

# Synthesis, Characterization and Anticancer Activity of Chitosan encapsulated Cobalt Nanoparticles

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## ABSTRACT

*Chitosan is a cationic natural polysaccharide which is derived from the chitin of crustaceans, prepared by the N – deacetylation of chitin. Chitosan is biocompatible and it shows the activities such as antimicrobial and antifungal activities, which makes it a favorable option for biomedical applications. The present study deals with the synthesis, characterization and anticancer activity of Chitosan – Cobalt nanoparticles. The anticancer activity has been stimulated in chitosan encapsulated Cobalt nanoparticles upon the addition of noni fruit extract as a reducing agent. The Cobalt loaded chitosan nanoparticles are characterized by UV-VIS, SEM, EDAX and XRD methods.*

**Keywords:** Cobalt-Chitosan nanoparticles, Anticancer, UV-VIS, SEM, EDAX and XRD.

## I. INTRODUCTION

Chitosan is derived from Chitin, the hard dirty white crystalline powder obtained from the exoskeleton of crustaceans. Chitosan is soluble in aqueous Acetic acid [1]. The presence of cationic NH<sub>2</sub> and alcoholic hydroxyl (OH) group makes the polymer more reactive and is responsible for the fungicidal activity [2]. The chitosan N-Benzyl Sulfonate derivatives were used as an adsorbent for heavy metals such as Hg<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup> [3]. Metallic nanoparticles find wide applications in catalysis, photonics, biomedicine, antimicrobial activity and anticancer activity [4]. Cobalt is a vital element for humans because it is a part of vitamin B<sub>12</sub>, (cyanocobalamin) which is essential for human beings. A very important application of Cobalt metal is in the field of cancer therapy, cellular separation and medical imaging [5].

Noni (*Morinda citrifolia* L.) is a medicinal plant and it is the crucial bio pharmaceutical used in folk medicine. It is commonly called starvation fruit [6] with bitter taste and fishy smell [7].

*M. citrifolia* fruits are rich sources of dietary fibre [8]. Hence it finds application in the treatment of diabetics.

When *M. citrifolia* juice alone is analyzed and compared with pulp powder, only vitamin C is retained in an amount (34 mg per 100 gram juice) that is 64% of the content of a raw navel orange (53 mg per 100 g or 89% of the Daily Value) [9]. Sodium levels in *M. citrifolia* juice (about 3% of Dietary Reference Intake, DRI) are high compared to an orange, and potassium content is moderate.

*M. citrifolia* fruit contains phytochemicals, including lignans, polysaccharides, flavonoids, fatty acids, scopoletin, catechin, betasitosterol, damcanthol, and alkaloids [10]. Plants are used as reducing agents in the synthesis of metal nanoparticles. Plant mediated metal nanoparticles aid to control the growth of fast growing tumour cells is due to the secondary metabolites and other non-metal composition in the synthesizing medium [11].

The present study deals with the synthesis of a bio pharmaceutical material, chitosan which is loaded with Cobalt and Noni fruit powder. The synthesized bio material is characterized by UV-Vis, EDAX, SEM and XRD methods. The anticancer activity of the synthesized chitosan encapsulated cobalt nanoparticles were demonstrated by MTT assay method.

## II. EXPERIMENTAL DETAILS

Chitin is isolated from the shells of crustaceans and deacetylation process on the isolated Chitin gives the Chitosan. The 0.5 g of Chitosan is dissolved in 15 ml of acetic acid and Cobalt sulphate solution is added further using burette. 1 gm of *Morinda citrifolia* (noni) dried fruit sample was treated to the Chitosan Cobalt solution with constant stirring, continued for 3 hours at room temperature. After stirring, the solution was filtered, and the filtrate was collected then added 2 drops of Sodium hydroxide. Then the pH was noted using Eutech pH meter. The solution was changed from blue to pink. The solution was kept overnight.

The formation of tiny nanoparticles are observed after keeping the solution overnight. Then the solution was filtered using Whatmann filter

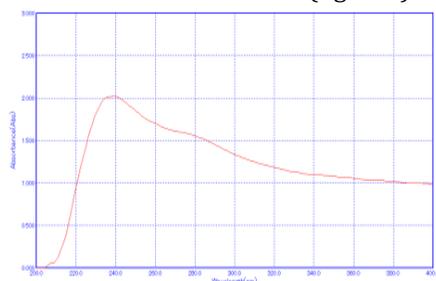
paper. The prepared Cobalt nanoparticles were kept at room temperature for two weeks. The dried sample was taken for further characterization study and anti cancer activity.

### III. RESULTS AND DISCUSSION

#### UV- Visible spectra:

UV-Visible spectroscopy is an important technique to determine the formation and stability of Cobalt Nanoparticle in aqueous solution. The reaction mixture changes its colour by adding various concentration of metal ions. After the addition of *Morinda citrifolia* fruit powder to the Cobalt loaded chitosan, the colour of the solution was changed from blue to pink indicating the formation of cobalt nanoparticles [12]. This colour change was due to the excitation of surface Plasmon vibrations of the Cobalt Nanoparticle.

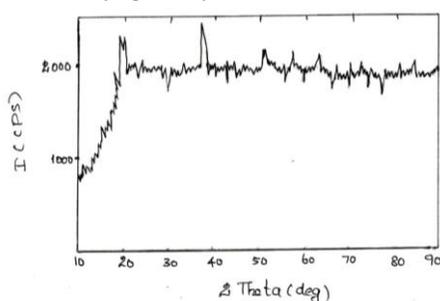
The SPR absorbance band of Cobalt nanoparticles was centered at 235 nm. (figure 1).



**Figure 1: UV-Visible spectrum of Cobalt loaded Chitosan nanoparticles**

#### X-Ray diffraction:

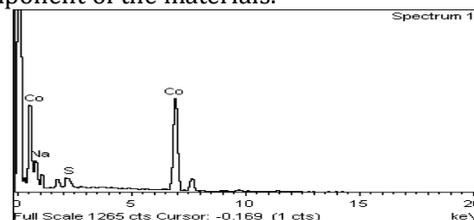
The phase identification and crystalline structures of the nanoparticles was characterized by X-Ray diffraction. Using Debye-Scherrer's formula, the crystalline size for the nanoparticles can be calculated. The X-Ray diffraction patterns obtained for the Cobalt nanoparticles synthesized using *Morinda Citrifolia* (flesh part) showed that there exists strong diffraction peaks with  $2\theta$  values of  $19.2^\circ$ ,  $38.2^\circ$ ,  $20.9^\circ$  corresponding to the crystal plane of (111), (222), (100) of crystalline Cobalt nanoparticles. (Figure 2)



**Figure 2: XRD spectrum of Cobalt loaded Chitosan nanoparticles**

#### Energy dispersive X-ray spectroscopy:

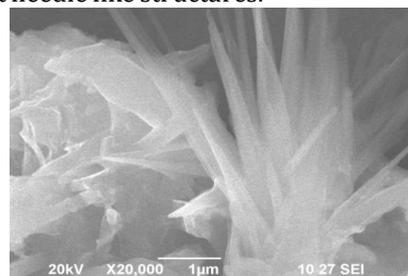
To confirm the presence of Cobalt in the synthesized materials, an elemental composition analysis was done by using energy dispersive X-Ray spectroscopy (EDX). Using this technique the elemental composition of the materials was analyzed with high resolution. EDX analysis data confirms the presence of Cobalt metal as a main component of the materials.



**Figure 3: Energy Dispersive X-rays spectrograph of Cobalt loaded Chitosan nanoparticles**

#### SEM

Scanning electron microscope is one of the powerful tools to identify the morphology of the nanoparticles. SEM image shows the arrangement of ions in the surface of the nanoparticles. Each individual nanoparticles was aggregated and exhibit needle like structures.



**Figure 4: SEM image of Cobalt loaded Chitosan nanoparticles**

#### INVITRO ANTIPROLIFERATIVE EFFECT DETERMINATION BY MTT ASSAY (ANTI CANCER ACTIVITY STUDY)

A549 (lung cancer) cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's modified Eagle's medium (Gibco, Invitrogen).

The cell line was cultured in 25 cm<sup>2</sup> tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO<sub>2</sub> incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

**Cells seeding in 96 well plate:**

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x10<sup>4</sup> cells/well) wasseeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

**Cytotoxicity Evaluation:**

After 24 hours the growth medium was removed, freshly prepared each plant extracts in 5% DMEM were five times serially diluted by two fold dilution (100µl, 50µl, 25µl, 12.5µl, 6.25µl in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

**Cytotoxicity Assay by Direct Microscopic observation:**

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

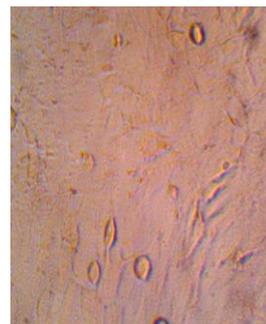
**Cytotoxicity Assay by MTT Method:**

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization..

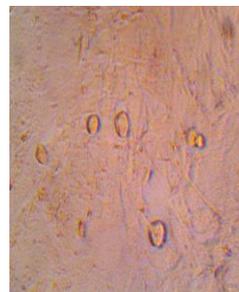
After 24 hours of incubation period, the sample content in wells were removed and 3 0µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution ( DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The bsorbance values were measured by using microplate reader at a wavelength of 570 nm (Laura B. Talarico et al., 2004).

| Sample volume (µl) | Average OD at 540nm | Percentage Viability |
|--------------------|---------------------|----------------------|
| Control            | 2.1405              |                      |
| 6.25               | 2.0501              | 95.7                 |
| 12.5               | 1.9989              | 93.3                 |
| 25                 | 1.7311              | 80.8                 |
| 50                 | 1.5637              | 73                   |
| 100                | 1.0096              | 47.1                 |

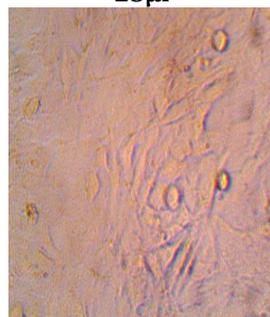
Table 1: LD 50 value - 97.83µg/ml



12.5µl



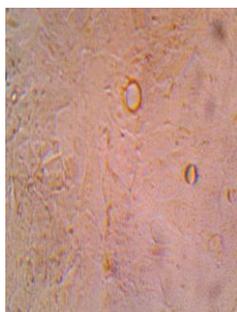
25µl



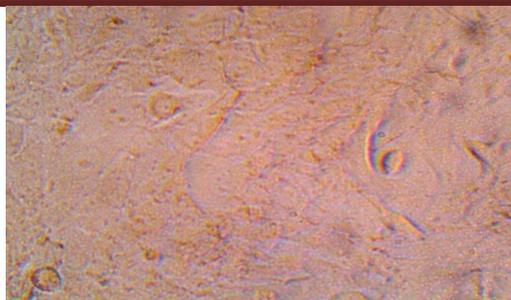
50 µl



100µl



6.25µl



control

#### IV. CONCLUSION

A novel biopolymer Chitosan encapsulated with Cobalt metal nanoparticles were synthesized using Morindacitrifolia fruit powder as a reducing agent. The nanoparticles were confirmed by UV spectral data. Elemental analysis was carried out to confirm the presence of Cobalt metal. From the XRD spectrum it is proved that the particles are crystalline in nature. Surface morphology was studied by SEM analysis which shows the aggregation of biopolymer molecules. Anti cancer activity is done by Direct Microscopic observation and MTT assay. The results shows that the percentage viability of cancer cells decreased by increasing the volume of test solution.

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