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**SAXS STUDIES OF ULTRASONICATED DISPERSIONS
OF BIOMINERAL PARTICLES PRODUCED
BY *KLEBSIELLA OXYTOCA***

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МУРР-исследования дисперсий биоминеральных наночастиц, продуцируемых бактериями *Klebsiella oxytoca*, предварительно обработанных ультразвуком

Методом малоуглового рассеяния рентгеновских лучей исследованы наночастицы ферригидрита, производимые бактериями *Klebsiella oxytoca*. Выполнены структурные исследования образцов водных дисперсий частиц разных концентраций, обработанных ультразвуком. Приведены результаты модельных расчетов, которые позволили выявить объекты вытянутой формы с радиусом гирации $6,73 \pm 0,16$ нм.

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SAXS Studies of Ultrasonicated Dispersions of Biomineral Particles Produced by *Klebsiella oxytoca*

A small-angle X-ray scattering experiment was performed on biogenic nanoparticles produced by bacteria *Klebsiella oxytoca*. Structural investigations of ultrasonic assisted samples of different concentrations of water dispersed particles were performed. Model calculations and fitting procedures revealed scattering objects of an elongated shape of 6.73 ± 0.16 nm radius of gyration.

The investigation has been performed at the Frank Laboratory of Neutron Physics, JINR.

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INTRODUCTION

Ferrihydrite is an iron oxyhydroxide with the molecular formula $\text{Fe}_5\text{HO}_8 \times 4\text{H}_2\text{O}$, although $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ and others have been accepted [1]. Structurally, it is believed to be based on simple chains of iron octahedral, although tetrahedrally coordinated iron has also been proposed. Ferrihydrite crystallographic modifications are commonly designated as «2-line» and «6-line» ones on the basis of the number of maxima observed in X-ray diffraction (XRD) patterns. Typically, ferrihydrite nanoparticles are 2–6 nm in size [2]; their large surface area/volume ratio imparts to them high sorptive capabilities, especially for heavy and transition metals. This makes the nanodisperse ferrihydrite an issue of considerable environmental and industrial importance [1].

In the literature, several methods of synthesis and purification of ferrihydrite are described [3]. Typically, ferrihydrite emerges in the situations, where Fe^{2+} is oxidized rapidly and/or where crystallization inhibitors are present. The oxidation can proceed via an inorganic pathway, but may also be assisted by microorganisms. This paper presents a study of structural parameters of biogenic nanoparticles produced by bacteria *Klebsiella oxytoca*. Ultrasonic assisted samples of different concentrations of water dispersed particles were analyzed by the small-angle X-ray scattering (SAXS) technique at room temperature.

Earlier investigations have shown that bacterium *Klebsiella oxytoca* creates two types of ferrihydrite nanoparticles depending on the microorganism's growth conditions [4, 5]. These two fractions are quite well identified by means of the Mössbauer spectroscopy [6, 7, 12], static magnetic measurement analysis [8, 9], scanning electron microscopy and small-angle X-ray scattering methods [10] on dry powder samples. SAXS studies on concentrated and nonultrasonicated samples of water dispersions of ferrihydrite have revealed a scattering object with the form factor of a cylinder of radius $R = 4.87 \pm 0.02$ nm and length $L = 2.12 \pm 0.04$ nm [11, 13] as obtained with the aid of FITTER program [14]. It was shown also by HRTEM analysis, that nanoparticles or their clusters bear on their surfaces some adsorbed protein molecules [10]. Indeed, due to its high sorption, biogenic ferrihydrite should be very difficult to purify from the organic matter and maintained dispersed in aqueous solution for a long time. Meanwhile, the latter requirements are necessary to meet for accurate investigation by

means of SAXS. In order to improve the aggregation stability (low ageing) of the investigated samples, we applied ultrasonic treatment as a physical method to disintegrate the particle agglomerates without causing changes in their chemical composition.

MATERIALS

Aqueous samples of biogenic particles of ferrihydrite were provided by the Siberian Federal University, Krasnoyarsk, Russia. The nanoparticles were obtained from the bacterial strain isolated from Borovoe Lake (Krasnoyarsk Region). Initial concentration of magnetic phase in the aqueous solution was 12.5 g/l (5 g of biogenic ferrihydrite powder dissolved in 400 ml distilled water).

Time stability of ultrasonic assisted samples of different concentrations was monitored by UV-vis spectroscopy in the wavelength range of 400–650 nm at room temperature (Fig. 1). The spectra of a biogenic ferrihydrite dispersion before and after ultrasonic treatment are shown in Fig. 2. Visible spectra of the samples show changes after the ultrasonic treatment as well. The decrease of the absorbance values might be related to the partial destroying of particle clusters and decreasing of the amount of the free organic material in solution due to its adsorption onto the particle surfaces. Thus, the ultrasonic treatment allows one to prepare and to perform small-angle X-ray scattering on the samples with different concentration of biogenic ferrihydrite nanoparticles.

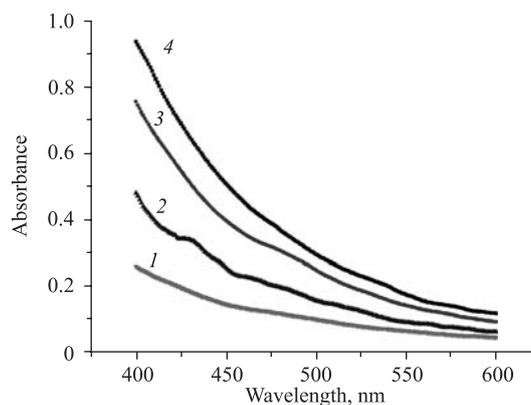


Fig. 1. Absorption spectrum vs. concentration of ferrihydrite: $1.85 \cdot 10^{-3}$ M (1), $3.70 \cdot 10^{-3}$ M (2), $5.54 \cdot 10^{-3}$ M (3), $7.41 \cdot 10^{-3}$ M (4)

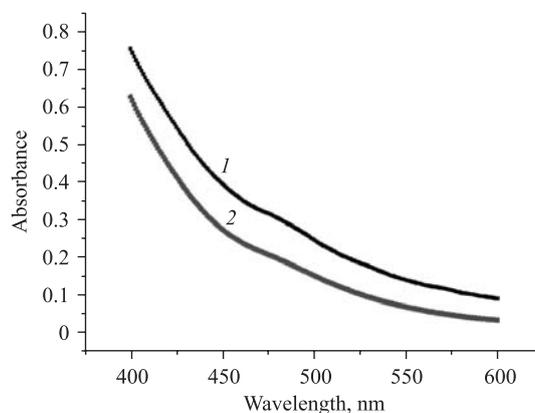


Fig. 2. Absorption spectrum of ferrihydrite sample with concentration $5.54 \cdot 10^{-3}$ M: before ultrasonic treatment (1) and after ultrasonic treatment (2)

EXPERIMENTAL

SAXS, as a probing technique, has a good potential for obtaining information from any sample; it requires modest sample preparation and it fits very well for probing and understanding the systems possessing substantial flexibility. SAXS can characterize macromolecular shapes and conformations in solution in a wide size range.

The X-ray scattering intensity is experimentally determined as a function of the scattering vector q whose modulus is given by $q = 4\pi \sin \theta / \lambda$, 2θ is the scattering angle and λ — the wavelength of the incident X-ray beam. The normalized scattering intensity is given by the product

$$I(q) = nP(q)S(q), \quad (1)$$

where $I(q)$ is the intensity as a function of the modulus of the scattering vector q ; n — the number of particles per unit volume; $P(q)$ is the orientation 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.

Analysis of the ferrihydrite nanoparticles was performed with the help of Brucker Nanostar SAXS spectrometer at the Institute of Synthetic Polymer Materials of RAS, Moscow. The experimental setup covered the q range of 0.007 – 0.23 \AA^{-1} . The experimental scattering curves presented in Fig.3 were analyzed

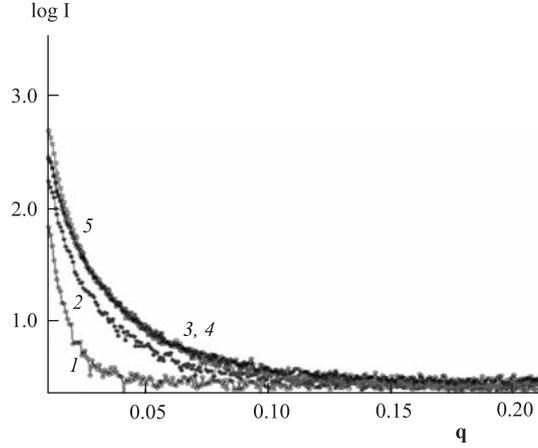


Fig. 3. SAXS scattering curves from ferrihydrite samples at different concentrations: buffer (1); ferrihydrite concentration $3.70 \cdot 10^{-3}$ M (2); $5.54 \cdot 10^{-3}$ M (3); $7.41 \cdot 10^{-3}$ M (4); $1.48 \cdot 10^{-2}$ M (5) plotted by PRIMUS; the logarithm of the scattering intensity $I(q)$ vs. modulus of the scattering vector q is shown

using model calculations from ATSAS Package (PRIMUS, GNOM and GASBOR) [14,15]. In order to eliminate concentration or aggregation influence on the modeled experimental data, a linear extrapolation to zero concentration was done for the scattering data obtained on the samples with different concentrations of ferrihydrite particles. In this case, the structure factor $S(q)$ becomes equal to 1 over the whole q range, and Eq. (1) reduces to

$$I(q) = nP(q). \quad (2)$$

Radius of gyration, R_G , which represents the distance of the scattering object parts from its centre of gravity, was calculated in a q range of $0.024\text{--}0.034 \text{ \AA}^{-1}$ using the Guinier approximation:

$$I(q) = I(0) \exp(-q^2 R_G^2/3). \quad (3)$$

A value of 6.41 ± 0.13 nm for the radius of gyration independently of the shape of the investigated particles was obtained.

In order to calculate more precisely the values of R_G , the pair distribution function of biomineral nanoparticles was computed using the fitting procedures included in GNOM software from ATSAS package. The $p(r)$ function or *pair-distance distribution function* describes the pairwise set of all distances between points within the scattering.

In SAXS, $p(r)$ is used to describe the spatial distribution of the electrons within the macromolecular structure, and is a useful tool for visibly detecting

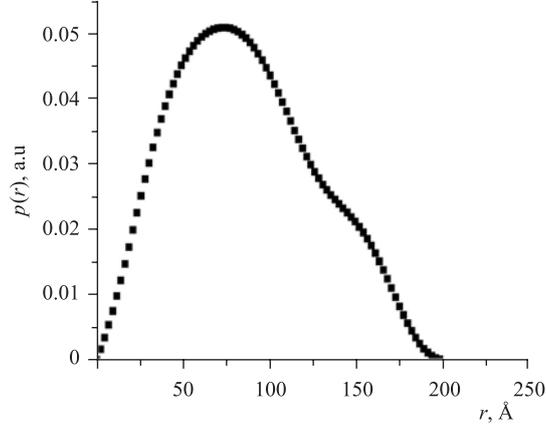


Fig. 4. Function $p(r)$ calculated from the scattering curve obtained from extrapolation to zero concentration

conformational changes within an ensemble of macromolecules. GNOM evaluates the particle distance distribution function through the indirect Fourier transform:

$$p(r) = \frac{r}{2\pi^2} \int_0^{\infty} qI(q) \sin(qr) dq, \quad (4)$$

where r is the distance vector between pairs of scatterers. Radius of gyration is obtained from the $p(r)$ function using the formula:

$$R_G^2 = \frac{\int_0^{D_{\max}} q^2 p(r) dr}{\int_0^{D_{\max}} p(r) dr}, \quad (5)$$

where D_{\max} denotes the maximum distance inside the scattering particle. This method to determine R_G takes into account all of the collected experimental data, not only those limited to small q domain, as is used in the Guinier approximation. Therefore, the obtained real space parameters are likely better estimated for the samples, where small amounts of aggregation can influence the accuracy of the information regarding the scattering profile. Calculated $p(r)$ distribution function in the q range of 0.02 – 0.26 \AA^{-1} is presented in Fig. 4.

There was not found any resemblances between the obtained $p(r)$ function and those corresponding to the objects of a specific shape (sphere, rode, core-shell, etc.) described in [17]. The elongated tail of the $p(r)$ function within the r range of 120 – 200 \AA indicates the presence of macromolecules in the scattering solutions. This fact confirms earlier results that biogenic nanoparticles removed from bacterium *Klebsiella oxytoca* are still wrapped in an organic sheath [11, 13].

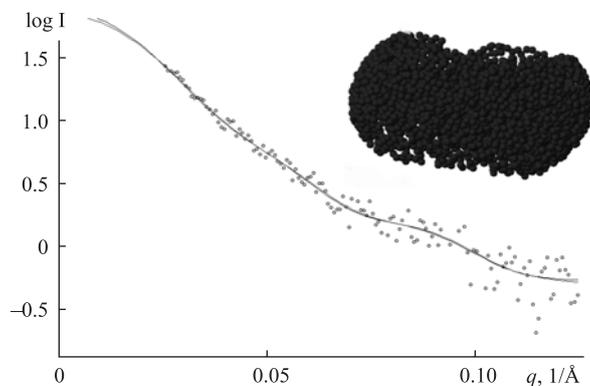


Fig. 5. *ab initio* reconstruction of water dispersed ferrihydrite nanoparticles

We remark that the value of radius of gyration of 6.73 ± 0.16 nm calculated from pair distribution function is close to those obtained from the Guinier approximation.

The overall shape of the particles was computed by the program GASBOR. This software performs an *ab initio* reconstruction of molecular structure by a chain-like ensemble of *dummy residues* [14].

Figure 5 displays the resulting plot for the scattering curve obtained from a sample of biogenic ferrihydrite nanoparticles. The identified elongated 3D object resembles quite closely the rod-like model reported earlier [11]. As any other method that generates 3D structures from the 1D scattering data, the resulting pattern is not unique and might be optimized with supplementary experimental data.

CONCLUSIONS

Characterization of ultrasonic assisted biogenic ferrihydrite particles dispersed in water was done by means of SAXS. Analysis of the scattering curves using the ATSAS software package revealed ferrihydrite particles of an elongated shape of 6.73 ± 0.16 nm radius of gyration. We note that the results obtained for diluted samples of ferrihydrite nanoparticles are close to the structural parameters determined previously by means of SAXS, HRTEM [11, 13] and magnetogranelometry [9] methods on concentrated samples. This fact allows us to surmise that ultrasonic treatment does not produce any essential physico-chemical changes of particles but facilitates to minimize the influence of aggregation on the scattering data. Higher q values contain more details regarding molecular shape. Thus, in order to obtain more precise information about the characteristic structure of ferrihydrite, further investigations by SAXS experiments at higher angles are necessary.

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