



Pectin Stabilized/Capped Ferromagnetic Co_3O_4 Nanoparticles with Antimicrobial Efficacy: A Greener Approach

POONAM RANA^{1*}, KAMALIKA BANERJEE¹, SAURABH SHARMA² and RAJNI SHARMA³

¹Indira Gandhi National Open University, New Delhi 110068 India.

²Natural Product Chemistry and Process Development Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur-176061, H. P, India.

³DAV College Sector 10, Chandigarh, India.

*Corresponding author E-mail: poonamrana1112@gmail.com

<http://dx.doi.org/10.13005/ojc/380201>

(Received: November 10, 2021; Accepted: March 23, 2022)

Abstract

The preparation of Co_3O_4 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, catalysis⁴⁻⁵ and biomedical applications⁶. 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, Co_3O_4 is the prominent nanostructure which has been synthesized with different morphologies using the previously mentioned methods.

The synthesis of Co_3O_4 nanoparticles using thermal decomposition of carbonatotetra(amine) cobalt(III) nitrate complex, $\{[\text{Co}(\text{NH}_3)_4\text{CO}_3]\text{NO}_3 \cdot \text{H}_2\text{O}\}$ at low temperature has been reported in literature¹³. The use of organic precursors like carboxylates, oxalates, citrates, tartrates and succinates for preparing Co_3O_4 nanoparticles has already been



achieved¹⁴. 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 routes¹⁵. 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 nanoparticle¹⁶. 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 frameworks¹⁷. 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 procedures⁶. The synthesis of magnetic cobalt oxide nanoparticles based on the use of non-toxic and biodegradable polymers like dextran¹⁸ starch¹⁹ are mainly reported. Pectin is a known heteropolysaccharide which is easily soluble in water and forms gels at certain specified pH values²⁰ but it is less explored as a capping agent for no known reasons. The source of this bio-polymer is apple pomace or citrus peel²¹ 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 $-\text{COOH}$ 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 agents²². The non-toxic, no waste generation and biocompatible nature of pectin makes it more reliable²³⁻²⁶. 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 nanoparticles²⁷. Interestingly, its usage as a precipitating agent²⁸ to get cobalt oxide nanoparticles is a less known fact. Herein we report thermal decomposition of $\text{Co}(\text{acac})_2$ 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) [$\text{Co}(\text{acac})_2$] 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 $\text{Co}(\text{acac})_2$ [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 [A°] 1.54060 with 2° theta from 10.0°–89.9° 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 method²⁹ 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)₂, 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 nanoparticles³⁰. 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.

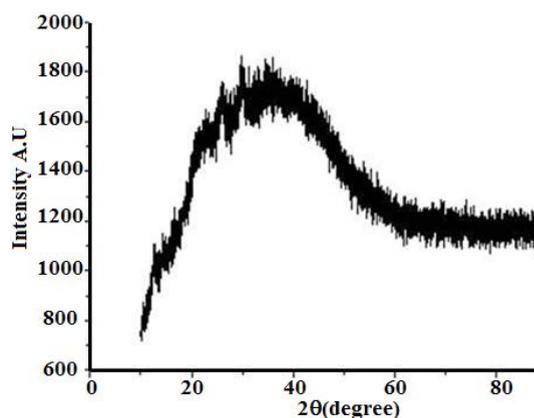


Fig. 1. X-ray diffractogram for the cobalt oxide nanoparticles
X-ray diffraction

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 noisier in appearance. The only peaks at 20.2°, 29.7° and 35.8°³¹ for Co₃O₄ could be noticed and were matched with cards [JCPDS:74-1656]³². The low temperature synthesis can be a reason for such kind of a wide range spectrum which appears to be amorphous in nature^{33,34}. Moreover, the use of pectin which is itself a less crystalline material also added up to above mentioned fact. The additional peak (12.6°) was also figured out which is marked as characteristic peak for pectin³⁵. 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 structure³⁵. 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 peaks³⁰ 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.³⁶

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.

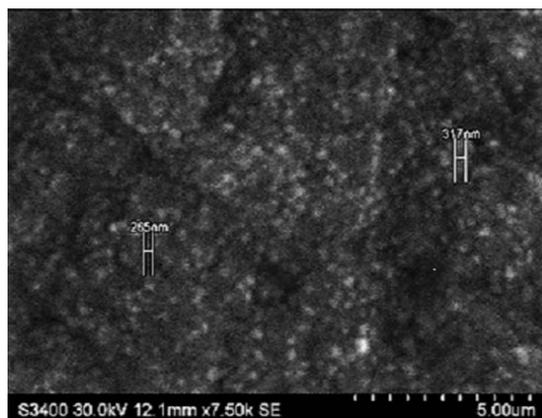


Fig. 2. SEM photomicrograph of cobalt oxide nanoparticles

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.

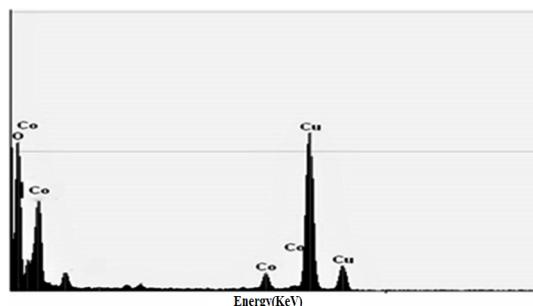


Fig. 3. EDX spectrum of cobalt oxide nanoparticles

The TEM micrograph, as in Fig. 4 shows less dispersed nanoparticles and a cloudy pattern due to the presence of pectin. The particles appear to have polycrystalline phases due to the compact arrangement of nanoparticles. The dense collectives of nanoparticles were noticed. The molecular weight and the size of pectin which is polymeric in nature can be the reason for this hazy micrograph. The compact arrangement of particles can be clearly seen. The particle size range is 10 nm -30 nm.

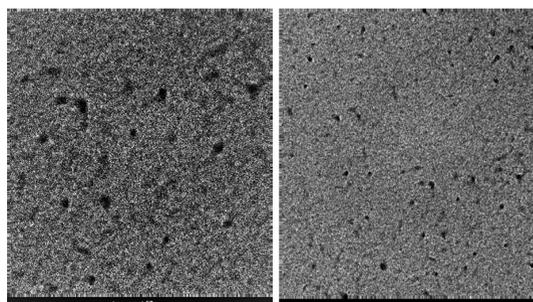


Fig. 4. TEM micrograph of cobalt oxide nanoparticles

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 Co_3O_4 nanoparticles³³. In the region 3000-3550 cm^{-1} are due 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 pectin³⁷. This FTIR

assured the presence of cobalt oxide nanoparticles and pectin together, therefore this inference was drawn that pectin has stabilized/capped Co_3O_4 nanoparticles effectively.

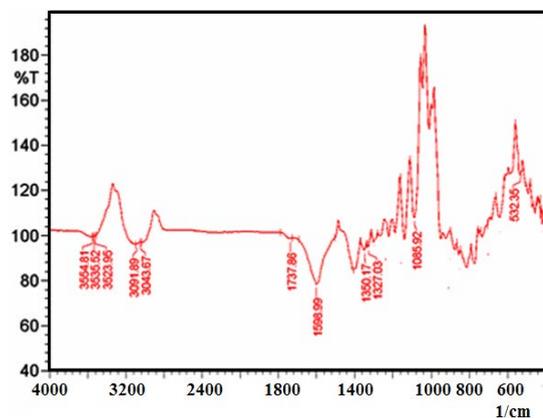


Fig. 5. FTIR spectrum of cobalt oxide nanoparticles

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 nm³⁸ and this confirmed the existence of cobalt oxide nanoparticles.

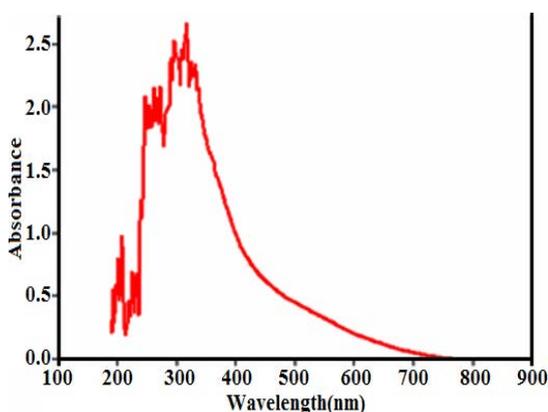


Fig. 6. UV-Visible spectrum of cobalt oxide nanoparticles

Magnetic properties

For the better understanding of the nature of these synthesized Co_3O_4 nanoparticles, the magnetic analysis was performed at room temperature (300 K). The magnetization curve

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

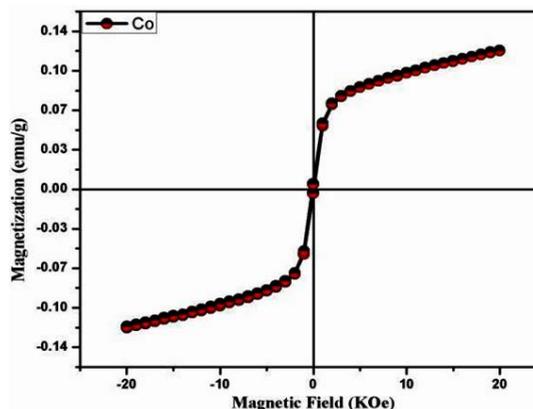


Fig. 7. Magnetic hysteresis (300 K) for cobalt oxide nanoparticles

The values of saturation magnetization (M_s) was found 0.119 emu/g with 83.09 of coercivity $H_c(\text{Oe})$ and zero remnant magnetization (M_r). All values are listed in Table 1.

Table 1: Saturation magnetization (M_s), Remanence magnetization (M_r), Coercivity (H_c), Remanence ratio (M_r/M_s)

Sample name	M_s (emu/g)	M_r (emu/g)	H_c (Oe)	M_r/M_s
Co	0.119	0.004	83.09	0.0336

The coercivity of these nanoparticles was very low as compared with the bulk Co_3O_4 which has antiferromagnetic behavior³⁹. The coercivity is known to vary linearly with the size of nanoparticles. The low M_s values confirm the change from antiferromagnetic bulk to ferromagnetic cobalt oxide nanoparticles⁴⁰. The cobalt oxide nanoparticles here have lower values of magnetization and M_r/M_s 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 M_s 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. aeruginosa*, *K. pneumoniae*, *B. cereus*. The Agar well diffusion⁴¹⁻⁴³ 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.

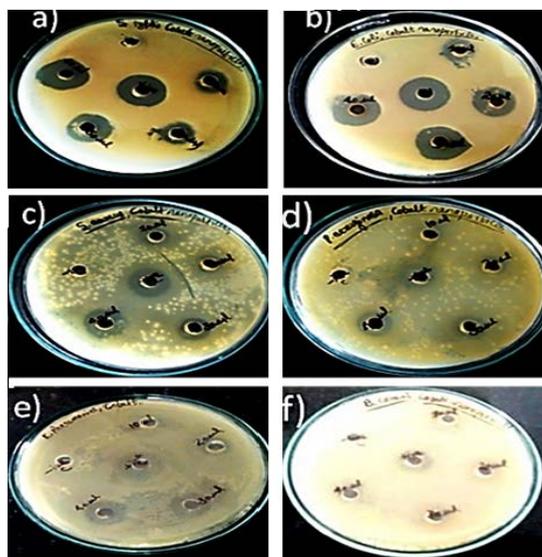


Fig. 8. ZOI (zone of inhibitions) for cobalt oxide nanoparticles a) *S. typhii* b) *E.coli* c) *S. aureus* d) *P. aeruginosa* e) *K. pneumoniae* f) *B. cereus*

Table 2: Antimicrobial behaviour of Co_3O_4 nanoparticles

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

We demonstrate here that pectin supported Co_3O_4 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 DNA⁴⁴. 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.

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 particles⁴⁵. 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.

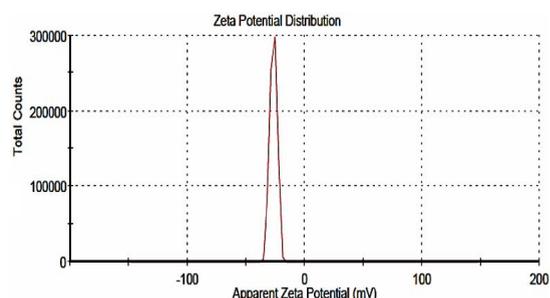


Fig. 9. Zeta potential of cobalt oxide nanoparticles at pH 5.5

It is known that nanoparticles with zeta potentials at IEP (Iso electric point) aggregate easily. So, it becomes necessary to see how stability is affected by pH. We did Zeta potential analysis and in our case of cobalt oxide nanoparticles the zeta potential at pH5.5 was recorded 26 mV. The lowering of pH to a value of 2.8 as shown in Fig.10 resulted in zero value of zeta potential which means the colloidal instability of the particles. Therefore, it was found that colloidal suspension was stable at the pH of 5.5 as shown in Figure 9.

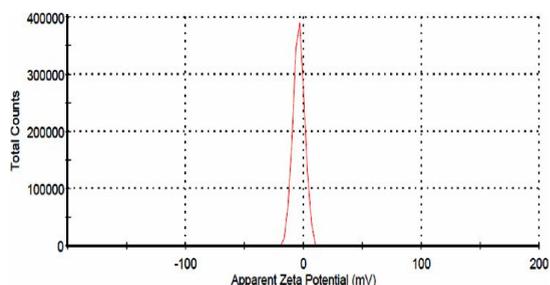


Fig. 10. Zeta potential of cobalt oxide nanoparticles after lowering the pH

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.

ACKNOWLEDGEMENT

The main idea was conceived by Dr. Poonam Rana and all authors contributed to the study and material preparation. The data was collected by Dr. Saurabh Sharma, Dr. Rajni Sharma and Dr. Kamalika Banerjee. The results were analyzed by Dr. Poonam Rana. All the authors gave their inputs and the first draft was written by Dr. Poonam Rana. All authors approved the final manuscript. We acknowledge Indira Gandhi National Open University (IGNOU) New Delhi 110068 for providing the necessary help. Our gratitude to the director of CSIR-IHBT Palampur H. P. India for supporting us with the different facilities for characterisation. For VSM sample analysis we are thankful to HP University Shimla H.P India.

Conflict of interest

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

REFERENCES

1. Yuan, W.; Xie, D.; Dong, Z.; Su, Q.; Zhang, J.; Du, G.; Xu, B. *Mater. Lett.*, **2013**, *97*, 129-132.
2. Sun, C.; Su, X.; Xiao, F.; Niu C.; Wang J. *Sens. Actuators B Chem.*, **2011**, *157*, 681-685.
3. Yuanchun, Q.; Yanbao, Z.; Zhishen, W. *Mater. Chem. Phys.*, **2008**, *110*, 457-462.
4. Dong, Y.; Wang, G.; Jiang, P.; Zhang, A.; Yue, L.; Zhang, X. *Bull Korean Chem Soc B.*, **2010**, *31*, 2830–2834.
5. Liang, Y.; Li, Y.; Wang, H.; Zhou, J.; Wang, J.; Regier, T.; Dai, H. *Nat. Mater.*, **2011**, *10*, 780–786.
6. Iravani, S.; Varma, R. S. *Green Chem.*, **2020**, *22*, 2643-61.
7. Lakshmi, B. B.; Patrissi, C. J.; Martin, C. R. *Chem. Mater.*, **1997**, *9*, 2544–2550.
8. Baydi, M. E.; Poillierat, G.; Rehspringer, J. L.; Gautier, J. L.; Koenig, J. F.; Chartier, P. *J. Solid State Chem.*, **1994**, *109*, 281.
9. Pirovano, C.; Trassatti, S. *J. Electroanal. Chem.*, **1984**, *180*, 171.
10. Sakamoto, S.; Yoshinaka, M.; Hirota, K.; Yamaguchi, O. *J. Am. Ceram. Soc.*, **1997**, *80*, 267-268.
11. Singh, R. N.; Koenig, J. F.; Poillierat, G.; Chartier, P. *J. Electroanal. Chem.*, **1990**, *137*, 1480-1413.
12. Singh, R. N.; Hamdani, M.; Koenig, J. F.; Poillierat, G.; Gautier, J. L.; Chartier, P. *J. Appl. Electrochem.*, **1990**, *20*, 442-446.
13. Farhadi, S.; Javanmard, M.; Nadri, G. *Acta Chim. Slov.*, **2016**, *63*, 335–343.
14. Smyrnioti, M.; Ioannides, T. Synthesis of Cobalt-Based Nanomaterials from Organic Precursors. chapter 3 page 49-69.
15. Kouotou, P. M.; Tian, Z. Y.; Mundloch, U.; Bahlawane, N.; Hoinghaus, K. K. *RSC Adv.*, **2012**, *2*, 10809-10812.
16. Rana, P.; Sharma, S.; Sharma, R.; Banerjee, K. *Mater. Sci. Technol.*, **2019**, *2*, 15-21.
17. Zhou, H.; Kitagawa, S. *Chem. Soc. Rev.*, **2014**, *43*, 5415-5418.
18. Yi-Yeoun, Kim.; Fiona, C. *Polym. Chem.*, **2011**, *2*, 1375-1379.
19. Vaya, D.; Das B. K. *Nanosci Nanotechnol.*, **2019**, *9*, 362-70.

20. 43. Gawkowska, D.; Cybulska, J.; Zdunek, A. *Polymers.*, **2018**, *10*, 762.
21. Thakur, B. R.; Singh, R. K.; Handa, A. K.; Rao M. A. *Crit. Rev. Food Sci. Nutr.*, **1997**, *37*, 47-73.
22. Claudia, L. E.; Elizabeth, C. M.; Rene, B. Q.; Yolanda, L. F.; Agustin, R. C. *Mol.*, **2018**, *23*, 942.
23. Sivagnanama, T. U.; Perumald, T. P. *RSC Adv.*, **2016**, *00*, 1-13.
24. Das, S.; Chaudhury, A.; Ng, K. Y. *J. Microencapsul.*, **2011**, *28*, 268–279.
25. Liu, L.; Fishman, M. L.; Kost, J.; Hicks, K. B. *Biomaterials.*, **2003**, *24*, 3333-3343.
26. Meneguina A. B.; Cury, B. S. F.; Evangelista, R. C. *Carbohydr. Polym.*, **2014**, *99*, 140-149.
27. Huang, K. C.; Ehrman, S. H. *Langmuir.*, **2007**, *23*, 1419-26.
28. Nayak, N. B.; Nayak, B. B. *Sci. Rep.*, **2016**, *6*, 23175.
29. Balouiri, M.; Sadiki, M.; Ibensouda, S. K. *J. Pharm. Anal.*, **2016**, *6*, 71–79.
30. Koli, P. B.; Kapadnis, K. H.; Deshpande, U.G. *J Nanostruct Chem.*, **2018**, *8*, 453-463.
31. Niasari, M. S.; Khansari, A.; Davar, F. *Inorganica Chim. Acta.*, **2009**, *362*, 4937-4942.
32. Sun, J.; Zhang, Q.; Wang, X.; Zhao, J.; Guo, J.; Zhou, Z.; Zhang, J.; Man, P.; Sun, J.; Li, Q.; Yao, Y. *J. Mater. Chem. A.*, **2017**, *5*, 21153-21160.
33. Muhammad, H.; Shaheen, R.; Akram, B.; Abdin, Z.; Haq, S.; Mahsud, S.; Ali, S.; Khan, R. *Mater. Res. Express.*, **2020**, *7*, 025019.
34. Haq, S.; Abbasi, F.; Ben Ali, M.; Hedfi, A.; Mezni, A.; Rehman, W.; Waseem, M.; Khan, A.R.; & Shaheen, H. *Mater. Res. Express.*, **2021**, *8*, 075009.
35. Lutz, R.; Aserin, A.; Wicker, L.; Garti, N. Structure and physical properties of pectins with block-wise distribution of carboxylic acid groups *Food Hydrocolloids.*, **2009**, *23*, 786–794.
36. Klug, H. P.; Alexander, L. E. *X-ray Diffraction Procedures.*, **1970**, *514*, Wiley, New York
37. Namanga, J.; Foba, J.; Ndinteh, D. T.; Yufanyi, D. M.; Krause, R. W. M. *J. Nanomater.*, **2013**, *87*, 1–8.
38. Xu, R.; Zeng, H. C. *Langmuir.*, **2004**, *20*, 9780–9790.
39. Farhadi, S.; Safabakhsh, J.; Zaringhadam, P. *J. Nanostructure Chem.*, **2013**, *3*, 69.
40. El-Okr, M. M.; Salem, M. A.; Salim, M. S.; El-Okr, R. M.; Ashoush, M.; Talaat, H. M. *J. Magn. Mater.*, **2011**, *323*, 920–926.
41. Magaldi, S.; Essayag, S. M.; Capriles, C. H. D.; Perez, C.; Colella, M. T.; Olaizola, C.; Ontiveros, Y. *IJID.*, **2004**, *8*, 39-45.
42. Valgas, C.; Souza, S. M. D.; Smania, EFA. *Braz. J. Microbiol.*, **2007**, *38*, 369-380.
43. Balouiri, M.; Sadiki, M.; Ibensouda, S. K. *J. Pharm. Anal.*, **2016**, *6*, 71–79.
44. Bhushan, M.; Kumar, Y.; Periyasamy, L.; Vishwanath, A. K. *Appl. Nanosci.*, **2018**, *8*, 137–153.
45. Mikolajczyk, A.; Gajewicz, A.; Rasulev, B.; Schaeublin, N.; Maurer-Gardner, E.; Hussain, S.; Puzyn, T. *Chem. Mater.*, **2015**, *27*, 2400–2407.