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Modified Method of ZnO Nanoparticles Preparation by Extracted Pyocyanin

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طريقة محورة لتحضير دقائق اكسيد الزنك النانوية بواسطة مستخلص البايوسيانين

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ABSTRACT

Background

Biosynthesis of nanoparticle mediated pigments has been widely used as an antimicrobial agent against microorganisms. ZnO nanoparticles were synthesized by using pyocyanin from locally isolated *Pseudomonas aeruginosa*, then characterized and utilized as antimicrobial activity against microorganisms.The goal of this study was to introduce a modified ZnO nanoparticles biosynthesized technique by pyocyanin pigment, which is safer, more efficient, and less expensive.

Materials and Methods

Luria Bertani broth was inoculated with the organism and kept at room temperature for 120 hours to get the highest production of pigment, chloroform was added to the culture broth at a 2:1 ratio, and a blue layer formed. The blue layer was collected in a dark glass bottle to prevent oxidation and directly used instead of drying the extract.

Results

The suggested technique is simple to use, requires no special laboratory equipment, and yields a good amount of pure pigment with a maximum spectral peak of 320 nm. The zinc oxid nanoparticles (ZnONPs) were characterized with Uv-VIS revealing a peak with maximum absorbance at 360 nm and Atomic Force Microscopy that indicated the average size 42.74 nm, as well as using FE-SEM and FTIR.

Conclusion

This study demonstrated a straight forward but efficient approach for biosynthesizing nanopatricles. By employing chloroform containing pyocyanin and simple processes based on biological methods, in which pyocyanin act as reducing agent and stabilizing mediator in the formation of nanostructured, so the nanoparticles were created in their purest form.

Key words: Zinc Oxide, Nanoparticles, Pyocyanin, Chloroform.

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INTRODUCTION

Nanoscience is one of the quickest developing study fields globally, enabling the manipulation of matter on a nanoscale. Engineered nanoparticles can be used in many practical human applications, including medical imaging, drug delivery systems, and cosmetics [1]. Metal-based nanoparticles are an effective antibacterial agent against a wide variety of common pathogenic pathogens. As a result, some nanoparticles, including silver, nickel oxide, zinc oxide and copper oxide are gaining significant interest as antimicrobials in consumer, health, and industrial products. most research have focused on zinc oxide NPs, because it has optical, magnetical, chemical, and mechanical properties that are clearly of corresponding bulk materials, distinctive chemical and physical features of ZnO for instance are higher, chemical stability higher, naturality and paramagnetism [2]. The production of nanoparticles using biological processes is relatively dependable and a viable alternative to more involved chemical synthetic processes [3]. The physical and chemical ways of synthesis are more expensive and less environmentally friendly than the biosynthetic process, which is safer, more biocompatible, and less destructive [4]. In addition, the biosynthetic route (which utilizes fungi, algae, bacteria, plants, and other organisms as precursors) is gaining traction due to their capacity to overcome toxicity [5]. Many naturally occurring compounds that bacteria produce have characteristics similar to those manufactured molecules. Different *Pseudomonas* strains produces a range of extra-cellular pigments, among them phenazines are the most important one. Production of the soluble pyocyanin pigment is *Pseudomonas aeruginosa's* most distinguishing property; a water soluble blue green phenazine compound[6]. When the nanoparticles are formed, they prefer to be stabilized before they can be used. Several reagents have been reported to act as stabilizing agents [7]. Pyocyanin act as reducing and stabilizing mediator in the formation of nanoparticles. When zinc oxide nanostructures are formed, pyocyanin function as a mediator that reduces and stabilizes the process [8].

MATERIALS AND METHODS

Isolation and Identification of Pyocyanin producing *P.aeruginosa*

Patients with wounds, burns, and urinary tract infections were obtained from private labs and Al-Kindy hospital to provide clinical samples, then were cultured on cetrimide agar for detection and identification of *P.aeruginosa*. The duration of the trial was from November 2021 and March 2022. The isolates were identified depending on the morphological features of culture media [9]. Moreover, the VITEK2 System was used to confirm the identification.

Detection of Pyocyanin Producing *P. aeruginosa*

Cetrimide agar was produced by the manufacturer's instructions. After 0.1 ml of active bacterial suspension was added to the medium, the medium was incubated for 5 days at 37 ºC.

Production and Extraction of Pyocyanin

With a few modifications, Elbargisy's method was used [10]. Nutrient agar samples were inoculated on Luria broth, and the cultures were allowed to develop for 120 hours at room

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temperature. After shaking the flask, the medium's surface developed a blue-green shading that later spread throughout the entire broth. According to [11]. 250ml of *Pseudomonas aeruginosa* broth culture was taken after five days of incubation. The media was then centrifuged at 5000 rpm for 15 minutes to extract the pyocyanin. Take the supernatant and filter it through a 0.2 µm filter before extracting it with chloroform. When chloroform was added to the culture broth at a 2:1 ratio, a blue layer formed. The blue layer was collected in a dark glass bottle to prevent oxidation and directly used instead of drying the extract.

Synthesis of Zinc Oxide Nanoparticles

Zinc acetate was used to prepare the zinc oxide nanoparticles. Zinc nanoparticles were synthesized according to a method described by [12] with some modifications. The method of synthesis involves mixing 150 ml of pyocyanin-containing chloroform with 15 g of zinc acetate and then sonicating the mixture for 10 minutes. The mixture is then shaken in a dark room for 24 hours, centrifuged for 15 minutes at 5000 rpm, and then thoroughly cleaned with deionized distilled water (DDW). Additionally, the solution was centrifuged at 5000 rpm, added DDW to the precipitate, and then kept in an incubator at 40˚C until it dried [13]. Then ZnO is stored in a dark container for further characterization and use[14].

Characterization techniques

1.**UV–VIS spectral analysis**

The ZnONPs were confirmed by measuring the wavelength of the reaction mixture in the UV-VIS spectrum of the spectrophotometer at a resolution of 1 nm in 2 ml quartz cuvette with 1 cm path length. The scanning range for the samples was 300-900 nm at a scan speed 500nm/min, by using a blank reference for correction of the spectrophotometer.

2.Atomic force microscopy (AFM) analysis

Atomic Force Microscopy was used to analyze the average diameter of ZnONPs. A thin film of the prepared sample was deposited on a silica glass plate by dropping a few drops of the sample on the plate and allows them to dry at room temperature in the dark. The deposited film glass plate was then scanned with the AFM.

3.Field Emission Scanning Electron Microscope (FESEM)

The scanning electron microscope technique was used to characterize the mean particle morphology and diameter of nanoparticles. The dried sample was sonicated with distilled water; small drop of this sample was placed on glass slide and allowed to dry. After that, a thin layer of platinum was coated to make the samples conductive.

4.Fourier Transform Infrared Spectroscopy (FTIR)

The dried biosynthesized sample was sonicated with deionized distilled water; small drop of this sample was placed on glass slide and allowed to dry. A wave number range of 4000 to 400 cm^{-1} was used to measure the spectral range of zinc oxide nanoparticles.

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RESULTS AND DISCUSSION

Identification of pyocyanin producing *P.aeruginosa*

Cetrimide agar is a selective/differential medium because it contains 0.03% cetrimide that acts as a quaternary ammonium cationic detergent (acetyl trimethyl ammonium bromide) and inhibits the growth of microorganisms (other than *P. aeruginosa*) by releasing phosphor and nitrogen from microorganisms [15]. This media that is utilized for *P. aeruginos* identification also increases the production of *Pseudomonas* pigments like pyocyanin and pyoverdine, which have characteristic blue-green and yellow-green colours, respectively [16].

Extraction of Pyocyanin pigment

Pseudomonas aeruginosa was grown at 37°C in the LB broth in the 120 hours rotary shaker incubator. *P. aeruginosa* developed pyocyanin after incubating it for five days, and it showed up on the broth's surface. The green pigment was dispersed in a broth medium after shaking the flask [10] **Figure 1**. The identical results reveal that within five days of the experiment, Pyocyanin was spontaneously produced from the *P. aeruginosa* strain [17]. Chloroform is used in the extraction of pyocyanin because it is a non-polar solvent that can dissolve the pigment and produce blue color [18]. This allows for the separation of pyocyanin from other compounds in the sample [19] **Figure 2**. The chloroform-containing pyocyanin solution was utilized without drying to minimize the cost of pyocyanin synthesis; this improved approach was quicker, simpler, and less expensive.

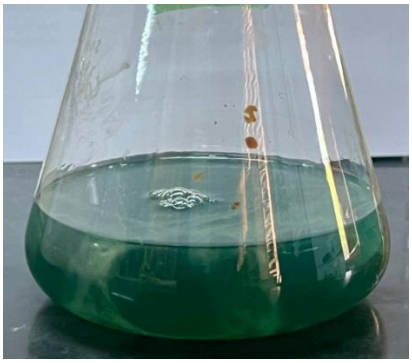

Figure 1. Blue-green pyocyanin produced *from Pseudomonas aeruginosa* on Luria Bertani broth.

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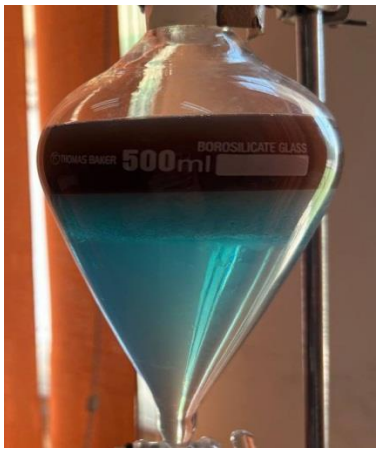

Figure 2. The Extracted pyocyanin.

Characterization of Pyocyanin by Ultraviolet Spectra

Scanning a UV-visible spectrophotometer (Shimadzu, Japan) is used to characterize the pyocyanin isolated from *Pseudomonas aeruginosa* **Figure 3.** to detect the maximum absorption. Absorbance is measured at 320 nm, similar result that indicate the UV-visible of pyocyanin are 316 nm [20].

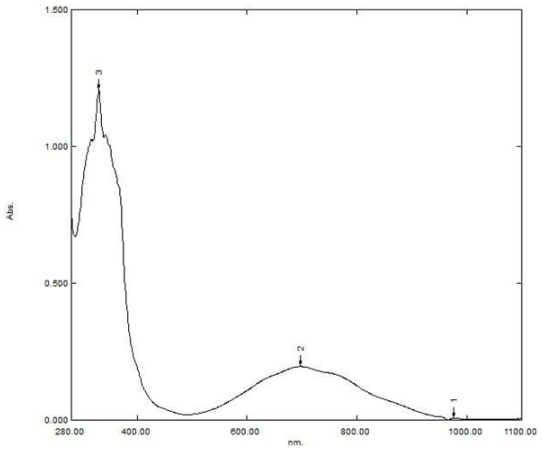

Figure 3. The UV-VIS of pyocyanin.

Zinc Oxide Nanoparticles Characterization

UV–VIS Spectral Analysis ZnO

By scanning with a UV-visible spectrophotometer to get the greatest absorbance, ZnO biosynthesis is identified. The outcome demonstrated that the greatest absorption peak of the biosynthesized ZnONPs was at 360 nm as shown in **Figure 4**. The absorption spectra at 360 nm show that nanoparticles of zinc oxide have formed [21]. The outcome was similar to a study [22], which demonstrated that the zinc nanoparticles absorbance peak was in the range of 350-385 nm.

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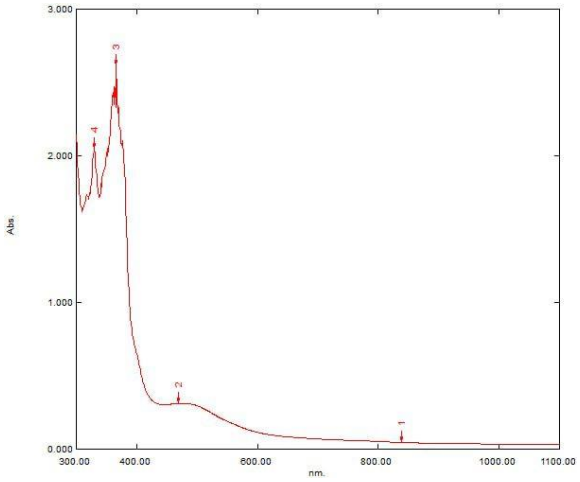


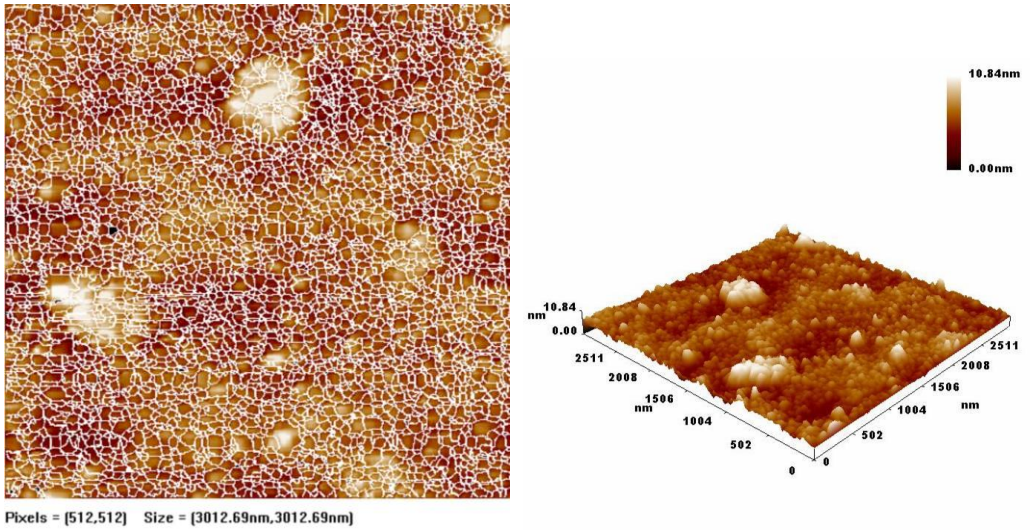
Figure 4. UV–Vis spectrophotometry of ZnONPs.

Atomic Force Microscopy (AFM) Analysis

Atomic force microscopy (AFM) was used to determine the size and surface morphology of ZnONPs nanoparticles, testing the contact forces between the tip and surface [23] **Figure 5** illustrates two and three dimensional AFM of ZnONPs which were all the same shape and size. Also, the average size of biosynthesized ZnONPS by pyocyanin according to **Table 1** was 39.26 nm.

Table 1. The Cumulation Size of Zinc Oxide Nanoparticles Biosynthesized by Pyocyanin by AFM technique.

Avg. Diameter:42.74 nm	**<=10% Diameter:18.00 nm**
<=50% Diameter:38.00 nm	**<=90% Diameter:68.00 nm**



(A)

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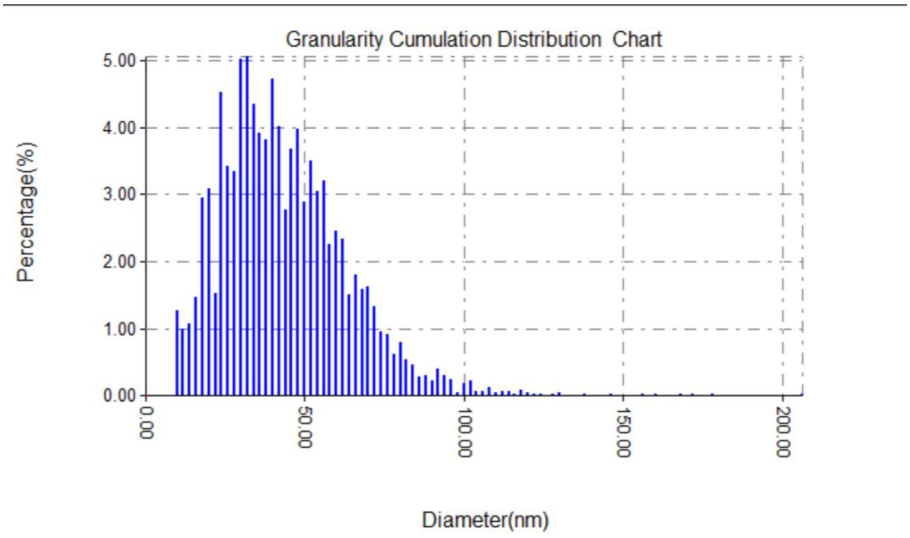

(B)

Figure 5. The biosynthesized ZnO NPs (A) 2D and 3D AFM of ZnO NPs (B)Chart Granularity Distribution of ZnONPs.

The result is in correlation with [24] AFM result which showed that the average Zinc oxide nanoparticles size was in a range from 31.63 to 84.80 nm.

Field Emission Scanning Electron Microscope (FESEM)

The FE-SEM image shows the homogeneous distribution and nearly spherically shaped ZnO NPs **Figure 6**. Which concurred with the AFM results. The average particle size of the NPs is about 41.28 nm, and some of them have spherical shapes.

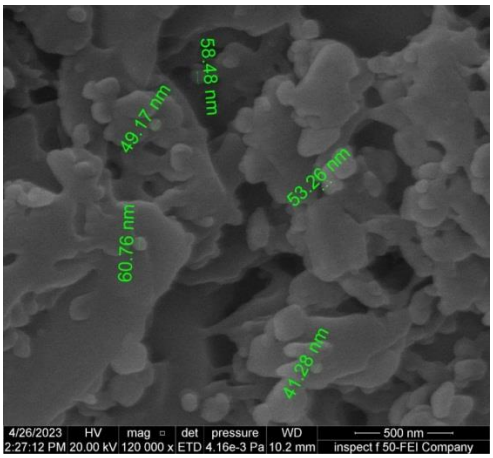

Figure 6. Field emission scanning electron microscopy images of ZnONPs**.**

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The result is in line with [25] which revealed that the biosynthesized ZnONPs had a spherical shape and showed less aggregation of particles with diameters of 50 nm.

Fourier Transform Infrared Analysis (FTIR)

FTIR spectrographic analysis is used to determine the vibration frequencies of the bonds in the molecule. The chemical functional group of ZnO nanoparticles within the synthesized ZnO nanoparticles supported the band value in the infrared radiation region. FTIR analysis confirmed the presence of ZnO bonding **Figure 7.** The result show a broadband around 3359.77-3103.25cm^{-1}is attributed to the stretching mode of the N-H Amine salt. In the meanwhile, other peaks located within the 1558.38 cm^{-1}are mainly caused by the stretching vibration of N-O (Nitrocompounds). Furthermore, peak perceived at 1446.51 cm^{-1} is attributed to the stretching mode of C-C(inring) aromatics, and 694.33 cm^{-1}are metal oxygen attributed to symmetrical as well as asymmetrical zinc carboxylate stretching[26].

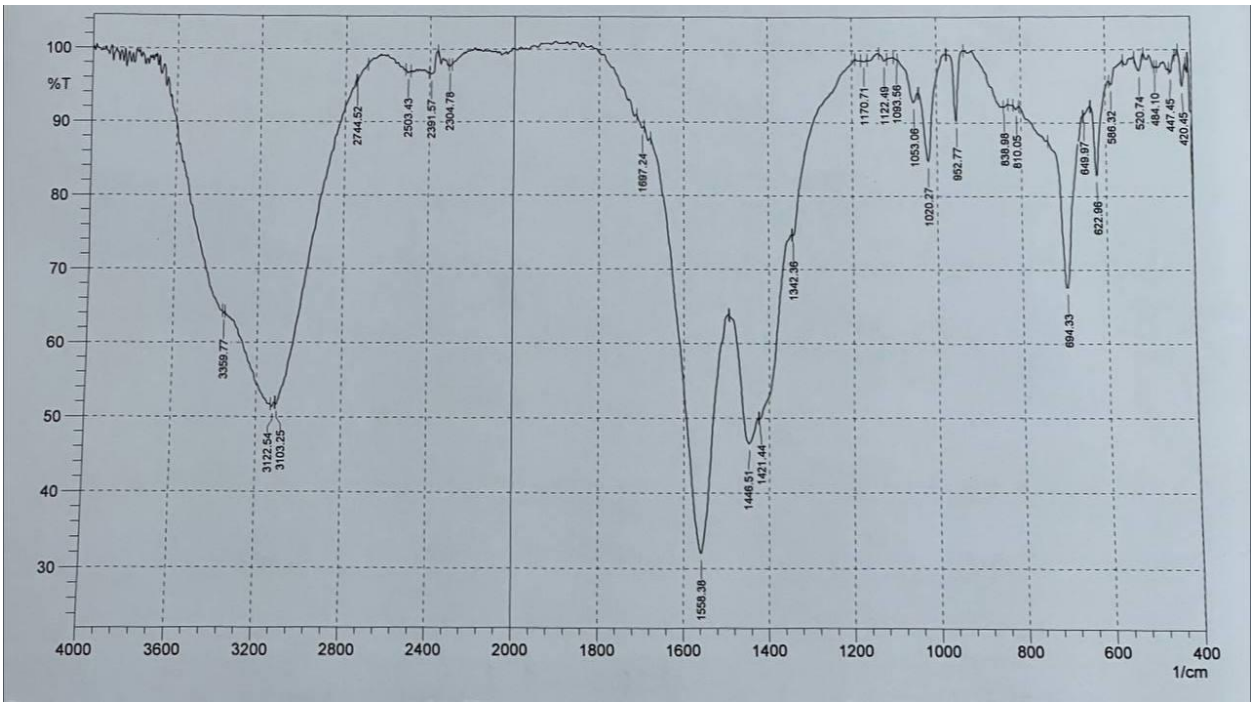

Figure 7. FTIR spectrum of the biosynthesized ZnO

Acknowledgments:
None

Conflict of interests.

The authors declare that they have no conflicts of interest

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الخلاصة

المقدمة

تم استخدام التخليق الحيوي للدقائق النانوية بواسطة الصبغات على نطاق واسع كعامل مضاد للميكروبات ضد الكائنات الحية الدقيقة.قد تم تصنيع جزيئات اكسيد الزنك النانوية باستخدام صبغة البايوسيانين المنتجة من بكتريا الزائفة الزنجارية المعزولة محليا,ثم تم توصيفها واستخدامها كعامل مضاد للميكروبات ضد الكائنات الحية الدقيقة.تم استخلاص البايوسيانين باستخدام مجموعة متنوعة من التقنيات, وبعضها يتطلب معدات متطورة.كان الهدف من هذه الدراسة هو تقديم تقنية محورة لتخليق دقائق الزنك النانوية بواسطة صبغة البايوسيانين والتي تكون اكثر امانا وكفاءة واقل كلفة.

طرق العمل:

تم تلقيح Luria bertani broth مع الكائن الحي وحفظه في درجة حرارة الغرفة لمدة 120 ساعة للحصول على أعلى إنتاج للصبغة، تمت إضافة الكلوروفورم بنسبة 2:1، وتشكلت طبقة زرقاء. جمعت الطبقة الزرقاء في قنينة زجاجية داكنة لمنع الأكسدة واستخدمت مباشرة بدلاً من تجفيف المستخلص.

النتائج:

التقنية المقترحة سهلة الاستخدام، ولا تتطلب أي معدات مختبرية خاصة، وتنتج كمية جيدة من الصبغة النقية مع ذروة طيفية قصوى تبلغ 320 نانومتر. تميزت جسيمات أكسيد الزنك النانوية (ZnONPs) بكشف الأشعة فوق البنفسجية (Uv–VIS) عن ذروة ذات امتصاص أقصى عند 360 نانومتر، ومجهر القوة الذرية الذي أشار إلى متوسط الحجم 42.74 نانومتر، بالإضافة إلى استخدام FE–SEM و FTIR.

الاستنتاجات:

اعطت هذه الدراسة نهجا مباشرا ولكن فعالا للتخليق الحيوي للدقائق النانوية. ومن خلال استخدام الكلوروفورم المحتوي على البيوسيانين وعمليات بسيطة تعتمد على الأساليب البيولوجية، حيث يعمل البايوسيانين كوسيط مختزل ومثبت في تكوين الدقائق النانوية. تم إنشاء الجسيمات النانوية في أنقى صورها. في هذا الإجراء المقترح، يتم إنشاء الجسيمات النانوية دون استخدام معدات كبيرة، بحيث يمكن للمختبرات من جميع الأحجام إعدادها بتكلفة منخفضة.

الكلمات المفتاحية: اوكسيد الزنك, الدقائق النانوية, البايوسيانين, الكلوروفورم