Original Research Article

Green Synthesis of Copper Chitosan Nanoparticles for Controlled Release of Pendimethalin

Abstract

Aim: In a bid to reduce the environmental impact from the use of herbicides, chitosan was used for the synthesis of copper nanoparticles and the controlled release formulations (CRFs) of pendimethalin copper-chitosan nanoparticles (Pend-CuCtsNPs).

Methodology: The synthesized nanoparticles were characterized using UV-visible spectroscopy, Fourier Transform Infra-Red (FT-IR), Powder X-ray diffraction (PXRD), Transmission electron microscopy (TEM) and Energy-dispersive x-ray (EDX). Average crystalline size of the nanoparticles was estimated from the Debye- Scherrer's equation.

Results: The yield of the synthesized CuCtsNPs increased linearly with the weight of the starting material with percentage yield of 93.8% for the 0.8% chitosan matrix. Encapsulation efficiency of the nano-formulation fell within 57.5 and 92.7%. The aqueous release studies of Pend-CuCtsNPs, monitored for 96 hours in a batch release mode were carried out in three different pH media and percentage herbicide released for all composites showed that the release in pH 5.5 (acidic) medium was higher and the lowest release was recorded for pH 7.0 (neutral) medium. There was a statistically significant difference between pH groups and time as determined by UNIANOVA ($p < .005$).

Conclusion: Findings from this study however shows that there is a controlled release of pendimethalin using nano-formulation over the conventional herbicide application.

Keywords: Chitosan; pendimethalin; encapsulation; controlled release; nanoparticles

1. INTRODUCTION

Plants constitute the world's primary food source and for this reason, there has been a tendency in recent years to maximize agricultural yields due to growth in human population. The increase in productivity has been achieved through the development of new high yield crops, the heavy use of fertilizers and pesticides/herbicides, and the use of heavy agricultural machinery [1]. The benefits obtained through the use of these agricultural improvements are irrefutable but several issues have been raised concerning the environmental damage generated from such processes. The intensive use of herbicides and other classes of chemicals products, in agricultural practice has resulted in serious impacts on the environment, causing an increase in the level of herbicide residues in natural water sources, soil, and foodstuff [2].

To reduce the influence of these factors on the environment (soil and surface/groundwater), one approach is to use controlled release formulations (CRFs). This represents an alternative to the conventional systems of herbicide application. It is a technology wherein an active ingredient is available for a specific goal at a concentration [3] and with a duration designed to achieve the intended effect, aiming at reaching optimal biological effectiveness and to reduce any harmful effects [4].

Copper nanoparticles (Cu-NPs), due to their excellent physical and chemical properties and low cost of preparation, have been of great interest compared to other metal nanoparticles. Copper have wide applications as heat transfer systems, antimicrobial materials, super strong materials, sensors and catalysts [5]. They are very reactive because of their high surface-to-volume ratios which can easily interact with other particles [6]. These attributes led to the development of alternative pathways to synthesize metal nanoparticles in the presence of polymers (eg,

polyvinylpyrrolidone, polyethylene glycol, and chitosan) and surfactants (acetyltrimethyl ammonium bromide) as stabilizers, and to form coatings on the surface of nanoparticles.

Chitosan is a natural product derived from chitin, a polysaccharide foundation in the exoskeletons of shellfish like shrimps and crabs [7]. It is a cationic polysaccharide composed of β-(1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine units. Generally, the use of chitosan as stabilizer for the synthesis of Cu-NPs is gaining momentum because of their availability, biocompatibility [8], hydrophilicity, nontoxicity, biodegradability, adsorption properties [9,10], bioactive polymer and drug absorption enhancement [11], environmental-friendly material with many superior properties with momentous amounts of amine and hydroxyl groups that can be easily engineered [8].

Pendimethalin (*N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidene) is a selective herbicide in both preemergence [2] and early postemergence to control most of annual grasses and certain broadleaf weeds in several crops [12] and grassy weed species in a number of crop and noncrop areas, on residential lawns and ornamentals [13]. Pendimethalin degrades slowly in aerobic soil and rapidly in anaerobic soil conditions and is classified as a non-leaching compound [14].

Encapsulation with nanoparticles (nanoencapsulation) is a process through which chemicals like pesticides are slowly but efficiently released to a particular host plant for pest control using nanoparticles. It allows for proper absorption of the chemicals into the plants [15]. Release mechanisms of nanoencapsulation include diffusion, dissolution, biodegradation and osmotic pressure with specific pH and is currently the most promising technology for protection of host plants against insect pests [16].

In this work, the direct, simple and effective green synthesis, characterization and aqueous release of Pendimethalin from copper chitosan nanoparticles is reported. Copper-chitosan nanoparticles was conjugated with Pendimethalin herbicide and characterized using TEM, EDX, FT-IR and XRD techniques. Batch release experiment of pendimethalin from copper chitosan nanoparticles were monitored using UV-visible spectrophotometer in a batch release studies in different buffer pH media.

2. MATERIALS AND METHODS

2.1 Materials

All chemicals procured for this work were obtained and used without further purification. Pendimethalin with trade name Pendilin® was supplied by Africa Agro commodities Ltd, Nigeria. Practical grade CuSO4·5H2O was obtained from Griffin and George (England). Chitosan (high molecular weight) and hydrazine (N2H4, 98.0%) were supplied by Sigma Aldrich (St. Louis, MO, USA). NaOH (99.0%) was supplied by Chem Light (India). Glacial acetic acid (99.5%), ascorbic acid and methanol are other reagents used. The release media pH 5.5, 7.0 and 8.0 buffers were prepared from potassium hydrogen phthalate, potassium dihydrogen orthophosphate, sodium hydroxide and hydrochloric acid. Characterizations were carried out using UV-Vis spectrophotometers (Jenway 7305 and Raylable 735), FEI Tecnai T20 FETEM (FEI company, USA), Bruker ALPHA FT-IR spectrophotometer (Bruker corporation, USA) and Philip Powder X-ray diffractometer (Netherlands, UK).

2.2 Methods

2.2.1 Synthesis of Cu-NPs in Chitosan by Chemical Reduction Method

Method earlier presented by Muhammad *et al.* [17] for synthesis of Cu-NPs was adopted in this research with slight modification. A solution of 0.4 M $CuSO_4 \cdot 5H_2O$ was prepared and 25 mL of solution was measured and added in drops to 100 mL chitosan solution with resultant light blue colour indicating copper chitosan complex formation. The mixture was stirred and heated using hotplate at 120 °C for about 20 min. A 2.5 mL aliquot of the 0.2 M ascorbic acid was added to the mixture with further stirring for 10 min without any colour change observed. Then, 5 mL of 0.6M NaOH was added to the mixture with further heating and stirring for 10 min to give a resultant green colour. Finally, 2.5 mL of 0.2 M hydrazine was added and the colour changed immediately to light red which gave a deep red colour with further heating showing the formation of copper nanoparticles. The solution was stirred for further 30 min for the reaction to go to completion. The reaction mixture was allowed to cool at ambient temperature. The solution was centrifuged at 3400 rpm for 20 min to obtain the Cu-NPs and the supernatant was decanted. The residual particles were properly washed with distilled water to ensure purity. The washed NPs were oven dried at 50°C for 24 hours.

2.2.2 Pendimethalin loading on Copper Chitosan Nanoparticles

A method previously described by Malathi *et al.* [18] was followed for the loading of Pendimethalin. Pendimethalin (0.05 g) i.e 0.1 mL of Pendilin® was added to 20 mL of methanol:water solution to form pendimethalin solution. Chitosan capped Cu-NPs (1.0 g) were dissolved in 10 mL of distilled water. The pendimethalin solution was added to CuCtsNPs aqueous solution. The resultant mixture was stirred and left standing overnight to remove the solvent. The Pendimethalin encapsulated CuCtsNPs were harvested by centrifugation at 3400 rpm for 30 min, washing was done thrice with distilled water and after final washing; Pend-CuCtsNPs obtained was dried in an oven at 40°C for 6 hours. The dried loaded NPs were properly stored in vacuum desiccator for further use.

2.3 Characterizations of synthesized and loaded nanoparticles

The maximum absorption band of CuCtsNPs and Pend-CuCtsNPs release studies were monitored using UV-visible spectrophotometer (Jenway 7305 and Raylable 735). The spectral were recorded over a range of 530-650 nm. The possible molecular interaction(s) between the plain chitosan, synthesized copper nanoparticles and the pendimethalin loaded nanoparticles was done using Bruker ALPHA FT-IR spectrophotometer (Bruker Corporation, USA) and source performing scan was carried out in the range of 430 – 4000 cm^{-1}. The crystallographic analysis was done by utilizing XRD. The diffractions of the sample were taken using Philips Powder X-ray diffractometer (Netherland, UK) with Cu Kα radiation (λ = 1.5146 Å) source performing scan range of 5 – 90° with 2θ scan step size of 0.001 and time of 1s. The morphology and particle size distribution of the synthesized copper nanoparticles and the Pendimethalin loaded nanoparticles were conducted using the FEI Tecnai T20 FETEM (FEI Company, USA) operating at an accelerating voltage of 200 kV. The instrument was further coupled with an Energy-dispersive X-ray (EDX) detector for elemental composition analysis.

2.4 Aqueous Release Experiments

Method earlier presented by Balcerzak and Mucha [19] was adopted with slight changes and the method was used both for the release in water and in different pH (pH 5.5, 7.0 and 8.0) media. 0.5 g of Pend-CuCtsNPs was placed in a vial containing 25 mL of gently mixed medium at ambient temperature. The mixture was shaken and at definite time intervals, sample of dissolution medium (3 mL) was withdrawn and replaced with fresh medium (3 mL) to maintain

sink condition. The time intervals were 5, 10, 30, 60, 1440 and 5760 min. The withdrawn mixture was analyzed by means of UV-Vis spectrophotometer for the amount released. Concentration of herbicide released from composite matrix at a characteristic pH was calculated from the calibration plot of pendimethalin.

3. RESULTS AND DISCUSSION

The formation of Cu-NPs was followed by a series of colour changes during synthesis. These different stages were characterized by colours which are not abnormal to the chemical reactions in the process.

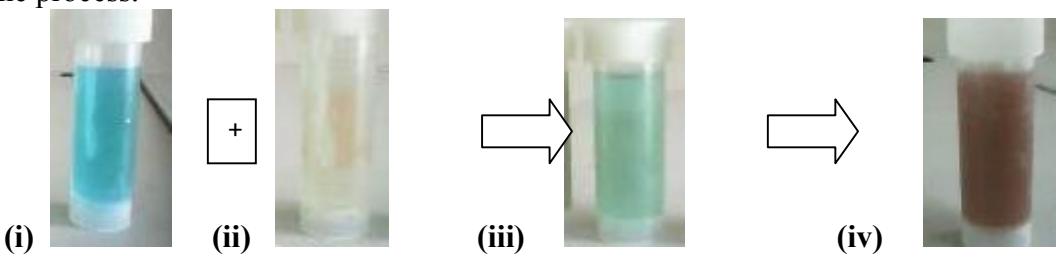


Plate 1: Colour Images of Reaction Mixture (Different stages of synthesis showing colour variations) in (i) $CuSO_4.5H_2O$(aq) (ii) Cts(aq) (iii) $[Cu(Cts)]^{2+}$(aq) (iv) [Cu(Cts)]NPs

During synthesis, addition of copper sulphate solution to chitosan solution in drops resulted in change of colour from blue to light blue (Plate 1). The light blue colour indicates the formation of copper chitosan complex $[Cu(Cts)]^{2+}$. The colour changed slightly to green on the addition of sodium hydroxide solution and a permanent deep red colour on addition of hydrazine. This was not different from that obtained by other researchers [8,17,20]. The formation of colour in reaction solution as observed in this work arises from excitation of Surface Plasmon vibration in the metal nanoparticles [21]. The choice of copper sulphate pentahydrate ($CuSO_4.5H_2O$) as precursor for this work was because of its easily oxidizable nature enhances nanoscale structures [20].

3.1 UV-Visible Spectrophotometric Analysis

The UV- visible spectrophotometric plot of the synthesized CuCtsNPs and Pend-CuCtsNPs are shown in Fig. 1 with maximum absorption band at 570 nm and 580 nm respectively. These may likely be a characteristic of Surface Plasmon Resonance (SPR) for copper nanoparticles. This was the first evidence to show that the product obtained was Cu-NPs [6]. It was generally suggested that Cu-NPs are known to show an SPR band in the 570- 600 nm since the precise SPR band position is not known [8,20]. The characteristic maximum absorption band observed for Pend-CuCtsNPs proves that it also falls within accepted range.

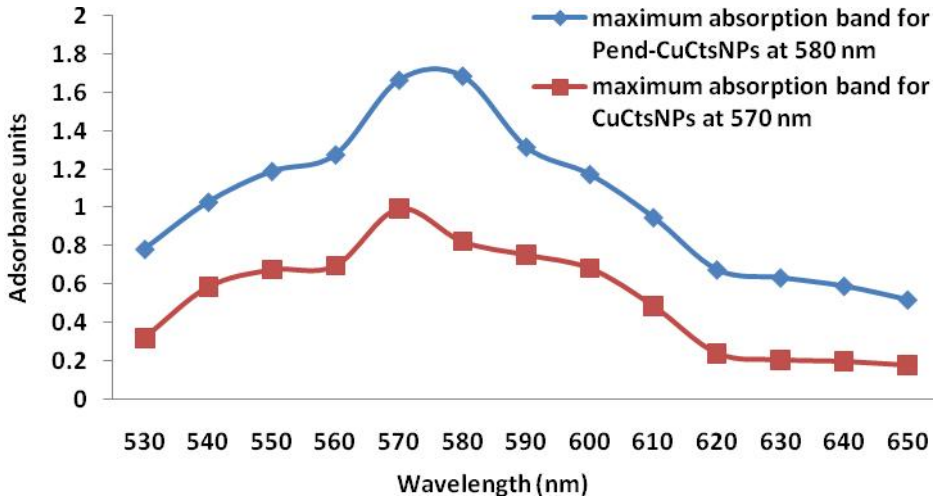


Fig. 1. UV-Visible Spectral of CuCtsNPs and Pend-CuCtsNPs

3.2 Fourier Transform Infra-Red (FT-IR) Analysis

Fourier Transform Infra-Red (FT-IR) spectroscopic characterization was carried out to determine the molecular interaction(s) between chitosan, the synthesized and loaded nanoparticles (Table 1). Chitosan shows a strong vibration peak at 3346 cm^{-1} which may be due overlapping of O – H and amine N – H stretching band and an N – H stretching vibration at 3265 cm^{-1}. It also shows a medium to strong peak at 2866 cm^{-1} which indicates the presence aliphatic C – H stretching. A very strong peak was also seen at 1641 cm^{-1} conforming to a C=O stretch in secondary amides while the peak at 1562 cm^{-1} indicates a very strong N – H deformation in amides. The peak at 1427 cm^{-1} indicates O – H in-plane bending. The medium vibration peak at 1376 cm^{-1} indicates C – H bending and a strong C – O skeletal stretching at 1023 cm^{-1}. The band around 1023 cm^{-1} suggests the existence of strong chemical bonding between the Cu in $CuSO_4$ and the -OH group of chitosan. These characteristic absorption peaks for $CuSO_4$ and chitosan shows that the composite nanoparticles contain both Cu and chitosan. The trend observed here was also confirmed by other researchers in their work [8,22].

For the CuCtsNPs and Pend-CuCtsNPs spectra, similar trends were observed with slight increase or decrease in peak values. A study of the spectrum shows a shift from 3346 cm^{-1} to 3337 cm^{-1} to 3328 cm^{-1} for the overlapped O – H and N – H stretching band. The disappearance of aliphatic C-H stretch at 2866 cm^{-1} was observed. Decrease in peak values were also observed for N – H stretch at 3265 cm^{-1} to 3183 cm^{-1} and a slight increase to 3229 cm^{-1}. C=O stretch at 1641 cm^{-1} were shifted to 1623 cm^{-1} while the peak for C=O stretch was not observed for Pend-CuCtsNPs. This peak at 1623 cm^{-1} occurs as a result of two bands from C=O stretch and N – H deformation [17,20]. FTIR studies confirmed that the band at 1623 cm^{-1} (amide II) found in Pend-CuCtsNPs was not in the FITR of chitosan indicating encapsulating evidence. Slight decreases were observed for O-H in-plane bend at 1471 cm^{-1} to O-H of 1426 cm^{-1} and to O-H of 1423 cm^{-1} , C-H bend at 1376 cm^{-1} was also shifted to C- H of 1376 cm^{-1} and C-O stretch at 1023 cm^{-1} drifted to C-O of 1060 cm^{-1} to 1025 cm^{-1} [23]. In addition, a new moderate absorption peak which might represent copper nanoparticles was observed at 610 and 601 cm^{-1} within the premises of the herbicide, pendimethalin. This corresponding vibration band can be related to the interaction between the chitosan, the Cu-NPs and the herbicide pendimethalin. Thus, it is an evidence that the capping/stabilizing ability of chitosan in the synthesized of copper nanoparticles and nano-herbicide formulation for controlled release study is very important, since all the peaks present

shows that Pend-CuCtsNPs is well attached to the groups available in chitosan and this enhances successful encapsulation of the herbicide [22,23].

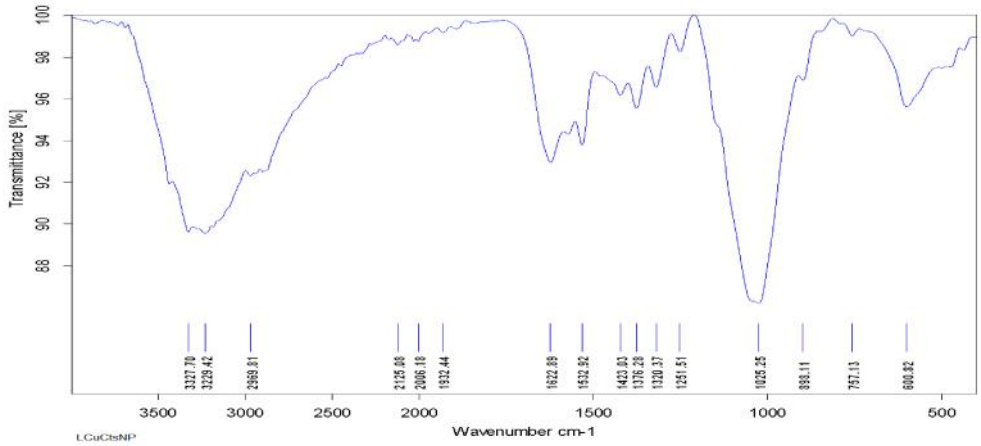


Fig. 2. FT-IR spectrum for Pend-CuCtsNPs

Table 1. FT – IR Functional Group Analysis for Plain Chitosan, CuCtsNPs and Pend-CuCtsNPs

Vibration assignment/ Functional group	Observed wave number (cm^{-1}) plain chitosan	CuCtsNPs	Pend-CuCtsNPs
O – H, N – H Overlap	3346(s)	3337(s)	3328(m)
N – H Stretch	3265(s)	3183(v.br)	3229 (s)
Aliphatic C – H Stretch	2866 (m-s)	-	-
C=O Stretch	1641(v.s)	1623(s)	-
N – H Deformation	1562(v.s)	1623(s)	1623(w-m)
O – H In-plane bend	1421(m)	1426 (m)	1423 (m)
C – H Bend	1376(m)	1376 (m)	1376 (m)
C – O Stretch	1023 (s)	1060 (v.s)	1025 (v.s)

Key: w = weak, m = medium, s = strong, v.s = very strong and v.br = very broad

3.3 Powder X-ray Diffraction Analysis

The XRD pattern of the synthesized CuCtsNPs and Pend-CuCtsNPs are shown in Fig. 3. A continuous scan mode was used to collect 2θ data from 5.00^o to 90°. The prominent diffraction peaks observed are indexed to (100), (110), (111), (200), (211) and (220) reflections. This confirms that the resultant nanoparticles are face centered cubic (fcc) of metal nanoparticles [21]. The highest intense peak for fcc materials is generally (111) reflection and this was observed in the synthesized nanoparticles. These planes observed were compared with the standard powder diffraction card of JCPDS copper file No. 04-0836 and copper X-ray diffraction reference No. 01-089-2838 [17,20].

The peak at 2θ value 17.98^o corresponding to (100) reflection is due to the presence of chitosan in the nanoparticles while the distinct XRD peak at 2θ value of 64.25^o corresponding (211) reflection in the spectrum of the nanoparticles may be due to the herbicide presence from the encapsulation process. A similar result was reported by [17, 22, 23] which is an indication of interaction between the nanoparticles and the stabilizing medium.

The broadened diffraction peaks suggest that the resultant nano particles have a very small crystalline size. This was evident from the size obtained using the Debye-Scherrer for calculation. The average crystalline size calculated for CuCtsNPs is 25.55 nm and Pend-CuCtsNPs was calculated to be 24.75 nm. These values indicate that the synthesized nanoparticles have high surface area to volume ratio [20]. The Debye-Scherrer equation is given as:

$$D = K\lambda/\beta\cos\theta \quad (1)$$

Where D is the mean nanoparticles diameter (Particle size), K is a constant with value 0.9, λ is the wavelength of X-ray (0.1541), β is full width at half maximum and θ is the differential angle.

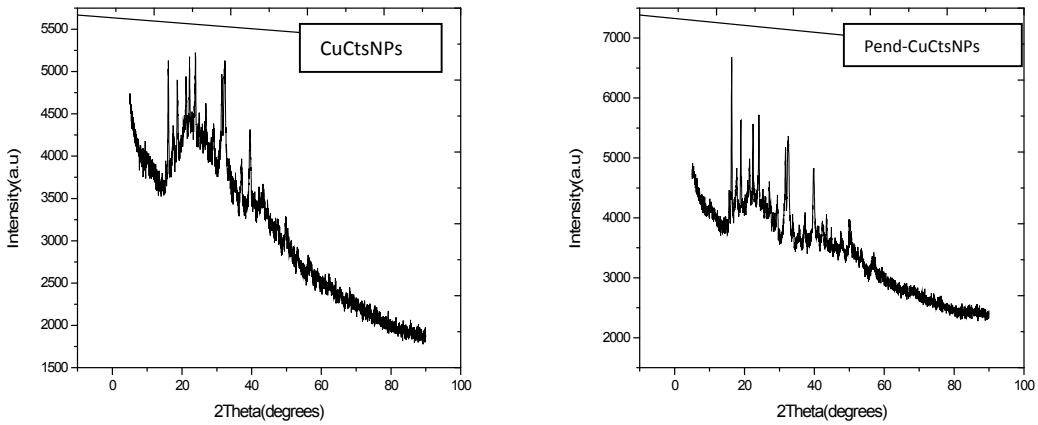
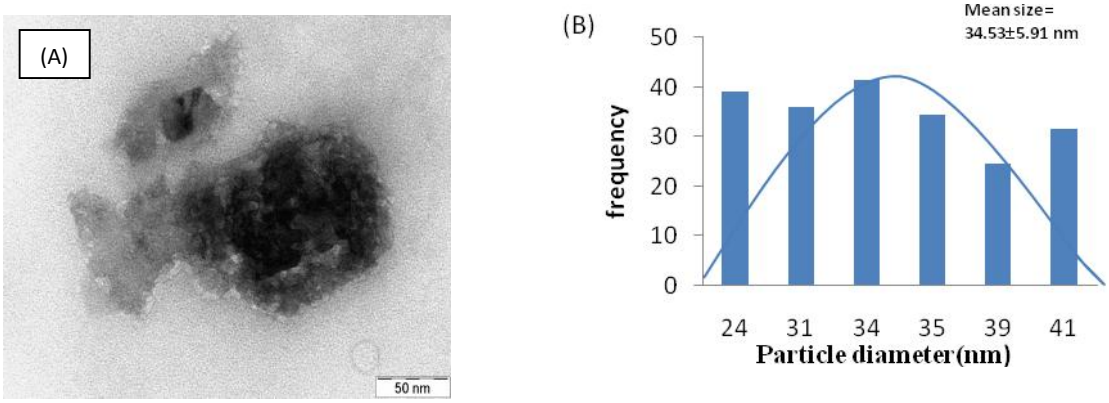


Fig. 3. PXRD Diffractogram of CuCtsNPs and Pend-CuCtsNPs

3.4 Transmission Electron Microscopy (TEM) Analysis

TEM analysis conducted for CuCtsNPs and the formulated Pend-CuCtsNPs revealed the image scale bar of 50 nm and shape from spherical to irregular (Fig. 4). Particle sizes were calculated using image J software with sizes ranging from 24.68 – 41.37 nm; mean particle size is 34.53 nm and standard deviation of 5.91 nm for CuCtsNPs and 27.05 – 43.48 nm; mean particle size to be 35.81 nm and standard deviation of 6.06 nm for Pend-CuCtsNPs. The images observed from the micrographs indicate polydispersed, larger-sized nanoparticles and higher agglomeration forming large flocks of particle aggregates [17].



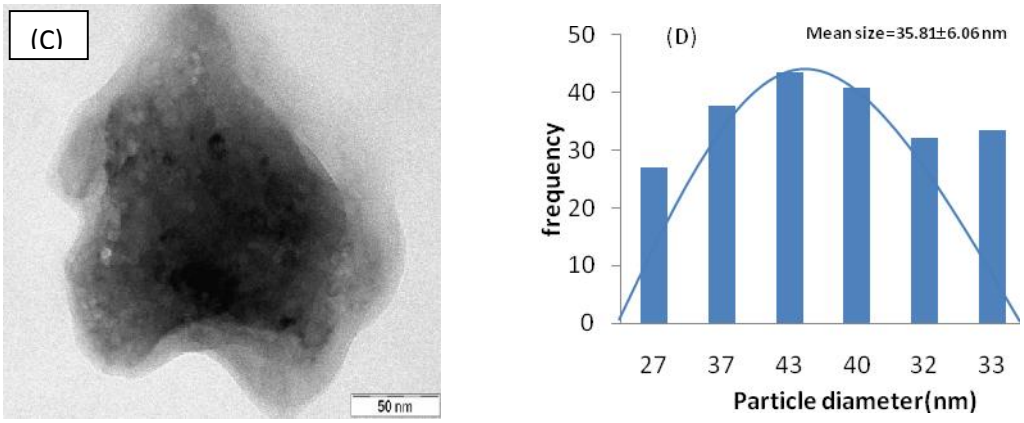


Fig. 4. TEM Images of the Synthesized CuCtsNPs and Pend-CuCtsNPs (A and C) and their Particle Size Distribution Histograms (B and D)

3.5 Energy-Dispersive X-ray (EDX) Analysis

EDX analysis was carried out to determine the elemental composition and purity of the samples by atom percentage of metal. The spectrum recorded for the CuCtsNPs and Pend-CuCtsNPs are shown in Fig. 5. The profile shows a strong copper signal at 8 keV along with oxygen and carbon peaks, which may have originated from the biopolymer bound to the surface of the copper nanoparticles. Gold and Aluminum or chromium peaks may be due to the same being present in the grids and elements like chlorine, iron, silicon and calcium may be due impurities from previous analyzed samples while sulphur may be from the precursor ($CuSO_4$) used. The micrographs explain the surface atomic distribution and chemical composition of the nanoparticles.

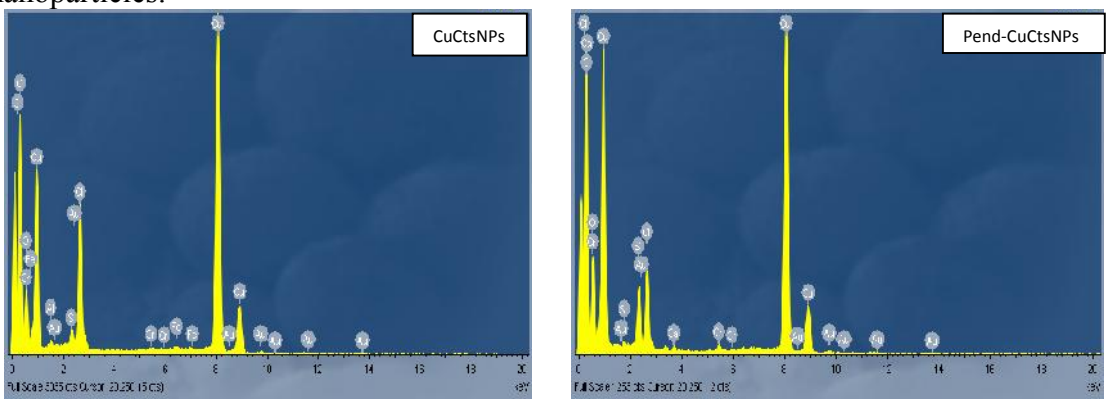


Fig. 5. EDX Spectra of the Synthesized CuCtsNPs and Pend-CuCtsNPs

3.6 Release Profile of formulated Pend-CuCtsNPs

The herbicide release from nanoparticles is usually a biphasic phenomenon although in some instances a triphasic trend is observed. The mode of release in the different media shows a steady release pattern as the time increases. All the herbicide release profiles exhibit an initial burst release, presumably from the particle surface, followed by a sustained release driven by diffusion of the active ingredient (a.i) through the polymer wall and polymer erosion [24]. This can be an advantage since it can control the sprouting of weed seeds and still exist to kill and inhibit new infestation [2,25]. Looking at different release profile in this work after 96 hours, it shows that the release process can still continue for a longer period compared to the time frame used in this work.

A close look at the release of herbicide from the nano-formulation for the two composite matrices (0.3 and 0.8%) shows that the highest release was observed in the pH 5.5 (acidic medium). This huge difference in the release rate for pH 5.5 can be linked to Pendimethalin enhanced solubility and stability under acidic condition as reported by WHO [26]. A Tukey Post Hoc tests were performed for the two variables at 95% confidence interval and herbicide concentration released at pH 5.5 gave mean difference when compared to those of both pH 7.0 and 8.0 at significance level ($P < 0.05$). The statistical treatment shows that there was no significance difference between pH 7.0 and 8.0 as the mean difference is non- significant ($P > 0.05$).

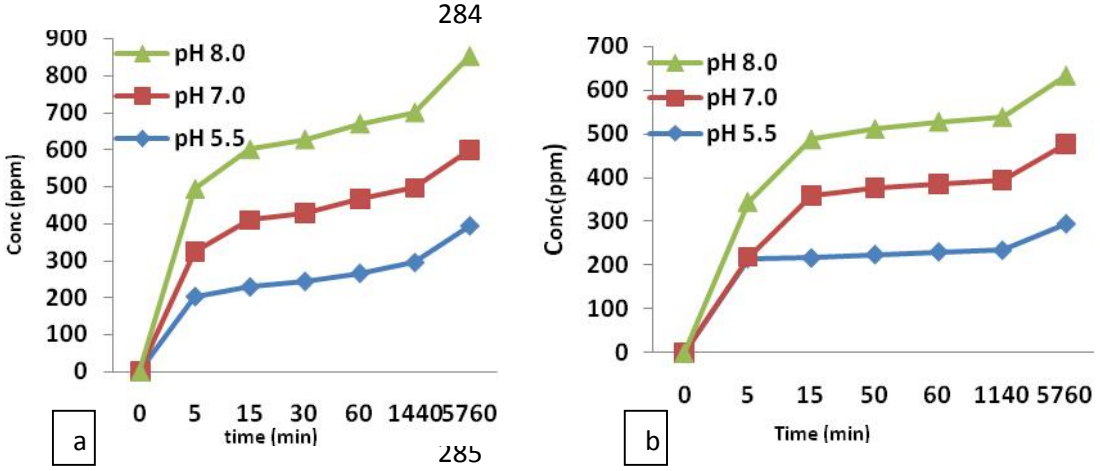


Fig. 6. Concentration of pendimethalin released from (a) 0.8% and (b) 0.3% loaded CuCtsNPs

4. CONCLUSION

This current work describes the successful development of metallic copper-chitosan nanoparticles via a green chemical reduction method. The green chemical approach toward the synthesis of Cu-NPs was simple, convenient, cheap and user friendly. Different characterization techniques such as UV-Visible spectrophotometer, FI-TR, TEM, EDX and XRD were employed to authenticate the effectiveness of the synthesized nanoparticles. The encapsulation efficiency (EE) of the herbicide on the 0.3 and 0.8% matrix is surface dependent (57.5 and 92.7%). Result however shows that there is a controlled release of nano-formulation over the conventional formulation.

REFERENCES

[1] Sopena F, Maqueda C, Esmeralda M. Controlled Release Formulations of Herbicides Based On Micro-Encapsulation. Ciencia Investigacion Agraria. 2009; 35 (1):27-42.

[2] Shehzad F, Shah J; Jan MR . Determination of Pendimethalin Herbicide in Water and Vegetable Samples by Microwave-Assisted Solvent Extraction and HPLC Method. Pakistan Journal of Weed Science and Resources. 2011; 17 (2): 175-185.

[3] Aouada FA, Moura MR, Orts WJ, Mattoso LHC. Polyacrylamide and Methylcellulose Hydrogel as Delivery Vehicle for the Controlled Release of Paraquat Pesticide. Journal of Material Science. 2010; 45**:** 4977-4985

[4] Ruegg WT, Quadranti M, Zoschke A. Herbicide Research and Development: Challenges and Opportunities. Weed Resources. 2007; 47: 271-275

[5] Barrabes N, Just J, Dafinov A, Medina F. Synthesis, Characterization, and Catalysis of Metal Nanoparticles. Application of Catalytic Biointerfaces. 2006; 62 (1 –2): 77–85

[6] Ipsa S, Nayak PL. Antimicrobial Activity of Copper Nanoparticles Synthesized from Ginger (Zingiber officinale) Extract. World Journal of Nano Science & Technology. 2013; 2 (1): 14-17

[7] Angham GH. Synthesis of Chitosan and its Use in Metal Removal. Chemistry and Materials Research. 2013; 3 (3): 21-26.

[8] Muhammad SU, Nor AI, Kamyar S, Norhazlin Z, Wan MD, Zin WY. Copper Nanoparticles Mediated by Chitosan: Synthesis and Characterization via Chemical Methods. Molecules. 2012; 17: 14928-14936.

[9] Rinaudo M. Chitin and Chitosan: Properties and Applications. Progress in Polymer Science. 2006; 31: 603–632.

[10] Adarsh KJ, Madhu G. Chitosan Coated Surface Modified Polypropylene Fabric for the Removal of Cd(II) and Zn(II) ions from Aqueous Solution. International Journal of Innovative Research in Science, Engineering and Technology. 2013; 2 (7): 2943-2951

[11] Bakiya LK, Sudha PN. Adsorption of Copper (II) ion onto Chitosan/Sisal/Banana Fiber Hybrid Composite. International Journal of Environmental Sciences. 2012; 3 (1): 453-470

[12] Veena BM, Preeti NT, Robertcyril SH, Sikandar IM, Harichandra ZN. Biodegradation of Pendimethalin by Bacillus circulans. Indian Journal of Biotechnology. 2010; 9: 173–177

[13] United States Environmental Protection Agency. Bulletin on Prevention, Pesticides and Toxic Substances. 1997; USEPA-738-F-97-007

[14] Kaleem S, Ansar M, Ali A, Ahmad S. Efficiency of Pendimethalin Herbicide against Trianthena Monogyna (Horse Purslane) Weeds in Cotton Crop. Pakistan Journal of Weed Science and Resources. 2006; 12: 177–182

[15] Scrinis G, Lyons K. The Emerging Nano-corporate Paradigm: Nanotechnology and the Transformation of Nature, Food and Agro-food Systems. International Journal Sociology Food and Agriculture. 2007; 15: 22-44

[16] Ram P, Vivek K, Kumar SP. Nanotechnology in Sustainable Agriculture: Present Concern and Future Aspects. African Journal Biotechnology. 2014; 13 (6): 705-713

[17] Muhammad SU, Mohamed EE, Kamyar S, Norhazlin Z, Mohamed S, Nor AI. Synthesis, Characterization and Antimicrobial Properties of Copper Nanoparticles. International Journal of Nano medicine. 2013; 8: 4467–4479

[18] Malathi S, Balakumaran MD, Kalaichelvan PT, Balasubramanian S. Green Synthesis of Gold Nanoparticles for Controlied Delivery. Advance Material Letter. 2013; 4 (12): 933-940

[19] Balcerzak J, Mucha M. Analysis of Model Drug Release Kinetics from Complex Matrices of Polylactide-Chitosan. Progress on Chemistry and Application of Chitin and its Derivatives. 2010; 15: 117-125

[20] Krithiga N, Jayachitra A. Synthesis of Copper Nanoparticle by Chemical Method and Analysis, its Siginificant Antibacterial Activity. International Journal for Research in Applied Sciences and Biotechnology. 2014; 1 (1): 22-27

[21] Selvarani M, Prema P. Evaluation of Antibacterial Efficacy of Chemically Synthesized Copper and Zerovalent Iron Nanoparticles. Asian Journal of Parmaceutical and Clinical research. 2013; 6 (2): 223-227

[22] Raut AR, Khairkar SR. Study of Chitosan Cross-linked with Glutaraldehyde as Biocomposite Material. World Journal of pharmaceutical Research. 2014; 3 (9): 523 – 532

[23] Ramya R, Sudha PN, Mahalakshi J. Preparation and Characterization of Chitosan Binary Blend. International Journal of Science and Research Publications. 2012; 2 (10): 1 – 9

[24] Bhattarai N, Ramay HR, Chou S, Zhang M. Chitosan and Lactic acid-grafted Chitosan Nanoparticles as Carriers for Prolonged Drug Delivery. International Journal of Nano medicine. 2006; 1 (2): 181-187

[25] Onyido I, Sha'Ato R, Nnamonu LA. Environmentally Friendly Formulations of Trifluralin Based on Alginate Modified Starch. Journal of Environmental protection. 2012; 3: 1085 – 1093

[26] World Health Organization. Pendimethalin in Drinking Water. WHO Bulletin on Guidelines for Drinking Water Quality, 2nd ed. 2003; 2: 1-3