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Structural investigation of biogenic ferrihydrite nanoparticles dispersion

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Structural properties of biogenic ferrihydrite nanoparticles produced by bacteria *Klebsiella oxytoca* are investigated. Investigations of morphology and size of particles dispersed in water by means of high-resolution transmission electron microscopy and small angle X-ray scattering measurements were performed. By model calculations followed by fitting procedure the structural parameters of a cylinder of radius $R = 4.87 \pm 0.02\ nm$ and height $L = 2.12 \pm 0.04\ nm$ are obtained.

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1. Introduction

Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology and comprises production, characterization and manipulation of nanoscale structures. Nanotechnology has a variety of applications in fields such as optics, electronics, bio-medicine, magnetics, mechanics, catalysis, energy science, etc. The development of techniques for the synthesis of nanoparticles of well-defined size, shape and composition is a challenge and an important area of research. Current chemical methods for the synthesis of nanoparticles are energy intensive, employ toxic chemicals, and often yield particles in nonpolar organic solutions, thereby precluding biomedical application. A promising new dimension in this field is the use of microorganisms for the production of inorganic nanoscale particles [1-7]. The clean, nontoxic and environmentally friendly ability of eukaryotic and prokaryotic microorganisms to form nanoparticles either intra- or extra-cellularly is particularly important in the development of nanobiotechnology. The synthesis of inorganic materials by biological systems is characterized by processes that occur at close to ambient temperatures, pressures and at neutral pH. Many microorganisms are known to produce inorganic nanostructures and nanoparticles with properties similar to chemically-synthesized materials, while exercising strict control over size, shape and composition of the particles. Examples include the formation of magnetic nanoparticles by magnetotactic bacteria, the production of silver particles by *Pseudomonas stutzeri*, synthesis of nanoscale, semiconducting CdS crystals in the yeast *Schizosaccharomyces pombe*, and the formation of palladium nanoparticles using sulphate reducing bacteria in the presence of an exogenous electron donor [1-7]. The ability of bacteria, fungi, actinomycetes, yeast, algae and plants to accumulate gold ions from solution has been reported and the synthesis of gold nanoparticles has been successfully demonstrated in a range of organisms including *Bacillus sp.*, fungal species such as *Verticillium* and *Fusarium*, actinomycete such as *Rhodococcus* and *Thermomonospora* and lactic acid bacteria [1-11].The interest also extends to the synthesis of nanostructures such as nanowires and the assembly of nanoparticles using biological templates such as S-layers and viruses [12, 13], DNA, proteins [14, 15].

New methods are developing to control disparity, chemical composition, the size, and the shape to get the best particles which can be well applied in different fields of science [1-16]. A growing need to understand the basics of this technique to facilitate application of the new methodology to laboratory and industrial needs is present.

In the present work structural properties of biogenic ferrihydrite nanoparticles produced by bacteria *Klebsiella oxytoca* are investigated. Earlier, it was shown that ferrihydrite nanoparticles produced by bacteria *Klebsiella oxytoca* in the course of biomineralization of iron salt solutions from natural medium [17] exhibit unique magnetic properties: they are characterized by both the antiferromagnetic order inherent in a bulk ferrihydrite and the spontaneous magnetic moment due to the decompensation of spins in sublattices of a nanoparticles [18]. Also, it was established that bacterium *Klebsiella oxytoca* creates two types of ferrihydrite nanoparticles as a result of variation of the growth conditions for the microorganisms (growth period, light exposition,

potassium citrate – ferric citrate rate, etc.), whose differences are accurately identified by means of Mossbauer spectroscopy [19, 20], static magnetic measurements analysis [20, 21] scanning electron microscopy and small angle X-ray scattering methods [22] on dry powder samples. The investigations in the direction of biomedical applications have revealed that the particles do not present cytotoxity and when attached to specific drugs present a weak antitumor activity against Ehrlich ascites carcinoma in mice [23].

Small Angle X-ray Scattering (SAXS) enables to measure structural features on length scales between 1 nm up to several hundred of nanometers by analyzing the scattering pattern at very low angles from the direct X-ray beam. By examining x-rays that are scattered at small angles to the primary X-ray beam, we can measure information that is directly proportional to the size and shape of nanometer-sized objects.

In order to avoid some structural overlay in the interpretation of experimental results obtained on dry powder samples, a small angle X-ray scattering study of the structure of biogenic ferrihydrite nanoparticles dispersed in aqueous solution is proposed and preliminary results are presented.

2. Samples and methods

Sample preparation

The microorganisms used in this study were isolated from sapropel from Lake Borovoe (Krasnoyarsk region).The lake is characterized by denitrification and ironreduction, with no sulfate reduction. The sapropel was passed through a magnetic separator. The microorganismsthus isolated were inoculated onto an agarized medium [17] and cultured under anaerobic conditions to obtain colonies [19]. The biomass grown in the liquid medium was checked for the presence of magnetic particles on an FMR spectrometer. In subsequent experiments, we used the *mbp*3 microorganism isolate. The *mbp*3 isolate retains its culturing properties and the bacterial biomass retains its magnetic properties for five years. In this study, the bacterial biomass of the *mbp*3 isolate was grown under microaerophilic conditions on a Lovley medium of composition (g/l) $NaHCO_3$, 2.5; $CaCl_2 \cdot H_2O$, 0.1; KCl, 0.1; NH_4Cl, 1.5; and $NaH_2PO_4 \cdot H_2O$, 0.6. The ferric citrate concentration was varied from 0.2 to 5 g/l, the yeast extract concentration was 0.05 g/l, and the benzoic acid concentration was varied from 0.2 to 0.5 g/l. Samples were collected 5–90 days after microorganisms had been inoculated into the culture medium. To isolate magnetic particles, the bacterial biomass was centrifuged (10 min at 10000 rpm) and then disrupted using a UZDN ultrasonic processor (1 min, 44 kHz, 20 W). The particles were recovered using a Sm–Co magnet.

The SEM (scanning electron microscopy) image of ferrihydrite powders prepared by drying the biomass (grown during 8 days) after it had been separated from the culture medium by centrifugation is presented in Fig. 1.

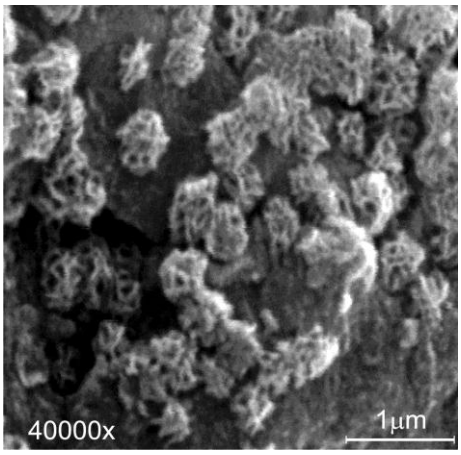
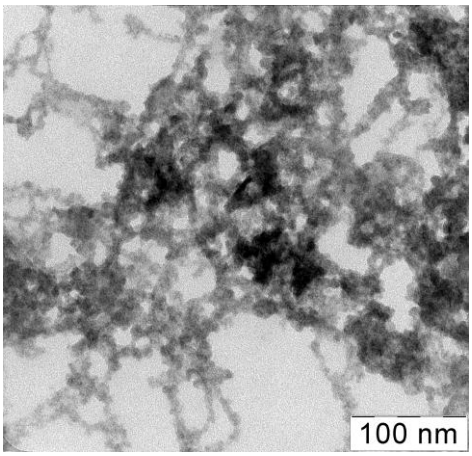
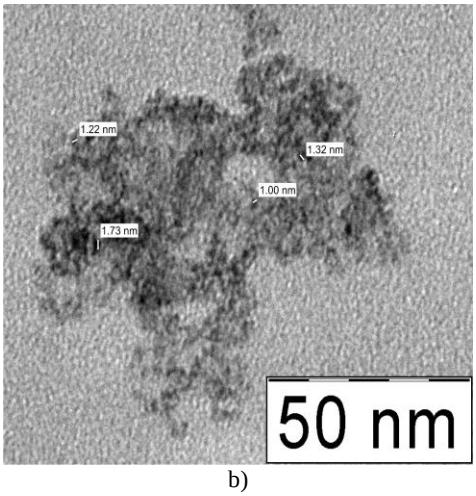


Fig. 1. SEM image of a sample containing ferrihydrite nanoparticles separated from the bacterial biomass grown during 8 days [22].

The sample of ferrihydrite nanoparticles aqueous dispersion was prepared with following composition: 5g, dry ferrihydrite powder, distillated water, 400 ml. In Fig. 2 (a, b) are presented two high-resolution transmission electron microscopy (HRTEM) images of this sample.



a)



b)

Fig. 2. HRTEM images of a sample of ferrihydrite nanoparticles dispersion in water: (a) and (b).

The presence of organic material is detected. The nanoparticles or clusters of nanoparticles are withheld in the organic network. Characteristic dimension of the particles estimated from HRTEM observations was found to be in between 1-2 nm.

Small angle X-ray scattering

In a SAXS instrument a monochromatic beam of X-rays is brought to a sample from which some of the X-rays scatter, while most simply go through the sample without interacting with it. The scattered X-rays form a scattering pattern which is then recorded by a detector which is typically a 2-dimensional flat X-ray detector situated behind the sample perpendicular to the direction of the primary beam that initially hit the sample. The scattering pattern contains the information on the structure of the sample.

The X-ray scattering intensity is experimentally determined as a function of the scattering vector Q whose modulus is given by $Q= (4\pi/\lambda)\sin\theta/2$, where λ is the X-ray wavelength, and θ is the scattering angle between the directions of the scattered and transmitted beams.

Macroscopically isotropic systems produce a scattering intensity which depends on the modulus of Q. In the special case of isotropic systems composed of isolated and identical particles embedded in a matrix with a constant electronic density, the normalized SAXS intensity is given by the product

$$I(Q) = n\,P(Q)\,S(Q), \qquad (1)$$

where $I(Q)$ is the intensity as a function of the magnitude q of the scattering vector; n is the number of particles per unit volume. $P(Q)$ is the orientational average of the particle form factor, i.e. the scattering function of a single isolated particle and may be modeled according to the geometry of the particle. $S(Q)$ is the orientation averaged effective structure factor that accounts for the short range spatial correlation between particles.

If the system is dilute, i.e. the particles are far away from each other and without spatial correlation, $S(Q) = 1$ over the whole Q range. Eq.1 simply becomes

$$I(Q) = n\,P(Q) \qquad (2)$$

3. Experimental results and discussion

Small angle X-ray scattering data

Quantitative investigations of morphology and size of the particles were made first by means SAXS measurements using the model calculations followed by fitting procedure. Experimental data were modeled in the whole Q region by using the form factors for different particle shapes to obtain the size parameters best describing the scattering signal. Form factors for several known geometries are available in [24].

Using the FITTER program [25] it was found a form factor for cylinder having diameter 2R and height L, described with the expression

$$P(Q) = 4\int_0^{\frac{\pi}{2}} \frac{\sin^2(QH\cos\alpha)}{(QH\cos\alpha)^2}\,\frac{J_1(QR\sin\alpha)}{(QR\cos\alpha)^2}\sin\alpha\,d\alpha$$

where, J_1 is the cylindrical Bessel function of order 1, best represents the experimental data.

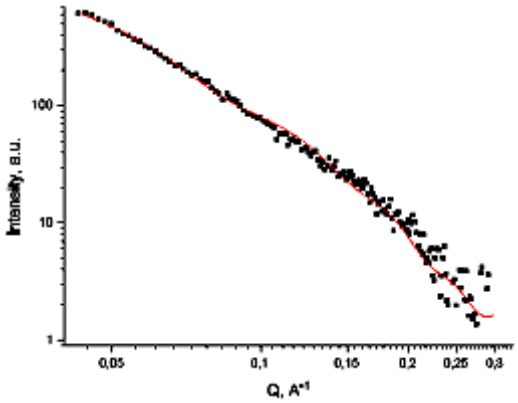


Fig. 3. Small angle X-ray scattering experimental curve from biogenic ferrihydrite nanoparticles dispersion (solid squares), obtained at Brucker Nanostar SAXS spectrometer at the Institute of Synthetic Polymer Materials RAS, Moscow and best fit for cylinder form factor (solid line).

The experimental data plotted as intensity in arbitrary units versus Q on a double logarithmic scale together with the best fit of the curve are shown in Fig.3. The parameters obtained from this fit are the cylinder of radius $R = 4.87 \pm 0.02\ nm$ and height $L = 2.12 \pm 0.04\ nm$.

4. Conclusions

Particle size analysis using high-resolution transmission electron microscopy images (HRTEM) combined with small angle X-ray scattering structure investigation of biogenic ferrihydrite aqueous suspensions are reported. Characteristic size of the particles (1-2 nm) estimated from HRTEM observations agrees with the height value of the objects identified by the small angle X-ray scattering data fit.

We note that quite close size estimations (1.5-1.8 nm) were deduced also from magnetogranulometry (analysis of magnetization curves) of the same particles [18].

The presence of organic material is detected by means of HRTEM. The nanoparticles or clusters of nanoparticles are withheld in the organic network.

Further steps in cleaning procedures of nanoparticles obtained from the bacterial metabolism will be developed,

together with methodological work on small angle X-ray scattering experiments of biogenic samples and mathematical modeling process.

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