

Coated NPK Fertilizer Based on Citric Acid-Crosslinked Chitosan/Alginate Encapsulant

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ABSTRACT

A coated nitrogen-phosphorus-potassium (NPK) fertilizer was prepared via crosslinking chitosan and alginate using citric acid. Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), particle size analysis, and zeta potential measurement showed the successful crosslinking, appropriate size, and colloidal stability of the coated NPK fertilizer. The encapsulation capability of the crosslinked chitosan/alginate (Chi/Alg) was studied using fluorescence spectroscopy and NPK analysis. The release behavior studies under various pH conditions showed that Chi/Alg NPK conformed to the standards of controlled release fertilizer with a maximum release rate of 40% for 30 days. The investigation of the release mechanism using Korsmeyer-Peppas mathematical model showed that the release of nutrients is governed by both coating material relaxation and diffusion process.

Keywords: controlled release, crosslinking, encapsulation, NPK fertilizer

INTRODUCTION

The nanostructured materials with novel properties that can be easily fine-tuned are ideal to make agriculture-based smart systems. Such smart materials, including encapsulation and controlled release systems, have revolutionized the agrochemical industries by providing more effective delivery of fertilizers (Azeem, KuShaari, Man, Basit, & Thanh, 2014; Jarosiewicz & Tomaszewska, 2003) and pesticides (Campos, de Oliveira, Fraceto, & Singh, 2015; Roy, Singh, Bajpai, & Bajpai, 2014) resulting in the enhanced nutrient absorption, lower loss of the active ingredient (Dasgupta, Madras, & Chatterjee, 2016; Roy, *et al.*, 2014) – thereby lowered possibility of leaching to the environment (more environment-friendly). These technologies can be further designed in such ways that they can respond to environmental changes – such systems are called smart materials (Ni, Liu, Lü, Xie, & Wang, 2011; Xiao, Zhou, & Qian, 2017). Generally, the controlled release fertilizers cost more than the conventional

(uncoated) fertilizers, such as urea. A controlled release fertilizer using natural polymers can provide a cheap but efficient nutrient management strategy (Azeem, *et al.*, 2014; Ni, *et al.*, 2011), not only because it can release the nutrient contents gradually and minimize the environmental pollution – but also the natural polymers abound in nature (Hu & Huang, 2013; Krishna Sailaja, Amareshwar, & Chakravarty, 2011; Rodríguez-Velázquez, Alatorre-Meda, & Mano, 2015) These biopolymers, especially in the form of hydrogels, are great candidate materials for the creation of encapsulation systems (Brown & McShane, 2006; Habibi, Kamaly, Memic, & Shafiee, 2016; Hori, Penaloza, Shundo, & Tanaka, 2012; Lima, Song, Blanco-Fernandez, Alvarez-Lorenzo, & Mano, 2011; Qu, Zhao, Ma, & Guo, 2017; Seiffert, Thiele, Abate, & Weitz, 2010; Shundo, *et al.*, 2014; Shundo, Penaloza, & Tanaka, 2013; Wang, Papadimitrakopoulos, & Burgess, 2013). The controlled release potential of hydrogels using crosslinked polymers is being explored for the environment-friendly and cost-effective coated

fertilizer applications (Bortolin, *et al.*, 2012; Bortolin, Aouada, Mattoso, & Ribeiro, 2013; Essawy, Ghazy, El-Hai, & Mohamed, 2016; Jamnongkan & Kaewpirom, 2010; Saraydin, Karadağ, & Güven, 2000). As an example, chitosan hydrogel was utilized by Jamnongkan and Kaewpirom to study the release kinetics of potassium (Jamnongkan & Kaewpirom, 2010).

While alginate is a preferred material for encapsulation applications (Das, Kasoju, & Bora, 2010; Gombotz & Wee, 1998; Rowley, Madlambayan, & Mooney, 1999; Sultana, *et al.*, 2000; Tan & Takeuchi, 2007), it is highly soluble in water, limiting its role as an encapsulating material. Such issue of instability can be addressed by crosslinking (Awadhiya, Kumar, & Verma, 2016; Demitri, *et al.*, 2008; Monteiro & Airoidi, 1999; Oryan, Kamali, Moshiri, Baharvand, & Daemi, 2018). Chitosan, on the other hand, is also a biopolymer of interest. It has been studied thoroughly as an active ingredient carrier since it is biodegradable, cost-effective, and less toxic (Dash, Chiellini, Ottenbrite, & Chiellini, 2011; Y. Liu, Shen, Zhou, Wang, & Deng, 2016; Stulzer, Tagliari, Parize, Silva, & Laranjeira, 2009; Zhao, Yim, Du, & Abu-Lebdeh, 2018). Chitosan could be derived from the crustacean waste material and the possible source would be its industrial waste, which reduces the costs (Stulzer, *et al.*, 2009).

In solution, chitosan has been found to form a complex with negatively charged polymers due to its cationic character, this overcomes the limitation of alginate as an encapsulant (Araújo, Romão, Doumer, & Mangrich, 2017; Picone & Cunha, 2013; Stulzer, *et al.*, 2009). The combination of chitosan and alginate, after being subjected to crosslinking using citric acid, could result in a more effective controlled release performance, compared to chitosan or alginate alone (Baysal, Aroguz, Adiguzel, & Baysal, 2013; Kumar, Chauhan, Gopal, Kumar, & Dilbaghi, 2015; Li, Dai, Zhang, Wang, & Wei, 2008; Zhang, Wei, Lv, Wang, & Ma, 2011). Citric acid is considered as one of the important organic acids that has a wide commercialization potential. Recently, major production of citric acid was conducted via microbial fermentation, as it is economical and easy to handle. Thus, using citric acid will reduce the cost of the typical controlled release fertilizer (Awadhiya, *et al.*, 2016; de Cuadro, *et al.*, 2015; Demitri, *et al.*, 2008; Ghorpade, Yadav, & Dias, 2016; H. J. Kim, Koo, Kim, Hwang, & Im, 2017; Y. Liu, *et al.*, 2016; Mali, Dhawale, & Dias, 2017; Ren, *et al.*, 2018).

In this regard, this study involved the investigation of the controlled release property of the crosslinked NPK fertilizer that is formed from the crosslinking of the chitosan/alginate (Chi/Alg) using citric acid (CA) encapsulating NPK fertilizer – hereby referred to as Chi/Alg/CA/NPK. Moreover, this study was able to determine the effect of the varying amounts of crosslinker (citric acid as the crosslinker) and polymer blend (Chi/Alg) ratio on the particle size and encapsulation efficiency. It also determined the controlled release behavior *in vitro* as well as through a pot experiment.

EXPERIMENTAL

Materials

Chitosan (low molecular weight, MW-90,000 g/mol), sodium alginate (low molecular weight, MW-80,000 g/mol) glacial acetic acid (AR, 99.7 v/v), citric acid monohydrate (AR, 99.5% w/w), potassium dihydrogen phosphate (AR, 99.5% w/w), calcium chloride dihydrate (AR, 99.5% w/w), ammonium chloride (AR, 99.5% w/w), pH buffers (AR 4.00, 7.00, 9.00), nitric acid (AR 69% v/v), hydrochloric acid (AR, 37% v/v), and sodium 8-anilino-1-naphthalene-sulfonate (AR, 97% w/w) were used. The deionized water was obtained from ELGA Pure Lab Deionizer at the Bureau of Soils and Water Management (BSWM) Laboratory.

Preparation of the chitosan/alginate encapsulated NPK fertilizer

The 3.0% (w/v) solution of sodium alginate was prepared by dissolving appropriate amounts of polymer in deionized water with continuous stirring up to 2 h, whereas the 3.0% (w/v) chitosan solution was prepared by dissolving an appropriate amount of polymer in 0.1M acetic acid, followed by dilution with deionized water. Then, both solutions were heated at 70°C and followed by the addition of NPK fertilizers in the form of NH_4Cl and KH_2PO_4 to the alginate solution. Subsequently, 10 mL of 2% (w/v) CaCl_2 solution was added to the alginate solution and was continuously stirred at room temperature. This was followed by the addition of citric acid as a crosslinker. Chitosan was then added dropwise to the alginate solution while being stirred at 1000 rpm.

Encapsulation efficiency of the coated chitosan/alginate (Chi/Alg) NPK fertilizer

This determination of the encapsulation efficiency was based on the method described by Kumar and his colleagues published elsewhere (Kumar, *et al.*, 2015) with some modifications. The colloidal dispersion was centrifuged at 10,000 rpm for 15 minutes; the residue was washed and filtered afterwards. The supernatant liquid was collected and added to the filtrate; then, it was diluted with deionized water and was subjected to the NPK analysis in terms of parts per million (ppm). The excess or uncoated mass of each element was calculated using total volume of liquid and concentration from the NPK analysis. Afterwards, the mass was subtracted to the total mass of NPK to obtain the mass encapsulated. Finally, the encapsulation efficiency was calculated as the ratio of the mass encapsulated and the total mass.

Characterization methodologies employed

The particle size distribution was performed to measure the average size of the synthesized particles and zeta potential for its stability in solution of each formulation was determined using Malvern ZetaSizer Nano ZS instrument. The samples were spun and filtered before pipetting into the cuvettes. The particle size was determined at a 90-degree angle using dynamic light scattering, while the zeta potential measurement utilized laser Doppler microelectrophoresis.

Fourier-transform infrared (FTIR) spectroscopy was utilized to monitor the reaction progress to prove the formation of the desired product. The FTIR spectra of the samples measured were obtained on a Thermo Scientific Class 1 Nicolet 6700 FTIR spectrometer in the range of 400–4000 cm^{-1} at 16 scans. Each of the samples tested was added with KBr and then pelletized prior to FTIR analysis. All samples from different stages of the crosslinking reaction were dried at 40°C for 12h. The different stages of the crosslinking process were as follows: the first portion was obtained from alginate-NPK mixture. The second was obtained by adding chitosan and the last one was obtained after the citric acid crosslinking at the end of the preparation process.

The encapsulation of a model encapsulant onto the matrix was investigated by fluorescence

spectroscopy using Spectrum ASCII Fluorescence spectrometer. A fluorescent dye, sodium 8-anilino-1-naphthalenesulfonate (ANS), served as a model encapsulant molecule. A 50-mL aliquot of the 5% (w/v) dye dispersion was prepared and divided into three 15-mL portions. The first portion contained only ANS dye, while the second and third were added with polymer blend (Chi/Alg) and crosslinked polymer blend (Chi/Alg/CA), respectively. They were stirred for an hour at 700 ppm then subjected to the aforementioned instrument.

The morphology of the crosslinked polymer system containing NPK fertilizer was characterized by scanning electron microscopy using JEOL 5300 Scanning Electron Microscope.

Controlled release studies of the coated chitosan/alginate (Chi/Alg) NPK fertilizer under different pH conditions

Three buffer solutions were used in the release study under various pH conditions: pH 5.0, 7.0, and 8.5. Exactly one gram polymer coated fertilizer (Chi/Alg/CA/NPK) formulation with the highest encapsulation efficiency was weighed and analyzed to determine the total content of NPK. Simultaneously, the same mass of polymer coated fertilizer was placed in each 100-mL buffer solution, dispersed, and were kept at room temperature. The NPK released was examined at different times: 5, 10, 15, 20, and 30 days by taking 5-mL buffer portion from each container and diluting it to 50 mL with deionized water prior to the NPK chemical analysis. Lastly, the percent release was plotted against time of each nutrient under various pH conditions and the release kinetics of the Chi/Alg/CA/NPK fertilizers was investigated using the Korsmeyer-Peppas equation.

Fertilizer efficacy evaluation of the coated chitosan/alginate (Chi/Alg) NPK fertilizer under different pH conditions

This involved three setups: (A) untreated; (B) with uncoated NPK fertilizer (Chi/Alg/NPK); (C) with coated NPK fertilizer (Chi/Alg/CA/NPK). Each setup was prepared in triplicates, where approximately 700 g loam soil was placed in each pot. Then, a soil sample extracted in each pot was subjected for total NPK analysis to ensure that they have a comparable nutrient content. The

mung bean seeds were swelled in water overnight. After noticeable enlargement of the seeds, five seeds were placed in each plot and observed for the germination process. After two days of sprouting, it was followed by the application of respective treatments. Finally, all pots were placed under direct sunlight and observed for growth. Plant height, leaf height and leaf width – all measurements were in cm. All of these growth parameters were obtained weekly up to eight weeks and the fruiting time was also noted for each setup.

RESULTS AND DISCUSSION

In general, the NPK fertilizer dissolution is necessary in order for the particle distribution to occur resulting to a better encapsulation upon crosslinking (Ma, Tan, Dai, & Zhou, 2013). However, modification was done because the presence of more water would reduce the encapsulation due to the NPK fertilizer solubility in aqueous solution, as some of them will escape the polymer crosslinking process (Ren, *et al.*, 2018). The heating process is needed for the citric acid crosslinking mechanism to form ester bonds with hydroxyl groups of polymers at about 80°C (Demetri, *et al.*, 2008), thus maximizing encapsulation process. Subsequently, the Ca^{+2} ions were introduced to induce the ionic gelation, then followed by polyelectrolyte complexation of chitosan and alginate which adds up to the encapsulating capacity of the polymer mixture (Kumar, *et al.*, 2015). Continuous stirring was done under heating for the citric acid crosslinking as well as to increase the gelling while preventing particle growth of resulting in colloidal dispersion.

Encapsulation efficiency of the coated chitosan/alginate (Chi/Alg) NPK fertilizer in various formulations

The encapsulation efficiency of various Chi/Alg/CA/NPK formulations obtained from varying the composition of polymers and amount of citric acid (CA) crosslinker is presented in Figure 1. It is clearly seen in the figure that an increase in CA has significantly increased the encapsulation efficiency of Chi/Alg. This is because crosslinking introduces a three-dimensional network structure (Chen, Gao, Qiu, & Hu, 2013) that will entrap the substance in the polymer matrix (Shi, *et al.*, 2008). Furthermore, when the amount of crosslinker was maintained at a constant level, the composition of the polymer blends has a significant effect on the encapsulation capacity. In Figure 1, variations of 1:2 and 2:1 have a smaller efficiency compared to 1:1, this is due to the fact that the imbalance of interaction caused by the dominance of one component results in less organized network which compromises the enhanced property created by combining two polymers (Riyajan & Nuim, 2013). It was noted that the higher ratio of alginate (1:2) gives a higher efficiency relative to 2:1 formulations, since alginate performs more interactions with calcium ions (Kumar, *et al.*, 2015) due to its sugar acid components giving carboxylate ions.

Particle size analysis of the coated chitosan/alginate (Chi/Alg)NPK fertilizer in various formulations

For the Chi/Alg/CA/NPK formulations, it is shown in Figure 2 that at constant crosslinker concentration, the imbalanced interaction given by 1:2 and 2:1 composition results in a larger size

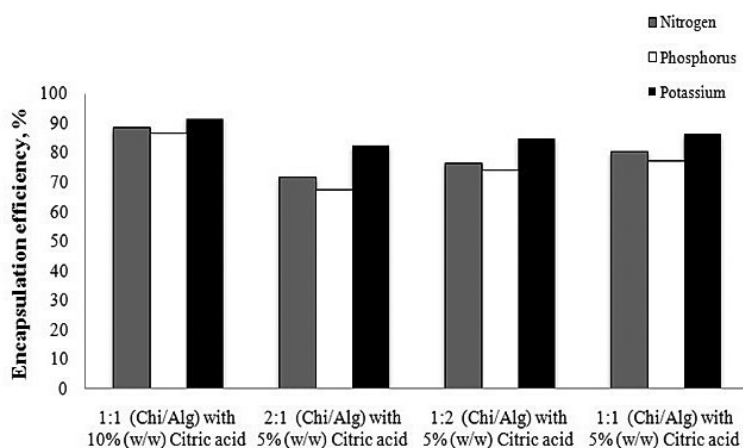


Figure 1. Encapsulation efficiency of various Chi/Alg/CA/NPK formulations.

because of the attractive forces given by oppositely charged polymers, chitosan and alginate as they will form a more compact structure (Kumar, *et al.*, 2015) resulting in smaller size like that of the 1:1 Chi/Alg formulation. Moreover, in the case of the Chi/Alg matrix, the excess alginate will form more gels with the Ca^{2+} ions, thus the average size is shifted to higher value. Generally, citric acid increases the size of the particle because of its crosslinking mechanism as described by Demitri and co-workers (Demitri, *et al.*, 2008) and Riyajan and Nuim (Riyajan & Nuim, 2013).

Zeta potential measurements of the coated chitosan/alginate (Chi/Alg) NPK fertilizer in various formulations

By observation, as in the case of Chi/Alg, the particles settled after about three days. Figure 3 supports the observation, the values lie above the -30 mV, which means that particles will settle

in a certain period of time. The reason for low zeta potential values is the dominance of van der Waals interaction over electrostatic repulsion (Zhang, *et al.*, 2011). In reference to the preparation method, the concentration of the polymers were 3% (w/v); hence, the higher amounts of polymer will introduce more van der Waals interaction overcoming the effect of electrostatic repulsion, since more particles are forming and the distance between them reduces. It can be observed from Figure 3 that the magnitude of zeta potential reduces as the amount of crosslinker is increased from 5% to 10% (w/v) of 1:1 formulations. That is accounted for in the formation of organized network upon crosslinking (Maitra & Shukla, 2014) and promotes more van der Waals interaction over electrostatic repulsion (Zhang, *et al.*, 2011) leading to the aggregation of particles. In general, the sign of zeta potential dictates the dominant ionic charge in the surface. The negative values obtained in this study are reasonable,

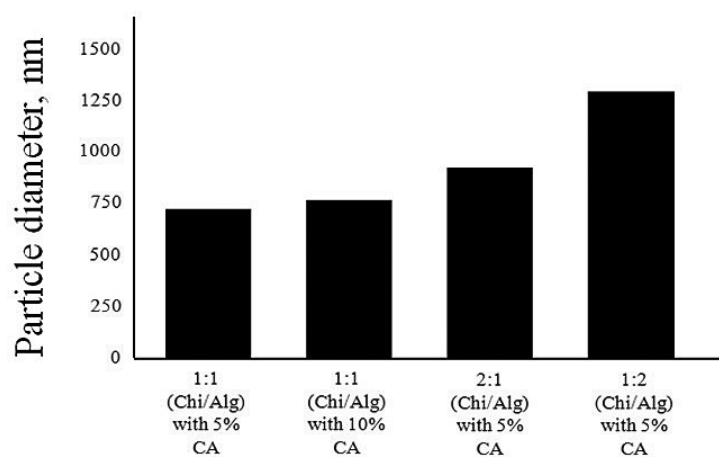


Figure 2. Average particle size of various Chi/Alg/CA/NPK formulations.

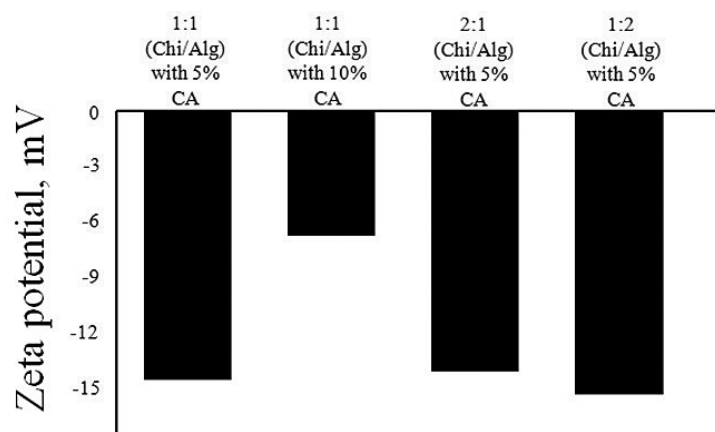


Figure 3. Zeta potential values of various Chi/Alg/CA/NPK formulations.

except for the Chi/Alg (2:1) formulation. This would be possible if by any chance the chitosan is distributed inward the dispersion particles upon measurement. Similarly as in the study by Silva and his colleagues (Silva, *et al.*, 2016), the alginate is oriented towards the surface of the multilayer chitosan/alginate nanocapsule. Additionally, the NPK fertilizer utilized in this study is in the form of ions which may cause the phosphate groups interact to the excess chitosan molecules creating a negative surface charge.

FTIR spectroscopy to follow the crosslinking process of the coated chitosan/alginate (Chi/Alg) NPK fertilizer

Figure 4 shows the changes in IR spectra as the reaction of Chi/Alg/CA/NPK (as a cross-linked fertilizer formulation) proceeds to completion from Figure 4A to Figure 4C. Figure 4A consists of alginate-NPK followed by an interaction of calcium-alginate and Chi/Alg interaction (Figure 4B) then ended up with citric acid crosslinking process (Figure 4C). The peak at 1086 cm^{-1} is maintained; however, its intensity decreases as the citric acid crosslinking happens, because the hydroxyl groups are being used for ester formation (Demitri, *et al.*, 2008); thus, some of them are converted to C-O stretch in ester resulting in

the appearance of band at 1210 cm^{-1} at the reaction stage C. The changes at 1430 cm^{-1} can be attributed to the Chi/Alg interaction as well as the interaction with calcium (Kumar, *et al.*, 2015) and the changes in the broad peak from 3800 to 2500 cm^{-1} can be accounted for the participation of both polymers in the crosslinking process. Lastly, the appearance of peak 1740 cm^{-1} indicates an ester formation; thus, a citric acid crosslinking occurs among the Chi/Alg molecules and it is also supported by the shifting of the citric acid peak (Figure 4D).

Fluorescence spectroscopy to evaluate encapsulation efficiency of the chitosan/alginate (Chi/Alg) materials

The experiment was done using sodium 8-anilino-1-naphthalenesulfonate (ANS) dye as a fluorescent agent. This dye is slightly soluble in pure water and provides a stronger fluorescence emission in a hydrophobic environment compared to that of hydrophilic one which means that the encapsulation process using Chi/Alg can be confirmed if the emission intensity maximizes, as the process proceeds to completion. In Figure 5, the ANS dye has its minimum emission intensity followed by uncrosslinked polymer which is just slightly higher than the emission of the ANS dye

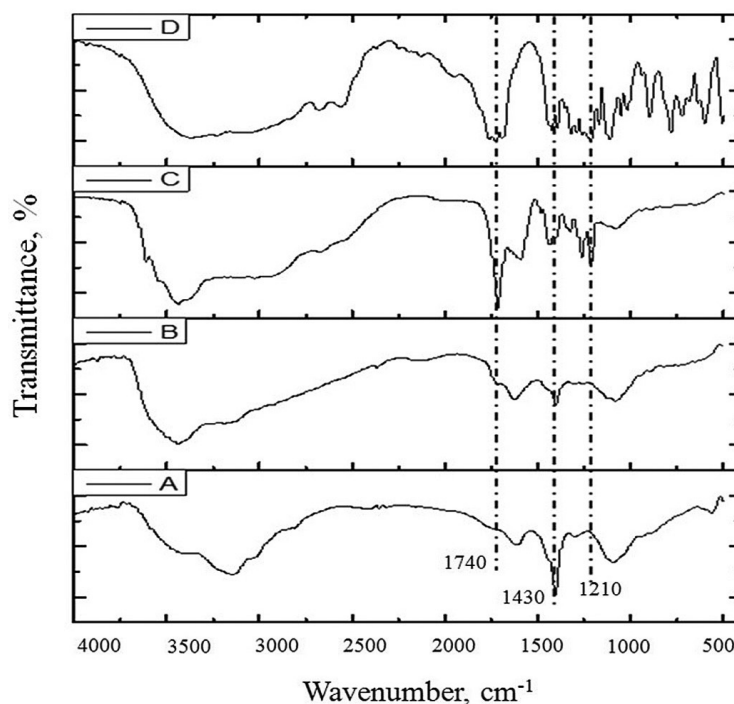


Figure 4. IR spectra of each reaction stage:(A) NPK fertilizer in Alginate solution; (B) Chitosan is added to Alginate and NPK; (C) Citric acid-crosslinked Chitosan/Alginate NPK. The citric acid spectrum is also shown for comparison (D).

solution; this indicates that the dye is not yet restricted to the hydrophobic part of the polymer. However, after the crosslinking process, there is an abrupt increase in emission intensity which means that the ANS dye is within the domain (Penaloza, *et al.*, 2013) and restricted in the polymer material resulting to extremely large values. Hence, the concept of encapsulation is confirmed.

Scanning electron microscopy of the coated chitosan/alginate (Chi/Alg) NPK fertilizer

The SEM image of Chi/Alg (1:1) with 10% (w/w) citric acid is shown in Figure 6. Most of the particles are globular and fall within the size range observed in the particle size analysis using dynamic light scattering measurements.

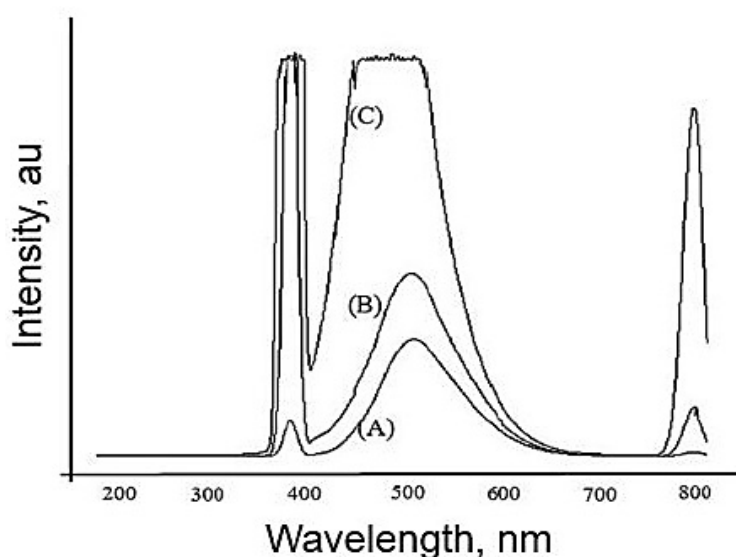


Figure 5. A fluorescent dye, 8-Anilino-1-naphthalenesulfonic acid (ANS), is used as a model active to be encapsulated. The fluorescence measurement of the ANS dye, in (A) water; (B) water containing chitosan/alginate (before crosslinking) and (C) water containing chitosan/alginate and citric acid (after crosslinking).

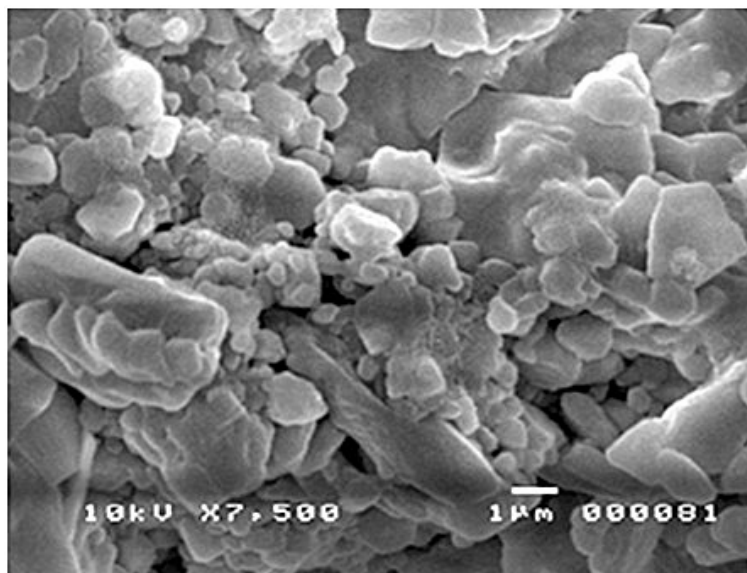


Figure 6. SEM image of the crosslinked polymer (chitosan/alginate reacted with citric acid) containing NPK fertilizer. The bar scale is 1 μm .

Release studies of the coated chitosan/alginate (Chi/Alg) NPK fertilizer under various pH conditions

The release profiles of Chi/Alg (1:1) with 10% w/w CA at various pH levels are shown in Figure 7. It is observed that the Chi/Alg NPK exhibits controlled release behavior. It can be seen by curve inspection that the release rate increases with pH. This demonstrates the solubility of polymer components under slightly alkaline conditions (M. S. Kim, Park, Gu, & Kim, 2012) and it also demonstrates the resistance of alginate at slightly acidic medium. It was shown in the curve that approximately 40% of NPK were released for the period of 30 days which is due to the chitosan-alginate interaction in the polyelectrolyte complex (Silva, *et al.*, 2016) and this complies with standard which is less than 70% for 28 days (C.-W. Liu, Sung, Chen, & Lai, 2014). The closeness of curves at different pH levels was also observed; the reason for this is due to the solubility of chitosan under slightly acidic conditions

(Kumar, *et al.*, 2015) that leads to the release of some entrapped nutrients in the matrix.

The release profiles for each nutrient at various pH values were fitted into Korsmeyer-Peppas model. Upon linearization of the said model, it generates an equation of the line in which y is log release, x is log time, y-intercept is log release rate constant, and slope is the release exponent, n which defines the release mechanism (Shariff, Pk, Klk, & M, 2007; Wang, *et al.*, 2017). On the basis of Figure 7, it can be seen that all nutrients and pH levels have slope that lies between 0.43 and 0.85, which is indication of both diffusion controlled release and matrix-based release (Li, *et al.*, 2008). In addition, the values of y-intercept increase with pH; hence, it supports the profile that release is highest at basic condition because of the solubility of the matrix.

Fertilizer efficacy evaluation of the coated chitosan/alginate (Chi/Alg) NPK fertilizer

In the first few weeks, the second, third and fourth setup continuously grows and their growth

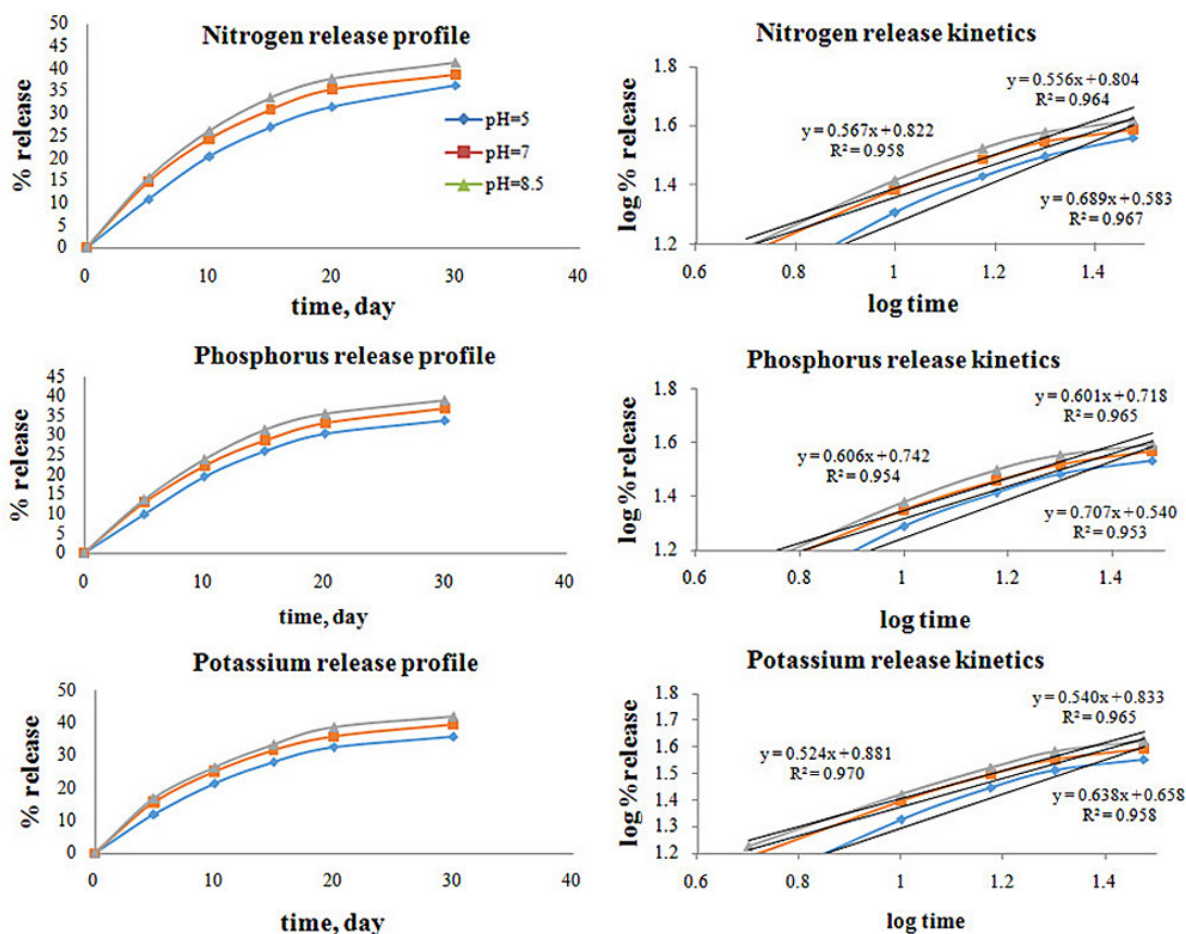


Figure 7. Release profiles (left) and kinetics (right) of Chitosan/Alginate (1:1) NPK with 10% (w/w) Citric acid at pH 5.00 (diamond plots), 7.00 (square plots), and 8.5 (triangle plots).

parameters (plant height, leaf length and leaf width) are seemingly identical. However, as the time goes by, the setup which contains the Chi/Alg NPK was continuously growing. It was also observed that the second setup growth slowed down due to the frequent rains (the trial was made during rainy season), where because of this, nutrients can be lost through run off (Saigusa, *et al.*, 2001). Figure 8 shows that the Chi/Alg coated fertilizer produces observable increase in terms of plant growth parameters after 8 weeks; this may be related to its release kinetics as described in the previous data. This may indicate that the controlled release is working on the given condition especially wherein frequent rains are observed which causes leaching. Hence, the controlled release process was observed.

CONCLUSION

The coated NPK fertilizer based on citric acid-crosslinked chitosan/alginate (Chi/Alg/CA/NPK) was successfully prepared as colloidal dispersion. The highest encapsulation efficiency, which ranges from 85–89%, was observed in 1:1 Chi/Alg ratio with 10% citric acid (per weight polymer) formulation. A SEM image revealed the existence of spherical particles, which supports particle size distribution and encapsulation phenomenon. FTIR showed successful citric acid crosslinking and interaction of individual components. Moreover, fluorescence spectroscopy confirms the existence of the encapsulation process. The encapsulated NPK formulation demonstrated the controlled release behavior at various pH levels and followed the Korsmeyer-Peppas model;

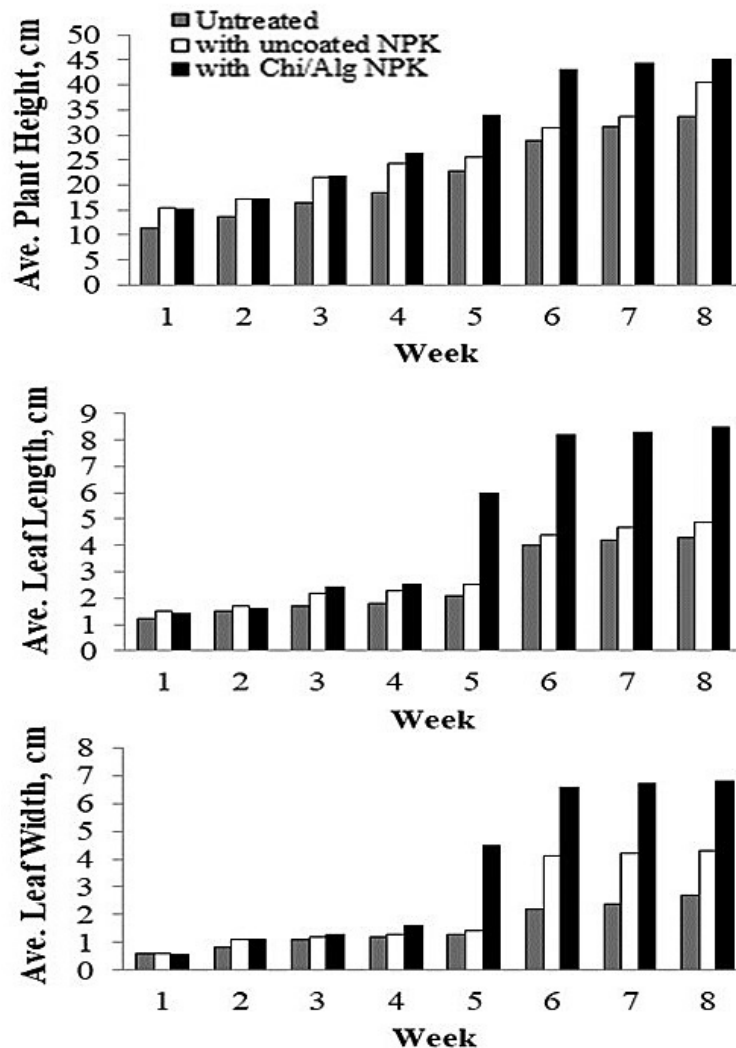


Figure 8. Growth parameters of Mung bean studied for eight weeks in various setups: Untreated (gray); with uncoated NPK fertilizer (white) and; with Chi/Alg coated NPK fertilizer (black). Plant height (Top); Leaf length (Middle) and; Leaf width (Bottom).

this shows that the release is both governed by polymer relaxation and diffusion. Lastly, these colloidal dispersions showed a good controlled release performance in the pot experiment and hence can be used in wide agricultural setting.

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