Characterization of bio-synthesized nanoparticles produced by *Klebsiella oxytoca*

L Anghel\textsuperscript{1,2,a}, M Balasoiu\textsuperscript{1,3,b}, L A Ishchenko\textsuperscript{4}, S V Stolyar\textsuperscript{4,5}, T S Kurkin\textsuperscript{6}, A V Rogachev\textsuperscript{1}, A I Kuklin\textsuperscript{1}, Yu S Kovalev\textsuperscript{7}, R S Iskhakov\textsuperscript{4,5}, G Duca\textsuperscript{8}

\textsuperscript{1}Joint Institute of Nuclear Research, Dubna, 141980, Russia
\textsuperscript{2}Institute of Chemistry of ASM, Chisinau, Republic of Moldova
\textsuperscript{3}Horia Hulubei National Institute of Physics and Nuclear Engineering, Bucharest, Romania
\textsuperscript{4}Siberian Federal University, 660041, Krasnoyarsk, Russia
\textsuperscript{5}Kirensky Institute of Physics, Siberian Branch of RAS, Krasnoyarsk, Russia
\textsuperscript{6}Institute of Synthetic Polymer Materials RAS, 117393, Moscow, Russia
\textsuperscript{7}Institute of Continuous Media Mechanics, Ural Branch of RAS, Perm, Russia
\textsuperscript{8}Academy of Science of Moldova, Chisinau, Republic of Moldova

Email: \textsuperscript{a}angel@nf.jinr.ru, \textsuperscript{b}balasoiumaria@yahoo.com

**Abstract.** Structural and morphological properties of biogenic ferrihydrite nanoparticles produced by bacteria *Klebsiella oxytoca* are investigated. The stability of water dispersions of biomineral particles produced by *Klebsiella oxytoca* was monitored by UV-Vis spectroscopy. Their chemical composition was determined by FT-IR spectroscopy. The vibrational spectra of biogenic ferrihydrite nanoparticles revealed typical absorption peaks of exopolysaccharides. Morphological analysis based on Raman spectroscopy indicated the presence of exopolysaccharides on the surface as well as inside the pores of the ferrihydrite nanoparticles. Structural investigations of ultrasonic assisted samples of different concentration of water dispersed particles were performed using small angle X-ray scattering analysis. Model calculations and fitting procedures revealed scattering objects of an elongated shape with 6.73±0.16 nm radius of gyration.

1. **Introduction**

New applications of nanomaterials are emerging rapidly. The synthesis of nanoparticles is a cornerstone of nanotechnology. New methods to study synthesis of nanoparticles are an important area of research. The methods currently being used encompass chemical routes. The by-products associated with metal production by these methods rise problems with respect to environmental pollution. Additionally, some of these processes are expensive, the synthetic procedures involving conditions such as high temperature, pressure and environmental inertness. Many chemical routes are known to use toxic chemicals for the synthesis of nanoparticles. The need-of-the-hour; however is to evolve the procedures for nanoparticles synthesis through environmental benign routes. Researchers in this field, therefore, have been eagerly looking at biological systems as alternative eco-friendly systems.

Microbial cells are highly-organized units, regarding morphology and metabolic pathways, capable of synthesizing well size-calibrated and well-structured particles. Furthermore, biogenic nanoparticles often are water-soluble and biocompatible, which is essential for many applications. Some well-known
examples of microorganisms synthesizing inorganic materials include magnetotactic bacteria (which synthesize magnetic nanoparticles) [1], diatoms (which synthesize siliceous materials) [2], and S-layer bacteria (which produces gypsum and calcium carbonate layers) [3].

Bacteria *Klebsiella oxytoca* produce two types of ferrihydrite nanoparticles depending on the microorganisms’ growth conditions [4,5]. Earlier investigations have quite well identified these two fractions by means of Mossbauer spectroscopy [6,7], 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 non-ultrasonicated samples of water dispersions of ferrihydrite have revealed scattering objects with the form factor of a cylinder of radius $R=4.87\pm0.02$ nm and length $L=2.12\pm0.04$ nm [11]. It was shown also by HRTEM analysis that nanoparticles or their clusters bear on their surfaces some adsorbed protein molecules [10]. However, in order to take full advantage of the obtained nanoparticles in establishing new technologies one needs to investigate more precisely structural characteristics and morphological properties of the biogenic ferrihydrite particles. In this paper, UV-Vis spectroscopy, FT-IR spectroscopy, Raman spectroscopy and small-angle X-ray scattering analysis were used to investigate the structure of bio-mineral nanoparticles produced by bacteria *Klebsiella oxytoca*.

2. Materials and methods

2.1. Sample preparation

Aqueous samples of biogenic particles of ferrihydrite were provided by Siberian Federal University, Krasnoyarsk, Russia. Initial concentration of biomineral nanoparticles in aqueous solutions was 12.5 g/l (5g of biomineral powder dissolved in 400 ml double distilled water).

Ferrihydrite concentration in the analyzed sample was determined using a spectrophotometric method based on the interaction of 1,10-phenantroline, a heterocyclic organic compound, and metal ions. Spectrophotometric measurements revealed the concentration of ferrihydrite in the analyzed sample of 2.68 g/l.

2.2. UV-Vis spectroscopy

UV-Vis spectra of the prepared samples were registered in the wavelength range of 400—650 nm at room temperature (Figure 1).

![Figure 1](image_url) **Figure 1.** The absorption spectrum versus concentration of ferrihydrite: $26.8\times10^{-2}$g/l (1), $20.1\times10^{-2}$g/l (2), $13.4\times10^{-2}$g/l (3), $6.7\times10^{-2}$g/l (4).

The spectra are rather nondescript with gradual absorbance decline from 400—650 nm and do not allow to determine the fraction of ferrihydrite in the analyzed samples at a specific wavelength.

![Figure 2](image_url) **Figure 2.** The absorption spectrum of ferrihydrite sample with concentration $13.4\times10^{-2}$g/l: before ultrasonic treatment (1) and after ultrasonic treatment (2).
Therefore, the changes produced by ultrasonic treatment of the samples were determined by comparison of the absorption spectra of the sample before and after ultrasonic treatment (Figure 2).

The decrease of absorbance values might be related to the partial destruction of the particle clusters and by the decrease of the amount of organic material on the surface of the particles. Time stability of ultrasonic-assisted samples of different concentrations was monitored using UV-Vis spectroscopy by comparison of the spectra, which were registered at 24h, 36h, 72h and one week after the ultrasonic treatment of the samples. The obtained data shows that the samples remained stable during one week. This study was useful for optimization the samples condition for small-angle X-ray scattering analysis.

2.3. FT-IR spectroscopy

In order to elucidate the structure and to identify the organic compounds which cover the ferrihydrite nanoparticles, a Fourier transform infrared (FT-IR) spectroscopic analysis was performed.

![FT-IR spectra](image)

**Figure 3.** FT-IR analysis of biogenic ferrihydrite nanoparticles produced by *Klebsiella oxytoca* cultivated in dark growth conditions (a) one week and (b) 3 weeks, showing similar spectra. Peaks: 1 and 2 correspond to alkenes =CH bond, 3 carboxylic and/or hydroxyl C-O; 4 indicates the presence of OCH, COH, CCH groups; 5 and 6 are characteristic to amide I and II of proteins; 9 corresponds to CH vibrations of Cl; 10 is characteristic of OH stretching vibrations.

Dry samples of ferrihydrite nanoparticles produced by bacteria *Klebsiella oxytoca* were subjected to FT-IR analysis using Perkin Elmer IR spectrometer at the Institute of Chemistry of ASM, Chisinau. The infrared spectra of biogenic ferrihydrite nanoparticles obtained from bacteria *Klebsiella oxytoca*
cultivated in dark growth conditions one week and three weeks are shown in Figure 3. (a) and (b), respectively.

FT-IR spectra of the ferrihydrite bio-mineral particles show a peak at 3255.0–3216.2 cm\(^{-1}\) characteristic of OH stretching vibrations. The peak at 2929.5–2926.8 cm\(^{-1}\) corresponds to CH vibrations of Cl, 1406.2 cm\(^{-1}\) indicates the presence of OCH, COH, CCH groups. These peaks clearly indicate the presence of glucose [12]. Further, the band at 1311.1 cm\(^{-1}\) indicates the CO bond of a polysaccharide. Strains of bacteria *Klebsiella oxytoca* are known to produce exopolysaccharides [13] and among these, several polysaccharides are specifically involved in the process of iron-binding [14]. Therefore, we assume that ferrihydrite nanoparticles isolated from bacteria *Klebsiella oxytoca* are wrapped by iron-binding exopolysaccharides. Furthermore, the bands at 1636.3 cm\(^{-1}\) and 1546.6 cm\(^{-1}\) confirmed the presence of amine I and II of proteins [15]. This suggests that iron-binding exopolysaccharides found in the analyzed samples are associated with the proteins residue.

2.4. Raman spectroscopy

The Raman spectroscopic characterization of biogenic ferrihydrite nanoparticles, from practical viewpoint, is useful for studying the trace deposits on substrates and is able to provide information regarding the particle chemical composition.

The Raman spectra of water dispersed biogenic ferrihydrite nanoparticles were obtained with a Laser Confocal Scanning Microscop SOLAR TII. The samples were studied at the wavelength of 441.6 nm, with the laser power of 100 MW.

![Figure 4](image_url) **Figure 4.** Raman spectra of distilled water (■) and of the multiple scan of a water dispersed sample of biogenic ferrihydrite with the concentration of 2.68 g/l: (▲) the scan of the nanoparticles in the sample; (▼) near to the surface of nanoparticles and (●) the scan deep in the sample.

The Raman spectrum of biogenic ferrihydrite nanoparticles in a single scan with the power set to 100 MW is shown in Figure 4. The spectrum consists of a broad weak band between 3650 cm\(^{-1}\) and 3000 cm\(^{-1}\). In Raman spectroscopy this is characteristic of OH bonds stretching vibrations in the exopolysaccharides covering the ferrihydrite nanoparticles and of the water molecules. The comparison of the spectrum of ferrihydrite nanoparticles obtained near the surface of the particles and the spectrum obtained deep in the sample revealed a small difference in the absorption band of OH. The spectra obtained for measurement deep in the sample presents a relatively greater intensity of the 3650 cm\(^{-1}\) - 3000 cm\(^{-1}\) broad band. Taking into account the fact that ferrihydrite has a high-specific surface area (approximately 200-840 m\(^{2}\)/g) [16], and that because of its pores and arrangement of the
nanoparticles, it has a large and accessible inner surface, the enhanced inner absorption can be related to the augmented quantity of exopolysaccharides absorbed in the ferrihydrite pores.

2.5. Small-angle X-ray scattering

The samples were also studied by small-angle X-ray scattering (SAXS) method on a Brucker Nanostar SAXS spectrometer available at Institute of Synthetic Polymer Materials RAS, Moscow. The experimental setup covered the \( q \) range 0.007 – 0.23 Å\(^{-1}\). The experimental scattering curves were analyzed using model calculations included in ATSAS Package (PRIMUS, GNOM and GASBOR) [17,18]. 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.

The radius of gyration, \( R_G \), which characterizes the distance of the scattering object parts from its centre of gravity, was calculated in the \( q \) range of 0.024-0.034Å\(^{-1}\) using the Guinier approximation:

\[
I(q) = I(0)\exp\left(q^2 R_G^2 / 3\right). \tag{1}
\]

This gave 6.41±0.13nm as an estimate for the radius of gyration independently of the shape of the investigated particles was obtained.

![Figure 5. Function \( p(r) \) calculated from the scattering curve obtained from extrapolation to zero concentration](image1)

![Figure 6. \textit{ab initio} reconstruction of water dispersed ferrihydrite nanoparticles.](image2)

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 conformational changes within an ensemble of macromolecules. Typically, the particle distance distribution function, \( p(r) = \gamma(r)r^2 \), where \( \gamma(r) \) is the characteristic function of the particles, is calculated by an indirect Fourier transformation to avoid problems due to the discrete sampling of the \( I(q) \) curve over a finite range [19]. The indirect Fourier transform essentially constructs trial \( p(r) \) functions that are Fourier transformed and evaluated in comparison with the experimental scattering. In the GNOM program [18], a regularizing multiplier is used to balance the smoothness of the trial \( p(r) \) function with the goodness of the fit to the data.

The radius of gyration is obtained from the \( p(r) \) function using formula:

\[
R_G^2 = \int_0^{R_{\text{max}}} r^2 p(r) \, dr / \int_0^{R_{\text{max}}} p(r) \, dr. \tag{2}
\]
In Eq. (2), $D_{\text{max}}$ denotes the maximum distance inside the scattering particle. This method of determining $R_G$ takes into accounts all the collected experimental data, not only those limited to small $q$ domains, as is used in the Guinier approximation [19]. 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 0.02 – 0.26 Å$^{-1}$ is presented in Figure 5.

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 the literature [19]. The elongated tail of the $p(r)$ function within the $r$-range 120 – 200 Å indicates the presence of macromolecules in the scattering solutions. This fact confirms the earlier results that the biogenic nanoparticles removed from bacterium *Klebsiella oxytoca* are still wrapped in an organic sheath [11].

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

The overall shape of the particles was determined with the aid of the program GASBOR. This software performs an *ab initio* reconstruction of molecular structure by a chain-like ensemble of dummy residues [18]. Figure 6 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.

3. Conclusions

Dry samples of ferrihydrite nanoparticles produced by bacteria *Klebsiella oxytoca* were subjected to FT-IR analysis. The FT-IR spectra revealed typical absorption peaks of exopolysaccharides. Additionally, the Raman spectra showed the presence of exopolysaccharides on the surface and inside the pores of the ferrihydrite nanoparticles. Characterization of ultrasonic assisted biogenic ferrihydrite particles dispersed in water was performed by SAXS. Analysis of the scattering curves using ATSAS software package suggests ferrihydrite particles of an elongated shape with the radius of gyration 6.73±0.16 nm. 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 magnetogranulometry [9] methods on concentrated samples. This fact allows us to surmise that ultrasonic treatment does not produce any essential physico-chemical changes to the particles but facilitates to minimize the influence of aggregation on the scattering data. Higher $q$ values contain more details regarding the 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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