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Biogenic Zinc Oxide Nano-structures Differentiation under Musical Sounds

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Abstract

A new emerging and green approach was used to synthesis nanomaterials. In the present study, we report the use of *Yarrowia lipolytica* supernatant in the synthesis of zinc oxide nanoparticles (ZnO NPs). During the synthesis, the reactional medium was subjected to different musical sounds (MSs) such as Quran sound, Tunisian music, and Flamingo. The obtained nanoparticles were analysed using UV–vis spectroscopy, which showed a peak near to 258 nm characteristic of ZnO NPs. The Fourier transform infrared spectrum was established to identify the functional groups that recorded absorption bands at 564 and 574 cm^{-1} confirming the presence of ZnO NPs. While, X-ray diffraction revealed the high purity crystalline ZnO NPs and indicated the effectiveness of MSs on the crystal size by having the smallest size on Quran sound (20.9 nm). Moreover, ZnO NPs morphology was affected by the MSs that varied from spherical agglomerated to nanotubular shapes. These results reveal the successful use of MSs in the synthesis of smaller and controlled nanoparticles.

Introduction

In line with rapid growth in the field of nanotechnology, the use of biological systems in nanotechnology gained greater interest due to their ecofriendly, convenient, low cost, non-toxic, and time saving [1]. Lately, plant-mediated synthetic methods have been demonstrated as potential and powerful alternative agents with capping, reducing, chelating, and stabilizing properties, which reduce metal ions [2]. It is noteworthy that natural biological systems like fruit extract [3], seed extract [4], root extract [5], olive mill wastewater extract [6], and leaves extract [7] have been employed for the synthesis of various nanoparticles due to their associated phytochemicals like phenols, terpenoids, ketones, aldehydes, and amides. Moreover, microorganisms-mediated synthesis like algae, cyanobacteria, fungi, and bacteria have been used for the biosynthesis because of their high production rate in a short time, ease cell growth manipulation, and high chelation features by extra/intracellular metabolites [8].

In the recent years, metal oxide nanoparticles have received significant interest because of their high surface area to volume ratio, variable size, shape, and high reactivity [9]. Zinc oxide nanoparticles (ZnO NPs) are one of the most widely used nanoparticles knowing to be non-toxic, biocompatible and owing unique chemical, physical, optical, electrical, and mechanical features [10]. ZnO NPs have been applied in many industries like biomedical as drug carriers [11], anticancer and antimicrobial agents [12], biosensing and bio-imaging [13]. As well as, these nanoparticles are mainly used in environmental, UV absorption, photocatalysis, agriculture, cosmetic, and food packaging [14–17]. Several methods have been developed for the synthesis of ZnO NPs, such as hydrothermal, laser ablation, combustion, electrochemical depositions, sol-gel, spray pyrolysis, chemical vapor deposition, thermal decomposition, co-precipitation, microwave-assisted, and mechanical milling [18]. However, these approaches suffer from drawbacks such as high-energy demand and the use of toxic and hazardous chemicals [19]. Taking inspiration from natural biological systems, researchers were able to adapt them for the synthesis of ZnO NPs. The green synthesis has been carried out by different biomaterials such as leaf extract of *Abutilon*

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indicum [20], fungi (*Aspergillus niger*) [21], bacteria (*Lactobacillus plantarum*) [22], and macro-Algae (*Gracilaria edulis*) [23].

Besides, the effect of ultrasound waves on the ZnO NPs synthesis is giving a new trend in green process. Bhatte et al. [24] reported the successful synthesis of ZnO NPs using ultrasound waves. Similarly, Balram et al. [25] synthesized ZnO NPs by sonochemical approach and functionalized multi-walled carbon nanotubes for the detection of toxic environmental pollutant 4-nitrophenol.

Therefore, the musical sounds (MSs) is knowing as audible sequence of sounds that are captured by auditory cells then transformed into electrical signal and interpreted to particular acoustic sound [26]. In the last years, several studies have carried out the effect of MSs on the cells. Mishra et al. [27] reported the positive effect of sound vibrations on plants by increasing the growth, the level of proteins, sugars, and regulating the transcription of certain genes. Moreover, Shaobin et al. [28] and Sarvaiya and Kothari [29] showed that exposing microorganisms to MSs promote the microbial growth, production of metabolites (pigments), and increase the antibiotic susceptibility. As well as, studies found that the MSs are be able to treat water and wastewater by removing chemical oxygen demand (COD), biological oxygen demand (BOD), phosphor, nitrate, and increasing the production of biogas [30, 31].

Here, the MSs were used for the synthesis of ZnO NPs. *Yarrowia lipolytica* supernatant was used as the biological agents owing reducing, stabilizing, and binding features for zinc ions. UV–Vis spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FTIR) were used to demonstrate and compare the characteristics of the obtained ZnO NPs.

Material And Methods

Microorganism and cultivation conditions

Yarrowia lipolytica strain (CLIB 40) was obtained from CIRM-levures, France. During this study, *Y. lipolytica* was routinely grown on Yeast extract-Peptone-Dextrose (YPD) broth [32]. The supernatant used for the synthesis of nanoparticles was obtained after centrifugation of the suspension at 8,000 rpm for 10 minutes.

Biosynthesis of zinc oxide nanoparticles

The supernatant obtained was added drop by drop into the reaction flask containing zinc nitrate ($ZnNO_3$ $6H_2O$) at different concentrations (0.001, 0.01 and 0.1 M). The reaction between the supernatant and Zn^{2+} ions was carried out in different optimized conditions for 48 h. After this time, the colloidal solution was centrifuged at 10,000 rpm for 10 min and the obtained pellets were washed with distilled water, and then dried in oven at at 100°C. The collected nanoparticles powder was grinded and stored at 4°C.

Optimization of parameters for the synthesis of zinc oxide nanoparticles

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The influence of different parameters on the synthesis of zinc oxide nanoparticles by using supernatant of *Yarrowia lipolytica* was demonstrated as follow: (1) the effect of pH was studied by using three different pH (4, 7, and 9), which was adjusted using HCl (0.1 M) and NaOH (0.1) M; (2) two differents temperatures were selected (29°C and 80°C); (3) three different concentrations were used (0.001, 0.01 and 0.1 M); and (4) the influence of static and agitated conditions was carried out.

Evaluation of the use of musical soundson the synthesis of zinc oxide nanoparticles

Combinatorial synthesis of zinc nanoparticles using *Yarrowia lipolytica* supernatant, zinc nitrate, and MSs was assessed. The reactional mixture was exposed to three different musical soundsfor 48 h at constant frequency of 44.1 kHz, which was analysed by NCH WavePad Sound Editor Masters Edition v. 5.5. For each selected sound: Quran sound, Tunisian music, and Flamingo, different decibels (dB) were noted – 4 dB, -1 dB, and 0 dB, respectively. Figure 1 shows the decibel distribution of the used sound waves.

Characterization of ZnO NPs

The biosynthesis of ZnO NPs was monitored by measuring the absorbance at a wavelength ranging from 200 to 700 nm using UV–vis spectrophotometer (Cary-Varian).

The spectra properties of the dried powder of the synthesized ZnO NPs were observed by Fourier transform infrared spectroscopy (FTIR) and were recorded on Nicolet IR200 FT-IR spectrometer in the spectral range from 400 to 4000 cm^{-1} at room temperature.

An X-ray diffraction (XRD) analysis of dry nanoparticles powder was carried out by X’Pert Pro MPD PANalytical device with a detector voltage of 40 kV and a current of 30 mA using CuKo radiation. Data were recorded from 10° to 70° (2θ) and the estimation of the sizes of particles was performed by Debye–Scherrer's formula.

The shape, size and elemental composition of ZnO NPs were characterized by using FEI Tecnai instrument G20 transmission electron microscopy (TEM) operating at 200 Kv (Lab_6) equipped with energy dispersive X-ray spectroscopy (EDX). Samples were prepared from white powder of ZnO NPs, which is dispersed in ethanol and sonicated for 10 min, then placed in formvar-coated TEM grid.

Results And Discussion

Optimization for the biosynthesis of ZnO NPs

In recent years, the biosynthesis of ZnO NPs using microorganisms has gained considerable attention due to the greener and lower cost approach. The supernatant containing bioactive compounds leads to the production of nanoparticles [33]. In this study, the supernatant of *Y.lipolytica* was used to reduce zinc

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ions into ZnO NPs, which is confirmed by color change of the reaction mixture from yellow to white (Fig. 2). Meanwhile, different parameters were investigated such as zinc nitrate concentration, temperature, agitation, and pH for the biosynthesis of ZnO NPs. As shown in Fig. 3, a clear surface plasmon resonance band at 258 nm was obtained for all spectrums that is typical characteristic of ZnO NPs and in agreement with the results reported by Talam et al. [34]. The spectrums of zinc nitrate concentrations (0.1, 0.01, and 0.001 M) showed a significant effect on the absorption peak with high intensity of the synthesized nanoparticles at 0.01 M (Fig. 3a). Jamdagni et al. [35] revealed that a concentration of 0.01 M was suitable for the synthesis ZnO NPs using *Nyctanthes arbor-tristis* extract. A slight shift of the absorption intensity at 0.1 M, which may explain by changing in the size and shape of the synthesized nanoparticles [36]. Additionally, three different pH were selected (3, 7, and 9) and revealed a remarkable absorption intensity of ZnO NPs while the highest peak was observed at pH 9 (Fig. 3b). Accordingly, many studies have reported the production of ZnO NPs at both lower and higher pH with more stability at basic pH [37, 38].

Further, at temperature of 29°C and 80°C, the ZnO NPs were synthesized and the UV spectrums showed two characteristics peaks at 258 nm and 381 nm (Fig. 3c). Increasing the temperature reveals a significant effect on the absorption peak causing high consume of zinc ions for the formation of zinc oxide nanoparticles [39]. Moreover, the variation from static to agitated conditions demonstrated a significant difference in UV spectrums (Fig. 3d). At static condition, only one highest peak at 258 nm was obtained corresponding to the synthesis of ZnO NPs. While at agitated conditions, a lower absorbance with a shift to 382 nm was indicated and explained by an agglomeration of the synthesized nanoparticles [40].

The presence of absorption peaks in all UV spectrums could be the result of high functional groups present on the *Y.lipolytica* supernatant. Mahdi et al. [8] demonstrated the involvement of proteins and functional groups (hydroxyl) of bacteria for the reduction of zinc ions. In addition, Pomastowski et al. [41] reported the presence of carboxyl groups in the supernatant of *Lactobacillus paracasei* as the reducing agent for zinc ions. While, previous study revealed the presence of alkaline proteins in the supernatant of *Y.lipolytica* [42]. Thus, it may be the reason for higher absorbance in alkaline conditions [43].

After determining the optimal conditions of each parameter (pH: 9, T: 80˚C, Zinc nitrate concentration: 0.01M, and in static condition), ZnO nanoparticles were synthesized and analyzed. At different time intervals, the synthesis of ZnO NPs showed a start of an intense peak after 24 hours at 258 nm (Fig. 4). Moreover, the absorbance is getting more intense after 48 hours, which indicated the stability and the potential bandgap adsorption of the synthesized ZnO NPs [36]. Similarly, previous studies reported the same range of the absorption peak of ZnO NPs [44, 45].

Musical sounds effects on the biosynthesized ZnO NPs

Different MSs were used for the synthesis of ZnO NPs mediated by supernatant of *Yarrowia lipolytica* and the features of the obtained nanoparticles are presented as follow:

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UV/Vis Spectroscopy

Figure 5 shows the absorption spectra of the synthesized ZnO NPs under various MSs. As it can be seen from Fig. 5a, an appearance of sharp absorption band around 258 nm after 5 min, which can be attributed to the formation of ZnO NPs. While, the other MSs did not present a clear absorption until 24 and 48 hours for Flamingo and Tunisian Ziara, respectively (Fig. 5b, c). As it is indicated above, the synthesis of ZnO NPs without MSs assisting gives an absorption peack after 24 hours. Importantly, Wani et al. [46] and Bhuyan et al. [47] found that the ultrasound waves serve as a viable alternative for the green synthesis of oxide nanoparticles. As well as, Bayrami et al. [48] showed the efficiency of ultrasound for the biosynthesis of ZnO NPs using Nasturtium officinale leaf extract. The sound wave in a liquid causes the formation and the growth of bubble via cavitation, which enhances biological processes and generates decomposition of molecules with formation of free effective radicals for the synthesis of nanoparticles [49]. Besides, the sensitive response of the nanoparticles to sound waves can be attributed to the level of decibel that stimulate the synthesis [50].

FT-IR spectrum analysis

The obtained ZnO NPs were subjected to FT-IR analysis to detect functional groups and the type of the existing bonds. Figure 6 shows the peaks characteristics of the synthesized ZnO NPs not assisted to MSs (Quran sound, Tunisian music, and Flamingo). The assignments of the observed bands are comparative to the data reported for nanoparticles [51].

The peaks at 3446 cm^{-1} is denoted to the stretching vibration of the hydroxyl groups (OH) present in the surface of the nanoparticles. This OH group originated from proteins and carbohydrates generate as metabolite from *Y.lipolytica*, which is therefore responsible for the reduction of Zn^{2+} ions and ZnO NPs synthesis [6]. The strong peaks from 1589 to 1619 cm^{-1} can be referred to stretching vibrations of C = C bonds of aromatic compounds. The bands at wavelength 1380 cm^{-1} have been reported to occur due to C – O stretching vibration. The absorption peaks observed from 1046 to 1061 cm^{-1} can be attributed to primary saturated alcohol (OH) in-plane-w vibration. The bands observed from 564 to 574 cm^{-1} correspond to Zn–O stretching bond, which confirms the synthesis of ZnO NPs [8, 52].

X-ray diffraction

In order to confirm the forme, the structure and the size of ZnO NPs, the obtained white powders were analyzed by X-ray diffraction and results are presented in Fig. 7. Analysis of XRD spectra showed well-defined peaks at 2θ values of 31.89°, 34.63°, 36.38°, 47.75°, 56.74°, 63.03°, 68.04° associated with the planes (100), (002), (101), (102), (110), (103), and (112), respectively. These results are well matched to the standard data of (JCPDS No. 36-3411), which confirm the formation of highly purified hexagonal crystalline ZnO NPs [48].

The averages of crystals sizes calculated by using Scherrer's formula applied on the major peaks (36.38°)

Loading [MathJax]/jax/output/CommonHTML/fonts/TeX/fontdata.js had the smallest size 20.9 nm. While, the other mean crystallite size were established to be 25.1, 27.9 nm for Tunisian Ziara, Flamingo waves, respectively. In addition, the ZnO NPs synthesized without using MSs had the highest average size (35.9 nm). Depending on the type and decibel properties of the sound waves, the decrease in the crystal size of nanoparticles can be attributed to the cavitational activity that helps to form smaller and uniform oxide particles [53].

Morphological and Elemental Analysis using TEM

The morphological shapes and elemental composition of the biosynthesized ZnO NPs were analyzed using TEM G20 associated with EDX. Figure 8 illustrates the TEM images of ZnO NPs fabricated without and with MSs assistance. Spherical agglomerated ZnO NPs were formed without the assessment of waves (Fig. 8A, A`). The ZnO NPs OF spherical shapes were obtained via green synthesis using fungus (Jain et al. 2013). In addition, Fakhari et al. [54] reported the biosynthesis spherical ZnO NPs using aqueous extract of *Laurus nobilis L.* leaves. On the other hand, the morphology of ZnO NPs is changed with the acoustic waves. Figure 8 (B, C) revealed that the spherical agglomerated shape is developed into nanotubular shape under Flamingo and Tunisian Ziara waves. Moreover, we observed the formation of nanotube ZnO NPs with fairly overlap narrow size distribution and uniform shape under Surat ElHadid (Fig. 8D, D`). This overlap is due to the polarity and electrostatic attraction of ZnO NPs [55]. It was previously reported that the morphology of biosynthesized ZnO NPs using *Lactobacillus plantarum* supernatant can be reshaped by modifying the reaction conditions [33]. In Fig. 9, the EDX analysis detected zinc and oxygen signals, which shows that the biosynthesis nanoparticles are in pure state of chemical nature [56]. Although, the biological synthesis of ZnO NPs has been taken over the past years, our results demonstrated the remarkable double role of microorganisms and musical soundson the biosynthesis of pure ZnO NPs.

Conclusion

The current study concerns the effects of sound more especially musical sounds upon the biosynthesis of ZnO NPs. The synthesis was accomplished by *Yarrowia lipolytica* supernatant and various MSs like Quran sound, Tunisian music, and Flamingo. Our results showed the accelerated formation of ZnO NPs, which were confirmed by UV-Vis and FTIR spectroscopies. The obtained ZnO NPs under MSs clearly indicates their highly pure crystalline structure through XRD spectrum with smallest average size of 20.9 nm. In addition, ZnO NPs morphology was changed from spherical agglomerated to nanotubular due to the effect of MSs. Overall, it has been established that the MSs may serve as viable alternative approach for numerous synthesis applications.

Declarations

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Conflicts of Interest: The authors report that they have no conflict of interests.

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Figures

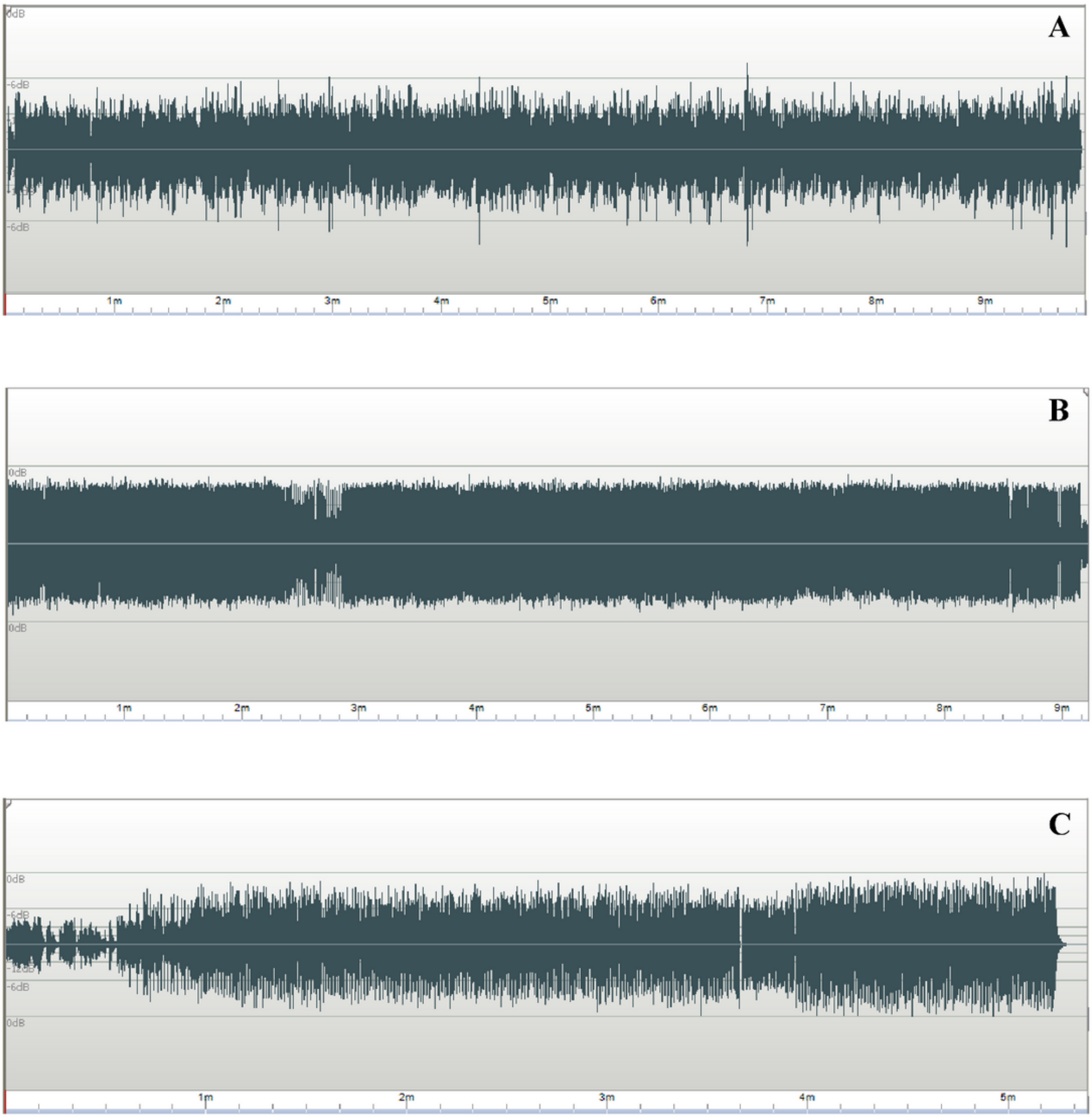

Figure 1

Decibel (dB) distribution over time for (A) Quran sound, (B) Tunisian music, and (C) Flamingo.

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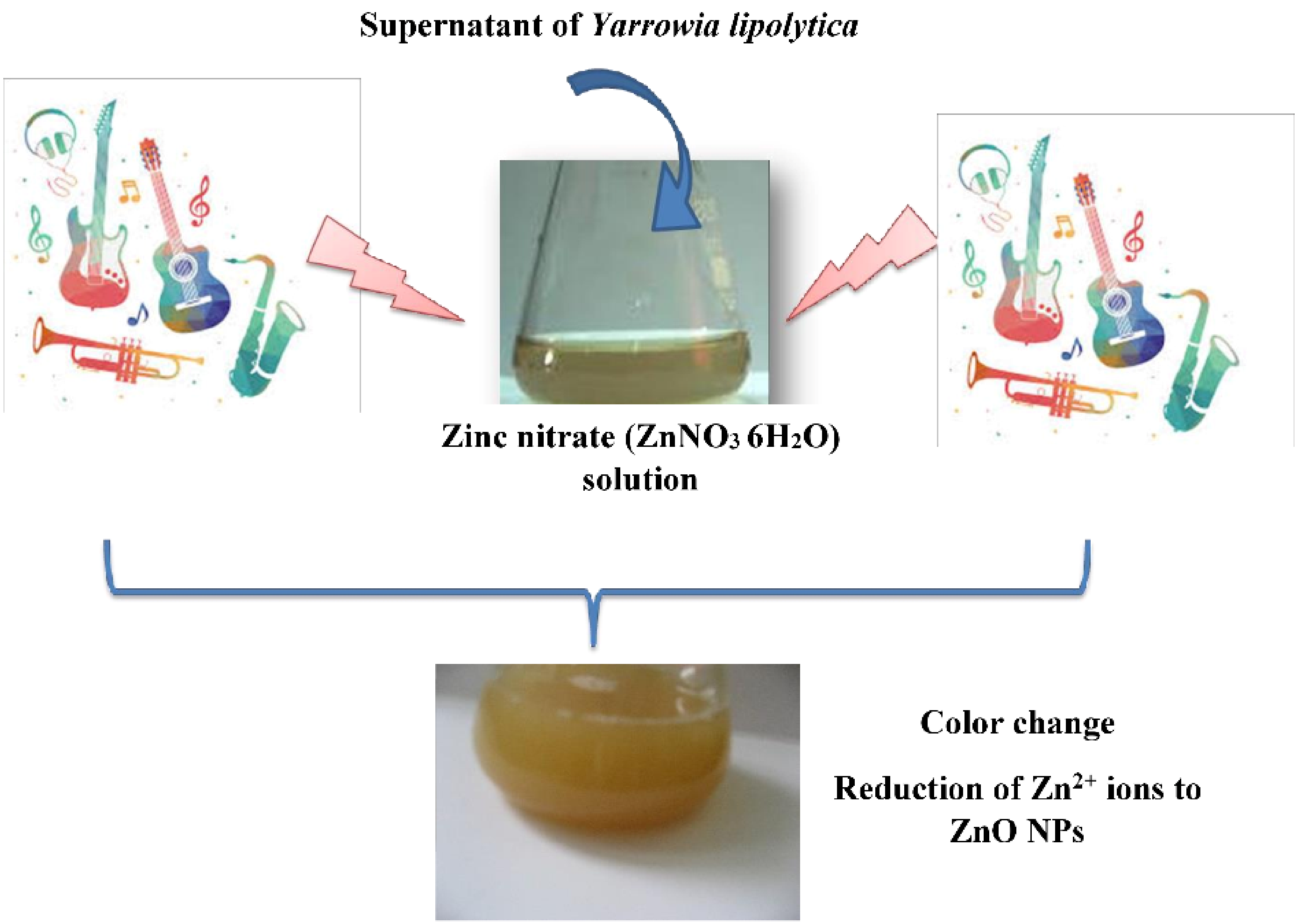

Figure 2

Color change of zinc nitrate solution by Yarrowia lipolytica supernatant Reduction.

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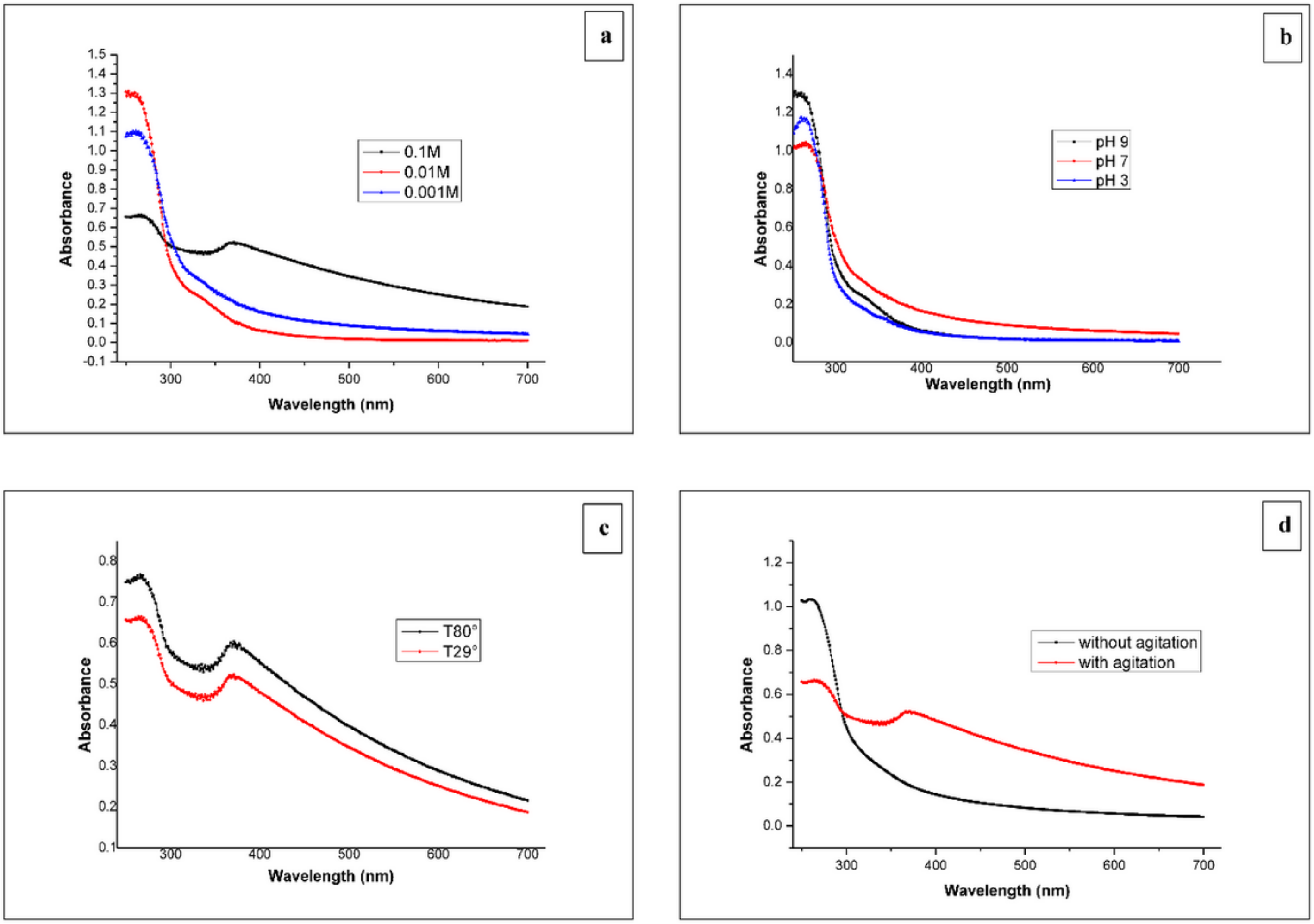

Figure 3

UV/Vis spectrums of ZnO NPs synthesized using supernatant of Yarrowia lipolytica under various (a) concentrations of zinc nitrate, (b) pH, (c) temperature, and (d) static and agitated conditions.

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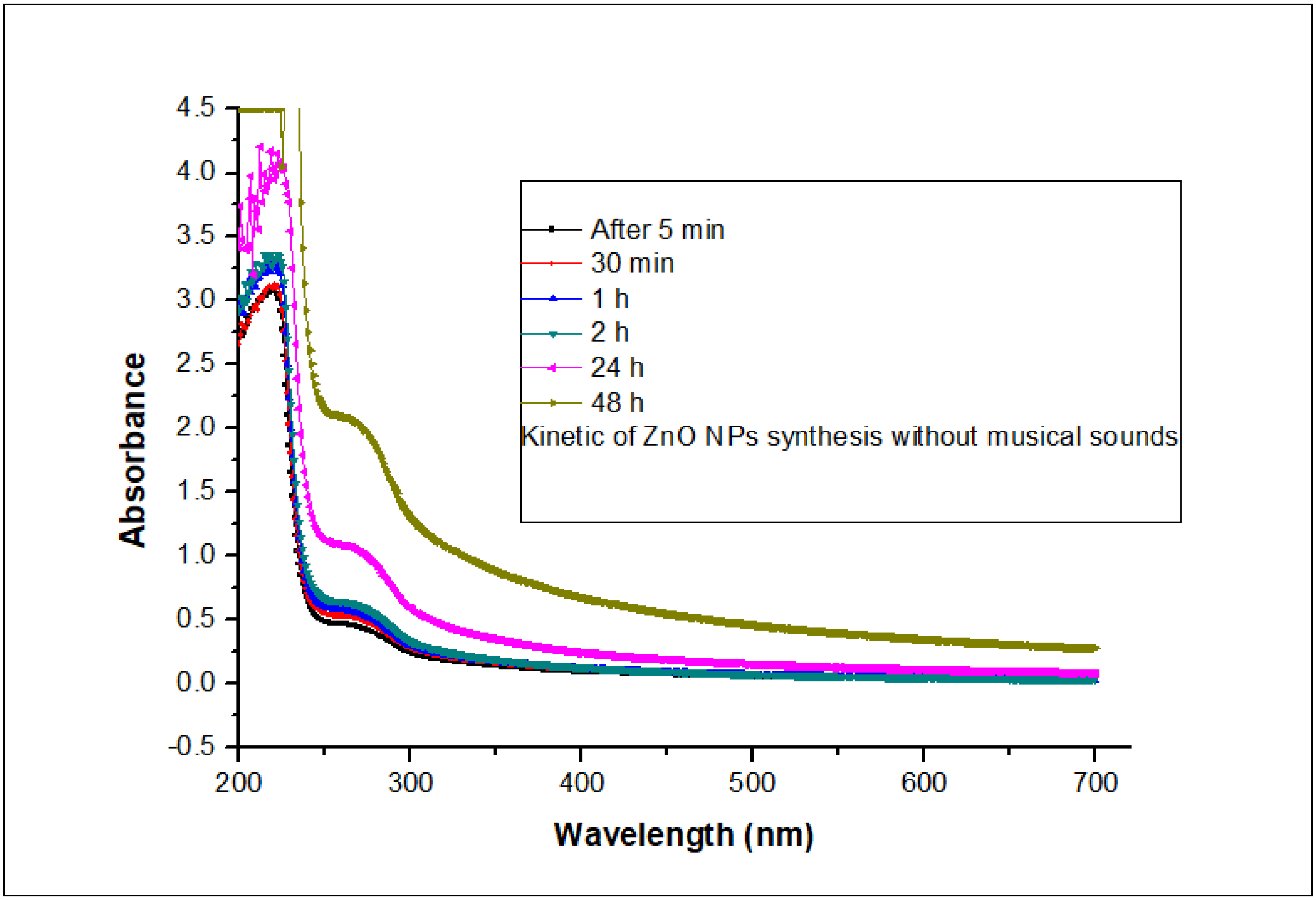

Figure 4

Kinetic of UV/Vis spectrums of ZnO NPs synthesized using supernatant of Yarrowia lipolytica under optimum condition without musical sounds.

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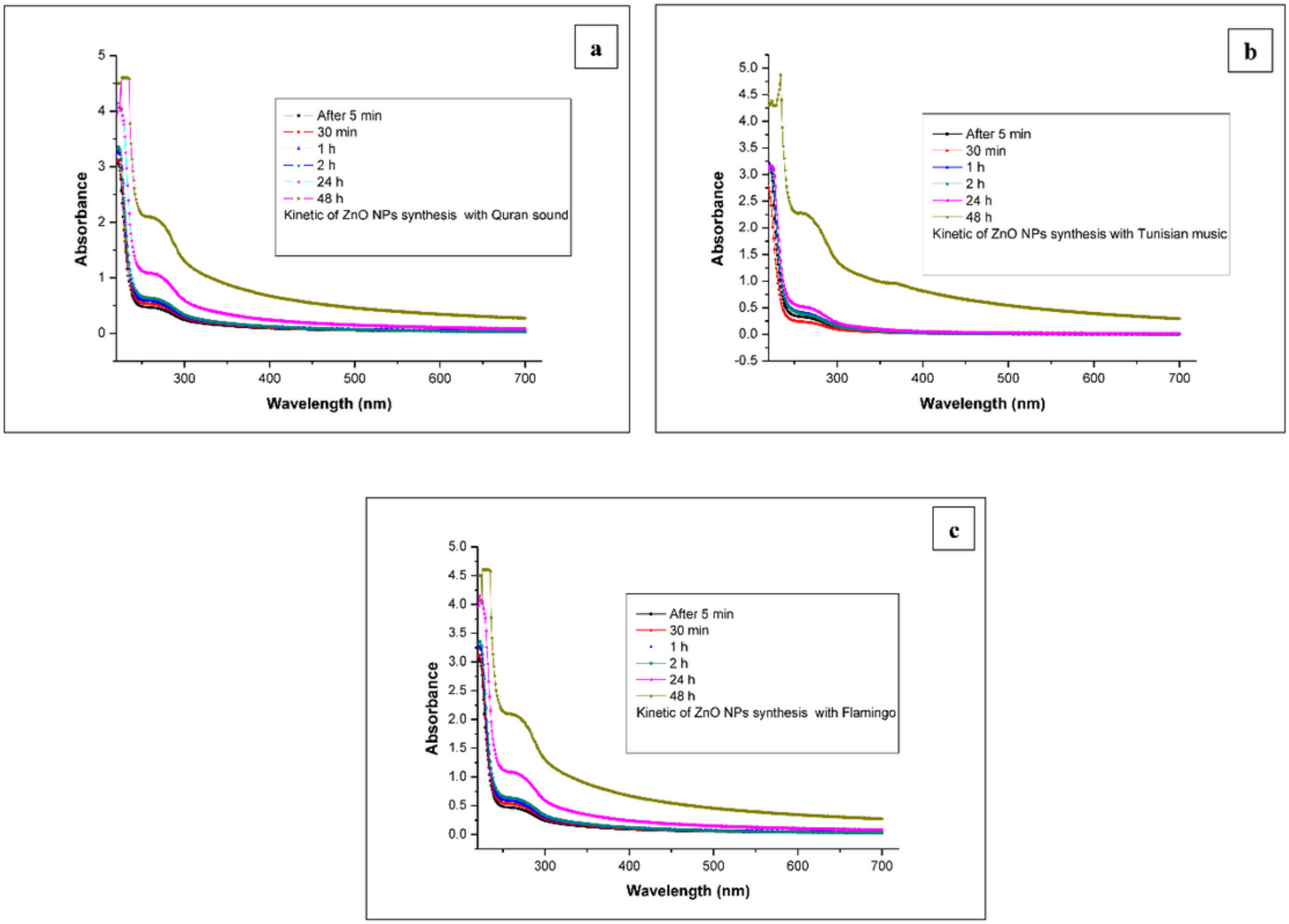

Figure 5

Kinetic of UV/Vis spectrums of ZnO NPs synthesized using supernatant of Yarrowia lipolytica and musical sounds(a) Quran sound, (b) Tunisian music, and (c) Flamingo.

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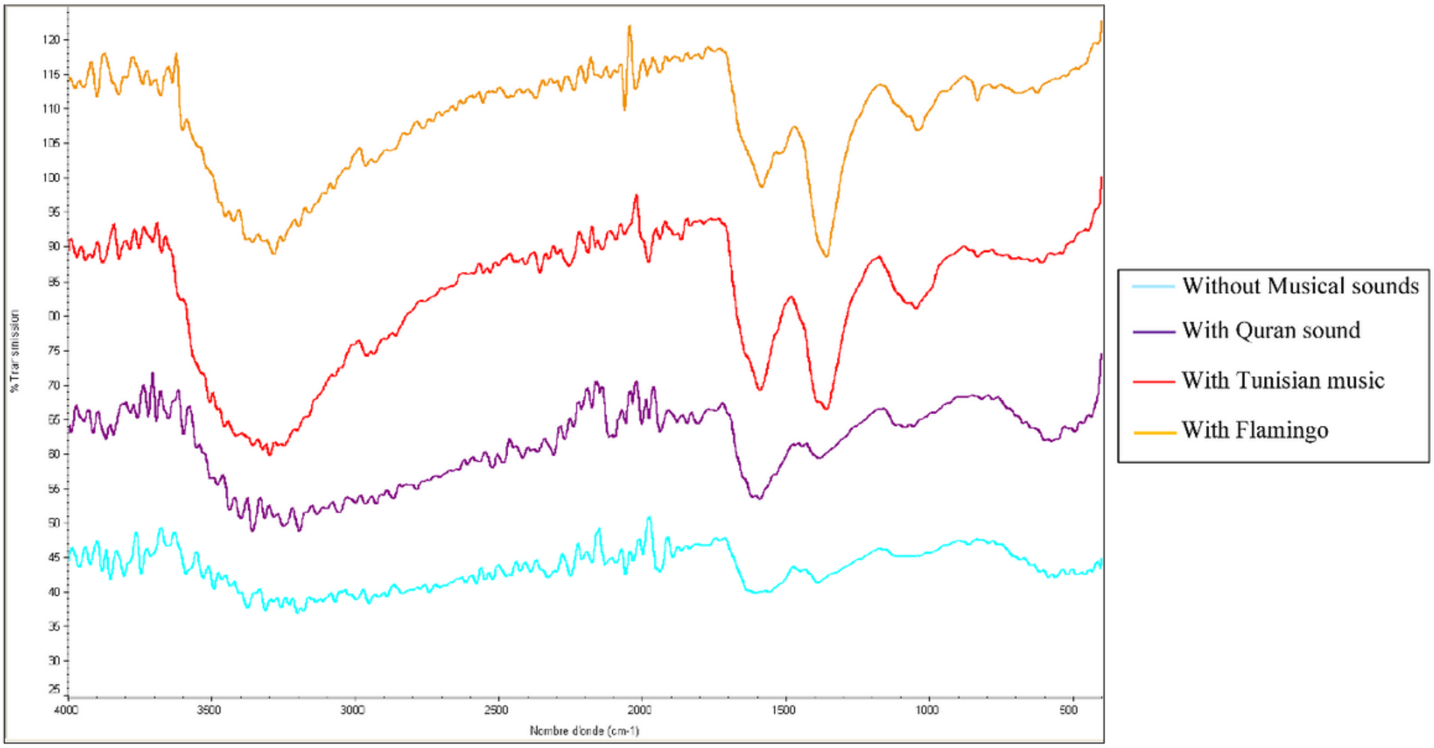
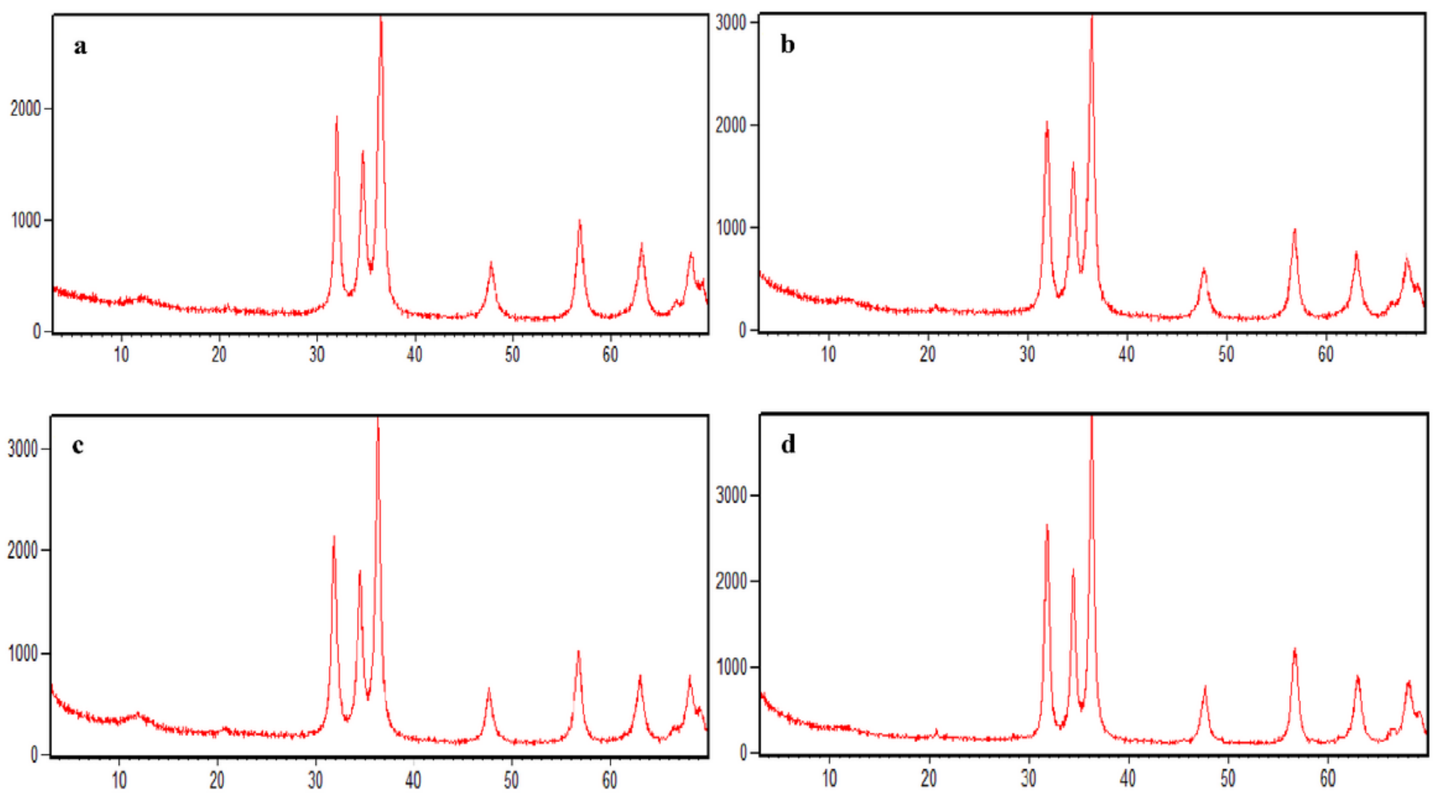


Figure 6

FT-IR spectrums of different ZnO NPs synthesized using supernatant of Yarrowia lipolytica.



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XRD graphs of biosynthesized ZnO NPs using supernatant of Yarrowia lipolytica (a) without musical sounds and with musical sounds (b) Quran sound, (c) Tunisian music, and (d) Flamingo.

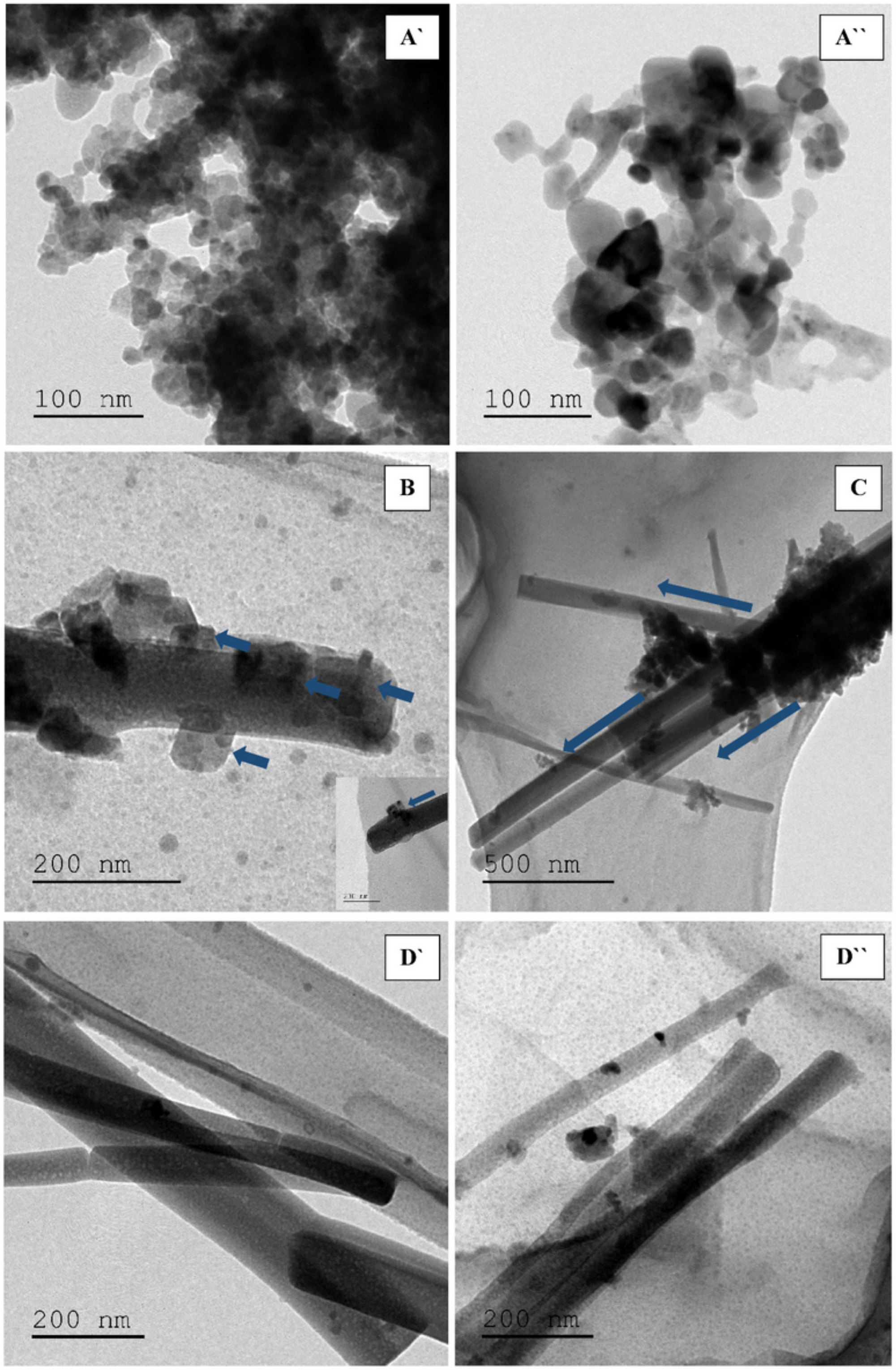

Figure 8

TEM images of the biosynthesized zinc oxide nanoparticles (A, A) $without \mu sical sounds$ and $with \mu sical sounds (B) Fla$ min go, $(C) Tunisian \mu sic$, and $(D,$ D)

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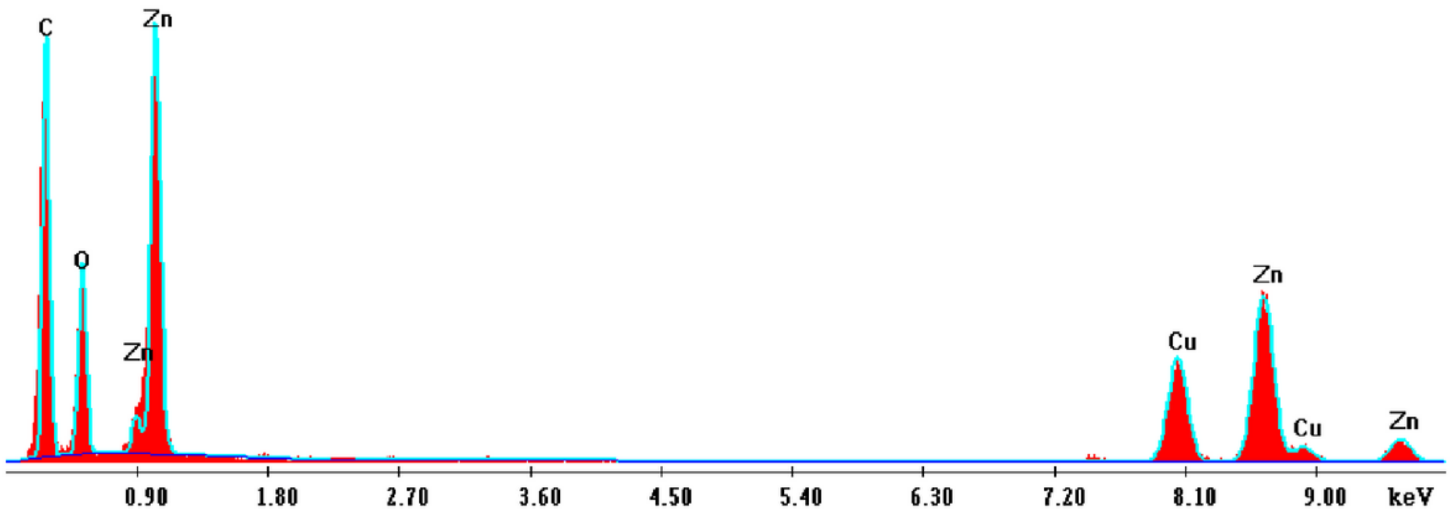

Figure 9

EDX spectrum of synthesized zinc oxide nanoparticles.

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