanism of particle formation in the sodium citrate/$HAuCl_4$ system occurs through stages of 1) formation of nanoclusters less than 5 nm, 2) formation of network of gold nanowires, 3) fragmentation of nanowires and subsequent 4) formation of individual Au NPs. The DLS data considered in this paper confirm this mechanism.

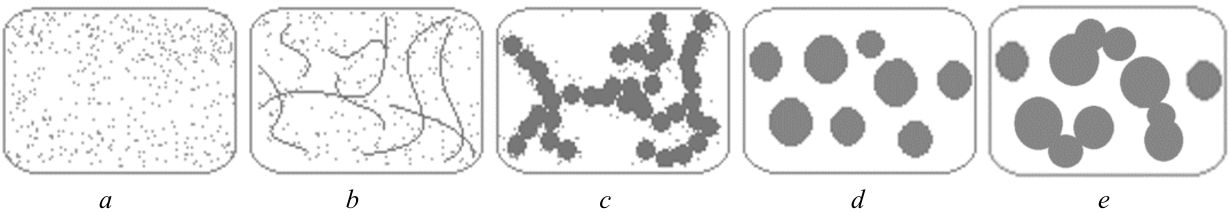


Fig. 8. Schematic representation of the formation of Au NPs in the presence of sodium citrate: the stage of formation of nanoclusters less than 5 nm (*a*), the formation of a network of nanowires (*b*, *c*), fragmentation of nanowires and subsequent formation of individual Au NPs (*d*), Au NPs aggregation (*e*)

According to the obtained data, depending on the experimental parameters, namely the ratio of components and heating temperature of the nanowire can be fragmented to form a stable colloid with individual Au NPs or interact between each other with their subsequent aggregations (Fig. 8).

Aiming to reach the stage of individual Au NPs (Fig. 8 *d*), we can stop on the stage of nanowires if the ratio is too small (Fig. 8 *b*), or if the temperature of reaction mixture is too low (Fig. 8 *c*). Additionally, we can distinguish the stage of further aggregation of the formed individual particles due to diffuse interactions (Fig. 8 *e*) when it is too prolonged heating. On the initial stage of cluster formation up to 5 nm (Fig. 8 *a*) we can influence increasing the pH of the medium.

THEORETICAL DESCRIPTION OF STABILITY OF Au NPS COLLOIDS CONSIDERING FRACTIONS OF INDIVIDUAL Au NPS AND AGGREGATES

One of the main and necessary properties of colloidal solutions of nanoparticles is their stability, which may be described by the colloid aging rate. The colloids aging occurs due to the nanoparticles' interactions, which lead to the nanoparticles adhere together, form aggregates and then sediment due to their mass. It is well-known that interactions in colloids according to the DLVO (Derjaguin–Landau–Verwey–Overbeek) theory [13] are mainly defined by two forces: attractive van der Waals force and repulsive force caused by the charged double layer around the nanoparticles. However, not only these two forces occur in colloidal solutions of nanoparticles. In part, there may be dispersion forces acting on the ions. As it was shown in [14], the ionic dispersion potentials can modify the interaction potential and the forces between the nanoparticles in colloid. These modifications depend on the salt concentration in the colloid and on zeta-potential and are not significant for systems with ions concentrations nearly and lower than 0.01 M and for zeta-potential lower than -10 mV. As the ions concentrations in this study are much lower than 0.01 M, in our calculations we may not take into account the ionic dispersion potentials.

There are many parameters influencing on the forces value (described *e.g.* in [15]), but here we consider the effect of the nanoparticles (or the nanoparticles aggregates) size on the colloids aging. Based on the DLVO theory without taking into account other interactions in colloids we conduct some calculations to theoretically confirm experimental results. For this purpose, let us calculate the interaction potential of two nanoparticles in the colloid, and the aggregation rate depending on their size.

As the aggregation occurs when two nanoparticles "meet" one another in the solution, its rate is proportional to the probability of this "meeting". This probability defines as the product of the number (or concentration) of the nanoparticles. Hence, the probability of aggregation of two average-sized nanoparticles is proportional to N_{ave}^2, where N_{ave} is the number (concentration) of the average-sized nanoparticles; the probability of the aggregation of two different-sized nanoparticles (one is of average size, and another one is of any other size r_i) is proportional to $N_{ave} \cdot N_{r_i}$. As it was shown in experiments, there is a main fraction of the average-sized nanoparticles in all the colloids, whereas number of nanoparticles of other sizes is much lower that the number of nanoparticles of this fraction: $N_{r_i} << N_{ave}$. This means that the aggregation of two equal-sized nanoparticles makes a major contribution to the colloid aging, as its probability (and rate as a consequence) is much higher than that of the aggregation of two different-sized nanoparticles.

Consequently, for the simplicity and with sufficient accuracy we can consider just the symmetric case of the aggregation of two equal-sized nanoparticles (aggregates), and put the size of the nanoparticles equal to the average size of the nanoparticles (aggregate) in the solution (Fig. 9).

Further calculations are conducted using common and well-known formulae of DLVO theory adapted from [16]. In the consideration we need to analyze the interaction potential of two nanoparticles and evaluate the aggregation rate.

The interaction free energy is estimated from the following equation:

$$U(d) = \int_{d}^{+\infty} F(x)dx, \qquad (1)$$

where $F(d)$ is the force acting between the nanoparticles:

$$F(d) = 2\pi R_{eff} W\left(d\right), \qquad (2)$$

with $R_{eff} = R/2$ is the effective radius for the system of two equal-sized nanoparticles, and $W(d)$ is the free energy of two plates per unit area. By means of the Derjaguin approximation

$W(d)$ consists of van der Waals $W_{vdW}(d)$ and double layer interactions $W_{dl}(d)$.

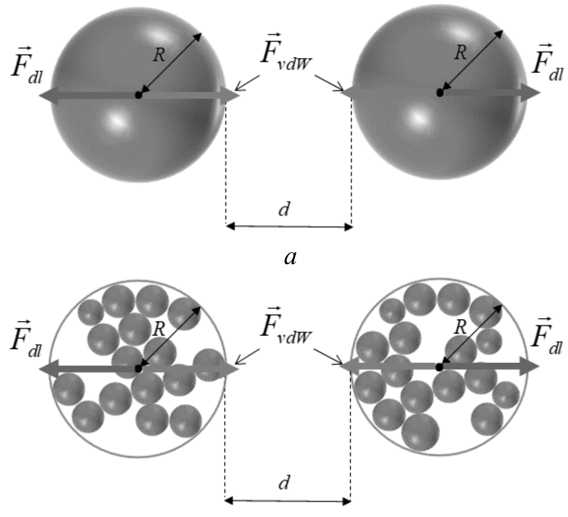
Van der Waals potential according to [17]:

$$W_{vdW}\left(d\right)=-\frac{A\left(R\right)}{6}\cdot\left(\frac{2R^2}{d\left(4R+d\right)}+\right.$$
$$\left.+\frac{2R^2}{\left(2R+d\right)^2}+\ln\left(\frac{d\left(4R+d\right)}{\left(2R+d\right)^2}\right)\right), \tag{3}$$

where $A(R)$ is Hamaker constant. This constant in classical colloidal systems is the property of the material and its surrounding. However, in nanosystems this property depends on the nanoparticle size [18]. For taking this into account we conducted the approximation based on the least squares method and received the following empiric formulae for the Hamaker constants of gold nanoparticles in water depending on their size:

$$A\left(R\right)_{Au}=125.562+11.18\cdot e^{-0.05R},$$
$$\varepsilon=0.36\cdot \tag{4}$$

In (4) the particle radius is assumed to be in nanometers, and the Hamaker constant is in zeptoJoules (10^{-21} J). The parameter ε is the standart error of the approximation. It can be seen that the relative error of the Hamaker constant approximation is less than 0.6 %, and (4) can be used for further calculations with sufficient accuracy.



a

b

Fig. 9. Systems under consideration: *a* – two equal-sized nanoparticles, *b* – two equal-sized aggregates of the nanoparticles. Here the main parameters of the system are presented: R is the radius of the nanoparticle (effective radius of the aggregate), d is the distance between the nanoparticles (aggregates) surfaces. F_{vdW} is the van der Waals force, F_{dl} is the force of the double-layer repulsion

The double layer interactions potential may be calculated as in [19]:

$$W_{dl}\left(d\right)=\frac{128\pi nkTR^2}{\kappa^2}\cdot\tanh^2\left(\frac{\sigma}{4}\right)\cdot\frac{\exp\left(-\kappa\cdot d\right)}{2R+d}, \tag{5}$$

where ε is the dielectric constant of the water, ε_0 is the dielectric permittivity of the vacuum, k is the Boltzmann constant, T is absolute temperature, n is number of ions per volume, σ is zeta-potential of the surface of the nanoparticle, κ is the reciprocal of Debye length calculated as follows:

$$\kappa=\sqrt{\frac{2000e^2N_AI}{\varepsilon\varepsilon_0kT}} \tag{6}$$

with the Avogadro's number N_A, electron charge e and ionic strength I.

Then the aggregation rate may be calculated as in [20]:

$$\alpha = \frac{2kT}{3\eta R_{eff}} \cdot \left(\int_0^{+\infty} \frac{1 + R/_{2h}}{(2R+h)} \cdot \exp\left(\frac{U(h)}{kT} \right) \cdot dh \right)^{-1} . \qquad (7)$$

Analyzing (3) it can be seen that the attractive force depends much on the nanoparticle size (Fig. 10).

The double-layer interaction potential dependence on the nanoparticle size is linear, and as it can be seen from Fig. 11, the difference in energy of repulsion of big and small nanoparticles is much smaller compared to the same difference in energy of interaction.

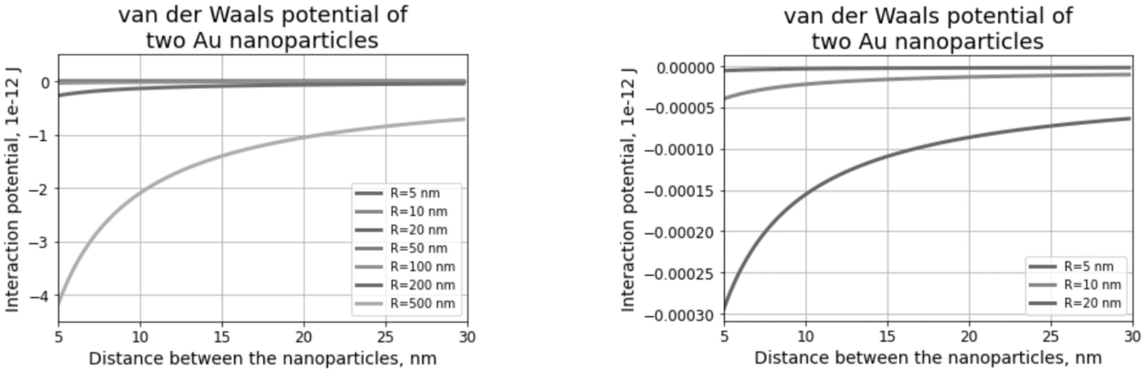


Fig. 10. Van der Waals interaction potential of two equal-sized nanoparticles for different nanoparticles (aggregates) sizes from 5 to 500 nm. On the right part of the figure results for three smallest nanoparticles are shown for comparison of the potential order of magnitude of different systems

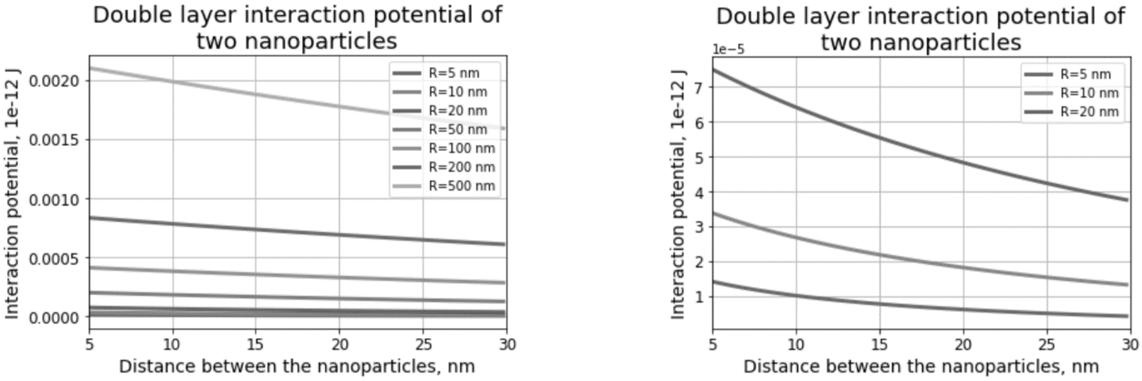


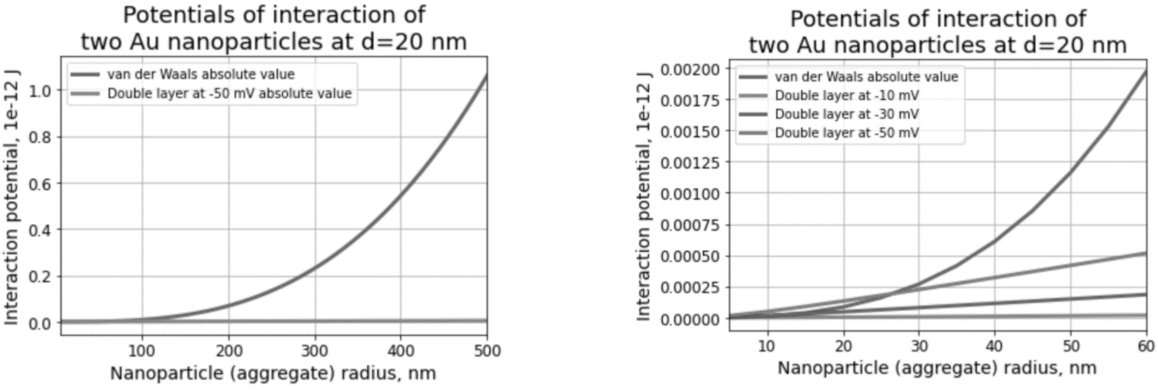
Fig. 11. Double layer interaction potential of two equal-sized nanoparticles for different nanoparticles (aggregates) sizes from 5 to 500 nm. On the right part of the figure results for three smallest nanoparticles are shown for comparison of the potential order of magnitude of different systems. Calculations were conducted for $T = 300$ K and $\sigma = -30$ mV

Comparison of both potentials values is presented in Fig. 12. From the Fig. 12 *a* it can be seen that for nanoparticles bigger than 100 nm the attractive forces are much bigger than repulsive, which corresponds to low stability of such colloids. From the Fig. 12 *b* it can be seen that for higher value of zeta-potential (-50 mV) repulsion prevails the attraction for bigger sizes of nanoparticles (up to 28 nm) for the considered distance. However, such comparison is not full as we need to analyze the shape of potential and the level of energy barrier in the system.

Let one compare of the interaction potentials for two nanoparticles of various sizes at different zeta-potential values (Fig. 13). It can be seen that for smaller nanoparticles there is an energetic barrier preventing the aggregation for zeta-potential values higher than -30 mV. For nanoparticles of 20 nm radius the barrier can be observed just for the case of zeta-potential of

-50 mV. And for nanoparticles (aggregates) of 100 nm radius there is no barrier at the interaction potential. Hence, we may state, that the colloids stability is well described in the frame of DLVO theory.

Finally, let one analyze dependences of aggregation rates in colloids on the nanoparticles sizes (Fig. 14 *a*).



A *b*

Fig. 12. Potentials of interaction of two nanoparticles at the distance 20 nm. Calculations were conducted for $T = 300$ K; (*a*) and (*b*) present same results but in different ranges

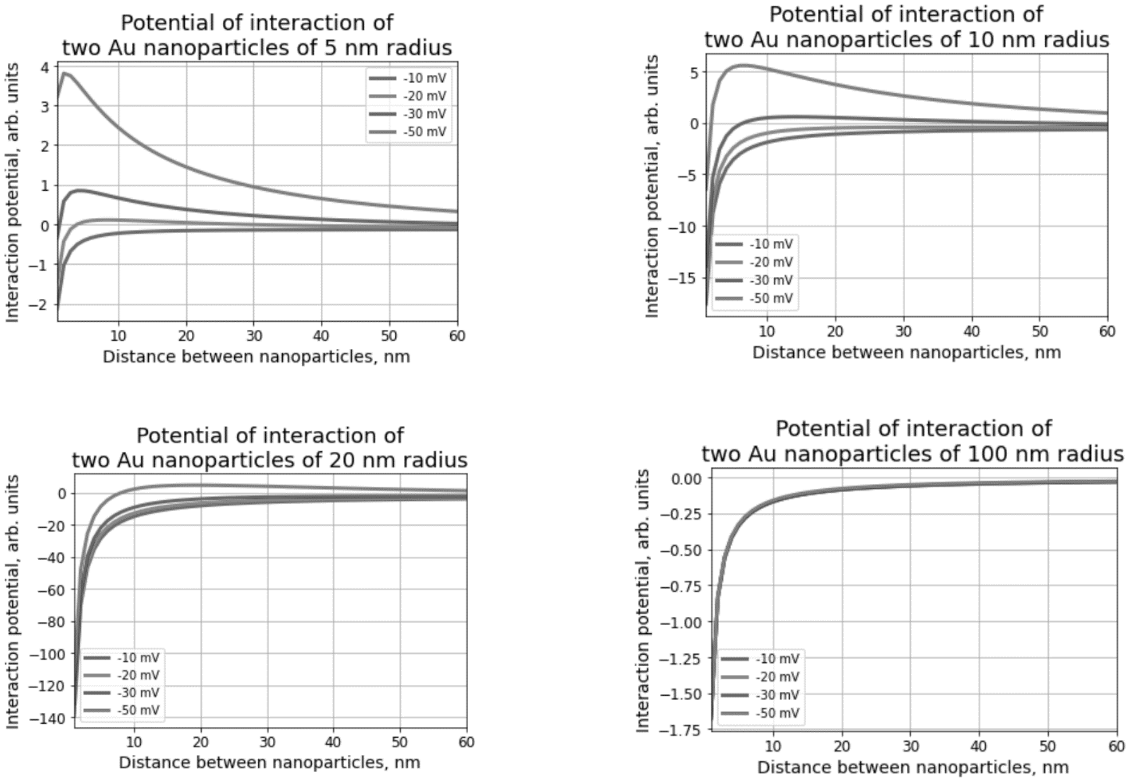
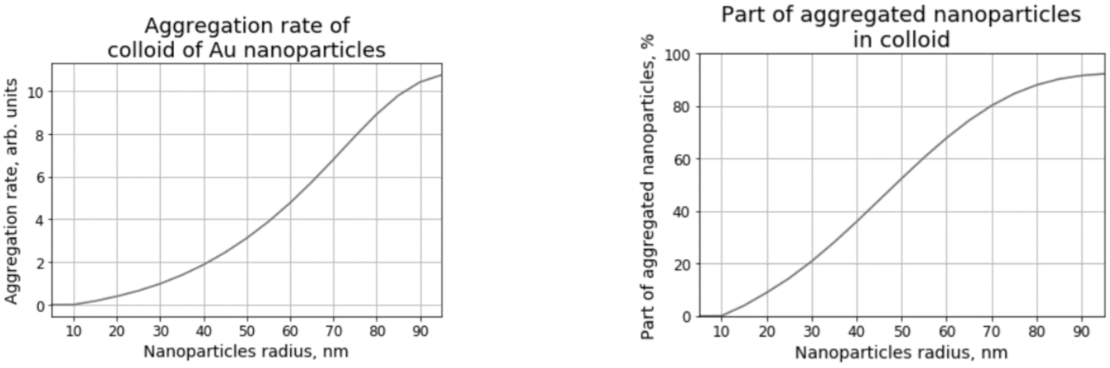


Fig. 13. Interaction potentials of two nanoparticles in the colloidal solution. Calculations were conducted for $T = 300$ K



A *b*

Fig. 14. Aggregation rate (*a*) and part of aggregated nanoparticles in colloid (*b*) at some time point t_0 depending on the nanoparticles average size. Calculations were conducted for $T = 300$ K, zeta-potential is -30 mV. Time point t_0 is the time when part of aggregated nanoparticles of average size 500 nm is goes to 100 %

It can be concluded that when the nanoparticles with average radius 100 nm are almost fully aggregated, there may be no significant changes in colloids with small nanoparticles (less than 10 nm), which is in good agreement with experimental results.

CONCLUSIONS

The formation of Au NPs strongly depends on the ratio of the functional groups of the molecule involved simultaneously in the reduction of metal ions, the binding to the surface of Au NPs and the formation of a charge for stabilization due to electrostatic repulsion. The change in the ratio of components is not enough to get a different size of Au NPs. Big concentration of the reagents mostly affects the aggregation process and colloid aging. Temperature is a critical activation factor, that should be about 100 °C, but prolonged heating causes collision induced aggregation. The initial stage of particles growth (the mechanism) can be affected with the change of pH of the system due to formation of deprotonated carboxyl groups and gold hydroxocomplexes. Applying size-dependent Hamaker constant to DLVO theory explains experimental results.

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Формування та стабільність наночастинок золота в колоїдах, отриманих цитратним методом

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Наночастинки золота (НЧ Au) знайшли широке застосування в різних галузях, зокрема в біомедичній практиці. Активність НЧ Au сильно залежить від розміру та асоціації частинок у колоїді, на які, в свою чергу, сильно впливають експериментальні параметри реакції. Отримання НЧ Au навіть за допомогою класичної процедури цитратного методу може бути складним завданням.

У даній роботі ми застосували різні експериментальні підходи для впливу на процес утворення НЧ Au в присутності цитрату натрію. НЧ Au були отримані з використанням різних експериментальних методик і *варіюванням співвідношення реагентів, їхніх концентрацій, температури реакції, тривалості нагрівання, порядку введення реагентів в реакційну суміш, pH і т.і. Порівняльний аналіз УФ-спектрів з даними ДРС за кількістю, об'ємом та інтенсивністю дозволив простежити зміни в колоїдах НЧ Au, знайти оптимальні умови проведення експерименту та спрогнозувати тривалу стабільність колоїдів. Застосування константи Гамакера, залежної від розміру, згідно теорії DLVO, пояснює експериментальні результати.*

Утворення НЧ Au сильно залежить від співвідношення функціональних груп молекули, що беруть участь одночасно у відновленні іонів металу, зв'язуванні з поверхнею НЧ Au і формуванні заряду для стабілізації за рахунок електростатичного відштовхування. Зміна співвідношення компонентів є недостатньою для отримання колоїдів з різними за розміром НЧ Au. Велика концентрація реагентів найбільше впливає на процес агрегації та старіння колоїдів. Температура є критичним фактором активації, яка повинна становити близько 100 °C, але тривале нагрівання спричиняє індуковану зіткненнями агрегацію. На початкову стадію росту частинок (механізм) може впливати зміна pH системи через утворення депротонованих карбоксильних груп і гідроксокомплексів золота.

***Ключові слова**: наночастинки золота, колоїдні розчини, цитрат натрію*

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