

Green Synthesis, Characterization and Aqueous Release of Paraquat Dichloride from Copper-Chitosan Nanoparticles

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Abstract

In a bid to decrease pesticide load in the environment, copper-chitosan nanoparticles synthesized by a green and standard chemical reduction method were loaded with paraquat dichloride, to determine possible controlled release of the herbicide. In one method, loading was accomplished by thermal cross-linking of the pesticide onto copper-chitosan nanoparticles with methylene bisphenyl isocyanate using *N,N*-dimethyl acetamide as solvent. Loading was achieved by simple equilibration in the second method. The paraquat-copper-chitosan nanoparticles were characterized using UV-visible spectroscopy, Scanning Electron Microscopy (SEM), and Fourier Transform-Infrared (FT-IR) spectroscopy. The UV-Visible spectrophotometry revealed prominent peaks for reduced copper at 525 nm and 501 nm for the herbicide loaded nanoparticles obtained from both methods, these values and FTIR bands indicating efficient loading of the nanoparticles. SEM of the lustrous particles revealed heterogeneous sizes and irregular shapes, pesticide loading was observed to reduce the nanoparticle sizes by up to 40 nm. Effect of pH on aqueous release was evaluated in three acetate buffer pH media, statistical analysis of the results giving best release profile for pH 7 buffer. Up to 50% delayed pesticide release was recorded.

Keywords: Chitosan, Copper, Nanoparticles, Paraquat dichloride, Release profiles

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I. Introduction

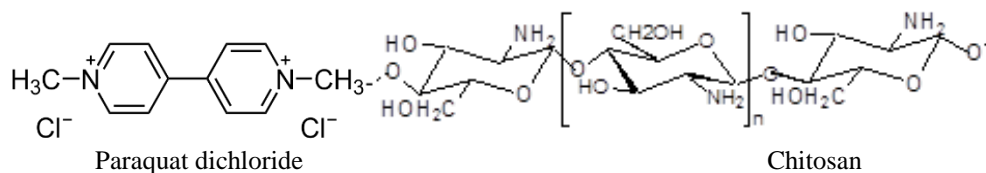
One third of the world's potential food supplies are estimated to be lost to pre- and post-harvest pests and diseases.¹ Plants formed the world's largest food source and in recent years, several efforts to increase agricultural yields due to demographical growth have been employed.² This has been achieved through the development of new high yield crops as a result of the use of fertilizers, pesticides, and heavy agricultural machinery.^{2,3} The use of synthetic pesticides has without doubt led to increased crop production.⁴ Although several benefits were obtained from the use of these agricultural improvements, many issues have arisen due to the attendant environmental damage like erosion, salinization and flooding of heavily irrigated soils, aquifer depletion, deforestation and environmental contamination due to excessive use of such pesticides.^{2,5} To combat these challenges, developing new biodegradable matrix based herbicides that can be slowly released yet maintaining possible effectiveness is required.^{6,7}

The application of nanotechnology in crop protection has drawn attention in recent years.⁸ It is a field which deals with production, manipulation and use of materials with sizes ranging in nanometers.⁹ Among the latest line of technological innovations, nanotechnology occupies a prominent position in transforming agriculture and food production.¹⁰ Preparation of metal nanoparticles is one of the major research areas in nanotechnology.¹¹ Among metals, copper nanoparticles have attracted much attention because of their catalytic and optical properties.¹² They are very reactive because of their high surface-to-volume ratios which can easily interact with other particles.¹³

Chitosan is a natural product obtained from chitin, a polysaccharide found in the exoskeleton of shellfish.¹⁴ Its ability to function as a chelating agent makes it a perfect material for the synthesis of metal nanoparticles (NPs).^{13,15,16,26} Chitosan is significant not only because it's biodegradable but it is a water absorber which helps in maintaining soil moisture. It also reduces soil acidity.⁶

The introduction of the herbicide paraquat dichloride (PQTD) in agriculture in the early 1960's greatly facilitated weed control.¹⁷ Paraquat, *N,N'*-dimethyl-4,4'-bipyridinium dichloride, is a bi-quaternary ammonium compound available as the dichloride salt.¹⁸ A non-selective contact herbicide, absorbed by foliage, with some translocation in the xylem¹⁹, it is sold under tradenames like *Gramoxone Extra*, *Cyclone*, *Spot Killer*, and *Sweep*. It is one of the most widely used herbicides for weed control worldwide,²⁰ partially inactivated upon contact with soil.²¹

The negative impacts of agricultural practices involving the use of synthetic herbicides on farmlands in promoting sustainable agriculture is of great concern. Reconciling the need for pesticide use in agricultural practices with environmental preservation, particularly in developing countries, is the biggest challenge that gave rise to this present study. Simple synthesis, characterization and aqueous release of paraquat dichloride from copper-chitosan nanoparticles is hereby reported.



II. Materials and Methods

2.1 Chemicals

All chemicals acquired were used without further purification and stored under appropriate conditions, as detailed in the manufacturers' instructions.

Chitosan (CHI, high molecular weight of 600,000–800,000 g/mol, $\geq 75\%$ deacetylated), hydrazine, 4,4'-methylenebisphenylisocyanate and *N,N*-dimethyl acetamide were purchased from Sigma-Aldrich Life Science, USA. Sodium hydroxide, ethanoic acid, copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), ascorbic acid, methanol and acetone were supplied by Emole Chemicals, Makurdi Nigeria. Paraquat dichloride solution, Weedlezz®, was supplied by Shandong Luba Chemical Co. Ltd, China. Standard acetate buffers pH 5, pH 7 and pH 9 were obtained from Kem Light Laboratories Nigeria.

2.2(a) Synthesis of Cu-Chitosan NP using chemical reduction method – Method 1

Synthesis of copper chitosan nanoparticles (Cu-CtsNPs) was carried out by adopting the method of Mohammed²² with little modification. An aqueous solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was made by dissolving 0.4 g granules in 10 mL distilled water, the solution was added dropwise to a solution (0.1 M ethanoic acid) of chitosan (40 mL) with resultant lightening of the dark blue colour. After heating and stirring at 120 °C for 20 min, 0.05 M ascorbic acid (0.5 mL) was added. With further stirring for 20 min, 0.6 M NaOH (2 mL) was added to the mixture, with no immediate colour change. On stirring for 30 min, a light green coloration developed. N_2H_4 (0.5 mL) was added to give a quick brown coloration which changed to dark red after 5 min of stirring. The mixture was further stirred for 30 min, allowed to cool at room temperature, centrifuged at 14,000 rpm for 15 min and the supernatant discarded. The Cu-Cts NP nanoparticles were repeatedly washed with deionized water and dried.

(b) Green Synthesis of Cu-Chitosan NP – Method 2

In a typical one-step synthetic process, 25 mL of 50 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was added to 50 mL of 0.75% (w/v) chitosan solution in 0.1% ethanoic acid and the mixture stirred at 70 °C for 12 h. The colloid was centrifuged at 10,000 rpm for 10 min and supernatant discarded. The precipitate was re-suspended in acetone (90%, v/v) and centrifuged, this process repeated three times to remove unreacted reagents. The solid was washed with deionized water and dried under vacuum overnight.²³

2.3(a) Loading of CuCtsNPs with paraquat dichloride- Method A

Paraquat dichloride (8.56 g, 0.04 moles) was dissolved in 100 mL of *N,N*-dimethyl acetamide (DIMAC) and added to 20.0 g of methylene bisphenylisocyanate in 150 mL DIMAC. The solution was maintained at 110 °C for two hours with stirring. 10.0 g of CuCts NPs was dissolved in 200 mL of *N,N*-dimethyl acetamide and added to the reaction mixture. After 3-5 minutes of fast stirring, a light-amber coloured gel was formed. The product was precipitated with methanol yielding a white microporous solid which was dried in a desiccator for 24 h as described by McCormick.²⁴

(b) Loading of CuCtsNPs with paraquat dichloride – Method B

Copper chitosan nanoparticles, 0.5 g was placed in a test tube and 25 mL of neat paraquat dichloride was added and manually shaken. The slurry was allowed to equilibrate for 24 hours, centrifuged for 30 minutes at 10,000 rpm and the supernatant decanted. The paraquat loaded copper chitosan nanoparticles were dried at room temperature.²⁵

2.4 Characterization of the synthesized and herbicide loaded nanoparticles

Formation of the NPs, pesticide loading and aqueous release studies of the synthesized PQTD loaded Cu-Chitosan nanoparticles were monitored using UV-Visible spectrophotometry (Jenway 6305

Spectrophotometer). Scanning was recorded over a range of 350 - 600 nm. FTIR spectra of the Cu-Chitosan NPs were obtained using PerkinElmer Spectrum Version 10.4.2. and FTIR-8400S Shimadzu, source performing scan was recorded in the range of 400 to 4000 cm^{-1} . Particle size and morphological analysis were carried out using Scanning Electron Microscopy (SEM). Nanoparticles of Cu-Chitosan (plain and loaded) were deposited on thin aluminium plates and dried at room temperature. The sample was directly placed on the stub without staining, exposed to accelerated voltage beam strength of 20.0 KV, and images captured at various magnifications (10,000 to 100,000x).

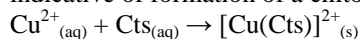
2.5 Aqueous Release study of Paraquat loaded Cu-CtsNPs

Batch release method reported by Faria²⁶ and Wuana¹⁶ was adopted with little modification for evaluation of the sustained release of pesticide from the polymeric system. In this method, 0.1 g of each sample was placed in 500 mL stoppered conical flasks. Release studies were carried out in distilled water and acetate buffers pH 5, 7 and 9 media over two weeks. At different time intervals, 3 mL aliquots were withdrawn and release progress was monitored by UV-Visible spectroscopy.

III. Results and Discussion

3.1 Synthesis and characteristics of Cu-CtsNPs

Method 1: Formation of Cu-CtsNPs was marked by a series of colour changes related to the chemical reactions taking place. The dropwise addition of bulk Cu solution to Cts solution resulted in a light blue coloration, indicative of formation of a chitosan-copper complex:



Ascorbic acid was added at this point to protect the yet to be formed NPs from oxidation, because of its good antioxidant capacity.²² No colour change was observed after this addition. A resultant colour change to light dirty blue was observed on adding NaOH. Copper chitosan nanoparticles ([Cu(Cts)]NPs) were formed following the addition of hydrazine with a quick light brown coloration which turned brick red (Plate 1). The same observation was reported by Mohammed²² although, a different observation was reported^{25, 27} where a yellow coloration was observed after addition of NaOH indicating the formation of Cu-NPs and Cu₂O NPs. The series of colours observed is attributable to excitation of surface plasmon vibration in the metal nanoparticles.²⁸

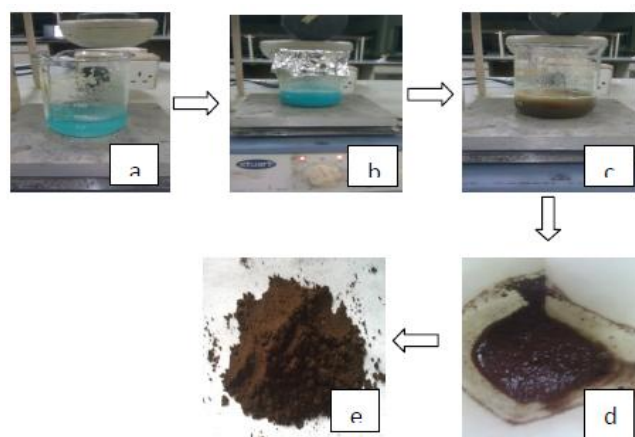


Plate 1: Colour changes observed during CuCtsNPs synthesis

[a] $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (aq), (b) $[\text{Cu}(\text{Cts})]^{2+}$, (c) $[\text{Cu}(\text{Cts})]\text{NP}(s)$, (d) wet and (e) dry finished product

Method 2: There was little colour change when acetone (reducing agent) was added after the first centrifugation. The colour remained blue even when dried and powdered (Plate 2).



Plate 2: Synthesis of CuCtsNPs via Green method

UV visible spectrophotometer was used to determine the absorption maxima of the synthesized Cu-Cts NPs. The samples were scanned within the range of 200-600 nm and λ_{max} was 525nm for Method 1 and 501nm for Method 2 indicating the reduction of Cu^{2+} ions in the presence of chitosan. These may likely be as a result on the shift of the surface plasmon resonance (SPR) of the metallic nanoparticles^{22,23}. Nanosized Cu particles are generally believed to exhibit an SPR between 500-600 nm.²⁹

Fourier Transform Infrared (FTIR) spectroscopy showed molecular interactions between chitosan and the other components of the synthesized NPs. Plain chitosan spectrum in Fig.1 shows an amide I C=O vibration band at 1648cm^{-1} .²² It also shows a strong vibration band at 3359cm^{-1} which may be due to an overlap of O-H and N-H stretch.³⁰ Other vibration bands were also observed at 2872cm^{-1} (aldehyde C-H stretching), 1647cm^{-1} (NH_2 bending), 1378cm^{-1} (alkane C-H bending), 1063cm^{-1} and 1024cm^{-1} (C-O-C skeletal stretching) respectively.³¹ A similar trend was observed in the Cu-Cts NPs spectra of M1 and M2 (Fig.1 b and c). Both show broad bands at 3331cm^{-1} and 3242cm^{-1} due to overlap of O-H and N-H stretching, 1561cm^{-1} and 1541cm^{-1} (NH_2 bending), and 1030cm^{-1} and 1031cm^{-1} (C-O-C skeletal stretching). A general decrease in band intensity was observed from plain chitosan to Cu-Cts NPs M1(b) to M2(c) comparing 3359 to 3331 to 3243cm^{-1} ; 1646 to 1560 to 1540cm^{-1} ; and 1064 to 1030 and 1031cm^{-1} . These bands show the presence of copper and chitosan in the synthesized nanoparticles. In Fig.1, bands were observed at 660cm^{-1} in M1(b) and 610cm^{-1} in M2(c). This vibration indicates an interaction between the Cu on the NP surface and the amino and hydroxyl groups of Cts. This confirms that the NPs were capped by the polymer as reported by other authors.^{22,23,32}

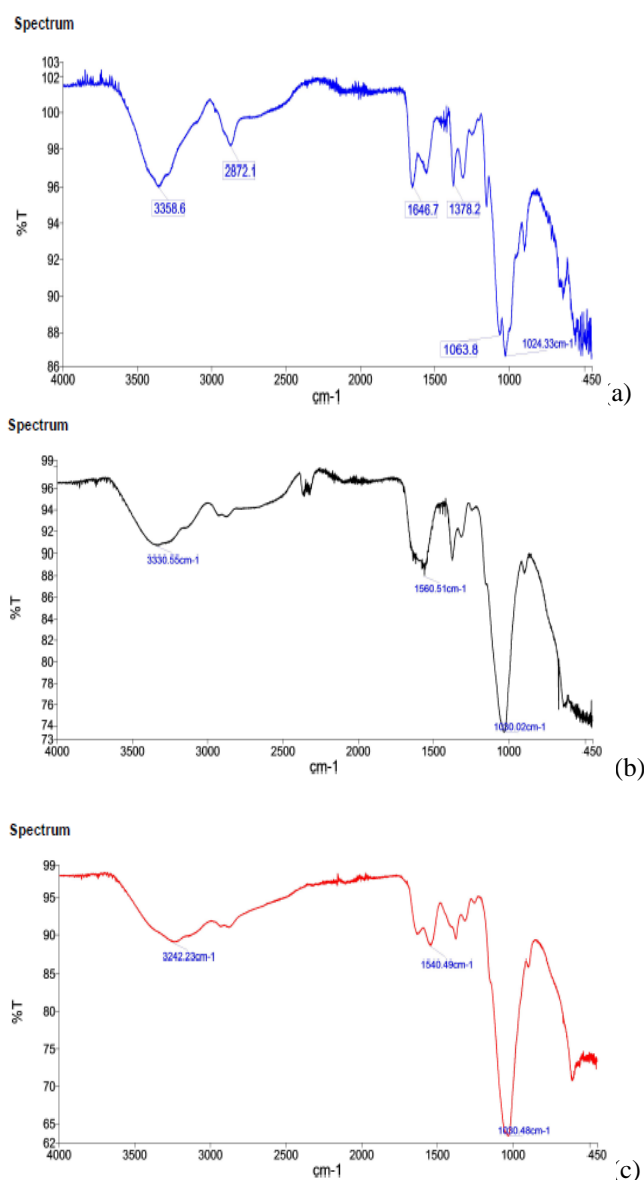


Fig.1: FTIR Spectra of (a) Pure chitosan (b) Cu-Cts NPs, M1 and (c) Cu-Cts NPs, M2

Morphological analysis of the synthesized NPs was carried out using SEM. The images of copper-chitosan NPs from Method 1 and Method 2 at different magnifications are shown in Plate 3 and Plate 4, revealing shapeless tiny, lustrous heterogeneous particles with rugose and wrinkled morphology. Particles from Method 1 are irregular in shape with sharp edges and dotted faces ranging from 0.01 μm to 0.9 μm , which is 10 - 90 nm (Plate 3). Particles from Method 2 are more discreet and have a more defined polygonal and crystalline structure with variable sizes in the range of 0.012 μm to 0.85 μm (Plate 4). Copper-chitosan nanoparticles usually have no specific shape. Literature reports spherical shape²² or dendrites having various branches with sizes in the range of a few microns (approximately 1 - 15 μm)³³.

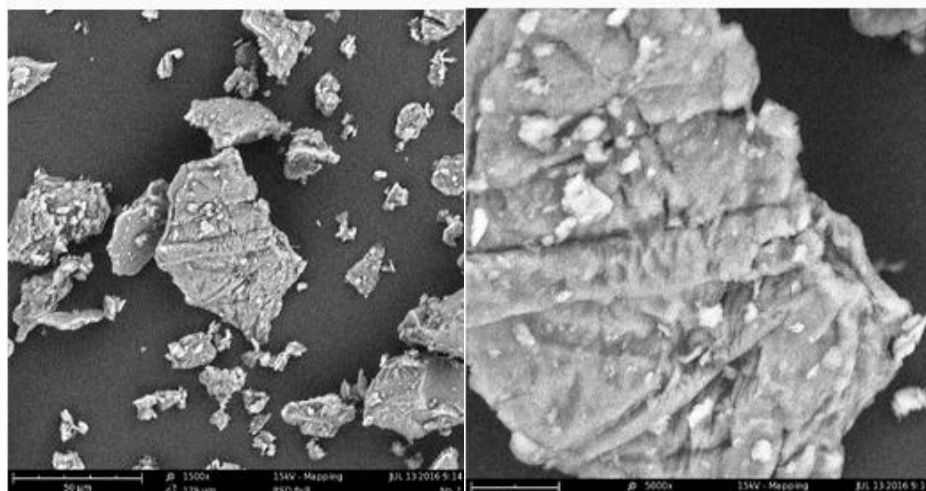


Plate 3: SEM Images of Cu-Cts NPs from Method 1 at Different Magnifications

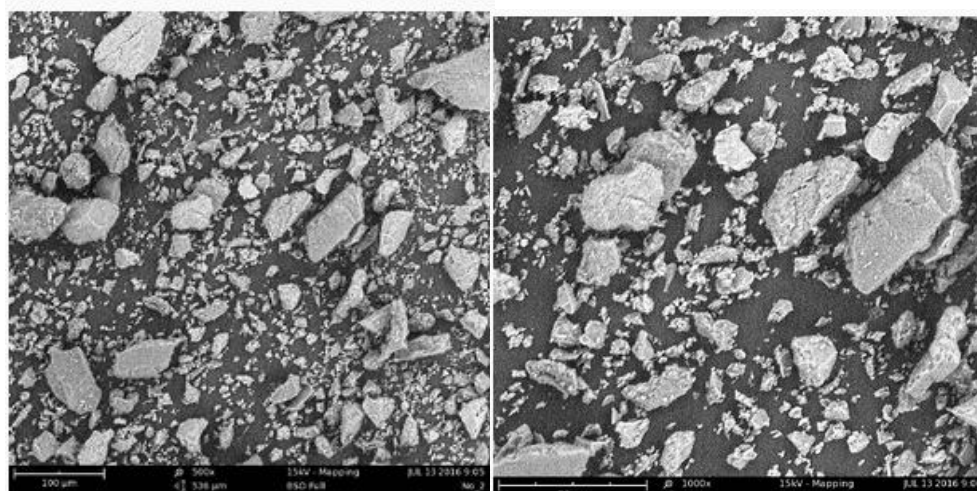


Plate 4: SEM Images of Cu-Cts NPs from Method 2 at Different Magnifications

3.2 Synthesis and characteristics of PQTD loaded nanoparticles

The Cu-Cts NPs obtained from Method 2 were used for Paraquat dichloride (PQTD) loading using two different methods for comparison. *Method A* of herbicide loading is a preparation of pesticide-polymer formulation for controlled release of pesticide under field conditions which comprises reacting a pesticide having a replaceable hydrogen with a multifunctional organic isocyanate to form a pesticide-isocyanate adduct and then reacting the adduct with a polymer having pendant groups containing a replaceable hydrogen to form a pesticide-polymer conjugate system in which the pesticide is linked chemically to the pendant groups by linkages which are cleaved under environmental conditions of use to controllably release an effective amount of the active pesticide. The replaceable hydrogen of the polymer (Cu-Cts NPs) is derived from hydroxyl groups.³²

Methylene bisphenylisocyanate (MBI) served as the reaction link between the pesticide and the copper chitosan nanoparticles. The dispersed Cu-Cts NPs were added with no colour change. An ash coloured microporous solid was precipitated immediately methanol was added indicative of pesticide incorporation.²⁴ (Plate 5).

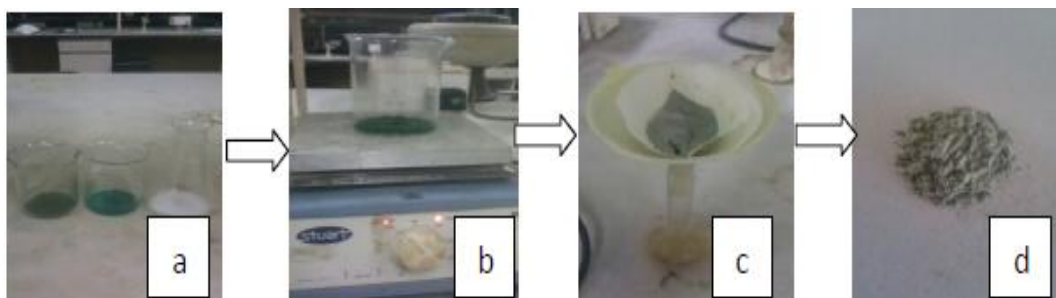
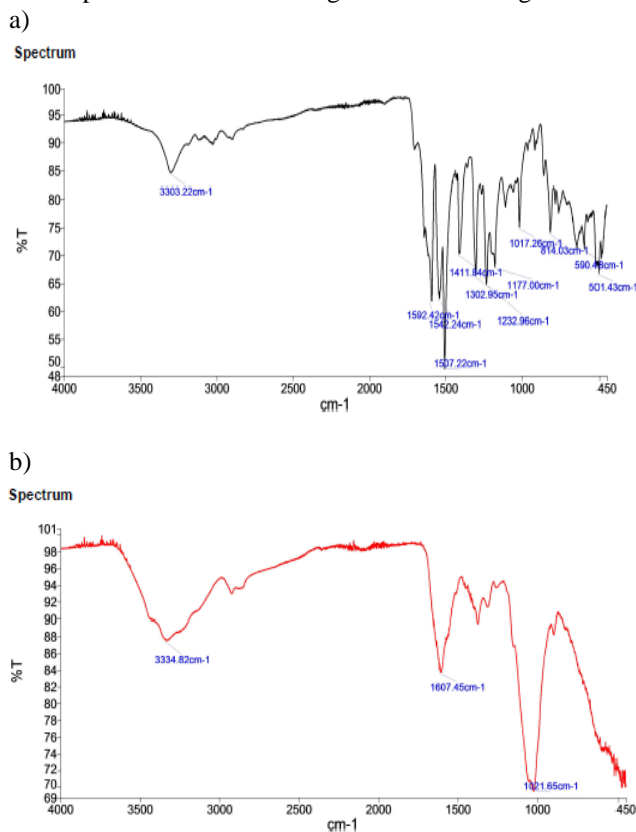


Plate 5: Loading of Paraquat Dichloride onto Cu-Cts NPs using Method A

(a) CuCtsNPs+ DIMAC, PQTD + DIMAC, MBI + DIMAC, (b) DIMAC (NPs + PQTD + MBI) +heat, (c) Wet product (d) dry Paraquat dichloride loaded CuCts NPs.

Method B involved equilibration of the pesticide-polymer matrix for 24 h, centrifugation, and drying with no attendant colour change observed.

FTIR analysis was carried out to determine the presence of any molecular interactions between PQTD and copper-chitosan nanoparticles. For PQTD, bands were observed at 3414, 2924, 1643, 1508, 1337, 1271, 1223, 1190 and 820 cm^{-1} (Fig. 2c). The FTIR spectrum obtained for the paraquat dichloride loaded complex in method A (Fig. 2a) was essentially a combination of the spectra of paraquat dichloride and that of Cu-CtsNPs. Bands appeared at 3303 cm^{-1} (corresponding to hydrogen-bonded alcohols, aromatic C-H, alkenes C-H stretching and N-H stretching) and absorptions at 2990 cm^{-1} (corresponding to an aromatic or alkene C-H, or alkane C-H band) and 1504 (corresponding to an aromatic C=C bond)³⁴, 1412 cm^{-1} (C-H bending of alkane), 1303 cm^{-1} and 1233 cm^{-1} (amine C-N), 1177 cm^{-1} and 1017 cm^{-1} (C-O-C stretching), 814 cm^{-1} and 591 cm^{-1} (C-X).³² A new band was observed at 501 cm^{-1} , possibly due to the interaction between Cu-Cts NPs and paraquat dichloride. This was evidence that all the components were fully incorporated in the reaction. The spectrum in Fig. 2b showed vibration bands for the loaded paraquat dichloride at 3335, 1607, and 1022 cm^{-1} but has no band at 1504 cm^{-1} (Method B). This is a pointer that Method A gave better loading than Method B.



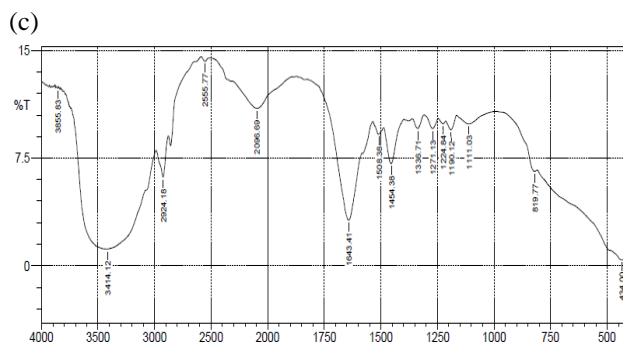


Fig. 2: FTIR Spectra of (a) Paraquat-Cu-Cts NPs, Method A (b) Paraquat-Cu-Cts NPs, Method B (c) Neat paraquat dichloride

SEM images of loaded nanoparticles from Methods A and B are displayed in Plates 5 and 6. The particles are of various sizes from 0.01 μm - 0.5 μm , being non-uniform and ranging from spherical to flat shapes for both methods A and B. Particles from method B are not significantly bigger than those from method A. SEM images showed that loaded NPs were of smaller sizes, 10 - 50 nm (Plate 5 and 6), than the unloaded NPs. General observation of SEM images acquired at all magnifications suggest that agglutination of the nanoparticles must have taken place. Loading of pesticide onto the NPs considerably reduced the average sizes of the unloaded NPs by up to 40 nm.

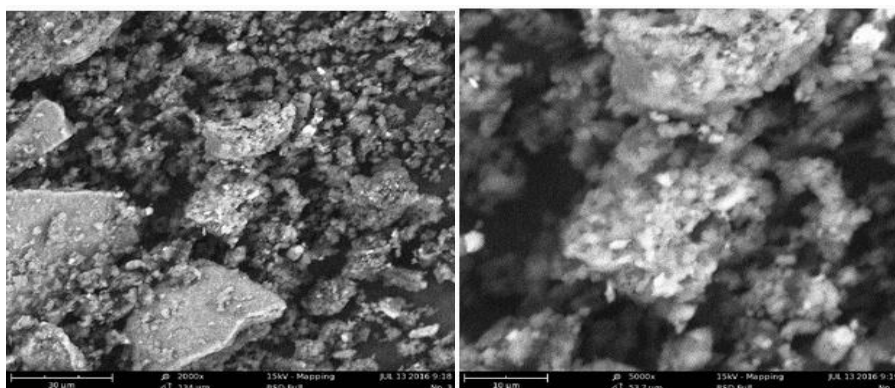


Plate 5: SEM Images of Cu-Cts NPs loaded with Paraquat dichloride (Method A)

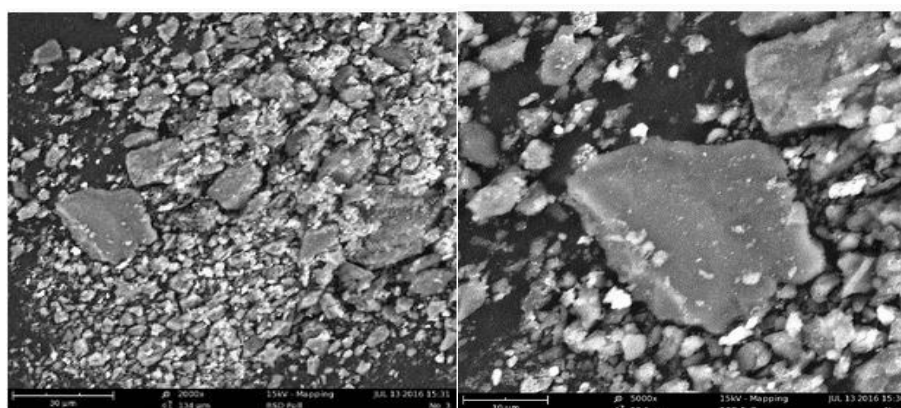


Plate 6: SEM Images of Cu-Cts NPs loaded with Paraquat dichloride (Method B)

3.3 Release studies

Aqueous release studies were carried out for the herbicide loaded Cu-Cts NPs in water and acetate buffer (pH 5, 7 and 9) media for fourteen days at the prevailing room temperature. The concentration of paraquat dichloride released was calculated using the linear regression equation obtained from the calibration curve. Paraquat release increased with time after an initial burst for all media presumably from the particle surface, and rising to a plateau (this process driven by polymer erosion and active ingredient diffusion through the porous material)^{6,16,26}

Compared to free paraquat dichloride, about 50% of herbicide was retained in the initial burst for the synthesized formulations in all pH media. This reduction in amount suggests that this formulation strategy remarkably delays active ingredient release. Amount of pesticide released and rate of attainment of the plateau was in the order pH 7 < pH 9 and water < pH 5. The amount released in the initial burst was highest for pH 5 and lowest for pH 7. Method B incorporation released pesticide faster than Method A.

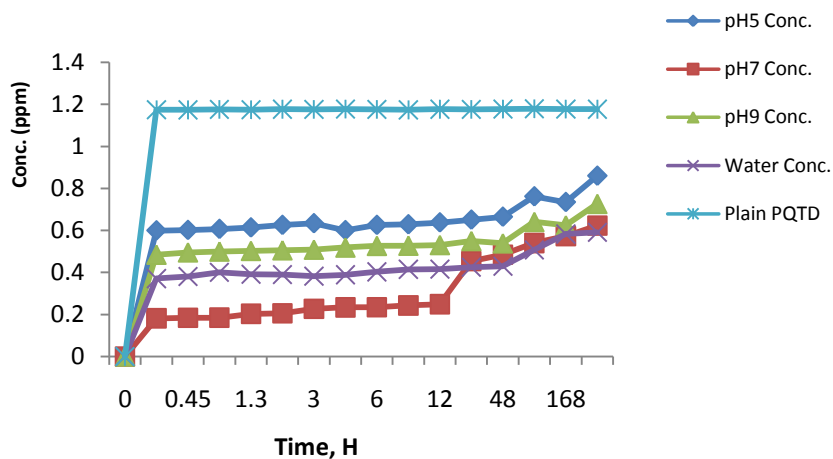


Fig. 3: Aqueous Release of Paraquat dichloride from CuCtsNPs Method A

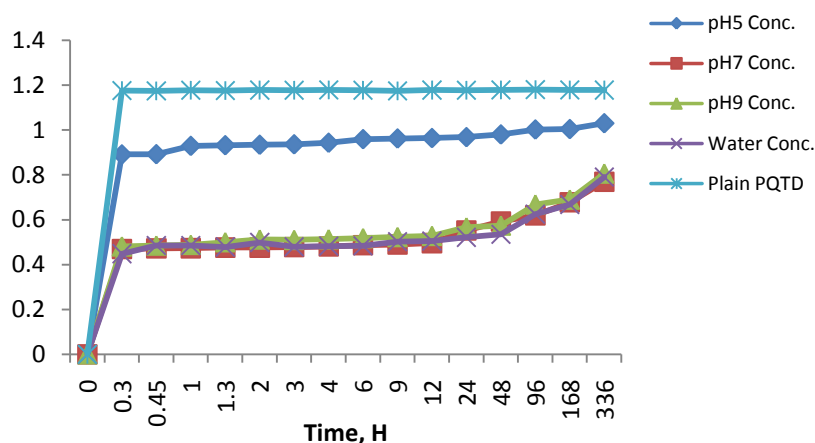


Fig. 4: Aqueous Release of Paraquat dichloride from Cu-CtsNPs Method B

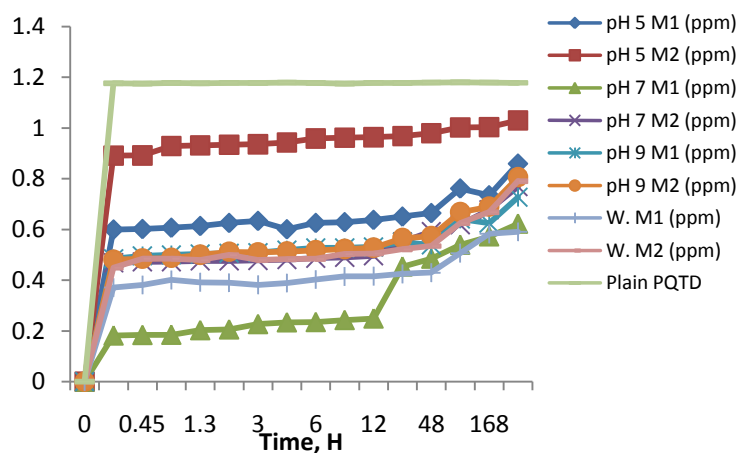


Fig. 5: Aqueous Release of Paraquatdichloride from Cu-CtsNPs Methods A & B

IV. Conclusion

Loading of paraquat dichloride on to copper capped chitosan nanoparticles synthesized by a green chemical reduction method, was achieved using methylene bisphenyl isocyanate as polymer-pesticide linking agent. Scanning electron microscopy and Fourier Transform infrared spectroscopy were applied in the analyses of the formulated paraquat-copper-chitosan nanomaterial, confirming good chemical interaction between polymer matrix, metal and active ingredient. Release studies in aqueous media, monitored by UV-Visible spectrophotometry, followed established trajectory of an initial burst culminating in a plateau with acetate buffer pH 7 producing the slowest and smallest active ingredient release and therefore the best release profile. Up to 50% paraquat dichloride was retained after the initial burst in both formulations in all media. These results reveal that loading of paraquat dichloride on to copper chitosan nanoparticles is a veritable method of achieving delayed and controlled release of the pesticide.

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