ХИМИЧЕСКИЕ НАУКИ

SYNTHESIS OF GOLD NANOPARTICLES VIA CITRATE REDUCTION AND THEIR CHARACTERIZATION

DOI: 10.31618/ESU.2413-9335.2019.3.69.492

Amanmadov A., Durdyyev R., Kotyrov M., Melebaev D.
Magtymguly Turkmen State University,
Saparmyrat Turkmenbashy Avenue 31, 744000, Ashgabat, Turkmenistan

АННОТАЦИЯ

В этой работе, коллоидные наночастицы золота (НЧЗ) были синтезированы путём химического восстановления кислого раствора золотохлороводородной кислоты ($\mathrm{HAuCl_4}$) в качестве прекурсора с щелочным раствором цитрата натрия ($\mathrm{Na_3C_6H_5O_7}$) в качестве восстановителя при температуре 80-100℃. В литературе этот метод известен как метод Туркевича. Оптическая спектроскопия и метод динамического рассеяния света (ДРС) были использованы для получения спектра поглощения и гидродинамических радиусов приготовленных наночастиц золота вместе с их распределением по размерам. Коллоидная стабильность образцов была получена посредством измерений дзета потенциала (ζ). Было установлено, что окончательный размер коллоидных наночастиц золота зависит от начального значения молярного отношения цитрата к золоту. Более того, было показано, что конечный размер коллоидных наночатиц золота может быть существенно уменьшен при определённом молярном соотношении.

ABSTRACT

In this work, colloidal gold nanoparticles (GNPs) were synthesized by the chemical reduction of an acid solution of tetrachloroauric acid ($\mathrm{HAuCl_4}$) as a precursor with a base solution of sodium citrate ($\mathrm{Na_3C_6H_5O_7}$) as a reducing agent at 80-100℃. In the literature, this method is known as the Turkevich method. UV-Vis spectrometry and Dynamic Light Scattering (DLS) were used to obtain the absorption spectra and the hydrodynamic radii of the prepared gold nanoparticles with their size distribution. The colloidal stability of the samples was obtained via Zeta potential (ζ) measurements. It was ascertained that the final size of colloidal gold nanoparticles varies with the initial value of the citrate-to-gold molar ratio. Moreover, it is shown that the terminal size of colloidal gold nanoparticles could be reduced significantly at the specific molar ratio.

Ключевые слова: наночастицы золота, золотохлороводородная кислота, метод цитратного восстановления, размер частицы, дзета потенциал, спектр поглощения.

Keywords: gold nanoparticles, tetrachloroauric acid, citrate reduction method, particle size, zeta potential, absorption spectra.

1.INTRODUCTION

Generally, particles with a size less than 100 nm are referred as nanoparticles (NPs). Nanoparticles of metals such as gold and silver were a field of interest for researchers in the last decades because of their optical, electronic, and chemical properties that are significantly different from the properties of bulk materials. Recent works have showed that the use of metallic nanoparticles, provides an opportunity for new antitumor therapies with reduced risk for development of resistance where several studies have proven nanoparticles' antitumor activity against breast, liver, colon, gastric, and lung cancers [1].

Gold nanoparticles (GNPs) are important in many applications of nanotechnology, because they display a variety of properties [2] and have important applications as diverse as cosmetics [3], electronics [4], therapeutics [5-7], imaging [8, 9], drug delivery [10, 11], pollution remediation [12, 13]. In biomedicine, especially in the cancer treatment, gold nanoparticles are very effective. However, different biomedical applications demands different sizes of gold nanoparticles. For example, in human cancer therapy, the required particle size is nearly 50 nm, while in cancer diagnosis it is approximately 20 nm [14]. It was demonstrated that cancer cells can be killed by excitation of internalized gold nanoparticles, which are excited by laser beam [15].

Performance and the physical, chemical, optical, electronic, catalytic properties of gold individual nanoparticles depend strongly on their size, shape, and surface morphology [16-20]. When the gold nanoparticles' size approaches the Fermi wavelength of electrons (<2 nm), molecule-like optical properties and size-dependent fluorescence appear [21, 22]. Thus, the synthesis of such nanomaterials with uniform size and morphology in a controlled manner remains a significant challenge. For the last decade different methods for synthesis of gold nanoparticles such as chemical [23], photo-reduction [24], microwave irradiation [25], γ-irradiation [26], and laser ablation [27] methods were investigated. Moreover, a number of biomimic processes have been used for the preparation of gold nanoparticles by using plants [28, 29], algae [30], fungi [31], bacteria [32], and yeast [33]. A variety of reductants such as sodium borohydride [34, 35], hydroxylamine [36, 37], ascorbic acid [38, 39] have been used to obtain different sized gold nanoparticles by reduction of chloroauric acid. Each of them has particular advantages and disadvantages.

However, the most popular one is the chemical reduction of choloroauric acid with sodium citrate, which plays the role of a reducing as well as a capping agent; it was introduced by Faraday [40] in 1857 and refined by Frens [41]. Nevertheless, this procedure is more known as the Turkevich method [42], who proposed a model for synthesis mechanism of gold nanoparticles. The synthesized gold nanoparticles produced via citrate reduction are polydisperse and often irreproducible. Moreover, it was shown that the polydispersity can reach 40% [43]. Thus, various theories have been proposed to explain this evolution. In 2007, Kumar and co-workers [44] developed a model for the synthesis based on the mechanism proposed by Turkevich et al. [45], but as it turned out, in several cases this model performed poorly. Consequently, Agunloye et al. presented a kinetic model based on the synthesis seed-mediated mechanistic description proposed by Wuithschick et al. [46].

In this article, we measured the sizes and polydispersity indices (PDI) of resulting gold nanoparticles via dynamic light scattering (DLS) spectroscopy. The nanoparticles were also confirmed by UV–Vis spectroscopy and the stability (ζ- zeta potential) of nanoparticles was measured by electrokinetic sonic amplitude (ESA).

2.EXPERIMENTAL

2.1. Material and reagents

For the synthesis of colloidal gold nanoparticles, we used tetrachloroauric acid ($HAuCl_4$) as gold precursor and sodium citrate ($Na_3C_6H_5O_7$) as a reducing agent. The concentration of $HAuCl_4$ aqueous solution was 12 mM. We used freshly prepared sodium citrate. The glassware used was all cleaned in a bath of freshly prepared aqua regia solution, then rinsed thoroughly with de-ionized water before use.

2.2Instruments

The preparation of colloidal gold nanoparticles was carried out by magnetic stirrer (AMTAST, MS-400). The UV-vis absorption spectra for colloidal gold nanoparticles were recorded by using UV-vis spectrophotometer (ОКБ Спектр, 130063 СФ-2000 Спектрофотометр). Size and size distribution of colloidal gold nanoparticles were analyzed by the dynamic light scattering method (PHOTOCOR Mini, 130130). The values of zeta potential of colloid gold nanoparticles were measured using zeta potential analyzer (PA field ESA, 0124F.49, PA Partikel - Analytik - Meßgeräte GmbH). To weigh chemicals and reagents, we used microscale (METTLER TOLEDO, MS204S). For the reactions, we used the chemical glassware (KLIN) and the pipette (Thermo Fisher Scientific) to transport a measured volume of liquids.

2.3. Preparation of gold nanoparticles via citrate method

Colloidal gold nanoparticles were synthesized by the Turkevich method [42]. Briefly, tetrachloroauric acid (HAuCl4) was reduced with sodium citrate. The HAuCl4 aqueous solution was stirred vigorously and heated under reflux. The sodium citrate was added when the HAuCl4 aqueous solution was boiling and left to refluxing for an additional time. Four different sets of experimental conditions for synthesized colloidal gold nanoparticles are shown at table 1.

Table 1.

Synthesis of colloidal gold nanoparticles

Metal	Reducing agent (capping agent)	Concentration and volume of reducing agent	Concentration and volume of precursor	Temperature	Refluxing time
Au	sodium citrate ($Na_3C_6H_5O_7$)	C=38.8 mM V=2 mL	C=1 mM V=20 mL	80^0C	10 min
Au	sodium citrate ($Na_3C_6H_5O_7$)	C=38.8 mM V=2 mL	C=1 mM V=20 mL	100^0C	10 min
Au	sodium citrate ($Na_3C_6H_5O_7$)	C=38.8 mM V=3 mL	C=0.3 mM V=100 mL	100^0C	15 min
Au	sodium citrate ($Na_3C_6H_5O_7$)	C=38.8 mM V=30 mL	C=0.5 mM V=300 mL	100^0C	30 min

3.RESULTS AND DISCUSSION

3.1 Particle size measurements of colloidal gold nanoparticles by dynamic light scattering (DLS)

Dynamic light scattering (DLS) provides a lot of possibilities to obtain information about such properties as size of nanoparticles and diffusion coefficient. The particle size was measured using a PHOTOCOR Mini 130130 equipped with a 25 mW temperature stabilized diode laser (654 nm) and operating at an angle of 90^0 and a temperature 18-22^0 C. A sample volume 2.5 ml was used in 10-mm-diameter cuvettes. The mean hydrodynamic radii of the particles, R_h, were computed from the intensity of the scattered light using the Photocor software package by multiple mode analysis, based on the theory of Brownian motion and the Stokes–Einstein equation:

$$D = \frac{k_B T}{6\pi\eta R_h}$$

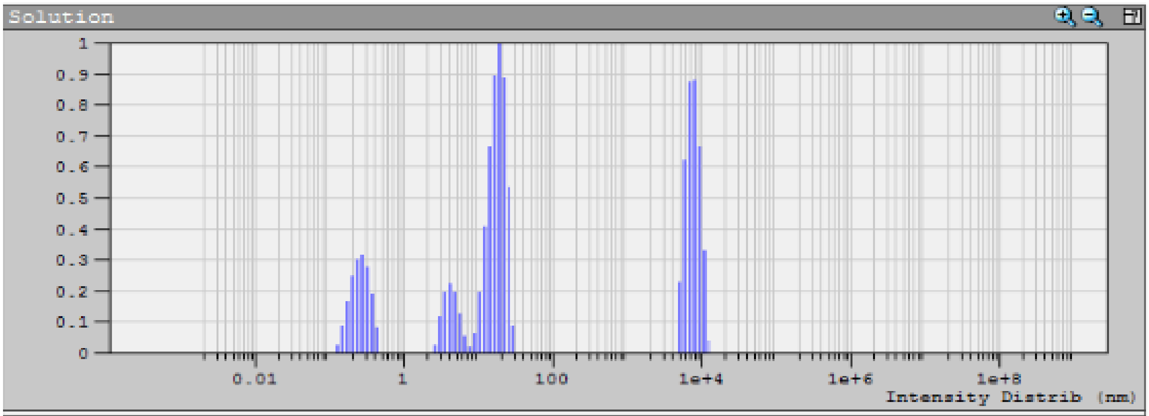
Where D is the diffusion coefficient (the primary parameter obtained from DLS measurements), k the Boltzmann constant, T the temperature, and η the solvent viscosity.

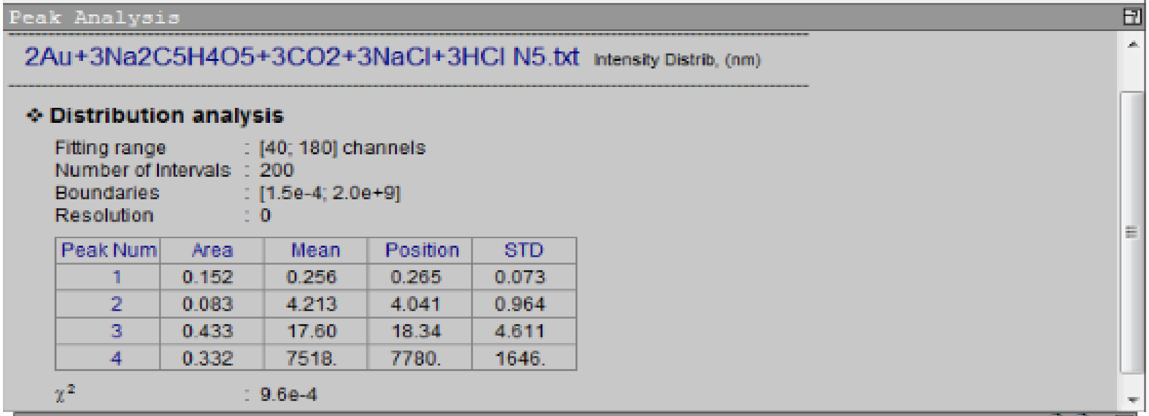
Table 2.

Particle size and PDI of colloidal gold nanoparticles synthesized under four different s ets of experimental conditions

Concentration and volume of reducing agent	Concentration and volume of precursor	Temperature	Refluxing time	Particle size (radius)	Polydispersity indices (PDI)
C=38.8 mM V=2 mL	C=1 mM V=20 mL	80^0C	10 min	64.18 nm	0.203
C=38.8 mM V=2 mL	C=1 mM V=20 mL	100^0C	10 min	61.15 nm	0.488
C=38.8 mM V=3 mL	C=0.3 mM V=100 mL	100^0C	15 min	18.34 nm	0.567
C=38.8 mM V=30 mL	C=0.5 mM V=300 mL	100^0C	30 min	15.27 nm	0.419

The DLS intensity size distribution of synthesized colloidal gold nanoparticles are shown in Figure 1. It is obvious that while increasing the amount of citrate used during synthesis decreases particle radius. The size and polydispersity of synthesized colloidal gold nanoparticles are 64.18 nm (0.203), 61.15 nm (0.488), 18.34 nm (0.567) and 15.27 nm (0.419) respectively.





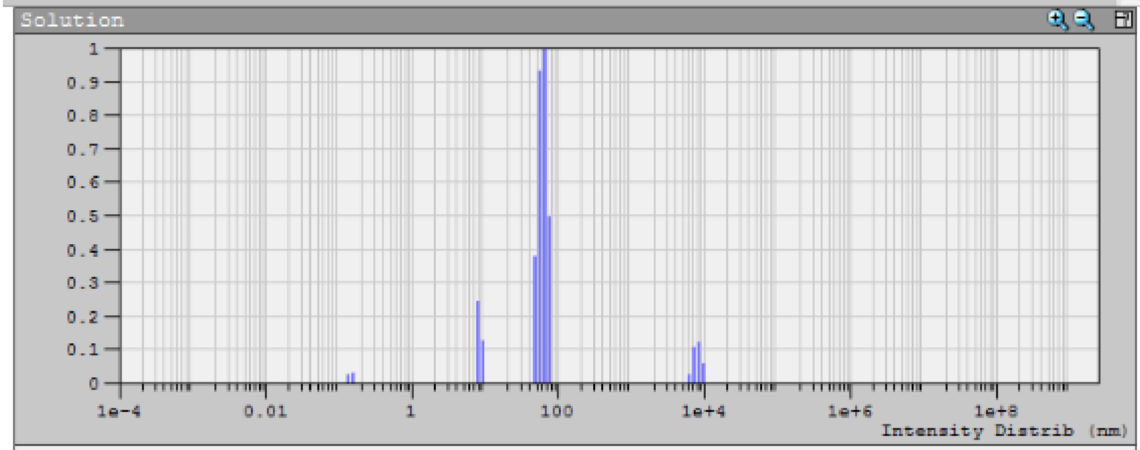
2Au+3Na2C5H4O5+3CO2+3NaCl+3HCl N5.txt Intensity Distrib, (nm)

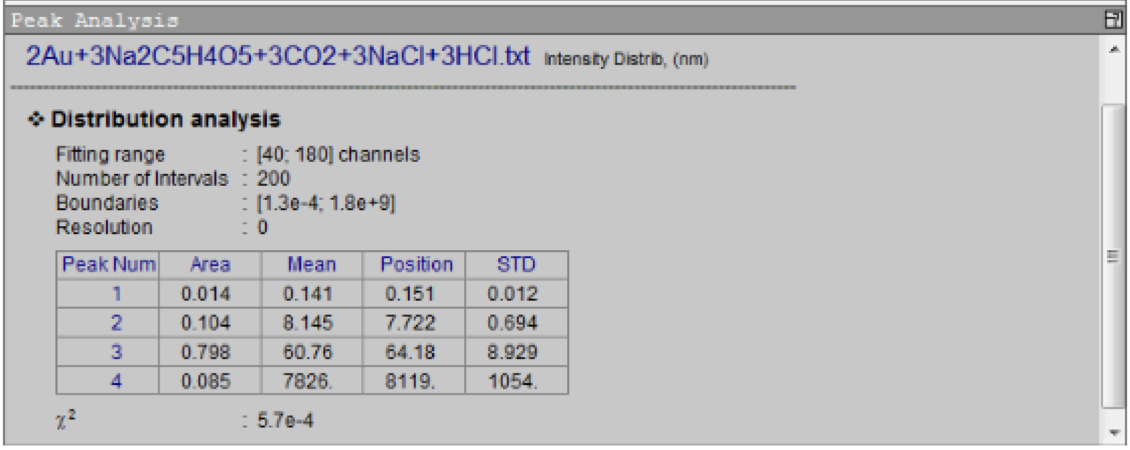
❖ Distribution analysis

Fitting range : [40; 180] channels
Number of Intervals : 200
Boundaries : [1.5e-4; 2.0e+9]
Resolution : 0

Peak Num	Area	Mean	Position	STD
1	0.152	0.256	0.265	0.073
2	0.083	4.213	4.041	0.964
3	0.433	17.60	18.34	4.611
4	0.332	7518.	7780.	1646.

χ^2 : 9.6e-4





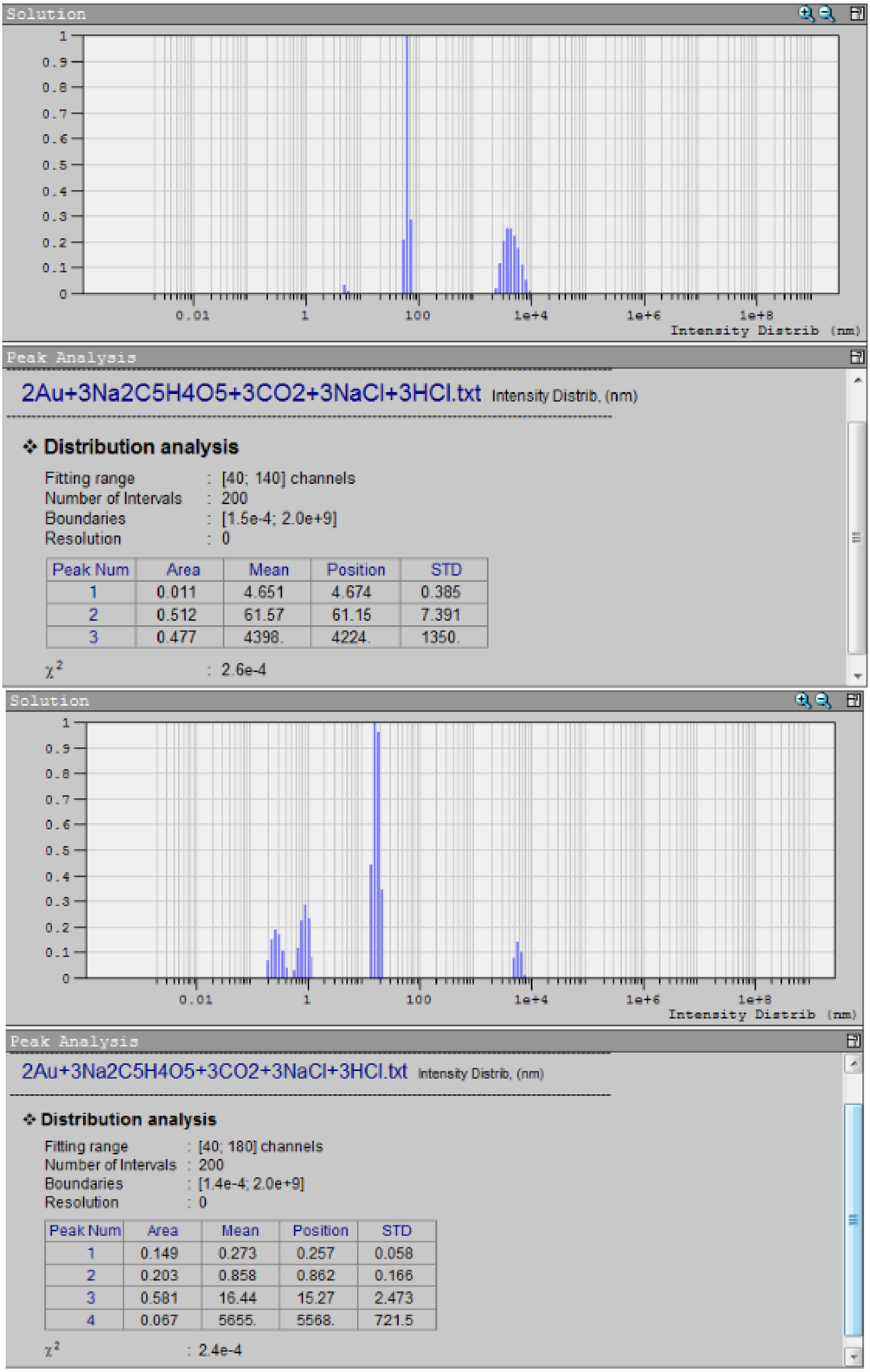
2Au+3Na2C5H4O5+3CO2+3NaCl+3HCl.txt Intensity Distrib, (nm)

❖ Distribution analysis

Fitting range : [40; 180] channels
Number of Intervals : 200
Boundaries : [1.3e-4; 1.8e+9]
Resolution : 0

Peak Num	Area	Mean	Position	STD
1	0.014	0.141	0.151	0.012
2	0.104	8.145	7.722	0.694
3	0.798	60.76	64.18	8.929
4	0.085	7826.	8119.	1054.

χ^2 : 5.7e-4



2Au+3Na2C5H4O5+3CO2+3NaCl+3HCl.txt Intensity Distrib, (nm)

❖ **Distribution analysis**

Fitting range : [40; 140] channels
Number of Intervals : 200
Boundaries : [1.5e-4; 2.0e+9]
Resolution : 0

Peak Num	Area	Mean	Position	STD
1	0.011	4.651	4.674	0.385
2	0.512	61.57	61.15	7.391
3	0.477	4398.	4224.	1350.

χ^2 : 2.6e-4

2Au+3Na2C5H4O5+3CO2+3NaCl+3HCl.txt Intensity Distrib, (nm)

❖ **Distribution analysis**

Fitting range : [40; 180] channels
Number of Intervals : 200
Boundaries : [1.4e-4; 2.0e+9]
Resolution : 0

Peak Num	Area	Mean	Position	STD
1	0.149	0.273	0.257	0.058
2	0.203	0.858	0.862	0.166
3	0.581	16.44	15.27	2.473
4	0.067	5655.	5568.	721.5

χ^2 : 2.4e-4

Figure 1. DLS Intensity % vs. Size graphs for synthesized colloidal gold nanoparticles

3.2 Absorption spectra of colloidal gold nanoparticles by UV-vis spectroscopy

The crystal lattice of gold, as other metals, is arranged in such a way that valence electrons are able to move throughout the volume of matter; as a result of this, metals have a high electrical conductivity. An alternating electric field of a light beam shifts the conduction electrons and an electric dipole forms on the surface of a nanoparticle, which oscillates with the field frequency of the incident light. This dipole oscillating near the surface of a nanoparticle is called the surface plasmon. A surface plasmon appears if a nanoparticle is much smaller than the length of the incident light.

The coincidence of the oscillation frequency of the surface plasmon and the oscillation frequency of the incident light causes a resonant absorption and scattering of light, which is called the surface plasmon resonance (SPR).

The absorption of light by a substance is calculated according to the Lambert-Behr law:

$$\lg\left(\frac{J_0}{J}\right) = \varepsilon \cdot C \cdot d$$

Here, J_0 and J are the intensities of the light before and after passing through a layer of thickness d (cm) with a concentration C (mol/l). The ratio $\frac{J_0}{J}$ and the value ε are called the extinction and the molar extinction coefficient, respectively.

Thus, a specific absorption band of the solution arises in the visible region due to the small particle size. A plasmon absorption band arises if the particle size becomes less than the mean free path of electrons in a bulk metal. Only for three metals (Au, Ag, and Cu) the plasmon frequency of the nanoparticles is shifted from the UV to the visible region of the spectrum; for all others, it is shifted to the UV region.

UV-vis absorption spectra of colloidal gold nanoparticles synthesized under four different sets of experimental conditions are shown in Figure 2. UV-vis absorption spectra were recorded from 400 nm to 900 nm using a СФ-2000 Спектрофотометр with an interval of 0.1 nm with a quartz glass 10 mm cuvette. The values of resonant plasmon absorption and Lambda max (**λ_{max}**) in the UV-vis spectrum are shown in Table 3

Table 3

Characterization of synthesized colloidal gold nanoparticles by UV-vis spectrum

Metal	Particle size (radius)	Absorbance	Lambda max (λ_{max})
Au	64.18 nm	2.28	525.5 nm
Au	61.15 nm	0.51	549.5 nm
Au	18.34 nm	0.89	533.5 nm
Au	15.27 nm	1.54	527.5 nm

3.3 Zeta potential measurements of colloidal gold nanoparticles by electrokinetic sonic amplitude (ESA)

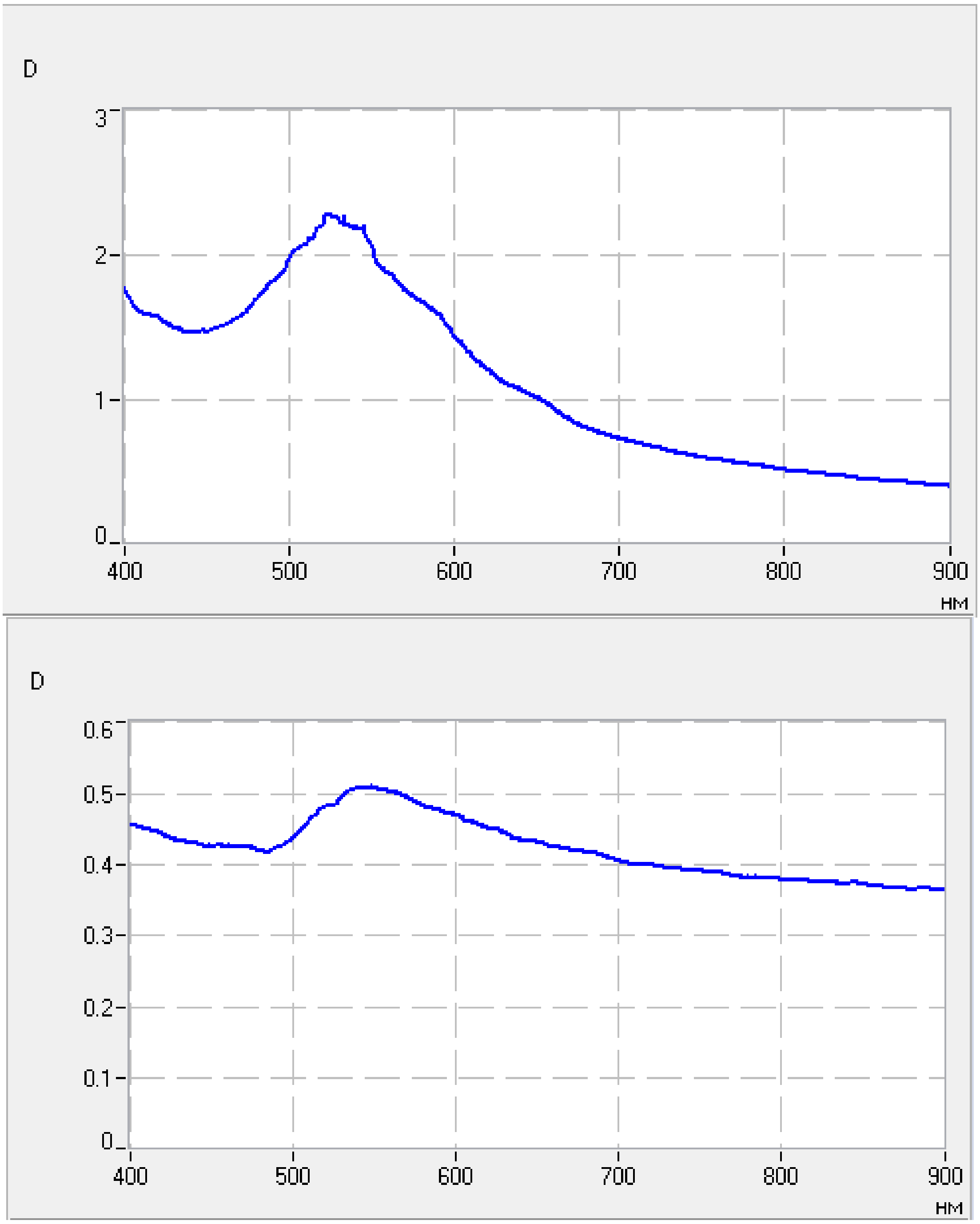
The effective charge or zeta potential of particles is classically measured as the speed at which they move in an external electric field (electrophoresis) by applying a direct current. In this case, the particles start moving towards one of the electrodes. The actual parameter that is measured is their dynamic mobility. The PA device employs alternating current to force the charged particles to move. In this case, they oscillate with the frequency of the external electric field. The oscillation of matter at frequencies of 200 KHz to 2 MHz produces sound waves with a net impulse transfer as long as there is sufficient density difference between the particles and the surrounding solvent. The amplitude of this wave or the electrokinetic sonic amplitude (ESA) is proportional through proven theory to the dynamic mobility and thus to the zeta potential of the particles in the dispersion.

Zeta potential values of synthesized colloidal gold nanoparticles are dependent on size and PDI of synthesized colloidal gold nanoparticles.

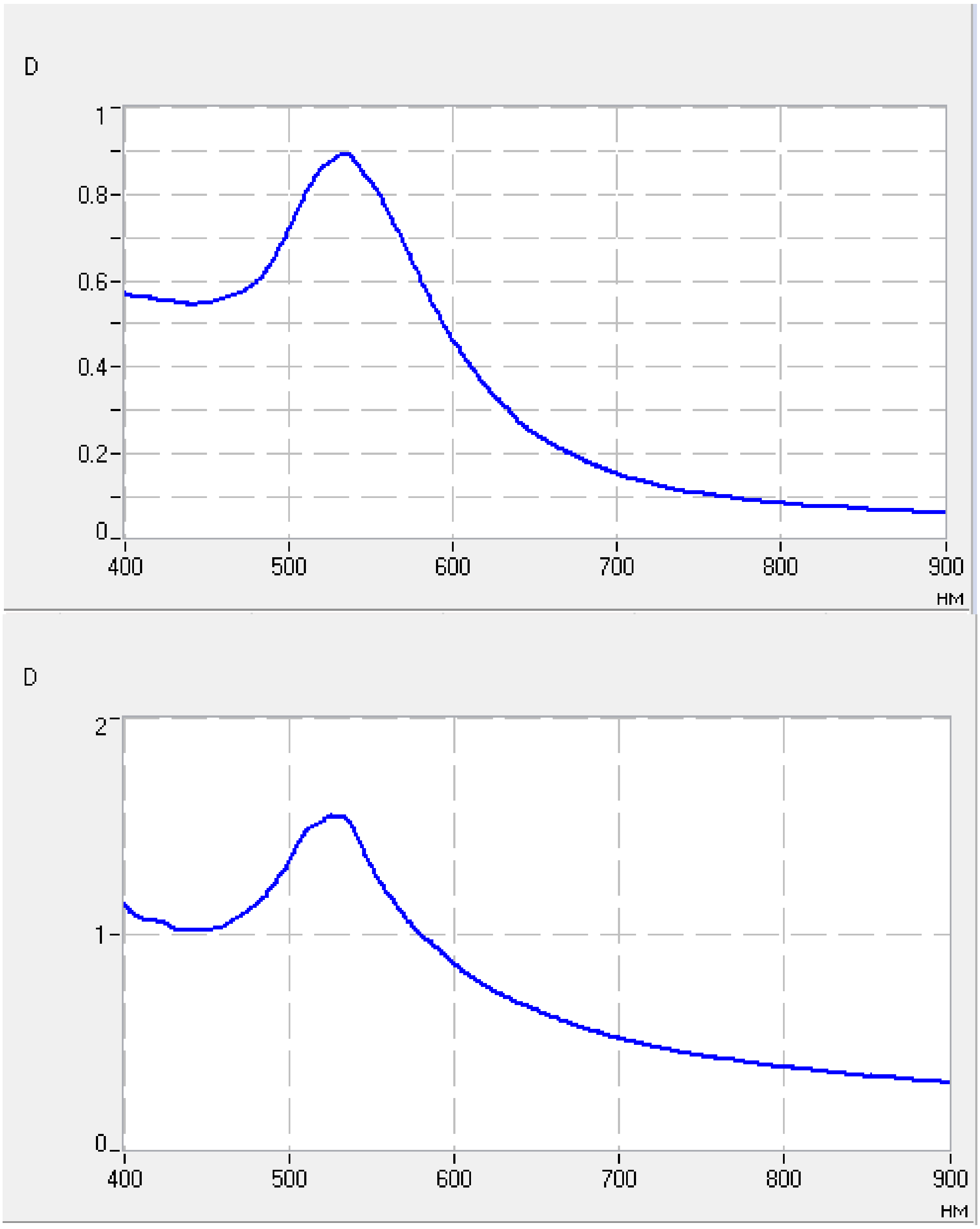
Table 4

Zeta potential of colloidal gold nanoparticles synthesized under four different sets of experimental conditions

Metal	Particle size (radius)	Zeta potential (ζ)	PDI
Au	64.18 nm	-4.2 mV	0.203
Au	61.15 nm	-3.5 mV	0.488
Au	18.34 nm	-37.8 mV	0.567
Au	15.27 nm	-33.6 mV	0.419



Absorption spectra of 64.18 nm gold nanoparticles b) Absorption spectra of 61.15 nm gold nanoparticles



c) Absorption spectra of 18.34 nm gold nanoparticles d) Absorption spectra of 15.27 nm gold nanoparticles
Figure 2. Absorption spectra of colloidal gold nanoparticles synthesized under four different sets of experimental conditions

4.CONCLUSION

At nanoscale, gold exhibits remarkably unusual physical, chemical and biological properties. In this research we have applied the citrate reduction of tetrachloroauric acid in an aqueous medium to obtain colloidal gold nanoparticles. To determine the size and the polydispersity of the nanoparticles we have used dynamic light scattering method, while to measure the zeta potential we have utilized the electrokitenic sonic amplitude method. The absorption spectra of all prepared samples were obtained using the UV-visible spectrophotometer. All samples showed a surface plasmon resonance peak which proves the formation of GNPs. We have showed that at the 7:1 citrate-to-gold molar ratio the final size can be reduced to 15 nm without losing the stability.

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