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CHITOSAN/UREA

BASED CONTROLLED RELEASE SYSTEMS FOR UREA: STRUCTURAL AND ENVIRONMENTAL ASPECTS

SISTEMAS DE LIBERACIÓN CONTROLADA BASADOS EN QUITOSANO/UREA: ASPECTOS ESTRUCTURALES, FUNCIONALES Y AMBIENTALES

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ABSTRACT

Slow-release systems have been proposed in agriculture to mitigate the environmental damage caused by nutrient losses during fertilization. This study evaluates the potential of chitosan as a biopolymer for the development of controlled-release urea systems aimed at achieving more efficient and sustainable agriculture. A Chitosan/TPP/Urea (3.5% w/v) system was synthesized through ionic gelation, achieving an encapsulation efficiency of 36.9 ± 3.1% and exhibiting a slow-release profile, with only 8.69% urea released after 120 minutes. This behavior confirms the formation of a dense polymeric matrix that delays nutrient diffusion. Potentiometric analysis revealed that chitosan concentration influences the protonation degree of amino groups and, consequently, the ionic crosslinking density with TPP. Compared with conventional urea fertilizers, the synthesized system improves nitrogen retention, reduces volatilization and leaching losses, and increases nitrogen use efficiency (NUE). From an environmental perspective, the proposed technology contributes to reducing the nitrogen footprint, promoting more resilient agricultural systems with lower ecological impact.

Keywords: Slow-release systems, Chitosan, Urea, Fertilizers, Sustainabilit.

RESUMEN

Los sistemas de liberación lenta han sido propuestos en la agricultura para mitigar los daños medioambientales causados por las pérdidas en la fertilización. El presente trabajo evalúa el potencial del quitosano como biopolímero para el desarrollo de sistemas de liberación controlada de urea, orientados hacia una agricultura más eficiente y sostenible. Se sintetizó un sistema Quitosano/TPP/Urea (3.5% p/v) mediante gelificación iónica, obteniéndose una eficiencia de

encapsulación de 36.9 ± 3.1% y un perfil de liberación lenta que alcanzó solo 8.69% tras 120 minutos. Este comportamiento confirma la formación de una matriz polimérica densa que retrasa la difusión del nutriente. El análisis potenciométrico mostró que la concentración de quitosano no influye en el grado de protonación de los grupos amino y, por tanto, en la densidad de entrecruzamiento iónico con el TPP. En comparación con los fertilizantes convencionales de urea, el sistema sintetizado mejora la retención de nitrógeno, reduce las pérdidas por volatilización y lixiviación, y aumenta la eficiencia en el uso del nitrógeno (NUE). Desde una perspectiva ambiental, la tecnología propuesta contribuye a la reducción de la huella de nitrógeno, promoviendo sistemas agrícolas más resilientes y con menor impacto ecológico.

Palabras clave: Sistemas de liberación lenta, Quitosano, Urea, fertilizantes, sustentabilidad.

INTRODUCTION

Chitosan is a naturally occurring polysaccharide found in the exoskeleton of crustaceans. It is characterized by being a biodegradable, biocompatible material with antimicrobial properties (Wang & Zhuang, 2022). Chitosan comes from the partial deacetylation of chitin and is formed of glucosamine and N-acetylglucosamine (Sivashankari & Prabaharan, 2017). The structure and properties of the material make chitosan a good candidate for drug delivery systems (Omer et al., 2021). Chitosan has amino groups with cationic characteristics, allowing it to form bonds with phosphate groups, resulting in spherical structures. Sodium tripolyphosphate (TPP) is used to create networks with chitosan (see Figure 1). However, the physical and chemical properties of the matrix are affected by the concentration of TPP as well as chitosan (Gutiérrez-Ruíz et al., 2024). In 2017, Lusiana et al. (2017) reported that incorporating TPP into chitosan matrices improves mechanical strength and water absorption, as well as enhances the number of active sites. More recently, Gutiérrez-Ruiz et al. (2024) optimized TPP concentration, reporting an optimal level of 0.1%.

In agriculture, chitosan has traditionally been used as a pesticide, but in recent years its application has expanded to slow-release systems (Eddarai et al., 2022). Slow-release fertilizers (SRFs) consist of materials that encapsulate or coat nutrients, creating a physical barrier that allows plants to access them gradually over time (Lakshani et al., 2023). This strategy is particularly relevant for nitrogen fertilizers, which represent the most limiting and essential nutrient in agriculture, as nitrogen participates in the formation of amino acids, proteins, secondary metabolites, and plant tissues (Mahmud et al., 2020). Among nitrogen fertilizers, urea is the most widely used due to its high nitrogen content (46%) and ease of application. However, conventional urea suffers severe losses (40–70%) through volatilization, leaching, and denitrification, leading not only to economic inefficiencies but also to environmental contamination of soil, water, and air (Beig et al., 2020; Tapia-Hernández et al., 2022).

The environmental impacts of nitrogen mismanagement are profound: nitrate leaching contaminates aquifers, ammonia volatilization contributes to air pollution, and nitrous oxide (N_2O), a potent greenhouse gas, exacerbates climate change (Sutton et al., 2022). The global nitrogen imbalance has been recognized as one of the most pressing sustainability challenges, threatening biodiversity, food security, and human health (Sutton et al., 2021). International initiatives, including the ***Breakthrough Agenda Report on Agriculture*** (Mukherji et al., 2024) have stressed the urgency of improving nitrogen use efficiency (NUE) as a pathway toward achieving climate-resilient and sustainable agrifood systems. Similarly, the UNECE has highlighted that up to 80% of anthropogenic reactive nitrogen is wasted, with losses valued at over $200 billion annually (Sutton et al., 2022). To address these inefficiencies, new technologies such as modified urea fertilizers, nanofertilizers, and chitosan-based encapsulation systems are being investigated. Modified urea fertilizers regulate nitrogen release, thereby reducing hydrolysis rates and minimizing volatilization and leaching losses (Swify et al., 2024). Nanofertilizers and controlled-release systems have demonstrated the ability to extend nutrient availability for weeks to months, improving crop uptake and yields while mitigating environmental impacts (Abhiram, 2023). Among these approaches, chitosan offers unique structural and functional properties that facilitate the formation of networks with urea, improving encapsulation efficiency and enabling controlled release (Kalia et al., 2017; Kondal et al., 2021).

Therefore, the development of optimized chitosan–urea slow-release systems represent a promising strategy to reduce nitrogen losses, enhance fertilizer efficiency, and mitigate environmental damage, directly contributing to Sustainable Development Goal 13 (climate action) and the broader objectives of sustainable agriculture (Eisa et al., 2022). Several recent studies have focused on improving nitrogen use efficiency through the development of controlled-release systems based on chitosan–urea matrices. These investigations aim to optimize both the material formulation and the synthesis conditions in order to increase encapsulation efficiency and extend fertilizer availability in the soil. The methodological approaches

vary significantly, and each method presents advantages and limitations associated with experimental complexity as well as the environmental safety of the process.

Table 1 summarizes the main advances reported in the literature regarding urea encapsulation methods using chitosan matrices, highlighting the materials employed, synthesis conditions, encapsulation efficiencies achieved, and their comparative advantages and disadvantages.

Table 1. Comparison of synthesis methods for chitosan–urea controlled-release systems.

Synthesis	Encapsulation Efficiency (%)	Advantages	Disadvantages	Reference
Emulsification	89%	High loading efficiency; good morphological stability; sustained release	Use of organic solvents	Hussain et al., 2012.
Emulsification	32–38%	Particle size control; non-Fickian release behavior	Use of organic solvents and aldehyde	Jayanudin et al., 2021
Emulsification	—	Accurate kinetic model (Case II); enables diffusion coefficient calculation	Does not experimentally evaluate encapsulation efficiency	Jayanudin et al., 2022
Emulsification	89%	Prolonged release; enhanced plant growth (Chinese cabbage)	pH and particle size not optimized; kinetic behavior not analyzed	Ma et al., 2023
Ionic gelation / Modified membrane (ionic crosslinking)	—	Improved mechanical properties, porosity, and swelling resistance	Quantitative encapsulation efficiency not reported	Cahyaningrum et al., 2024.
Ionic gelation (TPP) and ultrasound	~85%	High efficiency; reduced nitrogen volatilization; improved soil microflora	Requires nanoparticle size control; higher production cost	Kondal et al., 2021

Source: developed by the authors.

In this context, the selection of the synthesis method is a determining factor for achieving efficient encapsulation and controlled release. Among the methodologies reported, systems based on emulsification and cross-linking with glutaraldehyde exhibit high encapsulation efficiencies (≈80–90%); however, they involve the use of organic solvents and toxic aldehydes, as well as high experimental complexity (Hussain et al., 2012; Jayanudin et al., 2021). In contrast, ionic gelation methods using TPP offer a simpler, more reproducible, and environmentally safer alternative, as they avoid toxic agents and operate under ambient conditions (Kondal et al., 2021). Table 2 highlights the encapsulation principles, technical requirements, and environmental safety.

Table 2. Comparison of chitosan–urea synthesis methods reported.

Method	Experimental complexity	Environmental safety	Reference
Emulsification	High	Low	Hussain et al. (2012)
Emulsification	High	—	Jayanudin et al. (2022)
Ionic gelation with TPP	Low	High	Kondal et al. (2021)
Ionic gelation with TPP + Ca^{2+}	Medium	Medium–High	Cahyaningrum et al. (2024)

Source: developed by the authors.

Kondal et al. (2021) employed relatively low chitosan concentrations (≈1.5% w/v) to obtain urea nanocomposites through ionic gelation with TPP, achieving encapsulation efficiencies of approximately 85%. However, at low polymer concentrations, the chitosan network tends to exhibit a lower crosslinking density and a limited availability of protonated amino groups ($–NH_3^+$) capable of interacting with the carbonyl groups of urea, which may compromise the structural stability of the matrix and reduce fertilizer retention. Therefore, in this study, the chitosan concentration was increased to 3.5% w/v in order to evaluate the effect of a higher density of active functional groups on the encapsulation efficiency and the formation of ionic bonds with TPP.

This study aims to evaluate the interactions between urea and chitosan at different concentrations, exploring their encapsulation behavior and potential for controlled nitrogen release. By systematically analyzing key variables—such as chitosan concentration, urea content, pH, and TPP ratio—it seeks to identify optimal conditions for encapsulation, ultimately supporting more sustainable and efficient fertilization practices.

MATERIALS AND METHODS

Chitosan (molecular weight 50,000–190,000 Da; degree of deacetylation 75–85%), glacial acetic acid, sodium tripolyphosphate (TPP), and urea were purchased from Sigma-Aldrich and used without further purification. All solutions were prepared using deionized water.

Preparation of Chitosan/Urea Complexes

The material was synthesized by ionic gelation. Chitosan (3.5% w/v) and urea (5% w/v) were dissolved in 0.1% (v/v) acetic acid, previously adjusted to pH 4.7 to ensure protonation of the amine groups of chitosan ($–NH_3^+$). The mixture (50 mL total volume) was magnetically stirred at 600 rpm for 24 h at room temperature (25 ± 1 °C) and then filtered through Whatman No. 1 paper to remove undissolved residues.

A 0.1% (w/v) aqueous solution of TPP was prepared separately and stored at 4 °C. The TPP solution (25 mL) was added dropwise to the chitosan/urea solution at a controlled flow rate of 1 mL min^{-1} under constant stirring to promote ionic crosslinking between $–NH_3^+$ groups of chitosan and PO_4^{3-} groups of TPP. The resulting suspension was left to stabilize for 12 h and then frozen at –4 °C prior to dryin. Samples were lyophilized for 48 h using a Labconco freeze dryer (FreeZone 2.5 L, Kansas City, MO, USA) at a chamber pressure of 0.05 mbar and condenser temperature of –50 °C.

Additionally, a second chitosan/TPP/urea membrane was prepared under identical conditions but using a lower chitosan concentration (1.5% w/v) to evaluate the behavior of amino groups at different polymer concentrations. A chitosan/TPP matrix prepared under identical conditions but without urea was also synthesized and used to quantify background absorbance and determine the protonation degree of free amino groups.

Determination of encapsulation efficiency

The encapsulation percentage (%E) was calculated using Equation 1:

$$\%E = \frac{Ww - Wd}{Ww} x100 \quad (1)$$

Where Ww is the wet weight and Wd is the dry weight

Release study

The release profile was determined by immersing 0.1 g of the Ch/TPP/Urea material in 50 mL of phosphate buffer (pH 7.0) at 25 °C. After 5 min of equilibration, 0.125 mL of urease enzyme solution (1 mg mL^{-1} in phosphate buffer) was added. Aliquots of 1 mL were withdrawn every 15 min for 2 h, mixed with 3 mL of Nessler reagent, and analyzed at 480 nm using a UV–Vis spectrophotometer (Shimadzu UV-2600). A blank without urease was used to correct for background absorbance and material interference.

Potentiometric Titration

To evaluate the interaction between chitosan and urea, the supernatant obtained after encapsulation was titrated potentiometrically with 1 M NaOH. Inflection points in the titration curve were used to calculate the percentage of protonated amino groups, according to Equation 2:

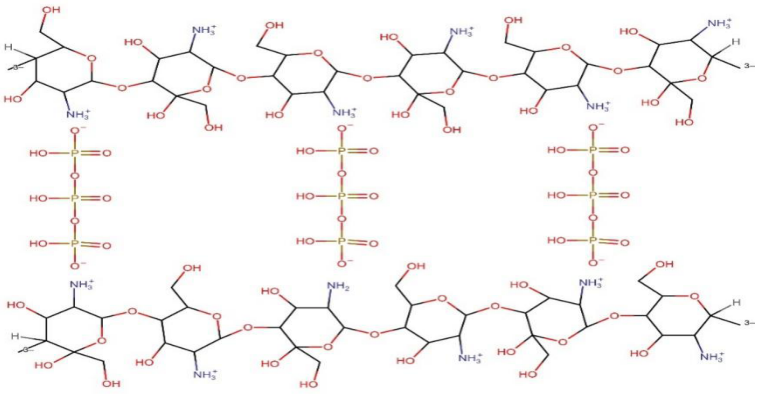
$$\%\ Protonated\ groups = \frac{16.1 * (y - x) * f}{w} \quad (2)$$

where ***Y*** is the major inflection point, "***x***" the minor inflection point, "***f***" the NaOH molarity, "***w***" the sample weight, and 16.1 is the equivalent weight of chitosan. Titrations were performed for both urea-loaded and urea-free matrices to quantify the reduction in available amino groups due to complexation.

RESULTS AND DISCUSSION

The protonation of amino groups in an acidic medium provides chitosan with a highly reactive character. These protonated groups ($–NH_3^+$) interact ionically with tripolyphosphate ions (PO_4^{3-}), forming micellar networks where urea becomes physically entrapped within the polymeric structure (Figure 1)

Fig 1. Schematic representation of ionic crosslinking between chitosan and TPP in acidic medium.



Source: developed by the authors.

The percentage of encapsulation was carried out by weight difference and is observed in Figure 2. The encapsulation percentage was 36.9 ± 3.1% of urea, this value is below that reported with other methodologies (Hussain et al,2012).

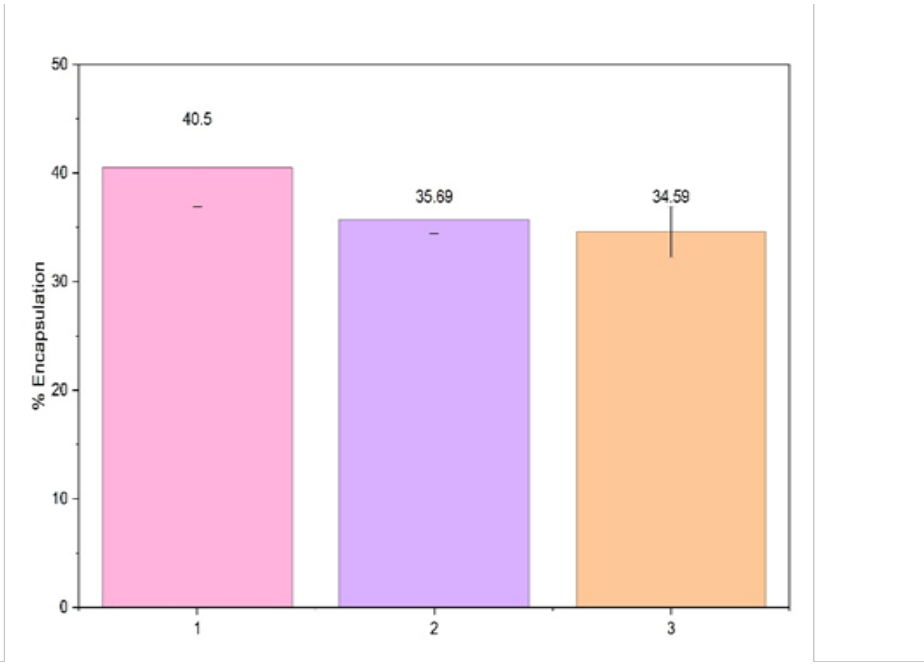
The results demonstrate that using a chitosan concentration higher than that reported in previous studies enables an effective encapsulation process. However, this process may be influenced by the increase in medium viscosity associated with the concentration used. Therefore, potentiometric titration proves to be a useful tool for analyzing the behavior of the crosslinking agent (TPP) in its interaction with chitosan in the presence of urea. Potentiometric titration evaluates the percentage of free amino groups present in chitosan.

The modification of the amino groups through potentiometric titration is an indicator of the interference generated by the presence of additional urea in the chitosan-TPP matrix. To evaluate the protonation behavior of the amino groups in chitosan and the effect of urea incorporation, potentiometric titrations were carried out using NaOH as the titrant. Figure 3 shows the pH variation as a function of the volume of NaOH added for two formulations with different chitosan concentrations (1.5% and 3.5% w/v), as well as for systems prepared with and without urea. The observed inflection points correspond to the neutralization of protonated amino groups ($–NH_3^+$) present in the chitosan backbone, reflecting the interaction strength between chitosan, tripolyphosphate (TPP), and urea within each formulation.

When comparing both plots, a distinct shift in the titration profiles can be observed. The formulations with higher chitosan concentration (3.5%) exhibit a sharper and earlier inflection point, indicative of a lower protonation capacity due to stronger polymer–polymer chain interactions. In contrast, the less concentrated systems (1.5%) show a delayed and broader transition, reflecting a greater number of accessible amino sites available for protonation. The presence of urea also slightly modifies the titration curve, suggesting specific interactions between its carbonyl groups and the protonated amine groups of chitosan, which in turn influence the crosslinking behavior with tripolyphosphate (TPP).

Table 3 summarizes the main parameters obtained from the potentiometric titration curves, including the first and second inflection points (X and Y), the corresponding volume difference (ΔV), and the calculated percentage of protonated amino groups. These results quantitatively support the observed trends in the titration profiles, showing that lower chitosan concentrations and the absence of urea favor higher protonation levels.

Fig 2. Encapsulation efficiency (%E) of Chitosan/TPP/Urea material.



Dry weight (Wd) and wet weight (Ww) in grams and percentage of encapsulation (%E) of Ch/TPP/Urea material using encapsulation percentage equation.

Sample	Ww	Wd	%E
1	88.3	52.5	40.54
2	90.5	58.2	35.69
3	97.4	63.7	34.59
Average	92.06	58.13	36.94

Source: developed by the authors.

Table 3. Calculated percentage of protonated amino groups for chitosan–TPP systems with different formulations

System	X (mL)	Y (mL)	ΔV = Y–X (mL)	Protonated groups %
W/Urea - 3.5 QS	21.0	22.0	1.0	53.67
Urea -3.5 QS	21.0	22.3	1.3	69.77
W/Urea -1.5 QS	21.0	22.5	1.5	80.50

Source: developed by the authors.

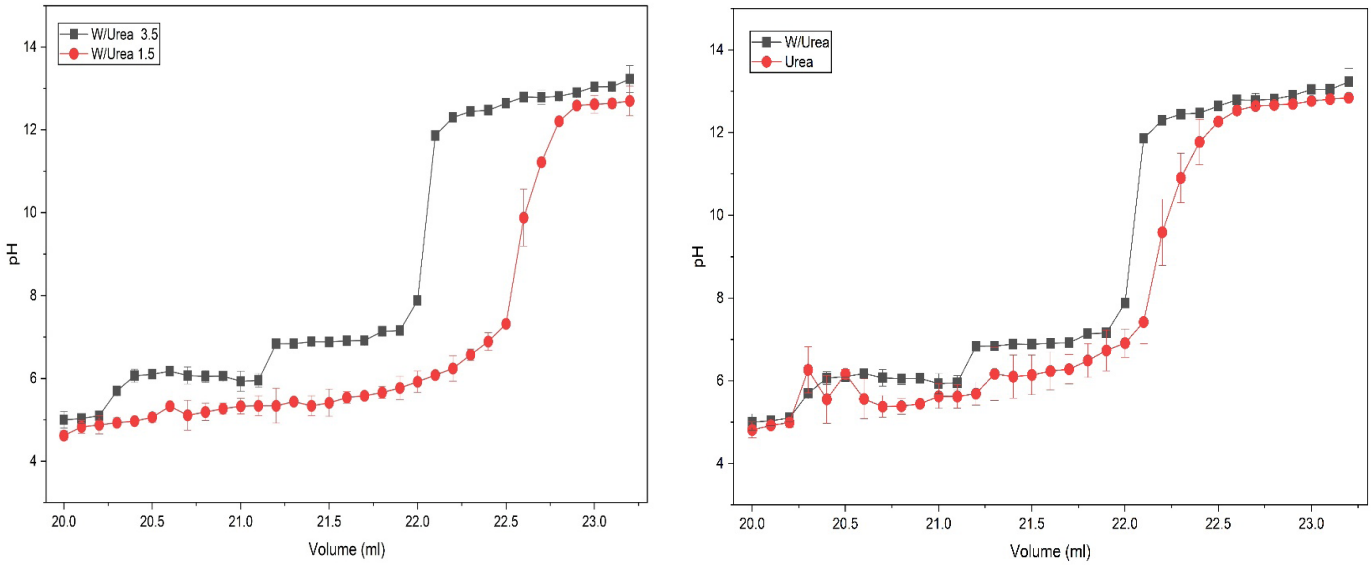
The incorporation of urea during synthesis produces an interference that may arise from the presence of its carbonyl groups, which compete with the amino groups of chitosan for the interaction sites with TPP. This partial interaction between urea and chitosan generates an apparent increase in free amino groups, as some of them remain oriented toward the medium without participating in the ionic crosslinking. As a result, the resulting polymeric network is less compact, which may reduce solute retention and, consequently, encapsulation efficiency.

This interpretation is consistent with the experimental values obtained: the encapsulation percentages, shown in Table 3, range between 34.59% and 40.54%, with an average of 36.94%, which is significantly lower than the values reported

by Kondal et al. (2021), who achieved efficiencies close to 85% using ionic gelation with 1.5% chitosan. The lower efficiency observed in this work may be attributed to the higher viscosity of the system generated by the chitosan concentration used (3.5% w/v).

Overall, the results suggest that a balance between chitosan concentration and urea content is essential to optimize the encapsulation process. A moderate chitosan concentration may maximize the availability of active amino groups without compromising the structural integrity of the gel, which could improve the encapsulation efficiency and bring the results closer to those reported in the literature for optimized ionic gelation processes.

Fig 3. Titration curve of chitosan with NaOH with urea and without urea.



Source: developed by the authors.

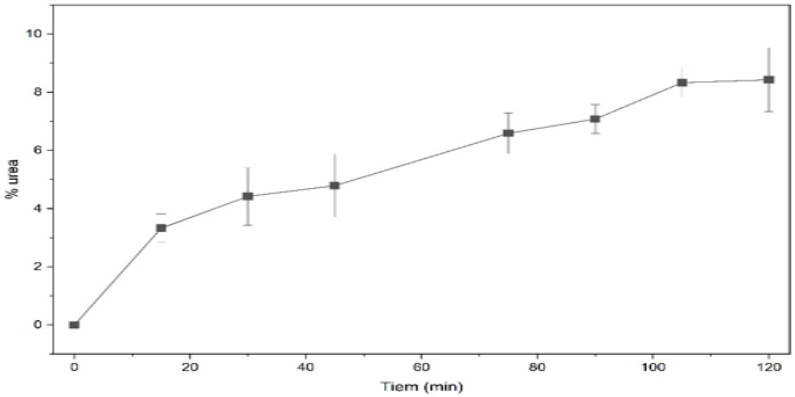
Figure 4 shows the urea release profile of the Chitosan/TPP/Urea system over a period of 120 minutes. The kinetic behavior exhibits three clearly defined stages: an initial rapid release during the first 20 minutes, followed by an intermediate phase governed by controlled diffusion, and finally, a tendency toward stabilization at 120 minutes, with a final release percentage of 8.69%. This value reflects a slow and non-linear release process, characteristic of chitosan-based polymeric matrices, in which solute transport is regulated by the structural organization of the material.

To contextualize this behavior, Table 4 compares the results obtained in this study with those reported in recent literature for similar chitosan systems. Under comparable time intervals, the urea release percentages reported by other authors are considerably higher, ranging from 32% to 60%. In contrast, the system developed in the present work exhibits a markedly slower release, suggesting the formation of a denser polymeric network with reduced nutrient permeability, likely resulting from the increased chitosan wall concentration.

The experimental results confirm that the ionic gelation method using sodium tripolyphosphate (TPP) effectively promotes the formation of chitosan–urea networks through electrostatic interactions between the protonated amino groups ($–NH_3^+$) of chitosan and the phosphate groups (PO_4^{3-}) of TPP. Potentiometric titration results demonstrated that the concentration of chitosan plays a critical role in determining the degree of protonation and the density of ionic crosslinking within the polymeric matrix. The observed decrease in protonation at higher chitosan concentrations suggests the formation of more compact and viscous networks, which in turn influence urea encapsulation and release kinetics. Although the encapsulation efficiency (36.9 ± 3.1%) was lower than that reported for other methods, the system developed in this study presents a clear environmental and methodological advantage: it avoids the use of toxic reagents and organic solvents. The resulting formulation exhibited a markedly slower urea release (8.69% after 120 min), confirming that increased chitosan concentration enhances matrix density and creates a more effective diffusion barrier. This slow

and sustained release behavior is particularly desirable for agricultural applications, as it can reduce nitrogen losses through volatilization and leaching, thereby improving nitrogen use efficiency (NUE).

Fig 4. Urea release profile of the Chitosan/TPP/Urea system.



Source: developed by the authors.

Table 4. Urea release performance of chitosan-based systems under different preparation conditions.

System	Experimental condition	Time evaluated	% of urea release	Reference
Emulsification	Crosslinked with glutaraldehyde	120 min	≈ 60 %	Hussain et al., 2012
Ionic gelation	Controlled stirring	180 min	32–37 %	Jayanudin et al., 2021
Emulsification	Different stirring speeds	180 min	≈ 35 %	Jayanudin et al., 2022
Ionic gelation	Chitosan 3.5%	120 min	8.69%	This work

Source: developed by the authors.

Overall, these findings demonstrate that controlling polymer concentration and crosslinking conditions enables the design of biopolymeric systems with tunable release profiles. The Chitosan/TPP/Urea formulation represents a sustainable and scalable alternative to conventional fertilizers, combining environmental safety with functional efficiency.

CONCLUSIONS

Chitosan proved to be an effective biopolymer for developing slow-release urea systems due to its biodegradability, biocompatibility, and ability to form stable ionic networks with sodium tripolyphosphate (TPP). The obtained formulation (Ch/TPP/Urea 3.5%) achieved an encapsulation efficiency of approximately 36.9% and exhibited a slow and sustained urea release, reaching only 8.69% after 120 minutes. This behavior indicates the formation of a dense polymeric matrix that effectively delays nutrient diffusion.

Compared to conventional urea fertilizers, this system demonstrates improved nitrogen retention, minimizing volatilization and leaching losses. Consequently, it enhances nitrogen use efficiency (NUE), promoting more efficient nutrient uptake and reducing environmental impacts such as groundwater contamination and greenhouse gas emissions.

From a sustainability perspective, chitosan-based controlled-release systems align with the objectives of the United Nations 2030 Agenda, particularly SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action). By reducing the nitrogen footprint and improving fertilizer efficiency, this technology contributes to the transition toward more sustainable and resilient agricultural systems.

In summary, the Chitosan/TPP/Urea system represents an environmentally responsible alternative for nitrogen management, offering a balance between agronomic efficiency and ecological protection, and positioning chitosan as a key material in the design of next-generation biofertilizers.

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