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Bio-Assisted Synthesis of Food-Grade FeOOH Nanoellipsoids as Promising Iron Supplements for Food Fortification

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Abstract

Background and Objective: Nanostructures of FeOOH are approved substitutions for iron salts in treatment of iron deficiencies. These particles can be promising additives to develop iron fortified foods. Researchers are interested to develop cost-effective techniques for the fabrication of food-grade FeOOH nanostructures. Relatively, polyethylenimine is commonly used to fabricate FeOOH nanoellipsoids. However, food industries need to develop novel protocols, which can be used in food processing. In this study, a simple economic technique was developed for the fabrication of food-grade FeOOH nanoellipsoids.

Material and Methods: Ferric chloride hexahydrate ($FeCl_3.6H_2O$) was used as iron precursor. BG-11 broth medium was used to cultivate *Chlorella vulgaris* microalgae. The *Chlorella vulgaris* culture supernatant was used for the fabrication of FeOOH nanostructures as an approved low-cost medium by the Food and Drug Administration. Nanoellipsoids of FeOOH were synthesized via hydrolysis of ferric ions in culture supernatants with no addition of other chemicals.

Results and Conclusion: Results showed that the prepared nanoellipsoids were β-FeOOH with 51.4-nm average length and 9.2-nm average width. The XRD analysis demonstrated that the secretory compound from *Chlorella vulgaris* included no negative effects on formation of FeOOH nanocrystals. The current developed technique can be introduced as a promising approach in fabrication of food-grade nanoparticles. Furthermore, the prepared structures can be used for the supplement formulation in pharmaceutical industries.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Iron deficiency anemia is one of the most common types of anemia in humans due to insufficient iron intake or disturbance in iron absorption. Recent worldwide investigations have revealed that one fourth of pregnant women suffer from iron deficiency; most of them are symptomatic [1]. Treatment of severe anemia is not easily possible with the common formulations and drugs and high doses are needed. Iron salts cannot be formulated at high doses due to their gastrointestinal side effects such as constipation, diarrhea and dark stool. These problems have promoted pharmaceutical industries to develop novel formulations with decreased or no side effects. Nanostructured iron is one of these novel formulations to provide high-dose iron supplements. Nanostructures of FeOOH are used as iron sources and are available as up to 150 mg of elemental iron per capsule. One capsule per day can provide the required iron to treat iron deficiency and iron deficiency anemia with no gastrointestinal side effects [2]. Food fortification with iron is the most effective approach against iron deficiency. This technique is used to increase iron intake in communities, especially in high-risk groups. There are evidences indicating that food fortification with iron increases hemoglobin and serum ferritin and decreases risks of anemia and iron deficiency [3].

In food products, ferrous compounds such as ferrous sulfate, ferrous lactate, ferrous succinate, ferrous gluconate and ferrous fumarate are the chemicals of choice [4]. Addition of ferrous compounds to foods can result in gastro-intestinal symptoms. Furthermore, this protocol includes drastic effects on food products and shelf-life. For example, ferrous ion enrichment of wheat flours changes their quality to unacceptable levels after four weeks of storage [5]. Another problem with added ferrous is oxidation through food processing and storage. Ferrous sources used in food industries are susceptible to oxidation and may produce brown precipitates [4]. Several studies have been carried out to develop modern technologies such as microencapsulation for the alleviation of drastic effects of ferrous in food products [6]. similar to pharmaceutical industries, food industries have also found elemental iron as a promising substitute for ferrous ion in iron-fortified foods [7]. For example, enrichment of wheat flours with elemental iron serve the product quality at acceptable levels after seven weeks of storage [5]. In recent years, investigations have been carried out to fabricate iron and other metals with high nutritional values in micro and nanostructures, which can be used in food fortification [8,9]. In these investigations, researchers have tried to eliminate chemical species from synthesis reactions and use food-grade compounds such as xanthan gum [8]. These compounds should include potentials to control synthesis reactions and fabricate structures with specific shapes or improved properties. This controlling role is regulated by controlling crystal growth patterns during the phase of nanoparticle growth.

Chlorella (*C.*) *vulgaris* is a green microalga that is accepted as GRAS (generally recognized as safe). The algal biomass is used as a valuable source for the bioactive compounds and food supplements. Nowadays, large industrial plants produce *C. vulgaris* biomasses, available as supplement tablets under various commercial brands. The culture supernatants are waste products of these plants, which are rich in carbohydrates, proteins and other secretory compounds [10]. Previous investigations have revealed that these waste products are able to properly control growth patterns of metal crystals in precipitation reactions [10,11]. As previously stated, FeOOH nanostructures are the choice for nano-iron supplement formulations. The FeOOH nanoellipsoids can be fabricated by hydrolysis of ferric ions. In absence of surfactants or controlling agents, large ellipsoid crystals of FeOOH are formed. Polyethylenimine (PEI) is the most used compound to control the hydrolysis reaction and fabrication of smaller ellipsoids. Size of the prepared ellipsoids depends on the PEI concentration. Higher concentrations of PEI result in decreases in ellipsoid sizes [12]. For the first time in this study, the *C. vulgaris* culture supernatant was introduced as a cheap food-grade controlling agent for the fabrication of FeOOH nanoellipsoids. Therefore, a simple approach was successfully developed for the production of nano-based iron supplements in food industries. The production processes can be carried out at ambient conditions with no needs to high-tech equipment. Therefore, this developed technique can be interesting and promising for the food industries in terms of iron-fortified products.

2. Materials and Methods

2.1 Materials

Ferrous chloride hexahydrate ($FeCl_{3.}$ $6H_2O$) was provided by Dr. Mojallali Chemical Complex, Iran (Catalog code: 412). The BG-11 broth medium was purchased from HiMedia, India (Catalog code, M1541). The *C. vulgaris* microalgae were previously isolated and identified in the laboratory of Prof. Ghasemi, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

2.2 Culture of Chlorella vulgaris cells

Briefly, BG-11 broth was used for the cultivation of *C. vulgaris* microalgae (10^7 cells ml^{-1}). Culture was illuminated using white fluorescent light at 27 °C. The light equipment was set to provide continuous illumination with 60 μEm^{-1} s^{-1} intensity. After 20 days, culture was centrifuged at 22132× g for 15 min and the supernatant was used for bio-assisted synthesis of nanoellipsoids [10].

2.3 Synthesis of FeOOH nanoellipsoids

Generally, FeOOH nanoellipsoids were fabricated via hydrolysis of ferric ions [13]. 5 ml of 1 M ferric chloride hexahydrate solution were mixed with 45 ml of the culture supernatant of *C. vulgaris* and heated to 80 °C for 2 h under stirring. Produced nanoellipsoids were harvested at 22132× g (Eppendorf 5910 R, Hamburg, Germany) and the precipitant was washed tree times and dried at 50 °C using oven.

2.4 Characterization of nanoellipsoids

Physicochemical properties of the prepared compound were evaluated as follows.

2.4.1 Shape and size analyses

To analyze the nanoellipsoids, field-emission scanning electron microscopy (FESEM, TESCAN MIRA3-XMU, the Czech Republic) was used with an electron energy of 20 kV at high vacuum. For sample preparation, films of the nanoellipsoids were prepared on glass slides using drop coating of the nanoellipsoid suspensions [14]. Size assessments (width and length measurements) were carried out using ImageJ Open Source Image Processing Program (http://imagej-net) developed by National Institutes of Health (NIH, USA) and results were analyzed using SPSS Software v.20 (IBM Analytics, USA).

2.4.2 X-ray diffractometry

Crystal structures of the particles were analyzed using powder X-ray diffraction (XRD) pattern and Siemens D5000 Diffractometer (Siemens, Karlsruhe, Germany) with Cu tube and Kα radiation operated at 40 keV and 40 mA.

Diffraction patterns were recorded in 2θ ranges of 10-90 degrees [15].

2.4.3 Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectroscopic analysis was carried out to assess chemical properties of nano-ellipsoids and possible biologic compounds incorporated in the particles [16]. Analysis was carried out using standard KBr pellet method and Bruker FTIR Spectrometer (Bruker Optics, Ettlingen, Germany). The IR absorption spectra were recorded at 400-4000 cm^{-1} and 0.01 cm^{-1} resolution.

3. Results and Discussion

3.1 Culture of Chlorella vulgaris cells

In the authors' previous investigations, the logarithmic phase of *C. vulgaris* growth was completed after 20 days of incubation. Therefore, this incubation time was selected for the current investigation to provide rich culture super-natants. During incubation, *C. vulgaris* cells were counted to reach a density of nearly 5×10^7 cells ml^{-1}. Previous investigations were successfully used this incubation time for bio-mediated fabrication of metal nanostructures using secretory compounds from *C. vulgaris* [10,11,17].

3.2 Synthesis of FeOOH nanoellipsoids

By hydrolysis of ferric ions, orange solution of ferric chloride converted to a brownish suspension. This change in the color and transparency could be a preliminary indication for the formation of FeOOH nanoellipsoids. Hydrolysis of ferric ions and formation of nanoellipsoids were developed as shown in Eq. 1 [18].

$$FeCl_3 + 2H_2O \rightarrow FeOOH + 3HCl \qquad \text{Eq. 1}$$

Additives used in food products should be fabricated in simple economic processes, which can be carried out in food industries with no needs to high-tech equipment. Use of organic solvents or toxic chemicals increases environmental concerns and approval difficulties for the final products. Therefore, a hydrolysis approach was chosen for the fabrication of FeOOH nanoellipsoids in this study. In this approach, hydrolysis of ferric ions was carried out in the aqueous media and ambient atmosphere with no addition of alkaline agents or toxic chemicals. The only difficulty within this reaction includes its need to a second compound for the size control of prepared nanoellipsoids [12,19]. This difficulty can be a critical point for the fabrication of food-grade nanoellipsoids. This difficulty can be addressed using secretory compounds from *C. vulgaris*. The *C. vulgaris* microalga is considered as GRAS by the Food and Drug Administration and its biomass is commercially available as food supplement. Supernatant of the *C. vulgaris* culture is a byproduct of biomass-producing industries with no extra costs and a food-grade compound for use as controlling agent in fabrication of food-grade nanomaterials [11,17]. Use of culture supernatants decreases needs to large volumes of water in the reaction. Based on Eq. 1, proton is the byproduct of FeOOH formation and thus the reaction mixture shifts to acidic pH. Acid production is the only unsolved disadvantage of the current method for the production of FeOOH nanoellipsoids in industrial settings. Acidic pH can harm metal containers and pipes. Moreover, acid-resistant stainless steels include a limited resistance to chlorine-containing media [20]. Therefore, acid resistance vessels are needed to carry out the synthesis reaction.

3.3 Characterization of nanoellipsoids

Physicochemical features of the prepared nanoellipsoids were analyzed to assess the currently developed technique for the fabrication of FeOOH nanoellipsoids.

3.3.1 Shape and size analyses

A FESEM micrograph of the prepared nanoellipsoids is shown in Fig. 1. As shown in the figure, particles are fairly uniform in size with flawless spindles. Size measurements were carried out for width and length of 100 randomly selected particles and corresponding histograms are demonstrated in Fig. 2. The widths of nanoellipsoids were measured as 4-13 nm with an average of 9.2 nm ±2.3. The lengths were 23-82 nm with an average of 51.4 nm ±13.9. The aspect ratio (the ratio of mean length to mean width) was calculated as 5.6. This aspect ratio was exactly equal to the nanoellipsoids synthesized in presence of polyethyl-eneimine (PEI) as a highly efficient and mostly used size controlling agent. Previous investigations revealed that use of PEI with various molecular weights and concentrations resulted in nanoellipsoids with length sizes ranging 25-235 nm. Resulted nanoellipsoids demonstrated an aspect ratio of 5 or 6 that is appropriate for medical uses and catalysis purposes [12]. Controls over the size of FeOOH nano-ellipsoids were also reported using various concentrations of HCl in hydrolysis reactions. Using 0.001-0.07 M HCl in reactions resulted in nearly 200-nm increases in length and 10-nm decreases in width of nanoellipsoids. These results have demonstrated that *C. vulgaris* microalgae are able to secret biologic compounds, which are promising candidates in economic fabrication of food-grade FeOOH nano-ellipsoids. Use of monosaccharides such as glucose in controlled fabrication of FeOOH nanorods was also led to similar results [19]. Previous reports have revealed shape controlling potentials of secretory carbohydrates from *C. vulgaris* in fabrication of FeOOH and other metal nano-particles [10,11,21].

3.3.2 X-ray diffractometry

Figure 3 illustrates XRD pattern of the prepared nano-ellipsoids. Recorded pattern was fit with characteristic diffraction peaks of akageneite crystals (β-FeOOH), previ-ously reported by Joint Committee on Powder Diffraction Standards (JCPDS Card No. 75-1549).

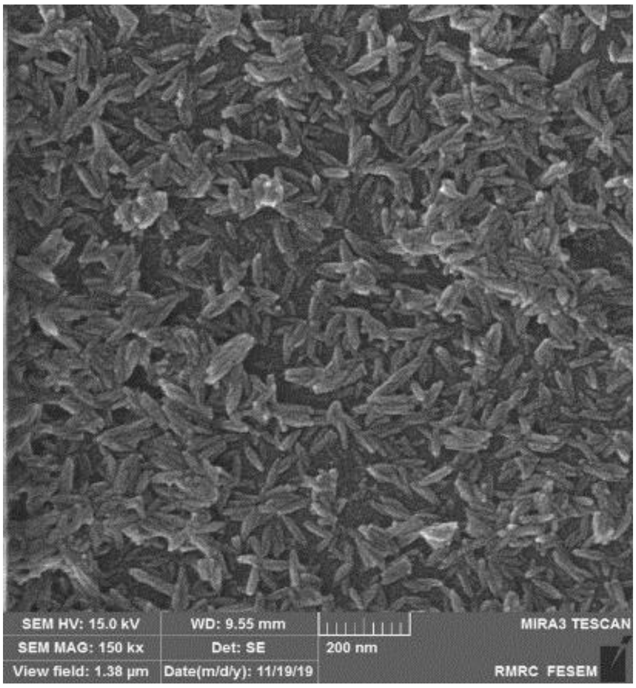

Figure 1. A field-emission scanning electron microscopy micrograph of the bio-assisted synthesized FeOOH nanoellipsoids

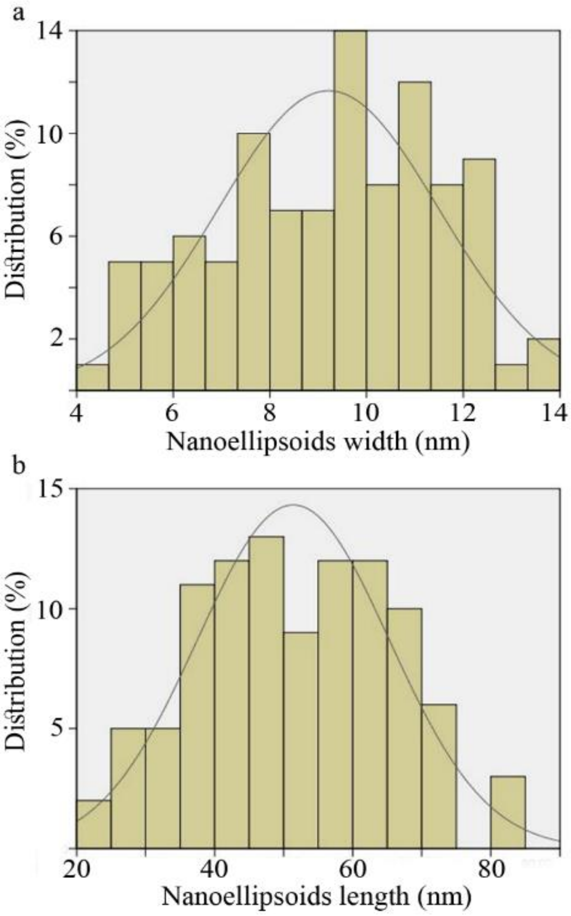

Figure 2. Nanoellipsoids width (a) and length (b) distributions. Measurements were carried out on field-emission scanning electron microscopy micrographs

Production of akageneite nanocrystals via ferric ions hydrolysis in presence of chemical and natural size controlling agents was also reported in other studies [12,19]. Compounds such as glucose can modify formation of nanocrystals to special structures [19]. Furthermore, synthesis parameters include significant effects on crystal structures and compositions of the resulting nanostructures [22]. In this study, presence of *C. vulgaris* secretory compounds included no disturbing effects on formation of β-FeOOH nanocrystals. Similar effects were reported for the controlled synthesis of spherical FeOOH nanoparticles using secretory compounds from *C. vulgaris*. The authors used *C. vulgaris* secretory compounds for the fabrication of FeOOH nanoparticles through precipitation reactions [11]. In the current study, FeOOH nanorods were synthesized via hydrolysis reactions. These results have shown that secretory compounds from *C. vulgaris* can play controlling roles in various synthesis approaches with no adverse effects on crystallinity.

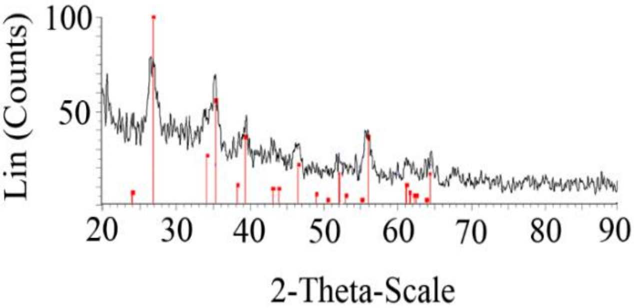

Figure 3. X-ray diffraction pattern of the prepared nanostructures, indicating characteristic peaks of akaganeite crystals. (■) β-FeOOH JCPDS Card No. 75-1549

3.3.3 Fourier transform infrared spectroscopy

The FTIR spectrum of the bio-assisted synthesized nano-ellipsoids is shown in Fig. 4. Two main peaks were recorded in this spectrum. The broad intense peak at 3446.6 cm^{-1} was due to stretching vibration of hydroxyl groups. The bending vibration of this group was identified at 1636.1 cm^{-1} [23]. Recorded spectra were indicated that no signs of biologic compounds from C. *vulgaris* was associated with the nanoellipsoids. Use of culture supernatants from *C. vulgaris* in fabrication of spherical FeOOH nanostructures resulted in formation of nanoparticles free from biological compounds [11]. A previous investigation showed that use of glucose for the fabrication of FeOOH nanorods resulted in carbon-rich nanostructures. Zhu et al. developed a glucose-assisted technique for the synthesis of FeOOH nanorods. They demonstrate that presence of glucose led to formation of carbon-rich FeOOH nanorods [19].

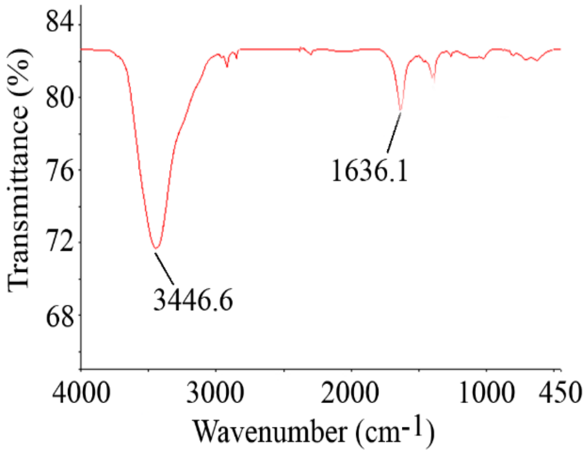

Figure 4. Fourier transform infrared spectrum of the bio-assisted synthesized FeOOH nanoellipsoids, indicating virgin nanostructures with no impurities

4. Conclusion

The *C. vulgaris* is a microalga introduced as GRAS microorganism and its biomass is commercially available as food supplement. The *C. vulgaris* supernatant is a byproduct of biomass and algal supplement producing plants. The supernatant is rich in bioactive compounds, mostly carbohydrates, which are secreted in the logarithmic phase of algal cell growth. These bioactive compounds are FDA-approved cost-effective materials that can be used as cheap sustainable fabrication materials for food-grade nanomaterials. In the current study, culture supernatants of *C. vulgaris* were successfully used as matrices for controlled hydrolysis of ferric ions to FeOOH nanoellipsoids. Hence, chemical polymers such as PEI and other chemical or natural compounds used to control growth of nanoellipsoids are necessary. Produced nanoellipsoids are food-grade and can be used for the iron enrichment of food products. This developed technique does not need high-tech equipment and can be carried out at ambient atmospheres. Purity of the produced particles is another advantage of the developed technique. In the current study, particles included pure FeOOH with no surface deposited biologic compounds or carbohydrates. In conclusion, this study has introduced a simple, ecofriendly economic approach for the fabrication of food-grade iron nano-supplements that can be developed in most food industries.

5. Acknowledgements

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6. Conflict of Interest

The authors declare no conflict of interest.

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ساخت کمک‌زیستی نانوذرات بیضوی شکل اکسی‌هیدروکسید آهن با درجه غذایی به‌عنوان مکمل آهن برای غنی‌سازی مواد غذایی

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چکیده

سابقه و هدف: ثابت شده است که نانوساختارهای اکسی‌هیدروکسید آهن جایگزین مناسبی در درمان کمبودهای آهن می‌باشند. این ذرات می‌توانند افزودنی‌های امیدبخشی برای توسعه مواد غذایی غنی شده با آهن باشند. محققان علاقه‌مند به توسعه فناوری‌های مقرون‌به‌صرفه برای ساخت نانوساختارهای اکسی‌هیدروکسید آهن با درجه غذایی می‌باشند. پلی‌اتیلن‌ایمین معمولا برای ساخت نانوذرات بیضوی شکل اکسی‌هیدروکسید آهن مورد استفاده قرار می-گیرند. اگرچه نیاز است صنایع غذایی به توسعه روش‌های نوینی بپردازند که بتوانند در فرایند مواد غذایی مورد استفاده قرار گیرند. در این مطالعه، روشی اقتصادی و ساده برای ساخت نانوذرات بیضوی شکل اکسی‌هیدروکسید آهن با درجه غذایی ابداع شد.

مواد و روش ها: فریک کلرید هگزاهیدرات ($FeCl_3.6H_2O$) به‌عنوان پیش‌ساز آهن مورد استفاده قرار گرفت. از محیط کشت آبگوشت BG-11 برای کشت ریزجلبک *کلرلا وولگاریس* استفاده شد. روماند[۱] کشت *کلرلا وولگاریس* برای ساخت نانوساختارهای اکسی‌هیدروکسید آهن به‌عنوان محیط کشتی ارزان و مورد تایید سازمان غذا و دارو مورد استفاده قرار گرفت. نانوذرات بیضوی شکل اکسی‌هیدروکسید آهن با آبکافت یون‌های فریک موجود در روماند کشت بدون افزودن ماده شیمیایی تولید شدند.

یافته‌ها و نتیجه‌گیری: نتایج نشان داد نانوذرات بیضوی شکل تهیه شده، بتا- اکسی‌هیدروکسید آهن با متوسط طول ۵۱/۴ نانومتر و متوسط عرض ۹/۲ نانومتر می‌باشند. آنالیز پراکنش پرتو ایکس[۲] نشان داد ترکیبات ترشح شده از *کلرلا وولگاریس* بر ساخت نانوبلورهای[۳] اکسی‌هیدروکسید آهن اثرات منفی ندارند. روش توسعه یافته حاضرمی‌تواند به‌عنوان روشی امیدبخش در تولید نانوذرات با درجه غذایی معرفی شود. علاوه براین، ساختارهای تهیه شده می‌توانند برای فرموله کردن مکمل ها در صنایع دارویی مورد استفاده قرارگیرند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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واژگان کلیدی

- نانوذرات بیضوی شکل آکاگانیت
- نانوذرارت با درجه غذایی
- غنی‌سازی با آهن
- نانوذرات آهن
- ترکیبات میکروبی

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[۱] Supernatant
[۲] X-ray diffractometry or XRD
[۳] Nanocrystal