Pectin Stabilized/Capped Ferromagnetic Co₃O₄ Nanoparticles with Antimicrobial Efficacy: A Greener Approach

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Abstract

The preparation of Co₃O₄ nanoparticles using thermal decomposition and their characterization by various physicochemical studies is being reported here. The focus is on the use of pectin as a capping/stabilizing agent and heading the methodology towards greener pathway, single pot and cost effective technique. The characterizations were done by using UV-Visible, FTIR, SEM, TEM and XRD analysis which confirmed the formation of nanoparticles. The FTIR spectrum revealed the presence of pectin along with the nanoparticles. The nanoparticles were found to be stable at pH 5.5 as evaluated by their zeta potential analysis. The magnetic measurements (VSM) revealed their ferromagnetic behavior. These nanoparticles indicated effective antimicrobial potency against six different strains of bacteria.

Keywords: Pectin, Cobalt oxide, Nanoparticles, Ferromagnetism, Zeta potential and antimicrobial potency.

INTRODUCTION

The transition metal oxide nanoparticles are an important class of nanomaterials that have been explored owing to their various uses in different areas like in lithium ion batteries¹ gas sensing² data storage³, catalysis⁴-⁶ and biomedical applications⁷. These kinds of nanoparticles can be synthesized by varied chemical and physical methods like Sol Gel⁷-⁸, thermal decomposition⁹-¹⁰ and chemical spray pyrolysis¹¹-¹² but most of these methods are either expensive or pose a threat to the environment.

Between the prepared cobalt nanomaterials, Co₂O₄ is the prominent nanostructure which has been synthesized with different morphologies using the previously mentioned methods.

The synthesis of Co₂O₄ nanoparticles using thermal decomposition of carbonatotetra(amine) cobalt(III) nitrate complex, {[(Co(NH₃)₄CO₃]NO₃·H₂O} at low temperature has been reported in literature¹³. The use of organic precursors like carboxylates, oxalates, citrates, tartrates and succinates for preparing Co₃O₄ nanoparticles has already been
achieved\textsuperscript{14}. A known class of compounds called Cobalt β-diketonates are precursors which are inexpensive, non-toxic and volatile. These are employed for the metal oxide nanoparticles synthesis via thermal decomposition routes\textsuperscript{15}. The synthesis of nanoparticles of metal oxide using metal acetylacetonates is easy to prepare. We have reported the studies of iron acetylacetonates for synthesising maghemite nanoparticles\textsuperscript{16}. Herein, we report the synthesis of cobalt oxide nanoparticles using cobalt acetylacetonates. The performance of these transition metal oxide nanoparticles can be further improved if nanoparticles are supported on some frameworks\textsuperscript{17}. Therefore, coating/capping is essential as it reduces the agglomeration of the nanoparticles and improves their colloidal stability. The use of supporting materials from natural resources like plant extracts is an area of interest due to their simplicity, time reducing, non-toxic, eco friendliness and fast alternatives as compared to well-known physicochemical procedures\textsuperscript{6}. The synthesis of magnetic cobalt oxide nanoparticles based on the use of non-toxic and biodegradable polymers like dextran\textsuperscript{18} starch\textsuperscript{19} are mainly reported. Pectin is a known heteropolysaccharide which is easily soluble in water and forms gels at certain specified pH values\textsuperscript{20} but it is less explored as a capping agent for no known reasons. The source of this bio-polymer is apple pomace or citrus peel\textsuperscript{21} which consists of a homogalacturonan backbone of esterified α-(1-4)-D-galacturonic acid residues. The degree of esterification is nearly up to 75% in apple pectin and therefore it is called high methoxy pectin. The presence of different –COOH and esterified-COOCH\(_3\) functional groups could be made applicable in different ways. The chemical and structural features of this polysaccharide enable it to bind with different metals and are more reliable than the chelating agents\textsuperscript{22}. The non-toxic, no waste generation and biocompatible nature of pectin makes it more reliable\textsuperscript{23-26}. The interactions among these functional groups of pectin and metal oxide could result in the formation of a stable complex. Hence, it engrossed the use of pectin as a stabilizing/capping material, a property that researchers profit from to form new nanomaterials with immediate applications. Since pectin is a water soluble powder therefore, it removes the possible toxicity due to organic solvents which are often required for making various metal nanoparticles. This work is focused on green, low temperature decomposition and a one pot methodology for synthesizing the nanoparticles and thus stabilizing them with plant based pectin. The exact composition and structure of pectin is still under debate. The high complexity and unpredictable nature of the molecule makes it more challenging to study. Nowadays metal oxide nanoparticles also have considerable attention in the field of biomedical sciences. Therefore, the use of biopolymers to stabilize nanoparticles is trending these days. Sodium borohydride is a well-known reductant. It is used for getting metallic nanoparticles\textsuperscript{27}. Interestingly, its usage as a precipitating agent\textsuperscript{28} to get cobalt oxide nanoparticles is a less known fact. Herein we report thermal decomposition of Co(acac)\(_2\) in the presence of pectin at low temperature for the preparation of cobalt oxide nanoparticles and try to explore the role of reducing agent in the formation of these metal oxide nanoparticles. It is a simplest approach which does not require sophisticated instrumental facility and is a short time process. Further, we have assessed and reported their antimicrobial activities against different strains of bacteria, magnetic behaviour and zeta potential in the upcoming sections of this text.

**MATERIALS AND METHODS**

**Materials**

Pectin and Cobalt acetylacetonate (II) [Co(acac)]\(_2\) were obtained and used as such from sigma Aldrich. Distilled water was used as solvent in the whole procedure. NaBH\(_4\) was purchased from SD fine chemicals and was also used during the process.

**Method of preparation**

Thermal decomposition method was adopted for synthesizing cobalt oxide nanoparticles. Distilled water (0.2 L) was taken in a 500 mL round bottom flask and Pectin (0.2 g) was made soluble in it with stirring followed by gradual addition of Co(acac)\(_2\) [20 mg/2 mL in dioxane] to the above dissolved pectin. The temperature was maintained at 100-120°C throughout the process. After continuous stirring a requisite amount of NaBH\(_4\) was added. As a result, a brisk reaction occurred. High volumes of effervescence due to evolution of hydrogen gas could be observed and the reaction mixture turned black in colour. It was found that the nanoparticles were sticking to the magnetic bar which infers they have magnetic properties. After two hours the colour of the reaction mixture altered slowly from black to...
light brown. To check the dispersion of nanoparticles, this solution was ultra-sonicated and no particles got deposited. An aqueous dispersion of the nanoparticles was achieved. The above mentioned was used for further characterizations.

A thin film was collected by evaporating the solvent and it was crushed to get powdered form. This powder was kept in hot air oven at 70-80°C and was completely dried. The XRD and VSM studies were performed using this powder.

Characterization techniques

We used IR Prestige-21 (CE) FTIR Shimadzu spectrophotometer to obtain the IR spectra of the prepared samples. The crystallographic aspect of the nanoparticles was explored using Xpert PRO K-alpha [Aº] 1.54060 with 2º theta from 10.0º–89.9º X-ray diffractometer. The size and surface morphology of nanoparticles was analysed using SEM (S.3400N, Hitachi, Japan) and TEM (Technai T20 twin, TEM 200 KV FEI Netherlands). UV-Visible spectra were also recorded using quartz cuvette with Nano drop 2000 spectrophotometer.

Antimicrobial Activity

We adopted agar well diffusion method to assess the antimicrobial behaviour of cobalt oxide nanoparticles. Freshly prepared Agar plates were taken and marked 6 mm diameter wells which were six in number, were gently punched. These wells were filled using Co₃O₄ nanoparticles (5 µL, 10 µL, 20 µL, 30 µL and 40 µL) and were subjected for overnight incubation at 37°C. The zone of inhibition (ZOI) was measured to evaluate the bactericidal activity of Co₃O₄ nanoparticles.

Magnetic measurements

Magnetic behavior was studied at room temperature using Microsense VSM.

Zeta Potential

The Zeta potential analysis was done using Malvern Zeta sizer ver.7.1 at room temperature.

RESULTS AND DISCUSSION

Cobalt oxide nanoparticles were fabricated by thermal decomposition starting with Co(acac)₃, this method of synthesis has been generally reported to be used along with higher temperature and pressure conditions for decomposing transition metal complexes but here we used rather low temperature and atmospheric pressure conditions. The adoption of green approach was our main focus therefore water in distilled form was used as a solvent and we hence bypassed the use of organic solvents completely. In continuation, on addition of sodium borohydride, a forceful reaction occurred possibly owing to the evolution of hydrogen gas resulting in effervescence. The colour of the reaction mixture became black it could be compared with the bare metal oxide nanoparticles. After a period of two hours of stirring, it became light brown in color. The possible explanation for color transformation can be due to the binding of these cobalt oxide nanoparticles with the biopolymer which is pectin in our case.

Fig. 1. X-ray diffractogram for the cobalt oxide nanoparticles

X-ray diffraction

The X-ray diffractogram of cobalt oxide nanoparticles is shown in Fig.1. The diffractogram shows very less number of sharp Bragg peaks and is noiser in appearance. The only peaks at 20.2º, 29.7º and 35.8º for Co₃O₄ could be noticed and were matched with cards [JCPDS:74-1656]. The low temperature synthesis can be a reason for such kind of a wide range spectrum which appears to be amorphous in nature. Moreover, the use of pectin which is itself a less crystalline material added up to above mentioned fact. The additional peak (12.6º) was also figured out which is marked as characteristic peak for pectin. This new combination of peaks presents for pectin and cobalt oxide nanoparticles marked the effective binding of these two entities.

Pectin from apple pomace is available in powdered form and is known to have less
crystalline structure. The preparation of cobalt oxide nanoparticle is done at low temperature and nanoparticles are stabilized by pectin, therefore some of the peaks in this spectrum do not appear clearly. In addition to this spectrum appears a bit bumpy and sharp peaks could not be seen. This kind of spectrum is recorded when the analyzed sample is less crystalline. All the above mentioned facts are clearly seen in the given XRD spectrum. The shift in the diffraction peaks is an additional phenomenon which is reported in literature for modified structure of nanoparticles. In our case this still remains unknown if there is a shifting of peaks due to any kind of structure modification.

This engrossed our attention and we proposed the capping/stabilizing of nanoparticles by pectin. The mean size of the particle was found to be 10.5 nm. as calculated using Scherrer’s equation.

**Morphological analysis using SEM and TEM**

The surface morphology and shape of the dispersed synthesized nanoparticles was studied using SEM. Some dense agglomerates with polycrystalline shapes were found due to large surface area as shown in Fig. 2. This appears as if nanoparticles are being caught in the clumsy network of pectin which is attributed to the dense and gel-like nature of pectin. Therefore, it was inferred that that less dispersive nanoparticles were formed and pectin effectively bound the nanoparticles. The shape of the nanoparticles was found to be close to spherical.

The chemical purity and stoichiometry was examined using EDX as shown in Fig. 3. The strong peaks for cobalt and oxygen were found in the spectrum. The peak for oxygen along with the cobalt confirms the sample as cobalt oxide. The Cu peaks appeared as we had used copper grids during the analysis.

**Fourier Transform Infrared Spectroscopic analysis**

The FTIR spectra of cobalt oxide nanoparticles was performed to figure out the different functional groups present. The FTIR spectrum as shown in Fig. 5 illustrates that the bands for Co-O bonds in the Far IR region of the spectra in between 640-400 cm⁻¹ are mainly the characteristics of the metal oxide bonds. The major absorption at 532 cm⁻¹ can be ascribed to Co₃O₄ nanoparticles. In the region 3000-3550 cm⁻¹ are due to the (O–H) stretching vibration of the hydroxyl group of water. The band at 1737 cm⁻¹ is assigned to the carbonyl (C=O) stretching vibration of an ester present in pectin. The band at 1085 cm⁻¹ is due to the bending vibration of C-O present in pectin. This FTIR
assured the presence of cobalt oxide nanoparticles and pectin together, therefore this inference was drawn that pectin has stabilized/capped Co$_3$O$_4$ nanoparticles effectively.

as can be seen in Fig. 7 shows that cobalt oxide nanoparticles have ferromagnetic behavior.

UV-Visible spectroscopy

The next Fig. 6 shows the UV-Visible spectrum of cobalt oxide nanoparticles. In this region of spectrum, the electronic transitions are generally studied. The UV-Visible spectra of metal oxide nanoparticles generally have a long tail extending towards the longer wavelength due to their quantum size effects. The similar behavior was noticed in the case of cobalt oxide nanoparticles when their UV-Visible spectra were recorded. The two bands were observed in the region of 250-350 nm and 400-600 nm$^2$ and this confirmed the existence of cobalt oxide nanoparticles.

Magnetic properties

For the better understanding of the nature of these synthesized Co$_3$O$_4$ nanoparticles, the magnetic analysis was performed at room temperature (300 K). The magnetization curve

The coercivity of these nanoparticles was very low as compared with the bulk Co$_3$O$_4$ which has antiferromagnetic behavior$^9$. The coercivity is known to vary linearly with the size of nanoparticles. The low Ms values confirm the change from antiferromagnetic bulk to ferromagnetic cobalt oxide nanoparticles$^{40}$. The cobalt oxide nanoparticles here have lower values of magnetization and Mr/Ms ratio. They exhibit ferromagnetic behavior at room temperature. These metal oxide nanoparticles consist of small crystalline domains. These magnetic domains are connected together by dipolar interactions; their sum is equal to the magnetic moment. This is well known that better crystallites support higher Ms values. We stabilized these nanoparticles using pectin, being less crystalline in nature it contributes towards the lowering of magnetization.

Antimicrobial activity

The nanoparticles these days are evaluated differently in many ways to check their antimicrobial activities and biomedical applicability. This has drawn our attention to investigate the antibacterial activity
for cobalt oxide nanoparticles against the following strains E. coli, S. typhii, S. aureus, P. aerugenosa, K. pneumoniae, B. cereus. The Agar well diffusion was adopted to investigate their antimicrobial behaviour. This was confirmed that cobalt oxide showed some antibacterial activity in case of all the strains of the bacteria. The results were most satisfactory against E. coli and S. typhii strains of bacteria. The results are listed below in Table 2.

Zeta potential analysis

ζ potential is the potential difference and it measures the surface charge of the colloidal particles and is an important parameter to know the stability of such particles. The analysis of zeta potential gives information regarding the stability of the colloidal particles. The particles which are small enough, there must be considerable value of their zeta potential to be stable. When the potential is low, there is more attraction and there will be agglomeration.

Table 2: Antimicrobial behaviour of Co₃O₄ nanoparticles

| S.No | Micro-organisms | Concentration (µL) and Zone of inhibition (mm) |
|------|-----------------|-----------------------------------------------|
|      |                 | Standard 10 µL 20 µL 30 µL 40 µL (5 µL)       |
| 1    | S. typhii       | 8 mm 3 mm 4 mm 10 mm 10 mm                     |
| 2    | E. coli         | 14 mm 5 mm 10 mm 12 mm 14 mm                   |
| 3    | S. aureus       | 10 mm 5 mm 5 mm 5 mm 8 mm                      |
| 4    | P. aeruginosa   | 5 mm 2 mm 2 mm 2 mm 5 mm                       |
| 5    | K. pneumoniae   | 5 mm 2 mm 4 mm 4 mm 5 mm                       |
| 6    | B. cereus       | 4 mm 2 mm 2 mm 2 mm 2 mm                       |

We demonstrate here that pectin supported Co₃O₄ nanoparticles show antibacterial potency against S. typhii and E. coli. The exact mechanism and action is speculative; it could be assumed that superoxides and peroxide are some ROS (Reactive oxygen species) which are thought to be responsible for the possible genetic transformations caused in the bacterial cell like proteins and DNA. The inorganic antibacterial agents are more stable at high temperature and pressures as compared to the other agents. We have observed here antimicrobial behavior of cobalt oxide nanoparticles synthesized at low temperature. The use of pectin in supporting these nanoparticles makes them more favorable for biomedical usage. The results of our study opens new prospects regarding the possible potential of cobalt oxide nanoparticles in biomedical applications.
CONCLUSION

Cobalt oxide (Co₃O₄) nanoparticles with a mean size of 10.5 nm were fabricated successfully using facile thermal decomposition at 100-120°C. Pectin as a capping agent was used to prevent the agglomeration of Co₃O₄ nanoparticles and it stabilized them very well. Based on the data obtained from FTIR and XRD the binding of pectin with the particles is confirmed and well elaborated. The UV-Visible absorption spectrum also relates with the other observations. The morphological characterization by SEM and TEM have shown dense agglomerates and some spherical shape of the particles. The magnetic studies using VSM helped in exploring the ferromagnetic behavior, thus their magnetic applications remain speculative. The other application of the nanoparticles was found in the biomedical area of science as they possess some anti-bactericidal properties. The use of apple pectin and distilled water throughout the synthesis makes it a green synthesis which is also eco-friendly and cost effective method. The Zeta potential analysis revealed the stability of colloidal dispersion in water, it was found that the nanoparticles dispersion is stable at pH 5.5. In future work we head towards exploring antimicrobial aspect in detail and drug delivery is another area to be covered. Furthermore, our keen focus will be on waste management and greener technology.

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Conflict of interest

The corresponding author states that there is no conflict of interest.

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