

## Original Research Article

# Research on a prolonged release system of urea powder potentially applied in sustainable agriculture

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## ABSTRACT

A prolonged release system (SLIP) of urea powder encapsulated in a wheat gluten matrix was investigated as a sustainable substitute for use in agriculture with the goal of lowering nitrogen losses in the soil. Thermogravimetry (TGA), Fourier Transform Infrared Spectroscopy (FT-IR), and Scanning Electron Microscopy (SEM) were the methods used to characterize SLIP. The urea release kinetics in water were then assessed. Very porous structures were produced, the thermal stability of SLIP was noted, and interactions between urea and gluten proteins through hydrogen bonds were verified. According to the kinetics, urea was released at a high rate (38%) in the first ten minutes and reached the diffusion equilibrium (86.35%) after 36 hours. There is a chance that urea SLIP will be employed as a sustainable substitute in agriculture.

**Keywords:** spectroscopy; wheat gluten; scanning electron microscopy; nitrogen; thermogravimetric

## 1. Introduction

Urea has been essential for all crops because of its high nitrogen content (N: 46%). This element is of vital importance in plant growth and development and is necessary for adequate yields<sup>[1]</sup>. However, nitrogen (N) is an unstable element that can be lost by leaching and volatilization before the plant absorbs it, reducing yield potential and crop quality<sup>[2,3]</sup>. It also has a polluting effect, which is produced by the emission of greenhouse gases with the release of NO<sub>2</sub>, the main anthropogenic pollutant from nitrogen fertilization<sup>[4,5]</sup>.

In recent years, research has focused on improving N efficiency in agricultural fields<sup>[6]</sup>. The use of controlled and extended release (SLIP) systems is being developed as an alternative to avoid N losses in the soil<sup>[7]</sup>. These SLIP systems are formulations that contain a plant nutrient in a form that increases its availability to the plant after application or allows its prolonged release for a longer period of time than a readily available fertilizer such as ammonium nitrate and ammonium phosphate, among others. Therefore, the development of urea fertilizers encapsulated or coated with sulfur, or urea with polymers, either with slow or prolonged release, has had good results in agricultural fields<sup>[8]</sup>.

Encapsulation, or coating, is a process in which small particles containing an active agent are surrounded

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by a coating to form a capsule. This allows the protection of the active agent against external agents, in addition to providing a decrease in the release rate<sup>[9]</sup>. Most encapsulant materials are organic polymers obtained from agricultural by-products such as proteins, starch, cellulose, and chitosan suitable for use as encapsulants<sup>[10]</sup>. These materials are 100% natural, biodegradable, low cost and highly available, which is a necessary characteristic to avoid soil contamination and obtain a low cost release system<sup>[11]</sup>.

Wheat gluten (GT) is a natural polymer that has these characteristics and is composed of low and high molecular weight proteins: gliadins (28,000–35,000 Da) and glutamines (70,000 to > 10 million Da)<sup>[12]</sup>. Both proteins have been investigated for their use in the preparation of nanoscale and microscale encapsulating materials, as well as for their high potential for application in agriculture<sup>[13,14]</sup>. Therefore, the objective of the study was to characterize and analyze a prolonged release system of urea powder as a sustainable alternative to be used in agriculture.

## **2. Materials and methods**

### **2.1. Urea powder SLIP design**

Commercial wheat gluten (WG) of the RocketteOR brand and urea (46%) were used to prepare the SLIP. SLIP was prepared according to the methodology described by Fessi et al.<sup>[15]</sup> and Barreras-Urbina et al.<sup>[14]</sup> with modifications to be applied in powder form. First, 1 molar (M) of urea was prepared (60.06 g of urea in 1.0 L of distilled water), of which 1.0 mL was added to 0.55 g of powdered GT. It was mixed with a spatula and manually kneaded into a paste. It was then frozen at  $-50\text{ }^{\circ}\text{C}$  in an ultra-freezer and freeze-dried. The freeze-dried paste was macerated in a mortar until a homogeneous powder was obtained and stored at room temperature until characterization.

### **2.2. Scanning electron microscopy**

1.0 g of the SLIP was weighed and placed in a JEOL 5410 LV scanning electron microscope operated at 20 kV to observe surface and internal structures, morphology, porosity, fiber size, granules, and pores.

### **2.3. Fourier transform infrared spectroscopy analysis**

Samples of 5.0 mg of SLIP were analyzed on a Perkin Elmer Spectrum Two infrared instrument. The runs were performed between  $4000$  and  $500\text{ cm}^{-1}$  with a diamond crystal attenuated total reflectance (ATR) accessory. The structures of the compounds and the hydrogen bond bridges formed between them were observed.

### **2.4. Thermogravimetric analysis**

3.0 mg of SLIP were weighed and analyzed in Perkin-Elmer TGA equipment, Model Pyris 1, in an N atmosphere at a flow rate of  $30\text{ }^{\circ}\text{C}$  per minute until  $600\text{ }^{\circ}\text{C}$  was reached. Subsequently, the thermal stability of the compounds and the degradation temperature of the materials used in the preparation of the SLP were measured.

### **2.5. Evaluation of prolonged release of urea in water**

SLIP powder was dissolved in a precipitated beaker with 1.0 L of distilled water, at pH 7, at  $25\text{ }^{\circ}\text{C}$  with magnetic stirring (IKA C-MAG HS7) at 110 rpm. The urea released was determined at the following times: 0, 5, 10, 20, 25, 30, and 60 min; also at 2, 4, 6, 8, 10, 12, 24, 36, and 48 h. At each time,  $10\text{ }\mu\text{L}$  of the aqueous medium was taken and replaced with another  $10\text{ }\mu\text{L}$  of distilled water according to Gulfam et al.<sup>[16]</sup>. In each sample, the concentration of urea released in the medium was quantified with Randox kit<sup>[17]</sup>, in UV-vis spectrophotometry equipment, VARIAN model Cary 50.

Reagent 1 (R1) was prepared by adding urease enzyme ( $\geq 5000 \text{ U L}^{-1}$ ) and reagent (R1b), formed by phosphate buffers (sodium salicylate:  $63.4 \text{ mmol L}^{-1}$ , sodium nitroprusside:  $5.00 \text{ mmol L}^{-1}$  and EDTA serum:  $1.5 \text{ mmol L}^{-1}$ ). Randomly, three vials were prepared: in the first one,  $1000 \mu\text{L}$  of R1 were added and used as a control; in the second one,  $10 \mu\text{L}$  of the standard included in the kit plus  $1000 \mu\text{L}$  of R1 were mixed to form the standard sample, and in the third one  $10 \mu\text{L}$  of the sample with  $1000 \mu\text{L}$  of R1 were added and mixed. The three vials were incubated for 5 min at  $25 \text{ }^\circ\text{C}$  and then  $200 \mu\text{L}$  of reagent R2 (Sodium hypochlorite:  $18 \text{ mmol L}^{-1}$ ) was added. The three samples obtained were incubated for 10 min at  $25 \text{ }^\circ\text{C}$ . Finally, absorbance was measured in triplicate for each sample at a wavelength of 695 nm.

With the absorbances and the urea calibration curve obtained, the concentration was determined, and the release curve was elaborated, in which the maximum point of diffusion of the urea released in time was determined. The data obtained were analyzed using descriptive statistics.

### 3. Results and discussion

A homogeneous powder with a particle size of approximately 2 mm was obtained, making it viable for application as SLIP. The size favors uniform distribution in the field, which can maximize the efficiency of fertilizer use and reduce time and the need for application energy<sup>[18,19]</sup>. Micrographs of commercial GT and SLIP are shown in **Figure 1**. For SLIP, 350x and 750x magnifications are shown in **Figure 1b** and **1c**, respectively. The SEM micrographs in **Figure 1a** show an agglomerated, non-porous, smooth-surfaced structure, as well as the presence of dense granules and irregularly structured compacts. These are composed of small granules on their surface, which is due to the conformational interaction of gluten proteins, glutenins and gliadins<sup>[20]</sup>. **Figure 1b** shows the porosity of their surface, which varies from  $18.8 \mu\text{m}$  to  $187.8 \mu\text{m}$  in diameter. These structures offer advantages, because they provide the ability to slowly and adequately release fertilizer<sup>[21,22]</sup>, which increases nitrogen efficiency and corroborates its potential for application as SLIP under field conditions<sup>[23,24]</sup>. While in **Figure 1b** and **1c**, the presence of fibers is not observed.

FT-IR spectroscopy (**Figure 2**) indicates an FT-IR spectrum of the urea powder in the band characteristic of the stretching of the N-H bond of the primary amide, which is composed of two peaks corresponding to the vibration of the N-H bond of the primary amide at  $3425$  and  $3318 \text{ cm}^{-1}$ . In addition, it shows a medium intensity band at  $1676 \text{ cm}^{-1}$ , which corresponds to the carbonyl group ( $\text{C} = \text{O}$ ). For the gluten powder (5b), a band of higher intensity was observed at  $3292 \text{ cm}^{-1}$  attributed to the stretching of the O-H bonds of the amino acids present in the wheat gluten proteins; with a band of medium intensity of  $2925 \text{ cm}^{-1}$ , corresponding to the stretching of the  $-\text{CH}_2-$  group. Likewise, two other bands of average intensities at  $\approx 1635 \text{ cm}^{-1}$  associated with the vibration of the amine I frequency band; this band is related to the vibrational stretching of the  $\text{C} = \text{O}$  carbonyl group, which is observed transposed behind the band. The second band at  $\approx 1520 \text{ cm}^{-1}$  is related to the vibrational frequency of the amine II band, which corresponds to the deformation of the N-H group. In the urea-loaded gluten composite powder (5C), the characteristic band of a primary amide was observed in the same region of the urea powder spectrum, with mean intensity of  $3428 \text{ cm}^{-1}$ , indicating that urea is present in the processed product. In addition, there is a band of average intensity corresponding to the carbonyl group ( $\text{C} = \text{O}$ ) of  $1671 \text{ cm}^{-1}$ , which was also observed in the urea and in the gluten powder. The description of these bands corresponds with the results reported by Castro-Enrez et al.<sup>[13]</sup> and Barreras-Urbina et al.<sup>[14]</sup>, who evaluated urea-loaded gluten microparticles prepared with electrospray and nanoprecipitation techniques. Also, changes in the position of the band were observed at  $5 \text{ cm}^{-1}$  for urea and  $41 \text{ cm}^{-1}$  for proteins; which can be attributed to a possible interaction produced by hydrogen bonds between the amino group of proteins and the carbonyl group of urea and between the amino group of urea and the carbonyl group of proteins<sup>[25]</sup>. In this regard, Tapia-Hernandez et al.<sup>[26]</sup> report that there is a strong interaction of the bond formed by the hydrogen

bonds of urea and gluten, indicating that the urea particles adhere to the gluten particles, which allows the urea not to be released quickly once in contact with the aqueous medium.

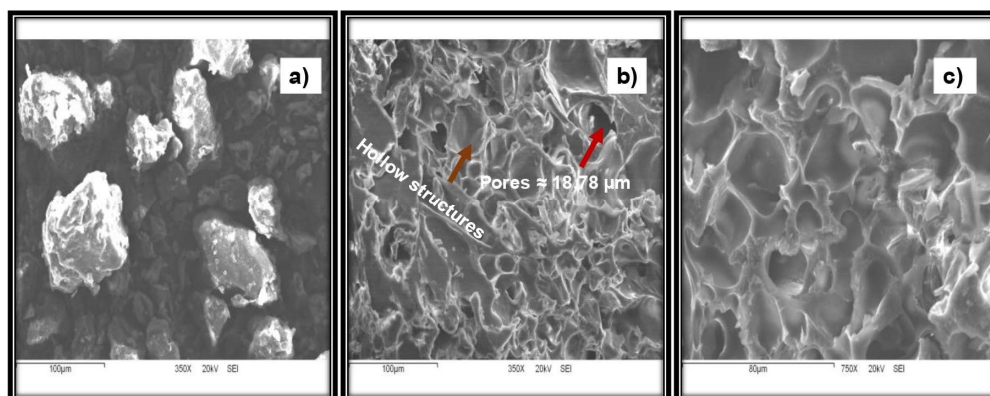


Figure 1. SEM micrographs of the evaluated materials: (a) wheat gluten powder (wg), (b) and (c) SLIP powder surface.

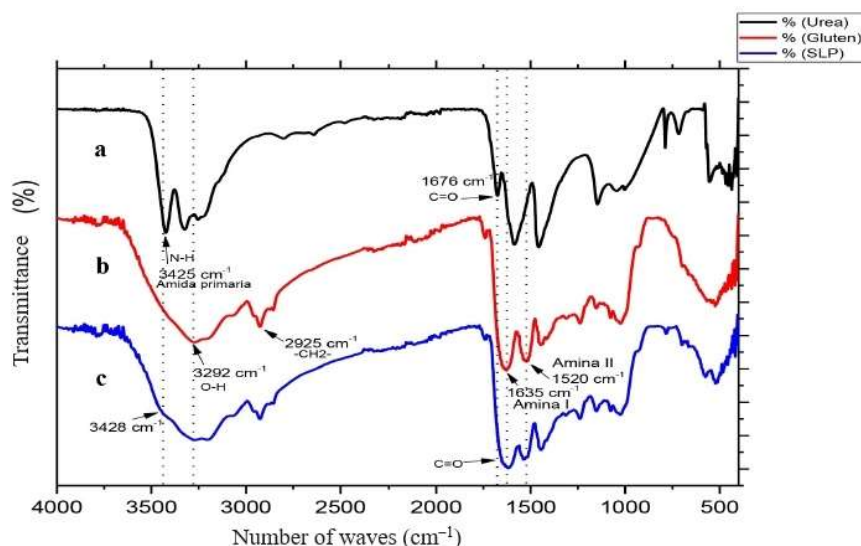
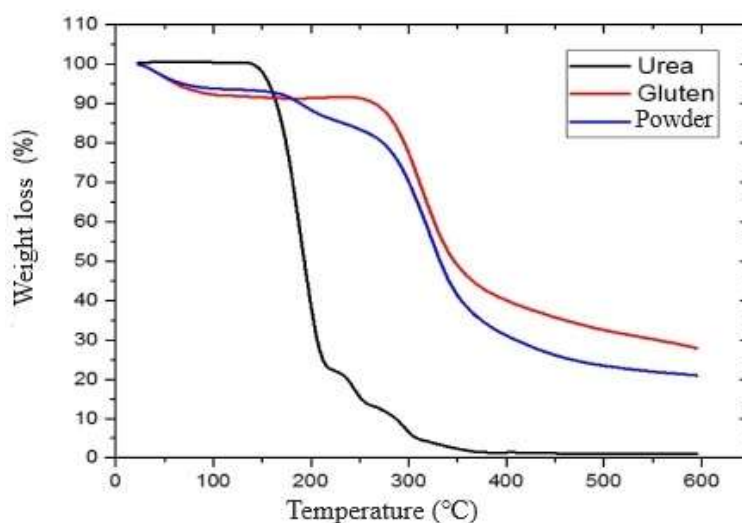


Figure 2. Analysis of the FT-IR spectra of the evaluated materials: (a) urea, (b) gluten powder, (c) SLIP powder.

The thermogravimetric analysis (TGA) is shown in **Figure 3**. **Table 1** shows the percentages of weight loss at different temperatures for urea, gluten and SLIP powder. Urea showed stability at 140 °C and from that point on, its degradation began, with the greatest weight loss (77%) at 215 °C and its degradation being complete at 600 °C. In this regard, Mathrmool et al.<sup>[27]</sup> report that the weight loss of urea is given in three stages, the first one related to the decomposition of urea to produce ammonium isocyanic acid (HNCO), which goes from 140 to 215 °C, being the highest weight loss. Then the ammonia evaporates and the gas is produced, due to the hydrolysis of the HNCO, which corresponds to the second weight loss that occurs from 215 to 308 °C. Finally, the chemical reaction of the raw materials is produced, which is related to the last weight loss and the total degradation. In the present study, it was observed that GT and SLIP started their degradation at 100 °C, which is attributed to the first loss of moisture, which could be related to the drying process of these samples before analysis. GT powder presented the highest weight loss between 24 and 1000 °C, in general, the highest weight loss of urea was 7% and of SLIP was 5% in the same temperature range. It was also observed that gluten powder had a weight loss of 55% between 253 and 367 °C, while at 600 °C, its degradation was 73%. This loss can be attributed to the breakdown of carbohydrates and polysaccharides present in the composite<sup>[28]</sup>.



**Figure 3.** Thermogravimetric analysis (TGA) of the evaluated materials: (a) urea, (b) gluten powder and (c) SLIP powder.

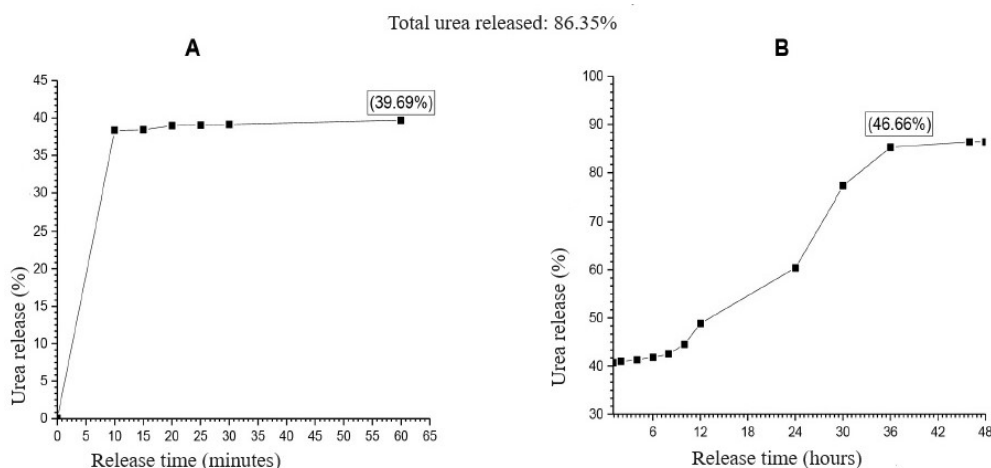
**Table 1.** Description of the thermal stability (TGA) of the materials evaluated: (a) urea, (b) gluten powder and (c) SLIP powder.

Samples	Thermogravimetric Analysis (TGA)	
	Temperature °C	Weight loss %
Urea	140–215	77
	215–254	85
	254–308	95
	308–600	100
Gluten	24–100	7
	246–367	55
	367–600	73
SLP powder	24–100	5
	100–204	15
	204–358	63
	358–600	79

For SLIP, a weight stability of 15% was observed at temperatures between 100 and 204 °C. Subsequently, its degradation continued up to 63% of the weight between 204 and 358 °C, and its degradation was completed at 79% at 600 °C. On the other hand, 27% by weight of the gluten powder and 21% by weight of the urea-loaded gluten powder did not degrade. For the powder system, it is observed that urea starts to degrade from 204 to 600 °C, at about 63%. This differs from that reported by Castro-Enríquez et al.<sup>[13]</sup>, who observed a weight loss of 19% at temperatures between 117 and 207 °C attributing it to urea degradation. While in the present study it was observed that urea began its degradation at 200 °C, which indicates greater stability of the SLIP obtained under high temperatures.

For the prolonged release kinetics of urea in water (**Figure 4**), it is observed that the release of urea occurs during the first 60 min, with rapid release from 5 to 10 min of 38% of the original concentration, which can be attributed to a bursting effect<sup>[29]</sup>. The rapid release may be due to the urea particles that were detached in the powder due to the mechanical process to which it was subjected, as well as the urea particles on the surface of the polymer matrix that are the first to be released upon contact with the aqueous medium. To continue with a slow release rate during the course of time. Similar results were obtained by Castro-Enríquez et al.<sup>[13]</sup>, who

observed a rapid release of urea in the first 10 min in urea-loaded GT membranes with equilibrium release at 5 h with 98% of urea released. The continuity of release (**Figure 4b**) indicates a slow and gradual release of fertilizer with respect to time, occurring during the first hour of a slow and gradual release to reach melt equilibrium at 86.35% total release at 36 h. In this regard, Barreras-Urbina et al.<sup>[14]</sup> with ethanol-soluble wheat and gluten protein microparticles report an approximate release of 50% of urea during the first hour and diffusion equilibrium at 12 h with 88% of the total release. Regarding the same, Bird et al.<sup>[30]</sup> report that urea diffusion equilibrium is reached when its concentration in the product is equal to the concentration in the aqueous medium. In the present study, the urea diffusion equilibrium was reached at 36 h, a longer time than reported, which could provide a favorable characteristic for the implementation of this system under field conditions. In this regard, Azeem et al.<sup>[7]</sup> report that a SLIP of sulfur-coated urea and wax-coated urea had a slow and prolonged release of urea with relevant results in the yield of the crops evaluated, and that SLP combinations of urea and conventional urea improve N efficiency in crops and decrease the cost of fertilization and management.



**Figure 4.** Release kinetics of SLIP in water. **A:** Release kinetics in minutes and **B:** Release kinetics in hours.

The use of SLIP is known to be associated with improved plant growth conditions, reduced stress, and toxicity resulting from an oversupply of nutrients to the near root zones of plants. In this regard, Trenke<sup>[31]</sup> reports that using a SLIP has the advantage of reducing toxicity caused by high nutrient concentrations. This allows the application of a larger quantity of fertilizer in a single application, which reduces the frequency of application and reduces working hours. This reduces the risk of environmental pollution and contributes to the reduction of gas emissions into the atmosphere. In addition to the fact that it increases fertilizer use efficiency<sup>[7]</sup>. SLIP of urea powder has potential characteristics that can be used as an extended release system in soil due to its porosity and hydrogen bonding interactions formed between urea and gluten. These interactions were confirmed by FT-IR and TGA determinations, indicating that the release system is thermally stable at high temperatures. In addition, the urea release test indicated its efficiency as a SLIP over time. Therefore, its application in the field can produce satisfactory results in terms of crop development since it can exceed expectations in yields, improve grain quality, reduce nutrient losses, and prevent damage to the environment.

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## Conflict of interest

The authors declare no conflict of interest.

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