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Rapid characterization of synthesized nanoparticles liquid dispersions using nanoparticle tracking analysis †

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Abstract: Obtaining such characteristics of dispersions of synthesized nanoparticles as concentration and particle size is an important task in nanotechnology, biomedicine, industry and many other fields. A rapidly developing method for such needs is nanoparticle tracking analysis (NTA). This technique enables visualization and recording of nanoparticles sized from dozens of nanometers to couple of micrometers moving under Brownian motion. The key point of getting precise information about a nanodispersion is video processing, which allows to analyze the sample quickly without damaging it. Samples of polystyrene and gold nanoparticles with different characteristics were dispersed in water and studied using NTA device. As the reference methods of nanoparticles characterization dynamic light scattering and transmission electron microscopy were used. This study also represents the main advantages and drawbacks of using NTA method in the study of nanoparticle samples of various concentration levels.

Keywords: nanoparticle sizing; nanoparticle tracking analysis; dynamic light scattering; video processing; Brownian motion

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1. Introduction

Due to their unique physical, chemical, and electronic properties, metal and polymer nanoparticles can be used in industry, energy, environmental studies, and biomedicine [1-3]. It is possible to tune the geometric, optical and surface properties of the synthesized nanomaterials for specific applications.

Liquid dispersions of nanoparticles have found their application as contrast agents in the imaging and treatment of tumors, in the detection of pathogens of infectious diseases, and in targeted drug delivery [4].

Gold nanoparticles are chemically stable, biocompatible with living tissues and non-toxic, like gold itself. [5] In addition, nanostructures are endowed with unique catalytic, ferromagnetic and optical properties. Such optical properties of colloidal gold as absorption, scattering, fluorescence and Raman scattering (SERS) are determined by plasmonic oscillations of electrons in the metal and can be tuned. During the interaction of a gold nanoparticle with the substance under study, its physicochemical properties, such as surface plasmon resonance, conductivity, and redox potential, can change. These properties have found application in the diagnosis and treatment of malignant tumors [6-7].

However, it was shown that only a small fraction of the nanoparticle injection dose (<0.7%) reaches the target [8]. It leads to conclusion that nanocarriers have some organism barriers to overcome before achieving therapeutic effect.

Under such circumstances, it is necessary to carefully control the monodispersity of the samples used. A large variation in the particle size distribution of a sample will lead to a decrease in the therapeutic effect. For such needs methods based on the light scattering can be applied.

Currently, the most common method for studying various nanostructures is transmission electron microscopy (TEM). Due to its high resolution capability, TEM allows visualization and characterization of nanoparticle size and shape, and enables the investigation of complex nanostructures. However, it is noted that TEM does not provide information about the hydrodynamic size of particles [9]. Therefore, if hydrodynamic size needs to be measured, an approximate value of the thickness of the stabilizing layer covering the particles is added to the measured size values. Moreover, it should be noted that when measuring by TEM, data obtained from a small sample is generalized to the entire sample. This implies that the obtained images do not allow for accurate assessment of particle concentration.

The scattering of light by particles is a very useful property that has found applications in many areas of science. One of these is the determination of the size of nanoparticles by analyzing the intensity function of the laser radiation scattered by them. Methods based on light scattering make it possible to analyze liquid dispersions of nanoparticles without complex sample preparation, which makes them most convenient for rapid analysis, for example, in technological processes or for tracking the dynamics of such processes in liquid dispersions of nanoparticles as particle aggregation, various chemical reactions, phase transitions, etc. There are various methods for this analysis. In this paper, we consider the well-studied method of dynamic light scattering and the recently widely used method of nanoparticle tracking analysis.

Dynamic light scattering (DLS) is a method used to measure the diffusion coefficient and hydrodynamic radius of nanoparticles in liquid dispersions by analyzing the temporal autocorrelation function of scattered light intensity. DLS is commonly used for determining particle sizes in transparent dispersions and low concentrations due to multiple scattering at higher concentrations. DLS measurements can be 90% higher than TEM readings because they give insight into the functional groups of particles located on the surface by measuring the hydrodynamic particle diameter [10].

The nanoparticle tracking analysis (NTA) method does not require complex sample preparation and allows for determination of nanoparticle size, as well as characterization of liquid dispersions such as particle concentration, presence of large impurities and particle aggregates, and degree of sample polydispersity. However, one of the unique and useful features of the method is the ability to simultaneously measure the light scattering intensity from individual particles, allowing for differentiation of particles based on material composition. Thus, particles in the same sample with the same size but different composition and refractive index can be distinguished [11].

The NTA method has a wide measurement range for nanoparticle sizes, a wide range of measurement for electrophoretic mobility, and the ability to determine geometric parameters of nonspherical particles within a measurement time of up to 10 minutes for one sample.

2. Methods and materials

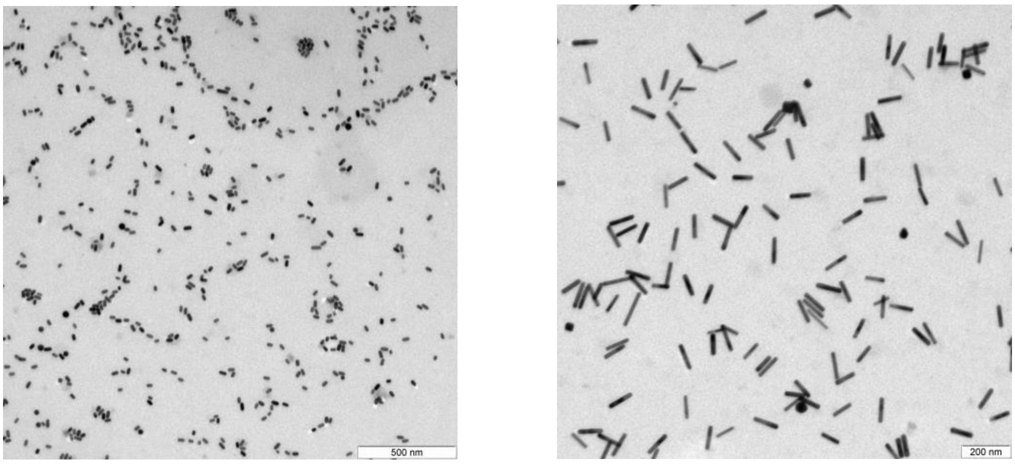
2.1. Nanoparticles samples

Experimental data were obtained by observing the movement of latex and gold nanoparticles of different sizes. Polystyrene latex nanoparticles with the diameter of 180 nm were used as the reference sample. Transmission electron microscopy (TEM) was used to determine the particle sizes. Nanoparticles were dispersed in deionized water to a concentration ranging from 10^7 to 10^9 particles per ml, in order to provide an acceptable concentration for measurements using the NTA method. Information about the sizes of gold nanoparticles is presented in Table 1.

Table 1. Gold nanoparticles specification.

Sample number	Shape	Size, nm	Production method
1	spherical	40	$HAuCl_4$ reduction
2	rods	12x24	selective etching
3	rods	12x36	selective etching
4	rods	12x48	selective etching
5	rods	12x60	selective etching
6	rods	12x72	selective etching
7	spherical	80	$HAuCl_4$ reduction
8	spherical	120	$HAuCl_4$ reduction

Samples of gold nanorods were studied as well as the spherical nanoparticles and although the DLS and NTA methods in their pure form are not suitable for measuring such nanoparticles, however, they allow indirect measurement of the characteristics of liquid dispersions (Figure 1). Samples 2-6 correspond to nanorods with aspect ratios of 2-6, respectively.



(**a**) (**b**)

Figure 1. TEM images of gold nanorods diluted samples: (**a**) aspect ratio of 2; (**b**) aspect ratio of 6. It should be mentioned that there were also detected quasi-spherical particle impurities, especially in samples with aspect ratio more than 3.

2.2. Dynamic light scattering

In the dynamic light scattering (DLS) method, the scattering coefficient of a liquid scattering of particles is determined by analyzing the correlation function of the scattered light intensity fluctuations caused by the Brownian motion of the particles. The hydrodynamic radius R can be obtained using the Stokes–Einstein equation [12].

A Photocor Complex dynamic and static multi-angle light scattering apparatus (Photocor Ltd., Russia) was used to measure the size of the nanoparticles using the DLS method.

During the experiment, the samples were irradiated with a laser beam with a wavelength of 657 nm. Water was used as a solvent; The temperature of the samples examined was 24 °C. Autocorrelation function measurements were made for a scattering angle of 90°.

The time-dependent correlation function accumulation time was about 20 s per sample. Correlation function analysis measured as a function of time was performed using the distribution analysis method.

2.3. Nanoparticle tracking analysis

Nanoparticle tracking analysis (NTA) is a relatively new technology that has become widespread in recent years and has shown that it can reliably measure the hydrodynamic diameter of many nanoparticle types. NTA is increasingly used in pharmaceuticals, biomedicine, research, and other fields of science and technology [13-14].

When the studied sample of liquid dispersion of nanoparticles is irradiated with laser beam, a camera with microscope objective captures a video of Brownian motion of particles. The analysing software determines the average distance moved by each particle in two directions (x,y). Due to the obtained data the particle diffusion *Dt* coefficient can be determined:

$$\frac{\overline{(x,y)^2}}{4} = Dt = \frac{KT}{3\pi\eta d}, \quad (1)$$

Here K – Boltzmann coefficient, T – absolute temperature, η – viscosity of medium and d – the sphere equivalent hydrodynamic diameter of a particle.

The NTA measurements were carried out using a nanoparticle tracking analyzer Photocor Nanotrack (Photocor LLC, Russia). The samples were irradiated with a laser with a wavelength of 405 nm. The measurement time was 30 seconds. The shutter speed and gain parameters for measuring each sample were selected individually to obtain the optimal signal-to-noise ratio in the resulting video recordings of the Brownian motion of particles. To achieve the optimal concentration for conducting studies by the NTA method, before the study, the samples were diluted with distilled water.

2.4 Results analysis

Statistical analysis of the obtained results was performed using MATLAB (MathWorks, USA) software package.

3. Results and Discussion

NTA method is more sensitive to changes in particle concentration compared to DLS. However, at low particle concentrations, it may be difficult to obtain reliable results, and at high concentrations, analysis of video files may become more complicated due to the large number of particles. The optimal concentration range for NTA is approximately from 10^7 to 10^{10} particles per ml. Additionally, when analyzing small particles (e.g., with a diameter of about 20 nm), difficulties arise due to the low sensitivity of the camera, which complicates focusing and analysis of the video files.

Samples of gold and polystyrene nanoparticles were measured. Research was conducted on three samples of liquid dispersions of gold nanoparticles and five samples of liquid dispersions of gold nanorods using the NTA method. The results were compared with measurements obtained using the DLS method. At least five measurements were conducted for each sample. The measurement results for spherical particles are presented in Table 2. The sizes obtained using the DLS and NTA methods exceeded the sizes of the TEM by 6-38%, since the hydrodynamic radius of the particles was determined in them

Table 2. Measured spherical gold nanoparticles sizes.

TEM particle size, nm	DLS			NTA		
	Mean size, nm	SD, nm	CV, %	Mean size, nm	SD, nm	CV, %
40	55	9	16	43	7	16
80	94	21	22	92	11	12
120	133	25	19	127	16	13

Mean size for all the samples measured by DLS was 10-30% larger than the one measured by NTA (**Figure 2**). This fact had place because DLS method is based on the ensemble measurements (a large number of particles measured at the same time) and larger particles scatter light significantly more intensely than the smaller ones. As a result, mean particle size in the sample measured by DLS biased towards larger particles.

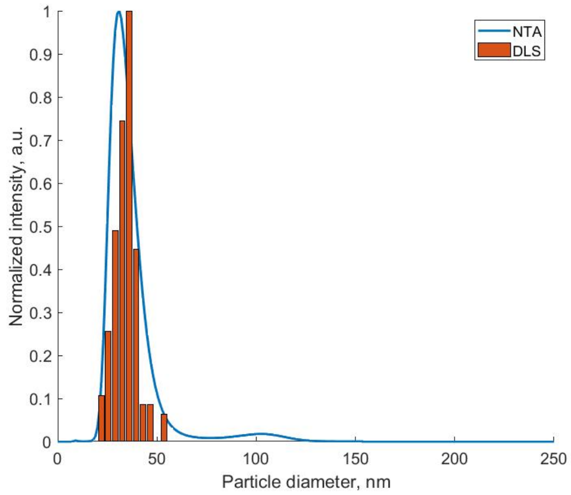


Figure 2. Particle size distributions for sample 1 of gold nanoparticles measured by NTA (blue) and DLS (orange) techniques

NTA can also detect the presence of large particles in a sample, but the limited number of particles analyzed in a single measurement makes it less sensitive to the presence of large particles in samples than DLS.

Another important characteristic of liquid dispersions of nanoparticles is the degree of monodispersity of the samples. Estimation of the spread in particle sizes in the samples based on the results of TEM image analysis showed that the studied samples can be considered monodisperse with relative deviations in length of 8–15% and in diameter of 7–12%. However, it is also necessary to take into account the fact that a very limited number of particles are taken into account when analyzing TEM images.

The DLS and NTA methods make it possible to obtain a more statistically reliable estimate of the particle size spread due to a larger number of analyzed particles. Despite the fact that the calculation of the hydrodynamic radius in both methods is carried out in the approximation for particles of a spherical shape, the values of the peak half-width in the particle size distribution made it possible to estimate the degree of monodispersity of the samples (Figure 3). It can be seen that the values obtained by different methods are in fairly good agreement with each other, however the NTA sizes had greater deviation due to the ability of analyzing tracks of each particle.

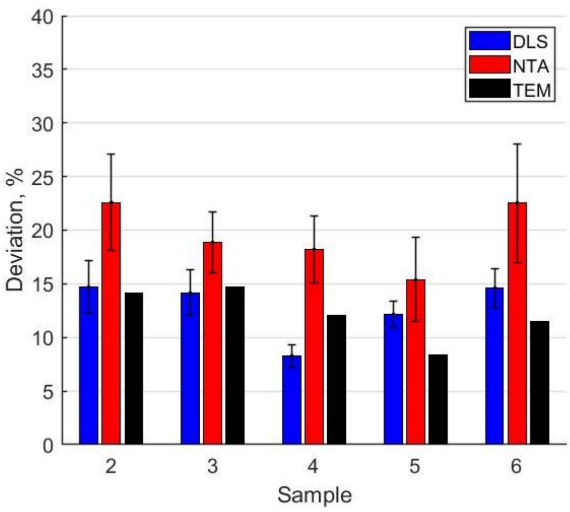


Figure 3. Relative deviation of particle sizes based on DLS, NTA and TEM measurements.

The measured concentration of nanorods in samples of liquid dispersions was from 10^7 to 10^9 particles per ml. Due to the generalization of data obtained from a study of a relatively small sample to the entire sample, the results of individual measurements of one sample can vary significantly. It was found that in order to obtain statistically reliable results of concentration measurements by the NTA method, a number of repeated measurements for one sample are required.

4. Conclusions

Methods based on the properties of Brownian motion and light scattering allow for the analysis of liquid dispersions of nanoparticles without the need for complicated sample preparation. Thanks to this, they are widely used for rapid and efficient nanoparticle analysis in many scientific fields. Dynamic light scattering and nanoparticle tracking analysis methods allow for the determination of particle sizes, as well as characteristics of liquid dispersions, such as the presence of large impurities and particle aggregates, sample polydispersity, and dynamics of internal processes.

The main drawback of both methods is that they are adapted for determining the sizes of exclusively spherical nanoparticles. In the case of non-spherical particles, the depolarized dynamic light scattering method can be applied, in which the rotational diffusion of particles is additionally measured. At the same time, there are a number of factors that can significantly distort measurement results.

Dynamic light scattering has a very limited resolving power, which is associated with solving an ill-posed inverse problem in data processing and complicates the processing of measurement results of particles in polydisperse systems. However, this method is more sensitive to the presence of large impurities or particle aggregates in the sample.

In the nanoparticle tracking analysis method, observation is made for each individual particle, which allows for more accurate information on particle sizes, including in polydisperse systems. In addition, it allows for the determination of the concentration of nanoparticles in liquid dispersion samples.

Mean size for all the samples measured by DLS was 10-30% larger than the one measured by NTA.

The obtained results can be used in the study of liquid dispersions of nanoparticles using dynamic light scattering and nanoparticle tracking analysis.

Author Contributions:

Conceptualization, P. Monakhova and P. Shalaev.; methodology, P. Shalaev; software, I. Gorev.; validation, P. Monakhova, P. Shalaev.; investigation, P. Monakhova; writing—original draft preparation, P. Monakhova and I. Gorev; writing—review and editing, P. Shalaev; visualization, I. Gorev. All authors have read and agreed to the published version of the manuscript.

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