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Facile Synthesis of Cobalt Oxide Nanoparticle for Biological Studies

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Abstract Currently Nanoparticles are utilized in various fields and have a different clinical application. In our research, we have developed the Cobalt oxide (Co_3O_4) NPs using the Sol-gel technique from the Cobalt chloride precursor. The synthesized nanoparticle was characterized by using X-Ray Diffraction (XRD), Scanning Electron Microscope (SEM), Dynamic Light Scattering (DLS), and Fourier Transform Infra-Red Spectroscopy (FTIR). The cytotoxicity studies were evaluated on the Vero cell line (African green monkey kidney cell line) by using MTT assay at 72 hrs. The Cell viability which was observed during the process depends upon the concentration and time exposure. Moreover, the In-vitro cytotoxic effects of Co_3O_4 NPs showed a better result at the concentration of 50 µg/100µl in Vero cell.

Keywords: *cobalt oxide NPs, sol-gel technique, MTT assay, cytotoxicity, vero cell line*

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1. Introduction

Nanotechnology has a rapid development area. It consists of many advantages namely the lowest surface free energy, high quality of stability, and unique properties in terms of optical, magnetic, and electrical activity. Structure of Nanoparticles and its function are dealing with a tiny scale level [1-6]. The uses of nanomaterials in the biological field have increased superior properties compared to their bulk counterparts due to they are having small scale probes easily interaction with biomolecules and environmentally friendly. It has been numerous application in the biomedical field such as drug and gene delivery [7,8], vaccine administration [9], tissue engineering [10], hyperthermia [11], protein detection [12], diagnostic and therapeutic purposes [13] etc. Cobalt oxide ($C_{O3}O_4$) nanoparticle is a multi-role material like p-type antiferromagnetic semiconducting material. Currently, several nanoparticles were used as cytotoxicity studies but these nanoparticles are a prominent necessity because Co_3O_4 NPs increased interact in day to day life [14,15,16] and having transversal application such as Ceramic, glass [17], solar cell [18], gas sensor [19], fuel cells [20], paint [21], ultraviolet [22], etc. Since the Co_3O_4NPs are one of the antioxidant agents and having attractive features include high stability, biocompatibility, large surface area, low cost, effective catalyst, etc. The study of cytotoxicity depends on some factors such as pH, size, and shape [23]. They are variety of method to developed Co_3O_4NPs include Hydrothermal [24], Co-precipitation [25], spray pyrolysis [26], solvothermal [27], Hydrolysis [28], ball milling [29], thermal decomposition methods [30]. Hence in this present work, Co_3O_4 NPs are synthesized from the Sol-gel technique with some modification. This technique was eco-friendly, high pressurized and high temperature possing involved, as well as investigated the cytotoxicity studies on the Vero cell line. [31,32]. These cells are derived from the African green monkey kidney.

2. Materials and Methods

To assess the Cytotoxicity studies about three of the following chemicals namely the $CoCl_2$, $C_2H_8N_2O_4$. Sodium hydroxide was used. These were purchased from Sigma-Aldrich. To evaluate cytotoxicity studies on Vero cell lines were purchased from the National Centre for Cell Science (NCCS). The purchased reagents which are used for this study were of analytical grade.

2.1. Synthesis Method

Cobalt oxide nanoparticle prepared using the Sol-gel reaction method. The 1g of $CoCl_2$ solution was added to the 50 ml of D.H_2O.0.5 g of $C_2H_8N_2O_4$ was added to the 50 ml of D.H_2O.The total volume of aqueous solution kept at the stirring condition for 20 minutes and the mixture was heated at 60°C for 30 minutes. The stable gel was

finally obtained. The obtained gel was washed with D.H_2O and dried at 70°C for 12hrs. The sample calcinated at 400°C for 4 hrs.

2.2. Characterization Techniques

To identify the crystallinity stage, we have characterized our sample through the X-ray Diffraction (XRD) in which the sample was recorded by using the *PhillipsPW1800* diffractometer along with the Cu Kα radiation. It was maintained at 40 kV for the identification of the crystalline. Using the *nano plus micromeritics*, the Dynamic Light Scattering (DLS) analysis was done to determine the particle size of Co_3O_4 NPs. To identify the morphology of the particles the analysis with the Scanning Electron Microscopy (SEM) was performed with TESCAN *(Model WEGA11)*. Here, the operation was done with an acceleration voltage of 25.0 kV. The spectrometer of *Perkin-Elmer* was used in the Fourier Transform Infrared Spectroscopy (FTIR) analysis of Co3O4 NPs. By the use of *Shimadzu Japan UV- spectrometer*, the Optical properties of Co3O4 NPs has been studied

3. Result and Discussion

3.1. X-ray Diffraction (XRD)

XRD pattern of Co_3O_4 NPs was shown in Figure 1. All the diffraction peaks are matched with standard JCPDS card No (78-0209). The diffraction peak position (2θ) have appeared at 31˚, 36˚, 59˚, 65˚ correlative to crystal planes (220), (311), (116), (214). As the result indicates that synthesized Co_3O_4 NPs are arranged in the rhombohedral phase.

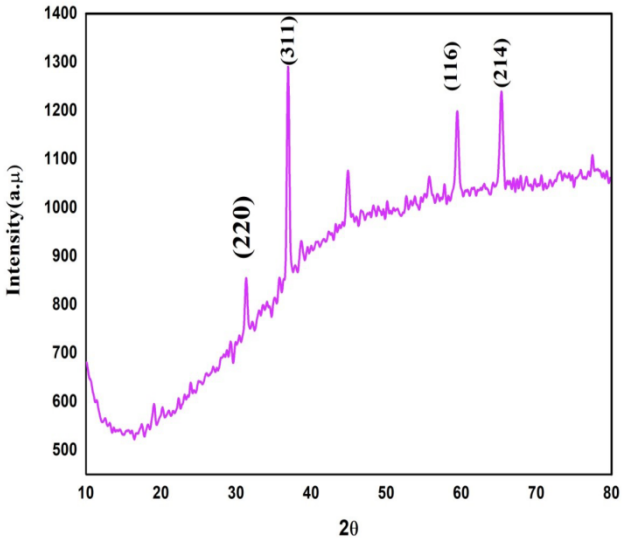

Figure 1. X-ray diffraction (XRD) analysis

3.2. Scanning Electron Microscopy

The morphology of Co_3O_4 NPs was obtained by SEM as shown in Figure 2a. reveals spherical nanoparticles and the size is 478 nm by using DLS. As shown in the instead of Figure 2b. They are good aggregation. Figure 2 (c) exhibits the EDAX spectrum. It can be shown that the Cobalt and Oxygen atom of Co_3O_4 NPs.

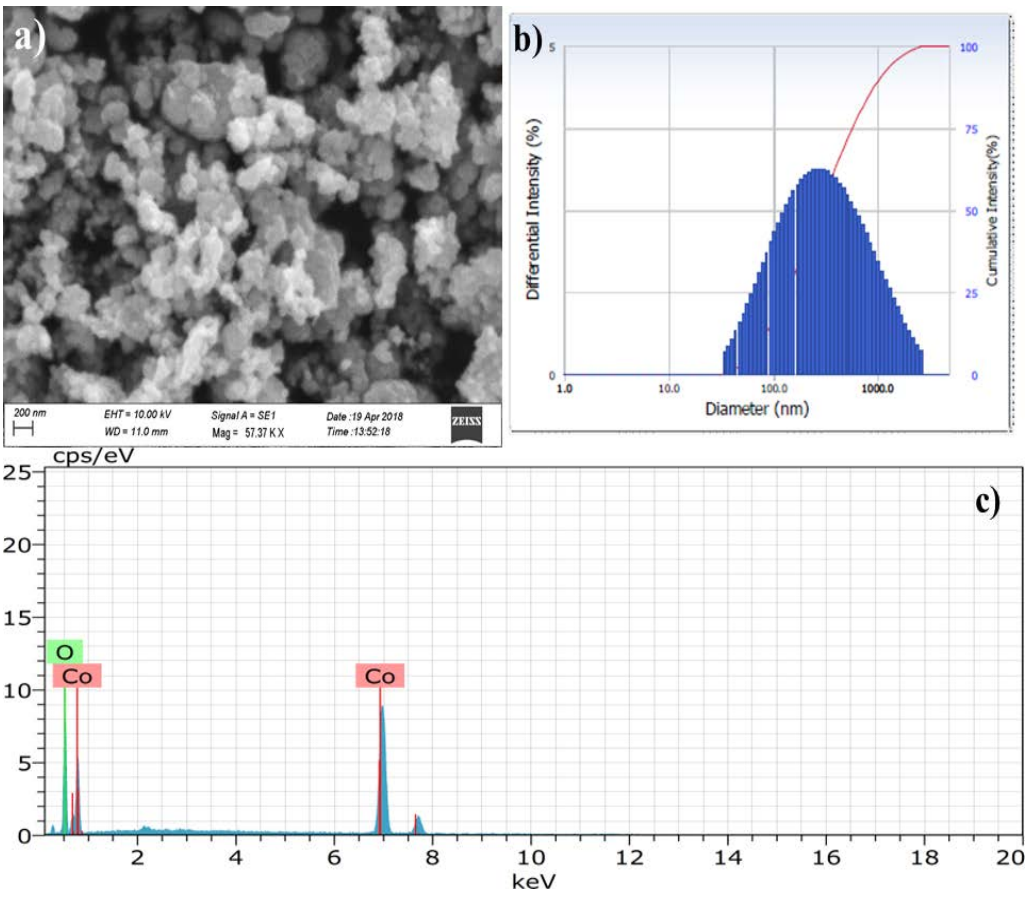

Figure 2. (a). SEM morphology of Co_3O_4 NPs, (b). DLS analysis of Co_3O_4 NPs, (c). EDAX analysis

3.3. Fourier-Transform Infrared Spectroscopy

FTIR was used to perform an analysis of the potential functional group of Co_3O_4 NPs. Figure 3. Broadband appeared at 3235 cm-$^{1\ is}$ corresponding to OH groups. The weak band at 2920 cm-^{1} are characteristic peaks of C-H symmetric stretching vibration.1843 cm^{-1}, 1580 cm^{-1}, 1400 cm^{-1}, 1337 cm^{-1}, 1043 cm^{-1} are assigned to bending of OH and C-O stretching vibration which are responsible for the process of being formed of Co_3O_4 NPs. The absorption band located in the spectral range of below 690 cm^{-1} which is the most assignment to a Co-O mode [34].

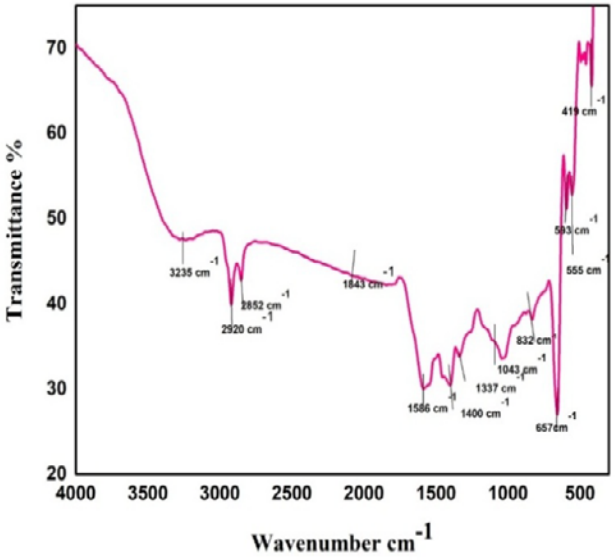

Figure 3. FTIR analysis of Co_3O_4 NPs

3.4. In-vitro Cytotoxic effect of Co_3O_4 NPs

To compete with the nanotoxicity effects, the toxicity studies of nanoparticles on Vero cells were increasingly investigated. In our research, we have assessed the toxicological effects of Co_3O_4 NPs which reveals itself as a biocompatible material. The obtained results were unveiled in Figure 4(a). The histogram is used to identify the viability percentage of Vero cell lines after 72 hrs incubation in different concentrations such as 50, 100, 200, 400, 500 (µg/mL). The given data was based on nanoparticle concentration and time exposure. At the concentration of 50 µg/mL of Co_3O_4 NPs, only the smaller amount of cell death was observed and it is proved to be as high biocompatible with Vero cells. At the concentration of 100 µg/mL, in cell membranes, the fewer toxicity was observed due to the binding of a high level of superoxide Mn^{+} ions. Conclusively, damaging the genetic material and the cell organelles. The morphological effects of Co_3O_4 NPs were analyzed by using an optical microscope and the obtained images were shown in Figure 4b. showed a Vero cell line in Co_3O_4 NPs. The shape existed in the form of a small rounded cell with a different size. Moreover, many studies are to be done to understand the biological properties.

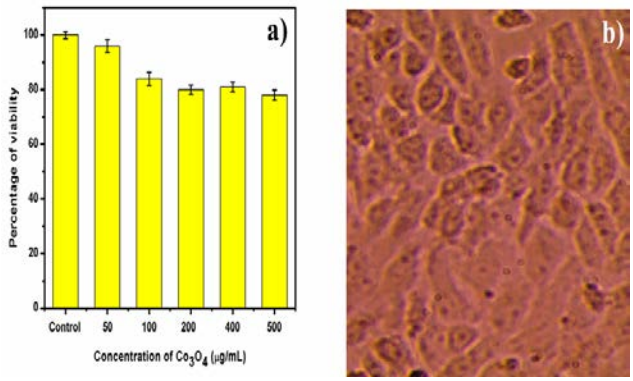

Figure 4. (a) bar diagram of Cell viability ofCo_3O_4 NPs (b) morphology image of Co_3O_4 NPs on Vero cell using optical microscopy

4. Conclusion

Formation of Co_3O_4 NPs is confirmed from XRD, further FTIR technique determines the functional group. The strong band of Co_3O_4 NPs was appeared at below 690 cm^{-1}. SEM analysis reveals various particle sizes with a spherical shape. The average particle size of 478 nm using DLS. EDAX result showed elements of Co_3O_4 NPs. The result reveals as prepared NPs enhance the Cell viability. It depends on the time and concentration of NPs. The Cytotoxicity study result showed Co_3O_4 Nps less toxicity as well as biocompatible materials and can be used for clinical purpose application.

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