



Characterization of Formulated Copper Chitosan Nanoparticles for the Controlled Release Studies of Dicamba Dimethylamine Herbicide

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ABSTRACT

In this study, a dicamba herbicide was encapsulated onto copper nanoparticles (CuNPs) via a green chemical reduction method in order to improve its herbicidal activity, with the intention of reducing its negative impacts on the environment. The synthesised Dicamba-copper chitosan nanoparticles (Dic-CuCtsNPs) was characterised using UV-visible spectroscopy, scanning electron microscopy (SEM), Fourier transform-infrared (FT-IR), powder x-ray diffraction (PXRD), differential scanning calorimetry (DSC), thermo gravimetric analysis/differential thermal analysis (TGA/DTA) and energy-dispersive x-ray spectroscopy (EDX). The maximum absorption peak of the Dic-CuCtsNPs was observed at 556 nm. The encapsulation efficiency of the Dic-CuCtsNPs formulation was 86 %. The average crystalline size of the nanoparticles, from its PXRD pattern, estimated using the Debye-Scherrer's equation was 45.94 nm. The SEM image obtained indicated particle size distribution of 16 – 64 nm. The DSC curve showed that the formulation exists in the crystalline state while the TGA data indicated that the formulation was thermally stable up to 425.75 °C. Aqueous release study of Dic-CuCtsNPs was carried out using three different pH media and two agricultural soils. The leaching profile of the formulated nano-herbicide through the University of Agriculture (UAM) and college of advance and professional studies (CAPS) soil layer index showed a high value for the UAM soil being 43.84 % higher than in the CAPS soil value of 26.92 %. Thus, an eco-friendly herbicide delivery system was formulated using CuCtsNPs, for the successful release of Dicamba in aqueous medium and agricultural soils.

Keywords: Controlled release, Dicamba, Encapsulation, Green synthesis, Herbicide, Soil

INTRODUCTION

Weeds cause greater damage to agriculture than pests and diseases together. Weeds constitute a major barrier to food production and economic development in many regions of the world because they compete for nutrient, water, sunlight and carbon dioxide with the cultivated crops, thereby reducing the main crop's productivity (Song, 2014; Meschede *et al.*, 2015). Weeds are unwanted vegetation, which are not intentionally planted, but continuously grow in unexpected areas within and around cultivated places (Mensah *et al.*, 2015). Herbicides, on the other hand, are a group of agrochemical substances known as plant protection products (PPP) that are utilised to specifically, partially or totally control or kill undesirable vegetation, such as weedy plants with the aim to significantly increase crop productivity by protecting the crop (McManus *et al.*, 2014). Herbicides are commonly formulated in the form of sprayed liquid and dry solids (Qurratu and Reehan, 2016; Maruyama *et al.*, 2016). Their mode of action is mostly a biochemical or a physical mechanism, which includes absorption into plant and translocation to the site action and also disrupting or altering one or more of their metabolic processes resulting in the weed's death (Norhayati *et al.*, 2014). Weeds always or

predominantly grow in situations clearly altered by man, presenting undesirable effects at a specific time and place (Sopena *et al.*, 2009; Mensah *et al.*, 2015).

According to Garcia *et al.* (2012), agriculture is the largest user of pesticide/herbicides, consuming 85% of the world production. This is aimed to chemically control the various pests that reduce the quantity and quality of food crops and other vegetables (Garcia *et al.*, 2012). This practice has greatly increased global food supply, but the excessive, inappropriate and uncontrolled use of synthetic agrochemicals for plant growth and protection (Sopena *et al.*, 2009; Ramadass and Thiagaraian, 2017). The side effects of this practice is, increased resistance in plant pathogenic microbes, negative impact on non-target organisms causing an increase in the level of herbicide residues in natural water, soil, and foodstuffs (Farhat-Un-Nisa *et al.*, 2011), deterioration of soil health and threatening to life and life supporting systems (Tornisielo *et al.*, 2013; Dhillon and Mukhopadyay, 2015).

Controlled release (CR) is defined as the permeation-regulated transfer of an active ingredient from a reservoir to a targeted surface in order to maintain a predetermined concentration level for a specified period of time (Roy *et al.*,

2014). Controlled release formulations (CRFs) can maintain an effective level of active ingredient (a.i) delivery for a controllable and longer time and represents an alternative to the conventional systems of herbicide application (Ruegg *et al.*, 2007; Sopena *et al.*, 2009; Faria *et al.*, 2017; Itodo *et al.*, 2017). This technology has been applied in various industries including the biomedical, food, agricultural, and pharmaceutical to deliver pesticides, herbicides, fertilizers, plant growth regulators, biomolecules, and drugs. Its main objectives are majorly more effective treatment, prolonged efficacy, enhanced safety and reliability and reduced side effects (Roy *et al.*, 2014). This thereby reduces the level of these substances in the environment, as lower amounts or fewer applications are required in order to achieve the pursued biological effect (Garrido *et al.*, 2014). Controlled-release formulations (CRFs) for agrochemicals are able to address problems such as leaching, volatilization and surface migration of the chemical substance because of their sustained-release properties and better performance than conventional formulations, even at lower dosages maintaining an effective concentration over a given interval of time (Aouada *et al.*, 2010; Nnamonu *et al.*, 2012; Choudhary *et al.*, 2017).

The application of nanotechnology to agriculture and food industries is getting greater attention nowadays. Changes in agricultural technology have been a major factor shaping modern agriculture (Biswal *et al.*, 2012). Among the latest line of technological innovations, nanotechnology occupies a prominent position in transforming agriculture and food production (Biswal *et al.*, 2012). Nanotechnology is a field of science which deals with production, manipulation and use of materials ranging in nanometers (Kavitha *et al.*, 2013). Nanotechnology has the potential to revolutionise the agricultural and food industry with novel tools for the molecular management of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients, among others (Ram *et al.*, 2014). Nanoencapsulation is a process through which pesticides are slowly, but efficiently, released to a particular host plant for pest control, using nanoparticles (Ashtaputrey *et al.*, 2017). It allows proper absorption of the pesticide active ingredient (a.i) into the plants (Itodo *et al.*, 2017). In recent times, most leading chemical companies focus on formulation of nanoscale pesticides for delivery into the target host tissue through nano-encapsulation (Ram *et al.*, 2014). Effective encapsulation of pesticides is required to provide a sustained and targeted delivery in order to minimize the amount of a.i used (Roy *et al.*, 2014). Dicamba is a broad-spectrum and synthetic auxin herbicide in the benzoic acid chemical family with the chemical name 3,6-dichloro-2-methoxybenzoic acid or 2-methoxy-3,6-dichlorobenzoic acid. This material was first registered in 1967 (NPIC, 2012;

Nishimura *et al.*, 2015). This chemical compound is a chlorinated derivative of o-Anisic acid, with different brand names for formulations which include Banvel, Diablo, Oracle and Vanquish (Grube *et al.*, 2011; Stone, 2013). It is a selective post-emergent herbicide used to control certain annual and perennial terrestrial broadleaf weeds and woody plants in grain crops and highlands (Beyki and Asadollahzadeh, 2016). Like other phenoxy herbicides, Dicamba mimics auxins, a type of plant hormone and causes abnormal growth by affecting cell division (Bunch and Gervais, 2012; Christoffoleti *et al.*, 2015; Beyki and Asadollahzadeh, 2016). Growth inhibition of stems, petioles, and leaves, senescence and tissue decay are usually the results of the entire process (Bunch and Gervais, 2012; Nishimura *et al.*, 2015). This abnormal plant growth disrupts cellular transport systems and eventually leads to the death of the plant. Non-target plants exposed to even a small quantity of Dicamba can experience phytotoxic effects like cupping, malformation, and necrosis of terminal leaves (EFSA, 2011; Egan and Mortensen, 2012). Under standard conditions, it usually exists as a crystalline solid and may also exist as a vapour or in the form of particulates (Bunch and Gervais, 2012; Nishimura *et al.*, 2015). Dicamba is moderately persistent in soil and is likely to be more rapidly degraded in soils with high microbial populations (Kah *et al.*, 2007; EFSA, 2011). It has a low organic carbon partition coefficient (K_{OC}) and therefore, has a low affinity for soil particles and suspended sediments. It has a low octanol/water partition coefficient (K_{OW}) and is resistant to oxidation and hydrolysis under most conditions (Strachan *et al.*, 2010).

The work reported here focused on providing a better and alternative solution to the several disadvantages associated with the conventional application of the Dicamba herbicide by formulating nanoparticles-based formulation. In this, chitosan was used as a matrix for the release of Dicamba dimethylamine herbicide, thereby reducing its harmful effect on farms and still maintains the highest possible effectiveness.

MATERIALS AND METHODS

Materials

All the chemicals procured for this study were used without further purification. High molecular weight chitosan and analytical grade of Dicamba (99.9 %) was supplied by Sigma Aldrich, Germany. Commercial formulation of Dicamba with trade name Banvel® (liquid formulation) was supplied by Syngenta Agro GmbH, Germany. $CuSO_4 \cdot 5H_2O$ (0.1 M), L-ascorbic acid (0.2 M), Acetic acid (1.0 M), $CaCl_2$ (0.01 M), NaOH (0.1 M) and distilled water were also used. The release media pH 5.5, 7.0 and 8.0 buffers were prepared from KHP (0.1 M) BDH Chemical Limited, Poole, England, KH_2PO_4 (0.1 M), NaOH (0.1 M) and HCl (0.1 M) supplied by JHD Chemicals, China. All

solutions were prepared using distilled water. Characterizations were carried out using UV-Visible spectrophotometer (Cary 300), Scanning electron microscopy (SEM), Fourier transform infrared (FT-IR), X-ray diffraction (XRD) Differential scanning calorimetry (DSC), spectroscopy, Thermo gravimetric analysis (TGA), differential thermal analysis (DTA), Energy-dispersive X-ray (EDX).

METHODOLOGY

Loading of dicamba herbicide onto CuCtsNPs

Synthesis of encapsulated dicamba herbicide onto CuCtsNPs was carried out in-situ using method according to Ihegwuagu (2015) and Faria *et al.* (2017), with slight modification. In this synthesis, 50 mL of 0.1 M CuSO₄.5H₂O aqueous solution was transferred into a conical flask and 100 mL of 3 % chitosan solution was added, changing the colour from blue to light blue. After 30 minutes of stirring, 25 mL of 0.2 M L-ascorbic acid solution was added drop wise into the flask and 210 ppm of Dicamba herbicide formulation was added to the stirring mixture as soon as the brown colour appeared and was heated for 2 hours. The resultant mixture was allowed to cool before being centrifuged at 2,000 rpm for 40 minutes. The supernatant was collected and analysed for the amount of unloaded or free a.i in the mixture, while the particles were washed thrice using distilled water. These were then oven dried at 40°C for 24 hours. The dried nanoparticles were grounded into fine powder, stored in an air tight sample vial and kept in a vacuum desiccator devoid of light for further use and characterization.

Encapsulation efficiency (EE, %) of Dic-CuCtsNPs

The encapsulation efficiency (EE, %) is expressed as the ratio between the amount of herbicide encapsulated by the nanoparticles and the total (100%) amount of herbicide used in the loading process. Method described by Faria *et al.* (2013) was adopted in this study to estimate the encapsulation efficiency of the loaded CuCtsNPs using equation 1.

$$\% EE = \frac{\text{total a.i used} - \text{a.i in supernatant}}{\text{total amount of a.i used}} \times 100 \quad (1)$$

Characterisation of the synthesised and encapsulated CuCtsNPs

The formation of CuCtsNPs and 2,4-D-CuCtsNPs was monitored using a UV-Visible spectrophotometer (Cary 300 UV-Visible spectrophotometer, software version 4.20). The spectrophotometer baseline correction was carried out using distilled water (blank). The particle size distribution and morphology of the loaded CuCtsNPs was carried out using an SEM technique (ESEM, FEI Quanta 200 FEG) operating at an accelerating voltage of 15 kV. The possible molecular interaction(s) between the synthesized and loaded CuCtsNPs was achieved using an FT-IR instrument (Shimadzu FTIR-8400S FT-IR spectrophotometer), recorded within the range of 4000 – 400 cm⁻¹, with 2 cm⁻¹ resolution and 10 scans. X-ray diffraction pattern was obtained for the loaded CuCtsNPs using a PANalytical X'Pert PRO diffractometer operated at 40 kV and 30 mA with Cu K α radiation ($\lambda = 1.5406$). Working conditions used include 2 θ scanning range of 5.0 – 80.0°, scanning step size of 0.02, speed of 1.00 s, sample length of 10.00 mm and a measurement temperature of 25 °C. Thermal characterization of the nanoparticles was carried out using a TGA instrument (PerkinElmer TGA 4000 thermogravimetric analyser) with operating condition of heating from 30 °C – 950 °C at 10.00 °C/min heating rate. DSC characterisation was carried out on the loaded CuCtsNPs using a DSC instrument (Mettler STAR[®] SW 13.00 differential scanning calorimeter) with a heating rate of 10.00 °C/min over a temperature range of 30 – 310 °C with 34.98 °C left limit and 300.21 °C right limit in an ambient air. The elemental composition for the synthesised and loaded CuCtsNPs was studied using an EDX-coupled with ESEM.

Preparation of herbicide stock solution and calibration curve

Dicamba stock solution was prepared using analytical grade of dicamba (PESTANAL[®]) To prepared 250 mg/L, 250 mg of dicamba was dissolved in a beaker, transferred into a 1000 ml volumetric flask and was made to mark with distilled water. Six point serial dilutions (20, 40, 60, 80, 100 and 120 ppm) of the herbicide were prepared from its stock solutions as working standards. Absorption readings for dicamba were scanned from 220-330 nm to obtain the absorbance maximum at 304 nm and a standard calibration curve was obtained by plotting concentration verses absorbance as shown in Figure 1.

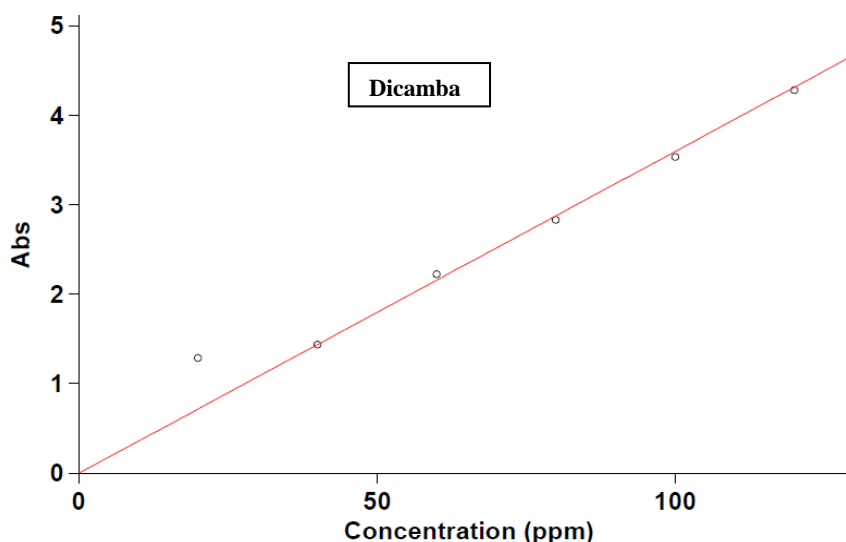


Figure 1: Standard calibration curve for dicamba at 304 nm

Aqueous Release Studies of Dic-CuCtsNPs

Methods earlier reported by Faria *et al.* (2017) and Itodo *et al.* (2017) were adopted for the release study in water. 0.5 g of the loaded nanoparticles was placed in a 15 mL vial at an ambient temperature of 25 ± 2 °C. The mixture was shaken and at definite time intervals, sample of dissolution medium (3 mL) was withdrawn and replaced with fresh solution (3 mL) to maintain sink condition. The time intervals ran from 0, 0.1, 0.3, 1, 2, 3, 4, 5, 6, 12, 24, 48, 72 hours, through 20 days. The withdrawn samples were analysed by means of UV-Vis spectrophotometer for the amount of a.i released. Concentration of herbicide released from Dic-CuCtsNPs was calculated from the calibration plot of Dicamba stock solutions.

Effect of pH on aqueous release of Dic-CuCtsNPs

The effect of pH on the release patterns was studied on the dicamba loaded nanoparticles using different (pH 5.5, 7.0 and 8.0) media to monitor the release process. This process was carried out for at ambient temperature (25 ± 2 °C) for 14 days. The absorbance of the resultant solution was measured with the aid of a UV-Visible spectrophotometer.

Aqueous release of Dic-CuCtsNPs in soils

The leaching potential of a.i released from both free and the encapsulated CuCtsNPs were carried out through soil leaching experiments, using soil leaching columns. Method earlier described by Jianfa *et al.* (2012) was adopted for this study, with slight modifications. Soil column tubes with an inner diameter 2.5 mm and a length of 15 cm were packed with glass wool to a height of approximately 4 cm at the bottom. This was to keep the soil, prevent losses and contamination of the leachates with the soil particles. 5 g of the untreated air-dried and 2 mm sieved soils were

manually packed into the glass columns, having length of 10 cm and inner diameter of 2.5 mm, with spatula under gentle column vibration, until no further sinking of the soil had occurred. This was to obtain uniform packing for reproducible results. The packed columns were pre-wetted with 15 mL of the artificial rain (0.01 M CaCl_2 aqueous solution) from top to bottom to displace available air in the soil pores by water. The columns were allowed to equilibrate and excess water was drained off. 0.5 g 2,4-D-CuCtsNPs was weighed and applied evenly over the surface of the wet soil in the columns. The columns were leached with 5 mL of the artificial rain, drop-wise, with the aid of an adjustable 25 mL burette, to mimic rain drop and to control the velocity of water flux. The leachate was collected into a fitted flat-bottom flask and transferred into a 5 mL sample vial. The soil column leaching experiments were carried out for 10 days to represent 10 successful rainfalls. The leachates collected daily from the soil columns were analysed using the UV-Visible spectrophotometer.

RESULTS AND DISCUSSION

Dicamba encapsulation efficiency

The herbicide encapsulation was aimed at controlling the liberation of the formulations a.i, to enable their use for specific purposes, at an intended concentration. This was also targeted to reduce their disadvantages and to maintain their highest possible effectiveness (Sopena *et al.*, 2009; Grillo *et al.*, 2014 and Norhayati *et al.*, 2014). The encapsulation process for the Dic-CuCts-NPs was successfully carried out using a 3 % matrix. From the encapsulation of dicamba on the 3 % chitosan matrix, it was observed that an increase in the herbicide amount had little or no change on encapsulation efficiency of the CuCtsNPs. This may likely reduce when the surface of the matrix is saturated. This is because the principle of active

ingredient (a.i) loading is surface dependent, as herbicide adsorption onto CuCtsNPs was carried out (Simar *et al.*, 2011; Itodo *et al.*, 2017). The percent EE observed for Dic-CuCtsNPs was 86 % when 150 ppm of dicamba was loaded onto the 3 % matrix. In herbicide encapsulation processes for agricultural applications, it is very important for a fraction of the herbicide active compound to remain unloaded with the polymeric matrix, as this free a.i can provide an initial effect for weed eradication before subsequent slow release from the loaded nanoparticles. The obtained encapsulation efficiencies in this study indicates that the initial weed eradication effects can be achieved using this system. The results obtained

were similar to the values reported by Silva *et al.* (2011) and Grillo *et al.* (2014).

UV-Visible spectrophotometric analysis of the Dic-CuCtsNPs

The formulated Dic-CuCtsNPs showed characteristic absorption peak at 556 nm as shown in Figure 2. The decrease in peak value from 560 nm for CuCtsNPs to 556 nm for dicamba may be as a result of the herbicide interaction in the process of encapsulation. The reduction of Cu^{2+} ions in the presence of chitosan and the use of L-ascorbic acid as capping and reducing agent leads to the formation of well-defined and highly polydisperse nano-herbicide (Mott *et al.*, 2007; Dang *et al.*, 2011; Zain *et al.*, 2014).

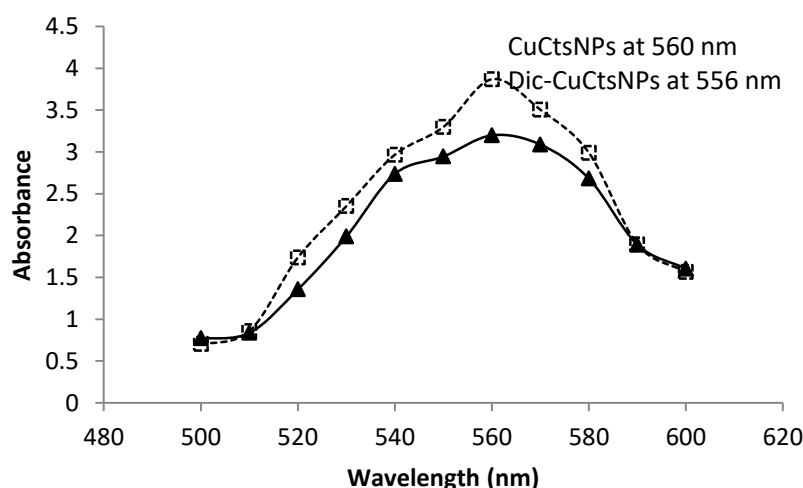


Figure 2: Absorption maxima for CuCtsNPs and Dic-CuCtsNPs

SEM analysis of Dic-CuCtsNPs

Scanning electron microscope (SEM) is one of the most widely employed techniques in nanomaterials and nanostructural characterization. An environmental SEM was employed to study the surface morphology of the formulated Dic-CuCtsNPs. The ESEM image obtained was shown on Figure 3. The image obtained for Dic-CuCtsNPs indicates clearly that the particle size ranged from 16 – 64 nm with shapes ranging from

spherical, rod to bud-shape (Ipsa and Nayak, 2013, Krithiga and Jayachitra, 2014; Suresh *et al.*, 2014; Sagadevan and Koteeswari, 2015). The image obtained for CuCtsNPs shows slight agglomeration which may probably be linked to the electrostatic interaction between the synthesized CuCtsNPs. The particle sizes obtained in this study were close to those obtained by other authors (Ayesha *et al.*, 2015; Sagadevan and Koteeswari, 2015; Phul *et al.*, 2018).

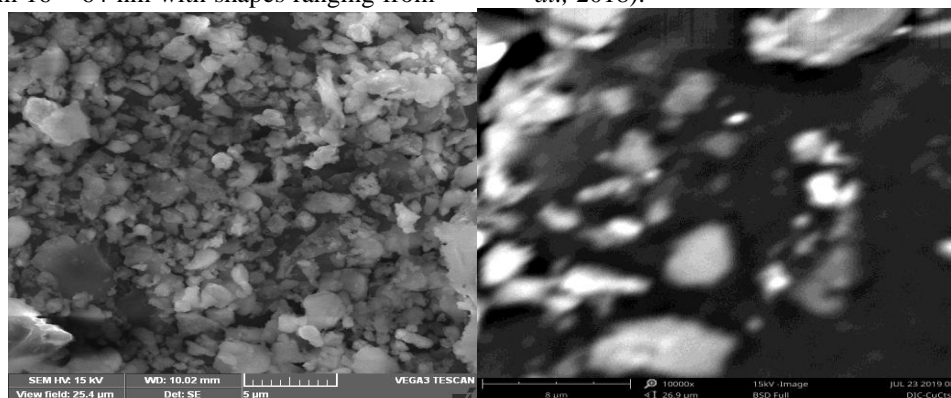


Figure 3: ESEM image for the formulated Dic-CuCtsNPs

FT-IR analysis of Dic-CuCtsNPs

Fourier transform infrared spectroscopic technique was employed to investigate the molecular interaction(s) among the different groups and the change in the chemical composition of the formulated Dic-CuCtsNPs. It provides information about the entities with their functional groups associated with the synthesized CuCtsNPs. The FT-IR spectrum for Dic-CuCtsNPs showing absorption bands at various wave numbers was presented in Figure 4 and is summarized in Table 1. The observed peaks at 3634 cm^{-1} and 3459 cm^{-1} arise due to free phenolic OH group and overlap of O – H and N – H stretch, respectively. This indicates that the encapsulation of Dic-CuCtsNPs unto the synthesized CuCtsNPs was successful (Shikha *et al.*, 2015; Sreeja *et al.*, 2015). The peaks observed at 3395 cm^{-1} and 3079 cm^{-1} correspond to intermolecular H-bonds and a strong OH stretch of

carboxylic acid respectively. The peak at 2824 cm^{-1} corresponds to a methoxy O – CH₃ stretch coming from the dicamba molecular structure. The peaks observed at 1705 cm^{-1} , 1412 cm^{-1} and 1250 cm^{-1} corresponds to C = O stretch, vinyl C – H in-plane bend and a phenolic C – O stretch respectively while the observed peak at 1126 cm^{-1} corresponds to a stretching vibration of C – O – C (Miao *et al.*, 2015; Khalid *et al.*, 2015) probably as a result of a conjugated carbonyl group. The observed peak at 1018 correspond to a C – O stretch while the peaks observed at 887 cm^{-1} and 795 cm^{-1} correspond to a di-substitution associated with C – Cl stretch of halo compounds (Ashtaaputrey *et al.*, 2017). The observed molecular interaction between the CuCtsNPs and loaded herbicide proved that the surface of the NPs was able to absorb dicamba as shown in Figure 4.

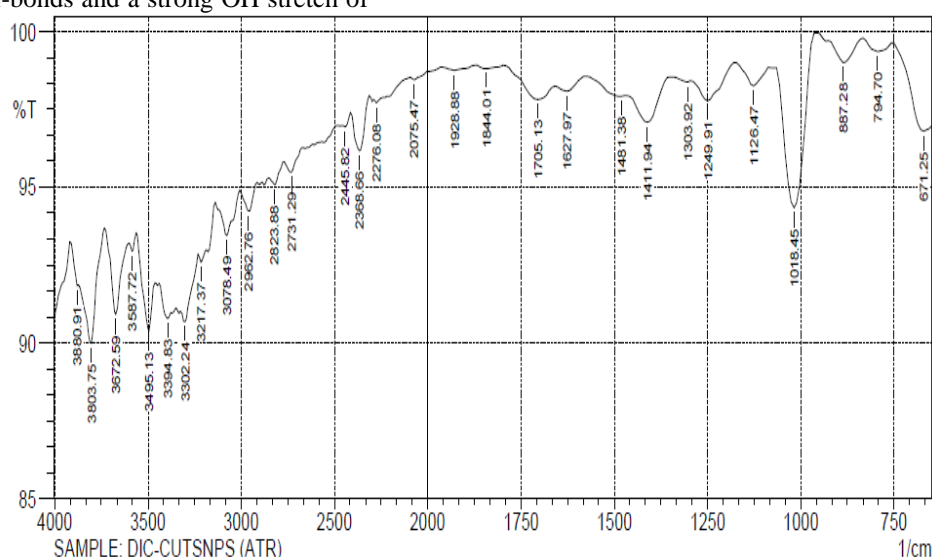


Figure 4: FT-IR spectrum for the synthesized Dic-CuCtsNPs

Table 1: FT – IR functional group analysis for Dic-CuCtsNPs

Group frequencies (cm^{-1})	Functional group	Observed frequencies (cm^{-1}) for;		Vibration assignment
		CuCtsNPs	Dic-CuCtsNPs	
3750 -3500	O – H	3572 (s,b),	3673 (m)	Hydroxyl group, H-bonded OH stretch
3570 - 3200	N – H, O – H	3449 (s,b)	3495 (s,b)	Amine, hydroxyl overlap
3300 - 2900	N – H	3163, 3009 (m)	3079 (m)	Amine stretch
3000 – 2700t	C – H	2862 (m)	-	Aliphatic C-H stretch
1900 - 1650	C = O	1790 (s)	1705 (s,b)	Carboxylic acid stretch
1680 - 1620	C = C	-	-	Alkenyl C=C stretch
1420 - 1410	C – H	-	1412 (m)	Vinyl C-H in-plane bend
1300 - 1200	C – O	-	1250 (m-s)	Phenol C-O-C stretch
1090 -1020	C – N, C – O	1034 (s,b)	1018 (m)	Primary mine CN, carbonyl CO stretch
900 – 860	C – Cl	-	887(s)	1,3-disubstituted
800 – 700	C - Cl	-	795 (s)	Chloro C-Cl stretch

PXRD analysis on Dic-CuCtsNPs

The XRD technique was employed to determine the crystalline state and the structural properties of the formulated Dic-CuCtsNPs. The XRD patterns obtained are presented in Figure 5. Prominent diffraction peaks were observed for copper from the loaded CuCtsNPs at around 2θ values of 39.18° , 43.56° , 50.76° and 74.31° . These correspond to (110), (111), (200) and (220) crystallographic planes of face-centered cubic (fcc) copper respectively. This confirms the cubic lattice of copper (Phul *et al.*, 2018; Deneke *et al.*, 2017; Sagadevan and Koteeswari, 2015; Theivasanthi and Alagar, 2010). The other diffraction peaks observed at 2θ values of 6.31° and 16.18° correspond to (001) and (020) may probably arise from the L-ascorbic acid and the chitosan used. All the diffraction peaks obtained in this work (Table

2) were in good agreement with the standard pattern for pure fcc phase of CuNPs -JCPDS No. 04-0836 and copper X-ray diffraction reference No. 01-089-2838 (Theivasanthi and M. Alagar, 2010, Usman *et al.*, 2013; Krithiga and Jayachitra, 2014; Suresh *et al.*, 2014; Deneke *et al.*, 2017). The highly intense peak for an fcc material is generally observed at (111) reflection and this was observed for the dicamba loaded CuCtsNPs. The significant broadening of the peaks was attributed to the nanocrystalline nature of the particles. This was evident from the size obtained using the Debye-Scherrer's equation with an average crystalline size of 46 nm, for Dic-CuCtsNPs indicating high surface to volume ratio and high surface charge (Krithiga and Jayachitra, 2014; Deneke *et al.*, 2017; Choudhary *et al.*, 2017).

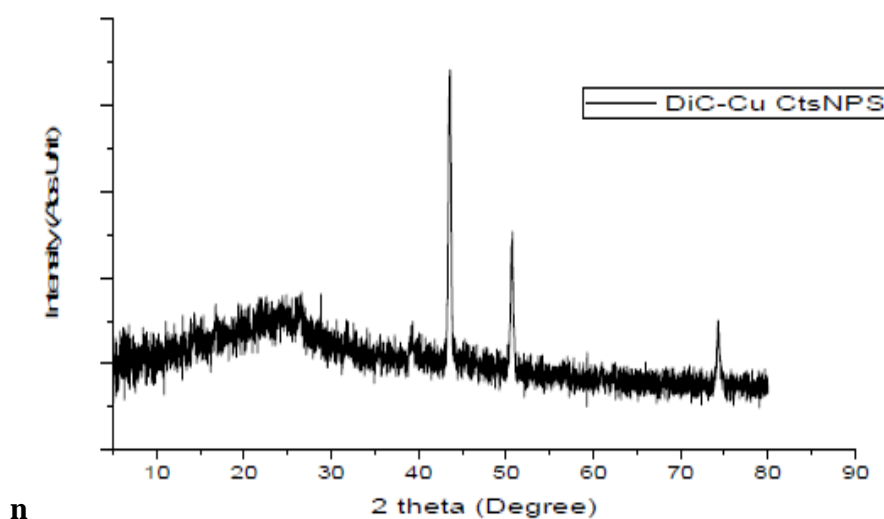


Figure 5: PXRD pattern for the formulated Dic-CuCtsNPs

Table 2: XRD Profile for the Formulated Dic-CuCtsNPs

Position [2θ]	Height (cts)	FWHM [2θ]	Crystalline size (nm)	hkl values	d-spacing (Å)	Rel. int. (%)
6.31	26.13	0.9446	16.84	001	14.0044	8.03
16.18	24.43	0.4723	33.97	020	5.2934	7.51
39.09	30.68	0.4723	35.68	110	2.2994	9.43
43.55	325.33	0.2755	62.08	111	2.0779	100.00
50.76	136.20	0.2755	63.81	200	1.7987	41.86
74.31	56.78	0.3149	63.28	220	1.2764	17.45

Average crystalline size = 45.94 nm

cts = counts, FWHM = full width at half maximum, hkl = miller indices and Rel. int = relative intensity

DSC for the formulated Dic-CuCtsNPs

DSC analysis was carried out on the formulated Dic-CuCtsNPs and the curve obtained is shown in Figure 6. The curve shows a T_g at 37.64°C and a single exothermic response which may be as a result of a new crystalline phase formation at 161.66°C . The onset temperature was seen at 158.62°C , with the endset of 165.55°C , while having a peak height of -61.89 mW . The

values obtained here were lower than the one obtained by Mott *et al.* (2007) which could be linked to herbicide type. The curve also shows another single peak corresponding to the melting of Dic-CuCtsNPs, starting at 229.41°C – with a maximum at 283.61°C , through 293.61°C . These indicate that the amorphous (pre-crystalline) content and the crystalline state where able to combine, thereby leading to its emelting, with a peak height of 22.39 mW (Shah *et al.*, 2006).

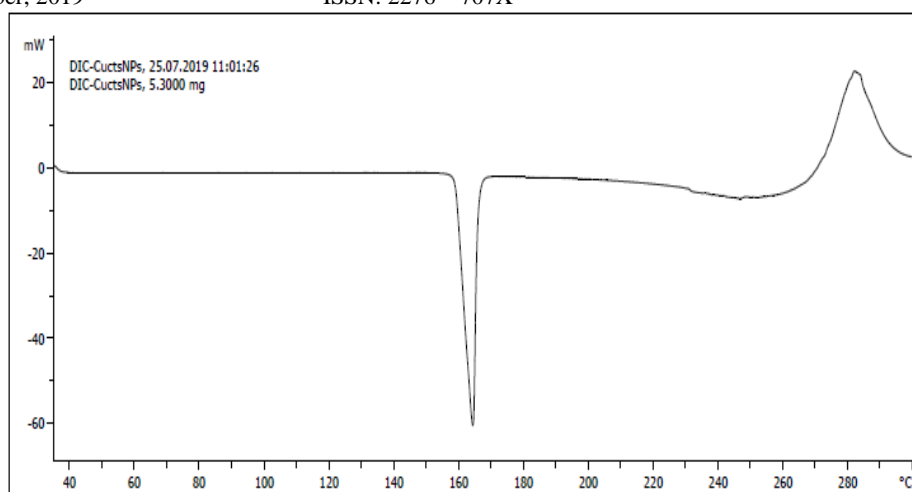


Figure 6: DSC curve the formulated Dic-CuCtsNPs

TGA/DTA analysis of Dic-CuCtsNPs

The formulated Dic-CuCtsNPs was subjected TGA and DTA analyses, with the results shown in Figure 7. The analyses were undertaken in order to study the changes in its composition and the extent of its stability with respect to temperature. The TGA curve shows three decomposition steps, with initial weight loss of 8.80 % from 26.48 °C to 273.50 °C. This is due to the present of moisture and other volatile matter. The decomposition of Dic-CuCtsNPs shows an onset between 26.48 – 273.50 °C, a mid-point between 273.50 – 425.75 °C, 389.94 °C and complete degradation between 469.19 – 678.18 °C. The gradual increase from 273.50 °C to the midpoint at 335.16 °C (37.30 %) and the

corresponding weight losses can be linked to pyrolysis and combustion of organic compounds from the starting materials that leave the material in the form CO_x ($x = 1,2$) (Mott *et al.*, 2007). The observation from this result was in line with well resolved exothermic peak in the DTA curve at an onset temperature of 178.45 °C to 548.69 °C, with a maximum peak at 412.77 °C. The values obtained from this study were higher than that obtained by Nattaya *et al.* (2014) which may be due to the nature of how the herbicide was loaded onto the nanoparticles. The 59.45 % complete degradation, as observed from the curve for the Dic-CuCtsNPs, shows that the material was thermally stable up to 425.75 °C (Chaudhary *et al.*, 2017).

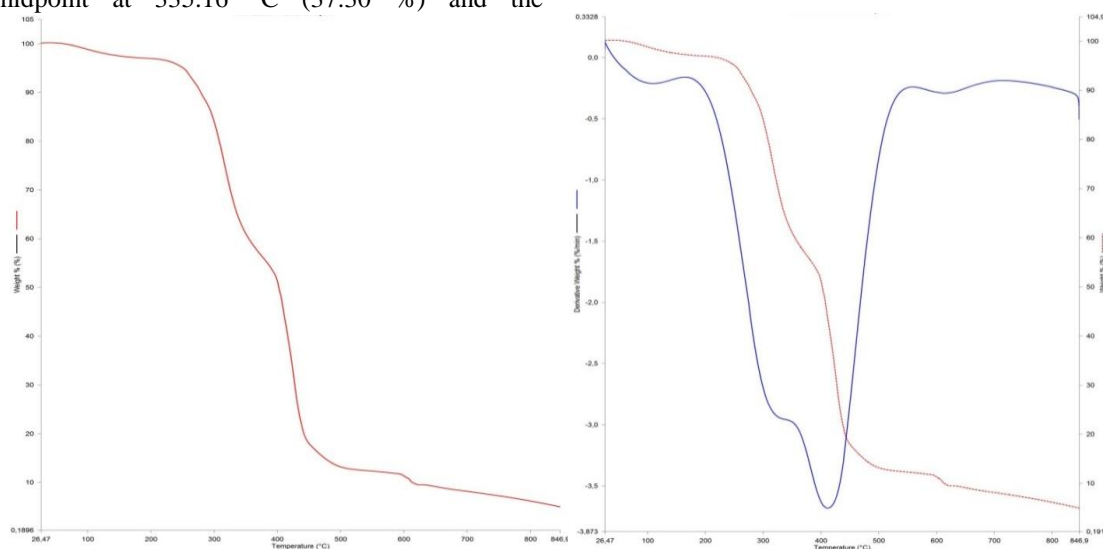


Figure 7: (a) TGA and (b) TGA/DTA thermographs for Dic-CuCtsNPs

EDX analysis on Dic-CuCtsNPs

The elemental composition for the formulated Dic-CuCtsNPs was characterised using ESEM coupled energy dispersive x-ray spectrometer (EDX). The EDX profile displayed

on Figure 8 indicates the presence of Cl and Cu as the major component. From the EDX pattern, the atomic concentration of Cl 39.22 % was observed and 33.93 % for Cu (Table 3). This implies that the chlorine in the herbicide was able to bind to the

surface of the CuNPs (Suresh *et al.*, 2014; Manikandan and Sathiyabama, 2015). It is very clear from the EDX profile that the formulated Dic-CuCtsNPs reduced the weight percentage of elemental copper from 60.37 % to less than 40.00

%. All other elements seen from the profiles may probably be as a result of left over impurities or excipients from herbicide’s formulation.

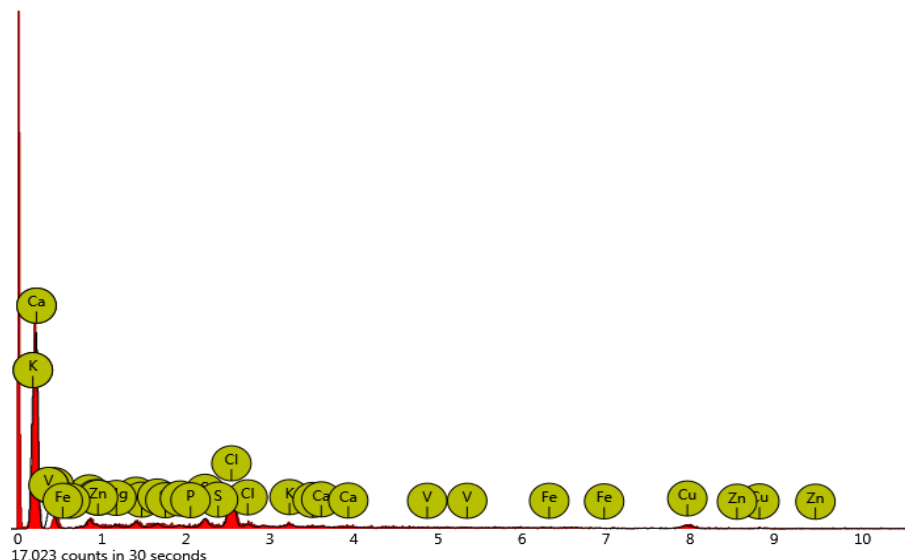


Figure 8: EDX Spectrum for Dic-CuCtsNPs

Table 3: Elemental composition of the formulated Dic-CuCtsNPs

Element Number	Element Symbol	Element Name	Atomic Conc. (%)	Weight Conc. (%)
17	Cl	Chlorine	39.22	35.10
29	Cu	Copper	21.15	33.93
16	S	Sulfur	8.55	6.92
13	Al	Aluminium	7.06	4.81
11	Na	Sodium	6.63	3.85
19	K	Potassium	3.81	3.76
30	Zn	Zinc	2.03	3.34
14	Si	Silicon	4.21	2.98
12	Mg	Magnesium	4.15	2.54
15	P	Phosphorus	2.07	1.62
20	Ca	Calcium	1.13	1.14

Aqueous release studies of Dic-CuCtsNPs

The aqueous release study of Dic-CuCtsNPs was carried out in water. The aim was to study the release the herbicide active ingredient (a.i) from the loaded polymeric matrix at a nanoscale level. Herbicide release from nanoparticles is usually a biphasic phenomenon although in some instances a triphasic trend is observed (Itodo *et al.*, 2017). The mode of release in water (Figure 9) shows a steady release pattern as the time increases. The herbicide release profile exhibits an initial burst release, presumably from the particle surface, followed by slow release driven by polymer erosion and diffusion of the a.i

through pores in the material (Sun *et al.*, 2012; Fernanda *et al.*, 2014; Faria *et al.*, 2017). This can be an advantage since it can control the sprouting of weed seeds and still exist to kill and inhibit new infestations (Silva *et al.*, 2011; Onyido *et al.*, 2012 and Grillo *et al.*, 2014). The release profile for the Dic-CuCtsNPs gave a reasonable, sustainable and steady release. The release of the herbicide’s a.i from the loaded CuCtsNPs is determined basically by the size of the nanoparticles, the nature of reagents employed, the molecular weight of the polymeric material and the type of interaction involved (Siepmann and Peppas, 2012; Peppas and Narasimham, 2014).

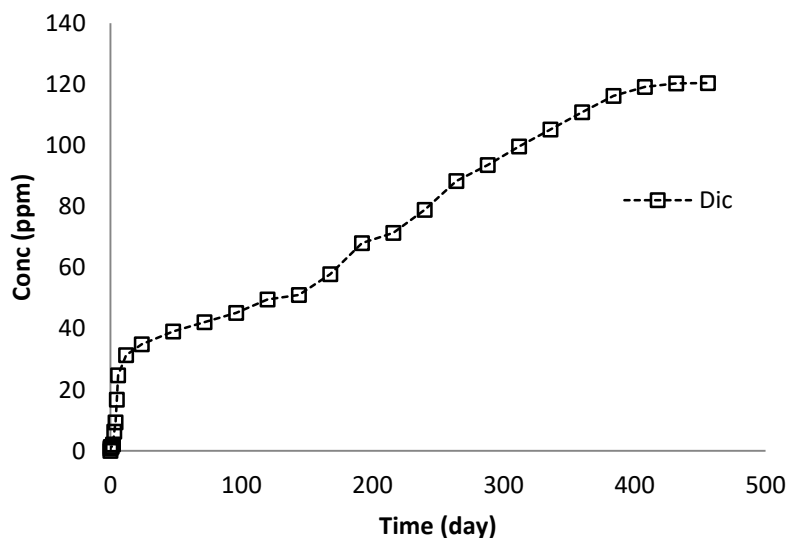


Figure 9: Aqueous release profile of Dic-CuCtsNPs

Effect of pH on aqueous release of Dic-CuCtsNPs

In order to study the effect of pH on the aqueous release process of the formulated Dic-CuCtsNPs, three different pH media i.e acidic (pH 5.5), neutral (pH 7.0) and alkaline (pH 8.0) were used. These media were used to represent the different environmental conditions. The results in Figure 10, obtained from the release of the herbicide a.i from the nano-encapsulation show a release at the neutral medium (pH 7.0) corresponding to 61.18 %. The release of dicamba

into the neutral medium was high compared to the release pattern observed in the pH 5.5 (acidic medium) with 58.47 %. The release of dicamba into acidic medium was moderately low while the alkaline environment (pH 8.0) was observed to have the lowest release percentage of 22.20 %. According to Nishimura *et al.* (2015), the release of dicamba into a neutral environment is microbially mediated and this could be the reason for the high value obtained in this study.

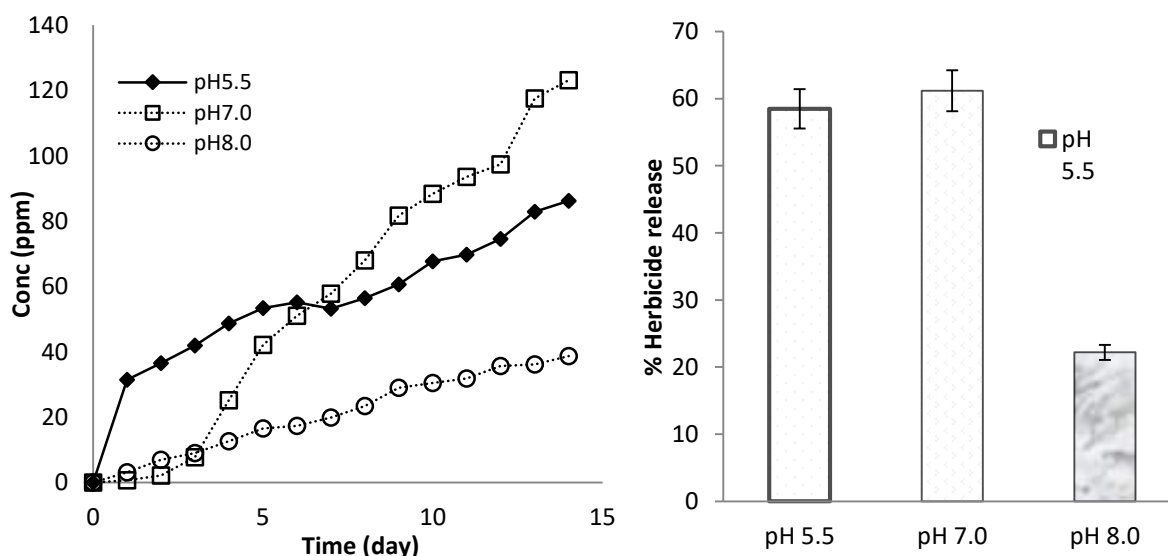


Figure 10: Effect of pH and Percentage release of a.i from of Dic-CuCtsNPs

Aqueous release of Dic-CuCtsNPs from Soil column

The leaching potential of the active ingredient (a.i) from dicamba into soil from the synthesized CuCtsNPs was studied. Pesticide

leaching is one of the major contaminant of groundwater and can be determined by the physicochemical properties of pesticide active ingredient, soils and rainfall (Włodarczyk and Siwek, 2016). Leaching profiles of the loaded

CuCtsNPs through the soil layer (UAM and CAPS) is shown in Figure 11. The percentage herbicide a.i release for the herbicide in the leachate after 10 irrigations on UAM soil was higher (43.84 %) than on CAPS soil (26.92 %). This observation could be as a result of the soil's ability to regulate the applied artificial rain and the amount of the a.i released from the matrix, which is dependent on the soil physicochemical properties. The results obtained in this study imply that encapsulating herbicides onto CuCtsNPs matrix can be a useful practice in reducing

herbicide leaching into soil since only small amount of a.i will be available for leaching. Similar observation has been reported from previous works carried out by Jianfa *et al.* (2012) and Włodarczyk and Siwek (2016). The percentage herbicide released showed that the process can still continue with time implying that nano-encapsulation of herbicides is beneficial in maintaining the biological effect of herbicides for a longer time, and also enhances their efficiency (Jianfa *et al.*, 2012).

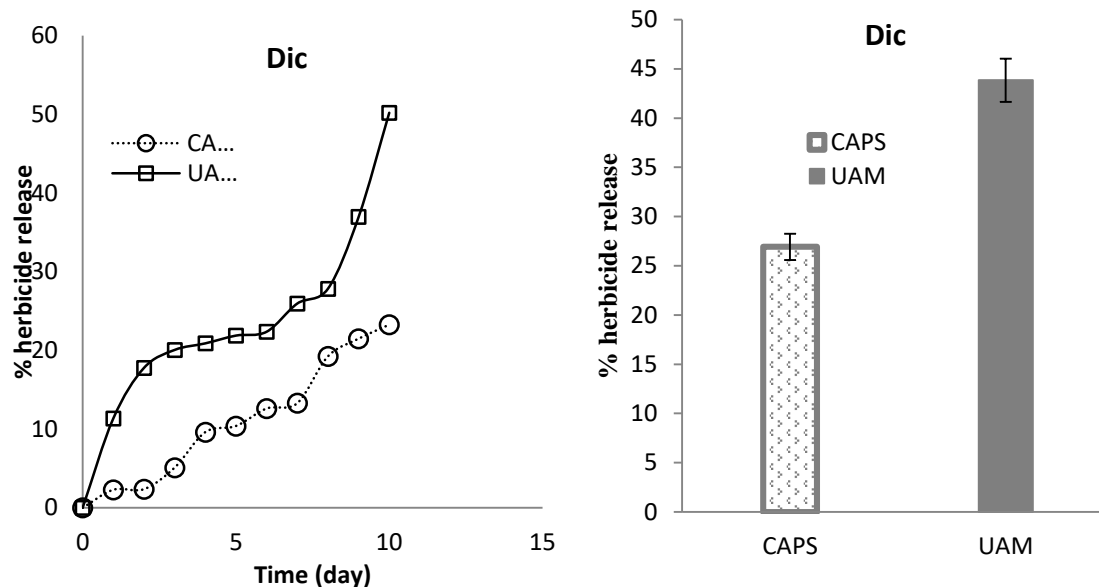


Figure 11: Aqueous release of herbicide a.i from Dic-CuCtsNPs in both CAPS and UAM soils

CONCLUSION

This study employed green chemical reduction method for the synthesis of Dic-CuCtsNPs and an environmentally friendly herbicide delivery system for the controlled release experiments that were undertaken. The method was simple, economical and effective. The encapsulated CuCtsNPs was subjected to different characterization techniques including UV-Visible spectrophotometry, SEM, FITR, XRD, DSC, TGA, DTA and EDX, in order to authenticate the encapsulation carried out and the effectiveness of the resultant material. The findings from this study show that the encapsulation of dicamba herbicides onto metallic-polymer nanoparticles can be used to reduce the negative impacts that can be associated with the use of synthetic herbicides on the environment as these threaten life and life supporting systems.

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