Investigation of the physicochemical properties of magnetic nanoparticles: size, magnetic properties, concentration

V N Kuryakov¹, I V Sergeev², O O Efanova² and O K Zheludkova²

¹ Oil and Gas Research Institute of the Russian Academy of Sciences, 3, Gubkin Street Moscow, 119991, Russian Federation
² DNA-Technology, Nauchnii Proezd 20, bld. 4, Moscow, 117246, Russian Federation
E-mail: vladimir.kuryakov@ipng.ru

Abstract. This work presents the results of studies of a series of samples of aqueous dispersions of magnetic nanoparticles. The particle sizes were measured for these samples by the dynamic scattering method. Using the method of ultramicroscopy, the number concentration of particles in the samples and the concentration of particles remaining in the volume of the samples after exposure to a magnetic field at various time intervals were measured.

1. Introduction

Magnetic nanoparticles (MNP) are in demand for solving various problems in various industries. There are great prospects for the use of MNPs in medicine, for example, for the control and diagnosis of oncological diseases [1-3]. The development of biosensors using MNPs is an urgent task in the field of Biomedical and biotechnological engineering [4, 5]. MNPs are in demand for the development of new and improvement of old methods of catalysis [6-8].

Magnetic nanoparticles are used in medical diagnostics as sorbents of nucleic acids (NA) for the isolation of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) from biological objects [9]. DNA molecules are adsorbed on the surface of magnetic particles coated with SiO₂, and such particles can be removed from the solution by a magnetic field and subsequently one can separate DNA molecules from magnetic nanoparticles. Therefore, one of the important factors for the efficient isolation of NAs is the number of nanoparticles involved in sorption and the number of particles that will be magnetically separated from the mixture. Experimental methods that make it possible to measure the number concentration of nanoparticles in a liquid are of interest to developers of magnetic nanoparticles. They can be used both in the process of synthesis of nanoparticles for production control, and as a tool for quality control of products at enterprises using magnetic nanoparticles.

The aim of this study was to evaluate the capabilities of an ultramicroscope-based device for measuring the number concentration of magnetic nanoparticles and to compare the physicochemical properties of magnetic nanoparticles with the efficiency of NA sorption.

2. Materials and Methods

Five samples (DNA-Technology, Russia) of aqueous dispersions of magnetic nanoparticles (MNP) of the composition Fe₂O₃@SiO₂ were selected for this study. Sample 1 (MCH 215-200121), sample 2 (MCH239-250221-MCH240-260221), sample 3 (G2901), sample 4 (F0211-2), sample 5 (F1707). The particles are the magnetic core of Fe₂O₃ and the shell of SiO₂. The concentration (by weight of the
components during preparation) of particles in an aqueous dispersion is 25 mg/ml. The dispersion medium is an aqueous solution of NaCl and NaN3 salts (C < 0.05M). No more than 6 months have passed since the synthesis of samples of aqueous dispersions of magnetic nanoparticles.

Dynamic Light Scattering (DLS) is an experimental technique that measures the size of nanoparticles in a liquid. This method is applicable for particles ranging in size from 0.5 nm to several micrometers and is based on measuring the correlation function of light scattering intensity fluctuations. In this work, measurements by the dynamic light scattering method were performed on Photocor Compact-Z equipment (Photocor, Russia). This instrument used a laser with a wavelength of 654 nm and a power of 30 mW [10].

The NPCounter device [11] was used to measure the number concentration of particles. The principle of operation of this device is similar to that of an ultramicroscope [12, 13]. The laser beam in this observation method is directed at an angle of 90 degrees to the optical axis of observation, i.e. does not hit the lens and the digital camera matrix. The field of view looks like a dark field. If there are nanoparticles in the sample, laser radiation will be scattered on them in all directions, including along the optical axis of the lens, which will result in scattered laser light hitting the matrix of the digital camera. In this case, the picture obtained from the matrix of a digital camera will be a dark field with luminous points, which are constantly moving (make Brownian motion). The dimensions of these luminous points are not directly the dimensions of the nanoparticles. In this optical scheme, we see the scattered light on the nanoparticles, and not the particles themselves. Thus, there is no effect of diffraction limit here. At the same time, it is not possible to determine the size of particles from one such picture, but it is possible to count the number of nanoparticles in the field of view. Knowing the volume in which the observation takes place, it is possible to measure the concentration of nanoparticles. To increase the reliability of the obtained results, concentration measurements are performed on hundreds of frames of video file obtained on the test sample. Special software analyzes each frame of the video and determines the number of nanoparticles in each frame.

A powerful neodymium magnet was used to study the influence of the magnetic field. Renewal medical water for injection (SoloPharm, Russia) was used to dilute the samples. For controlled with a high accuracy of dilution of samples, we used dispensers (pipettes) of variable volume 0.1-1 ml and 0.01-0.1 ml (Eppendorf). Sartorius BP 301S analytical balance (resolution of 0.1 mg, Germany) was employed for the sample component weighing.

3. Results and discussion
For the studied samples of dispersions of magnetic nanoparticles, the efficiency of their isolation of nucleic acids was determined. For this, for each sample, the number of amplification cycles required for the detection of a test sample of an amino acid was determined. Sample number 5 was found to be the most effective (minimum number of cycles), and sample number 2 was the most ineffective.

To carry out measurements on the NP Counter instrument, it is necessary to dilute the sample, so that there are 10-100 particles in the instrument's field of view (the instrument's field of view is approximately 100x100 µm and the observation depth in the sample is about 10 µm). For this, 4 samples with a volume of about 3 ml with a particle concentration of 10, 100, 1000, and 10,000 times less were prepared from each sample of magnetic nanoparticle dispersions. Controlled dilution was performed with water for injection (bidistilled water) using pipettes. Sample G2901 diluted in this way is shown in Figure 1. For all samples, a dilution of 10,000 allowed to use NP Counter for measurements.
The DLS method was used to estimate the particle size in the samples. This method allows measurements in opaque samples (backscattering procedure [10]), but this is a rather complicated procedure and the measurement accuracy may decrease with this procedure of measurement. Therefore, measurements by the DLS method were carried out on samples with a dilution of 1000 and 10000 times. For each sample, 5 measurements were carried out and the average value was found. The results are shown in Table 1.

### Table 1. DLS measurements (mean hydrodynamic radius).

| Sample    | Dilution of 1000 | Dilution of 10000 |
|-----------|------------------|-------------------|
| Sample 1  | 215±26 nm        | 219±16 nm         |
| Sample 2  | 290±70 nm        | 310±70 nm         |
| Sample 3  | 460±40 nm        | 125±7 nm and 1390±420 nm |
| Sample 4  | 56±13 nm and 400±30 nm | 355±40 nm       |
| Sample 5  | 420±50 nm        | 470±30 nm         |

In some samples, the DLS method detected only one average size, some samples showed a bimodal distribution. At the same time, in all samples by the DLS method, the presence of a small number of large particles of several micrometers was detected - such particles are measured with a poor accuracy by the DLS method and their concentration is very small, therefore, they were not included in the table as measurement results. Also, by the type of correlation functions measured by the DLS method, and the scatter of measurement results on each of the samples, it can be argued that among the studied samples there are no samples with a narrow particle size distribution.

For all samples diluted by a factor of 10,000, the number concentration of particles was measured and from these measurements the concentration in the initial samples was calculated. The results are shown in Table 2.

### Table 2. Results of measuring the number concentration (particles/mL) in the samples of magnetic nanoparticles dispersions.

| Sample    | Sample 1     | Sample 2     | Sample 3     | Sample 4     | Sample 5     |
|-----------|---------------|---------------|---------------|---------------|---------------|
|           | 45.9·10¹⁰    | 57.5·10¹⁰    | 54.3·10¹⁰    | 49.9·10¹⁰    | 39.1·10¹⁰    |

It can be said that, taking into account the measurement accuracy, no significant difference in the number concentration is observed in all the samples studied.

Also, for each sample, a measurement was made of the concentration of particles that remain in the water in the cuvette after 1 minute of attracting the particles to the cuvette wall by magnet (Table 3). The location of the magnet and the cuvette (with the initial sample of the nanoparticle solution after 1 minute of exposure) during such an experiment are shown in Figure 2.
Figure 2. Interaction of the magnet and the sample of MNP dispersion.

Table 3. Number concentration (particles/mL) of particles in the liquid after 1 minute of effect of the magnet.

| Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|----------|----------|----------|----------|----------|
| 55.4·10⁶ | 65.1·10⁶ | 54.3·10⁶ | 48.8·10⁶ | 59.7·10⁶ |

Table 3 shows that the concentration of particles in all samples after 1 minute of effect of the magnet is approximately the same.

For sample G2901 (and partially for 0211), additional studies were carried out:
- measurements of the concentration of residual particles in the original sample with a magnet exposure time of 20 and 40 seconds;
- for diluted samples 10, 100, 1000 and 10000 times (samples in Fig. 1) measurement of the number concentration and radius (by the DLS method) of residual particles after exposure to a magnet for 1 minute;
- measurement of the concentration of residuals particles after 1-minute effect of the magnet, provided that the liquid is replaced with clean water each time after attracting particles to the wall of the vial.

Figure 3. Dependence of the number concentration of residual particles (residual concentration) in a liquid (sample G2901) on the time of magnet effect.
Figure 3 shows a plot of the number concentration of residual particles in a liquid (sample G2901) versus time of magnet effect. One can see that in the first 20 seconds of exposure to the magnet, the concentration of particles in the sample drops by three orders of magnitude. Over the next 40 seconds of exposure to the magnet, the concentration drops by one order of magnitude.

Table 4 shows the values of the initial number concentration of particles and the concentration of residual particles after exposure to the magnet for 1 minute for samples prepared from G2901 by dilutions of 10, 100, 1000 and 10,000 times.

| Dilution ratio | Without magnet (determined by dilution) | 1 minute with magnet (measured) |
|---------------|----------------------------------------|--------------------------------|
| Original      | 54.25·10^10                          | 54.0·10^6                     |
| 10            | 54.25·10^9                           | 1.05·10^9                     |
| 100           | 54.25·10^8                           | 6.24·10^8                     |
| 1000          | 54.25·10^7                           | 2.55·10^7                     |
| 10000         | 54.25·10^6                           | 10.05·10^9                    |

For clarity, the data from the table can be presented in the form of a graph (Figure 4), on which the X-axis represents the dilution factor of the initial sample, and the Y-axis – how many times the concentration of residual particles is less than the initial one (the ratio of the concentrations of two columns). It can be seen that the relative "efficiency" of magnetization decreases for diluted samples. In diluted samples, a larger percentage of particles remain in water.

Figure 4. Effect of a magnet on diluted samples Sample 3 and Sample 4. Relative residual concentration.

Figure 5 presents the data from Table 4 showing the number concentration of residual MNP in the diluted samples. Figure 5 shows that in the initial sample (highly concentrated) the effect of attracting for particles by magnet is high: relative to the initial number of particles in the volume, only a small number of particles remain unattracted by magnet after being exposed to a magnet for 1 minute. For 10 times diluted sample the absolute residual concentration is higher. This effect was rechecked and repeated. Dilution can lead to an increase in concentration of residual particles.
Figure 5. Effect of magnet on diluted samples G2901 and 0211. Residual concentration in diluted samples.

Figure 6 shows the mean radius (DLS measurement) of the residual particles after 1-minute effect of the magnet on diluted samples. It can be seen that there is a slight increase in the average size depending on the dilution ratio.

Figure 6. Mean particle radius of residual particles for samples G2901 and 0211.

For sample G2901, the following experiment was performed: a sample was prepared with a 10-fold dilution; this sample was magnetically exposed for 1 minute; the residual concentration is measured for it. The water from the vial is drained, clean water of the same volume is poured (during such procedures magnetic particles were attracted by a magnet to the vial wall); the magnet was then removed from the vial and the sample is intensively mixed; the procedure is repeated again several times. The results of measuring the residual concentration versus the number of cycles of water changes in the sample after effect of the magnet are shown in Figure 7.
Figure 7. Dependence of the number concentration on the number of water change procedures in sample G2901 after effect of the magnet for 1 minute.

The zero cycle in Figure 7 corresponds to the first measurement in the sample without changing the water. Figure 7 shows that changing the water after each effect of the magnet leads to an increase in the residual concentration in the sample. Although in this case, a decrease in the residual concentration was rather expected, since with each change of water, poorly attracted by magnet particles were removed from the sample, and those that were well attracted should remain in the sample.

4. Conclusions
From the studies carried out, it can be concluded that the NP Counter can be used to measure the concentration of magnetic nanoparticles (MNP) with good accuracy. It is possible to measure both the number concentration of MNPs in the samples, as a parameter of product quality control, and the concentration in the sample after various effects on the sample, for example, by a magnetic field.

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References
[1] Ferreira M, Sousa J, Pais A and Vitorino C 2020 The Role of Magnetic Nanoparticles in Cancer Nanotheranostics Materials (Basel). 13 266
[2] Fathi Karkan S, Mohammadhosseini M, Panahi Y, Milani M, Zarghami N, Akbarzadeh A, Abasi E, Hosseini A and Davaran S 2017 Magnetic nanoparticles in cancer diagnosis and treatment: a review Artif. Cells, Nanomedicine, Biotechnol. 45 1–5
[3] Minbashi M, Kordbacheh A A, Ghobadi A and Tuchin V V. 2020 Optimization of power used in liver cancer microwave therapy by injection of Magnetic Nanoparticles (MNPs) Comput. Biol. Med. 120 103741
[4] Adeoye A O M, Kayode J F, Oladapo B I and Afolabi S O 2017 Experimental analysis and optimization of synthesized magnetic nanoparticles coated with PMAMPC-MNPs for bioengineering application St. Petersbg. Polytech. Univ. J. Phys. Math. 3 333–8
[5] Niya H F, Hazeri N, Fatahpour M, Roudini P and Shirzaei M 2021 Immobilizing Pd nanoparticles on Fe3O4@tris (hydroxymethyl) aminomethane MNPs as a novel catalyst for the synthesis of bis (pyrazolyl)methane derivatives J. Mol. Struct. 1239 130400

[6] Niya H F, Hazeri N, Fatahpour M, Roudini P and Shirzaei M 2021 Immobilizing Pd nanoparticles on Fe3O4@tris (hydroxymethyl) aminomethane MNPs as a novel catalyst for the synthesis of bis (pyrazolyl)methane derivatives J. Mol. Struct. 1239 130400

[7] Rostami H and Shiri L 2021 One-pot synthesis of thiazole-2-imine derivatives by CoFe2O4@SiO2-PA-CC-Guanidine-SA MNPs as an efficient and recyclable catalyst Mater. Today Chem. 20 100400

[8] Zahedifar M, Seyedi N, Salajeghe M and Shafiei S 2020 Nanomagnetic biochar dots coated silver NPs (BCDs-Ag/MNPs): A highly efficient catalyst for reduction of organic dyes Mater. Chem. Phys. 246 122789

[9] Rahnama H, Sattarzadeh A, Kazemi F, Ahmadi N, Sanjarian F and Zand Z 2016 Comparative study of three magnetic nano-particles (FeSO4, FeSO4/SiO2, FeSO4/SiO2/TiO2) in plasmid DNA extraction Anal. Biochem. 513 68–76

[10] Particle size, zeta potential and molecular weight analyzers, Photocor LLC, accessed 01 July 2021, https://www.photocor.com/

[11] NanoParticle Counter, NP Vision LLC, accessed 01 July 2021, <http://npcounter.ru/>

[12] Mcfadyen P and Smith A L 1973 An automatic flow ultramicroscope for submicron particle counting and size analysis J. Colloid Interface Sci. 45 573–83

[13] Endo Y, Kousaka Y and Takato K 1998 A new sizing technique of aerosol particles — line start sedimentation type using an ultramicroscope Adv. Powder Technol. 9 141–52