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Dielectric Monitoring the Effect Green Copper Oxide Nanoparticles Incubation with Baker›s Yeast Cells Suspension

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ABSTRACT

The current era of emerging nano-technology has immersed us in a sea of nanomaterials used in different fields of life. Copper oxide nanoparticles (CuONPs) are used in medical issues. Studies were conducted upon CuONPs cytotoxicity. The monitoring of cell viability was carried out by several methods mainly depends on cytolysis or membrane leakage. Many of these assays are invasive and the others are toxic. Electrical impedance measurement was a rational tool to investigate the biological materials by the mean of two electrodes. The current attempt aims at dielectric monitoring in form permittivity and conductivity spectrum of green (CuONPs) incubation effect with baker's yeast cells suspension. CuONPs are characterized by electron microscopy and Fourier transformer infrared (FTIR) spectroscopy was investigated under room condition.

KEYWORDS

Dielectric, Green Synthesis, Copper Oxide, Nanoparticles, Yeast cells.

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INTRODUCTION

Among metal oxide, copper oxide nanoparticles (CuONPs) are used in electronic and optoelectronic industries as semiconductor, gas sensor, and solar cell thin films. CuoNPs are incorporated in marine antifouling paints and agricultural biocides that can become airborne and deposit in soil. Eventually CuO NPs can be released into the ecosystem from power stations, smelters, metal foundries, asphalt, inkjet printers and rubber tires and can undergo oxidation as active form **(Jagathesan *et al*., 2018)**.

CuONPs also have a valuable trait in medical applications. CuONPs have antimicrobial activity against fungi and bacteria (**Rossetto *et al*., 2014**). They also are used as contrast agent for magnetic resonance imaging ultrasound dual imaging. Therefore, for appropriate utilization, the physiological behavior of CuONP should be fully understood to minimize the cytotoxic effects (**Galván Márquez *et al*., 2018**).

Retrospectively, several studies were conducted upon CuONPs cytotoxicity. The monitoring of cell viability was carried out by several methods mainly depends on cytolysis or membrane leakage (trypan blue), mitochondrial activity or caspase assay (Resazurin and Formosan) , functional assay (hemoglobin assay), genomic and proteomics assay, and flow cytometry assay **(Aslantürk, 2017)**.

Many of these assays are invasive and the others are toxic upon chemical kits like trypan blue, orange red viability test. The accuracy and reproducibility are limited. Moreover these methods are still expensive. A surge for new reliable and available monitoring methods was a challenge. During earlier decade, the electrical impedance measurement (EIM) was a rational tool to investigate the biological materials by the mean of two electrodes (**Schwan,1963**). (EIM) is noninvasive, real time methods for monitoring impedance changes of living biological cells (**Ogawa *et al.*, 2020**).

Several studies have reported a correlation between cell viability and impedance magnitude (**Roohvand *et al*., 2017).** Non-invasive physical methods were stepping up such as dielectric spectroscopy (DS), which provides simple, sensitive, real time, high throughput, and sterility maintenance for monitoring cell electrical **(Ogawa *et al*., 2020)**. Dielectric spectroscopy mainly measures the dielectric permittivity of cell population that is the ability of cell to store electric charges over the scanning frequency using two electrodes **(Roa, 2013**).

The current attempt proposes to dielectric monitoring (permittivity and conductivity) of green copper oxide nanoparticles incubation effect with baker's yeast cells suspension under room condition.

MATERIAL AND METHODS

Green Synthesis of Cu oxide nanoparticles (CuONPs)

The synthesis procedure of nanoparticles was adopted from earlier work of (**Awad *et al.,* 2013**). A constant volume of the aqueous guava leaves extract of (about 10 ml) was mixed with Copper Sulphate ($CuSO4·5H2O$) 5ml solution (1 mM) with concomitant stirring at room temperature. The mixture was sonicated, then filtered to separate plant debris. Appearance of a dark color indicated the formation of CuONP. Then, nanoparticles were precipitated by centrifugation at 13,000 rpm for 10 minutes and stored at -4 °C for further use.

Electron Microscopy

Surface and size morphology of yielded nanoparticles were monitored by using electron microscopy, transmission (JEOL1400 Plus, Japan) and scanning (JSM-5300, JEOL, Japan) electron microscopy. Sample preparation of nanoparticles was performed by drop coating method was on carbon coated copper grids under vacuum conditions. The average diameters of particles were deduced from captured photographs, as shown in Figure 1.

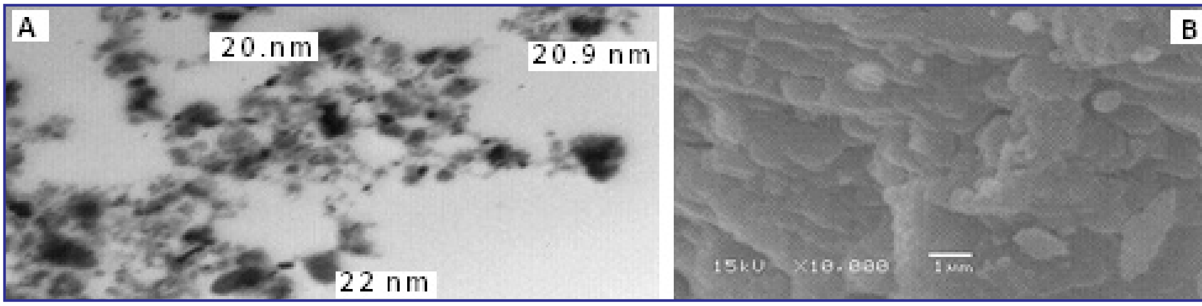

Fig. (1): Photographs of electron microscope, (A) transmission and (B) scanning, for the prepared CuONPs by the current green methods with corresponding screen deduced size.

Fourier Transform Infrared Analysis (FT–IR)

FT-IR (model: 8400s, Shimadzu corporation, Japan) spectroscopic technique was used to detect functional active groups bonding of the prepared nanoparticles via IR irradiation.

Yeast cell preparation and CuNPs incubation

Yeast cells are well studied. They are simple unicellular cells with unique features for ease of growth and genetic manipulation making it an exceptional host microbe and good candidates for medical application (***Roohvand et al.*, 2017**). Bakery dry yeast cells were purchased from local markets. Two grams yeast powder was added to 10 ml distilled water pretreated with traces of table sugar (to maintain yeast cell viability) with concomitant stirring at 37°C.

Dielectric measurement

Resistance and capacitance of yeast cells suspension was monitored at 5 and 50 kHz frequencies via two square form silver electrodes connected to LCR meter (Hioki, 3532, LCR Hitester Japan, Physics Department, Faculty of Science, Damanhour University, Egypt) at room temperature (24 ± 0.1°C). Measurement cell was designed as two parallel cubic silver electrodes of 1x1 mm2 area (A) and 5 mm apart (d). The permittivity (ε) and conductivity (σ) of yeast cells suspension were calculated from (**Equation 1** and **Equation 2)** as a function of capacitance (C) and conductance (G) (reciprocal f resistance R) at frequencies range of 1 kHz -5 MHz.

$$\varepsilon = C.\frac{\mathrm{d}}{\mathrm{A}} \qquad \textbf{Equation 1}$$

$$\sigma = \frac{1}{R}.\frac{\mathrm{d}}{\mathrm{A}} \qquad \textbf{Equation 2}$$

RESULTS

FT-IR peaks

In the Figure 2, peaks at 1600 cm-1 may concern the asymmetric and symmetric stretching vibration of COO– (10), which supports the presence of protein in the plant extract reducing agent for met-al precursors. The band at 1141 cm-1 can be for the symmetric C–O vibration associated with C–O– SO3 group. In addition, signals at 3900 cm-1 (OH stretching), 2420 cm-1 (CH stretching) were noticed. The presence of Cu elements can be seen by two strong absorption bands at 620 and 770 cm-1 **(Sharma *et al.*, 2017)**.

For all study cases a frequency dependence responses curves were noticed in Figure 3 Concerning permittivity, the trend begins with step-like increment followed by decrease till plateau stage. A contrary behavior occurred for the conductivity curves. At the current microwave frequency range, only β-dispersion of dipole relaxation may be mainly concerned during the permittivity response, α-dispersion (ionic relaxation) could not occur as it is related to lower frequency range (**Schwan, 1963**).

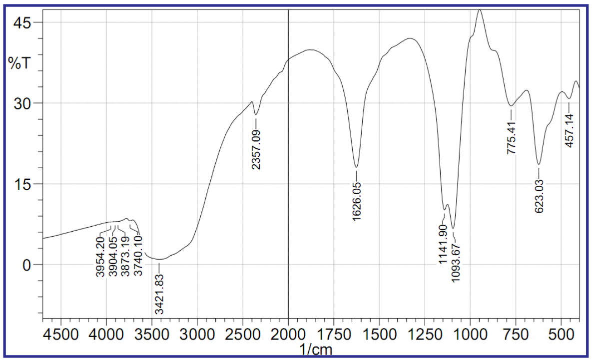

Fig. (2): Print-out of FT-IR shows values of transmission peaks of green CuONP powder.

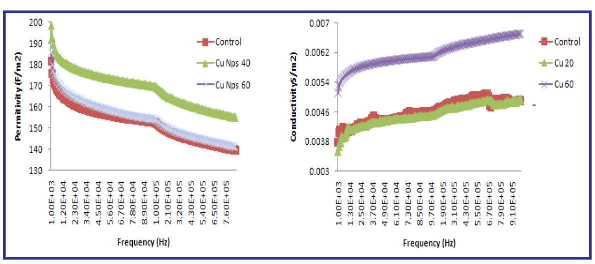

Fig. (3): Graphical representation of dielectric spectrum of yeast cell suspention at different CuONPs concentrations (20, 40, 60 ppm) (a) dielectric constant and (b) conductance.

As we noticed in the dielectric relaxation curve, higher permittivity at low frequency due to ionic conductivity and presence of free ions conductivity contributed to the dipole polarization (ionic and electronic), while at higher frequency the dipoles responses decrease which indicate normal dielectric behavior **(Downey *et al.,* 2014)**.

According to current results, the permittivity peak yeast cells decreases with increasing the concentration of (CuNPs). This observation may be due to the higher Cu ions presence in the sample. As the concentration of Cu ions increase, electrolytic conduction exists in the presence of a solvent and permittivity declines as frequency increase. The opposite behavior is noticed in conductivity spectrum. This behavior is typical characteristics of the polar dielectrics (**Yao *et al.*, 2019**).

CONCLUSION

In brief, the present work employed the electrical characteristics of living yeast cells (electrical permittivity and conductivity) to monitor to cell integrity and viability under the influence of copper oxide nanoparticles incubation. The dielectric spectrum as impedance spectroscopy, showed sensitive, fast, affordable, real time and screening to cell ionic change under the influence of external effect. The opposite behavior is noticed in conductivity spectrum. This behavior is typical characteristics of the polar dielectrics

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