

Synthesis and Characterization of Glutaraldehyde-Crosslinked Chitosan for Urea Controlled Release

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Abstract: Urea exhibits high nitrogen content (~46%), which is essential for plant growth. However, significant nitrogen losses occur through decomposition, leaching, denitrification, and volatilization. Employing controlled-release systems, such as chitosan membranes crosslinked with glutaraldehyde, can mitigate these losses and enhance fertilizer efficiency. Therefore, this study aimed to develop and characterize chitosan-glutaraldehyde membranes as controlled-release fertilizer systems for urea. Chitosan membranes were crosslinked with varying glutaraldehyde concentrations (0%, 0.4%, 0.9%, 1.3%, and 1.8%) and used to encapsulate urea. The

membrane produced was characterized using FTIR and swelling degree assay. The potency of glutaraldehyde-crosslinked chitosan as a urea coating agent was also studied spectrophotometrically using the Nessler reagent. FTIR analysis revealed low intensity of the C=N stretching vibration, indicating limited crosslinking reaction. Additionally, both swelling degree and urea release increased proportionally with increasing glutaraldehyde concentration. These findings suggest limited crosslinking reactions occurred between glutaraldehyde and chitosan within the studied concentration range.

Keywords: Urea, chitosan, glutaraldehyde, crosslinking.

INTRODUCTION

The agricultural sector plays a crucial role in the Indonesian economy, sustaining nearly 42.8 million households [1]. In agricultural practices, fertilizers play a crucial role in supplying essential nutrients to plants, enhancing crop quality and promoting higher yields [2]. Among them, urea is the most important nitrogenous fertilizer for plant nutrition [3]. It contains a high amount of nitrogen, $\pm 46\%$, which is essential for the growth of leaves and stems in plants [2], [4]. However, losses from decomposition, leaching, denitrification, and ammonium volatilization [5], significantly reduce the efficiency of nitrogen uptake by plants, limiting it to 20-30% [2]. Furthermore, the emission from those process, such as nitrate (NO_3^-), nitrogen dioxide (NO_2), and ammonia (NH_3) can contaminate water resources, contribute to ozone depletion, and act as a potent greenhouse gas [6], [7]. These losses not only diminish crop yields, but also have significant environmental consequences.

Recently, controlled release systems (CRFs) have the potential to enhance urea utilization efficiency by slowing down their dissolution rate in water [8]. CRFs are formulated by coating or encapsulating the fertilizer within polymeric or biopolymeric matrices, which exhibit resistance to soil, climatic, and weather influences, thus ensuring efficient fertilizer release and reducing nutrient loss [9]. Chitosan (CA), a biomass-derived polymer, is extensively used in coating and encapsulation applications due to its advantageous properties. These properties include high yield, ease of processing, biodegradability, non-toxicity [10], and the ability to form water-insoluble layers and porous structures [11].

However, chitosan-based CRF coatings are not reactive enough to provide sufficient interaction with the target compound, urea [12]. Additionally, this compound exhibits inadequate mechanical strength, which is unsuitable for load-bearing applications, and easily degrades under harsh conditions such as high pH and pollution [13]. To address these limitations, modification approach such as crosslinking strategies are needed to enhance the material's properties [12], [14]. Glutaraldehyde

(GA), a common cross-linker, effectively enhances chitosan's properties, including improved chemical stability, degradation resistance, mechanical strength, and hydrophilicity [13], [15]. Their suitable interaction is attributed to the cationic nature of chitosan, derived from its amine and hydroxyl groups, which facilitate interactions with various compounds [16], [17]. This glutaraldehyde cross-linked chitosan has been demonstrated in diverse applications, including wastewater treatment [15], [18], [19], drug absorption [13], [20], phosphor coatings [21], and tissue engineering scaffolds [14].

Given the description above, the glutaraldehyde-crosslinked chitosan system presents a promising alternative as a urea coating agent to enhance nutrient efficiency in plants. This study aims to develop and characterize chitosan crosslinked with varying concentrations of glutaraldehyde and evaluate its efficacy as a controlled-release fertilizer system for urea. The findings of this research are anticipated to contribute significantly to advancements in agricultural practices by providing a novel and sustainable approach to fertilizer management.

METHODS

Materials

All chemicals used in this experiment are analytical grade and purchased from Merck, Germany, which include chitosan (degree of deacetylation 80%, medium molecular weight), acetic acid, glutaraldehyde urea, sodium hydroxide, hydrogen chloride (37%), ammonium chloride, potassium iodide, mercury (II) iodide.

Preparation of Urea Fertilizer Matrix

Chitosan-based controlled-release fertilizers were prepared following the method described by Jamnongkan & Kaewpirom (2010) [21]. A 1 g chitosan sample was dissolved in 50 mL of

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2% acetic acid solution and stirred for 20 hours. Subsequently, 5 mL of glutaraldehyde solution at varying concentrations (0%, 0.4%, 0.9%, 1.3%, and 1.8%) was added to the chitosan solution and stirred for 2 hours under magnetic stirring. Thereafter, 5 mL of 5% urea solution was dripped into the mixture and stirred for an additional 2 hours to ensure homogeneity. The resulting mixture was poured into petri dishes and dried in an oven at 35 °C for 2 days to obtain a gel-like membrane.

FTIR Characterization

The structure of glutaraldehyde-crosslinked chitosan matrix was characterized using a Thermo-Scientific Nicolet iS10 FTIR spectrophotometer (USA). The samples were prepared in a powder state, then formed with KBr and pressed to form pellets. FTIR spectra were determined at a wavenumber between 400-4000 cm^{-1} .

Determination of Water Absorption

The water absorption capacity of the glutaraldehyde-crosslinked chitosan matrix ($3 \times 3 \text{ cm}^2$) was determined gravimetrically, following the methods by Salah El-Din (2012) [22]. Initially, the membranes were dried and weighed (referred to as W0). Subsequently, the samples were immersed in excess distilled water for 24 hours for complete water absorption. The membranes were then removed from the water bath, dried to a constant weight, and reweighed (referred to as W1). The water absorption was calculated using the following equation:

$$\% \text{ Swelling} = \frac{W1 - W0}{W0} \times 100\%$$

Urea Release Study

Urea release from the membrane was assessed by measuring the concentration of ammonium ions, a product of urea hydrolysis and ammonification, using a colorimetric method involving Nessler's reagent (HgI_2) [5]. The 0.5-gram dried urea matrix was cut into small pieces and immersed in 160 mL of distilled water. Then, the urea release was observed every day for 5 days using the Nessler method. A 25 mL sample solution was collected daily for 5 days and subjected to analysis using the Nessler methods. Each sample was mixed with 30 mL of 1 M NaOH and distilled for approximately 30 minutes into a receiving flask containing 20 mL of 0.1 N HCl. The distillate was treated with 1 mL of Nessler's reagent and diluted to a final volume of 50 mL with distilled water. After incubation for 30 minutes at room temperature, the absorbance of the solution was measured at 385 nm using a Genesys 10S UV-Vis spectrophotometer (USA), with distilled water as the blank

RESULT AND DISCUSSION

Synthesis of Glutaraldehyde-Crosslinked Chitosan Membrane

Membrane technology can be a promising strategy to control urea release, thus enhancing nitrogen uptake by plants. The coating or encapsulating fertilizer can be done using low-water solubility and porous polymer, such as chitosan, which serves as a controlled-release barrier, regulating the diffusion of nutrients from the fertilizer core [23], [24]. As mentioned before, this compound is a linear structure [25] composed of cationic nature from its amine and hydroxyl groups, which facilitate interactions with various compounds [16].

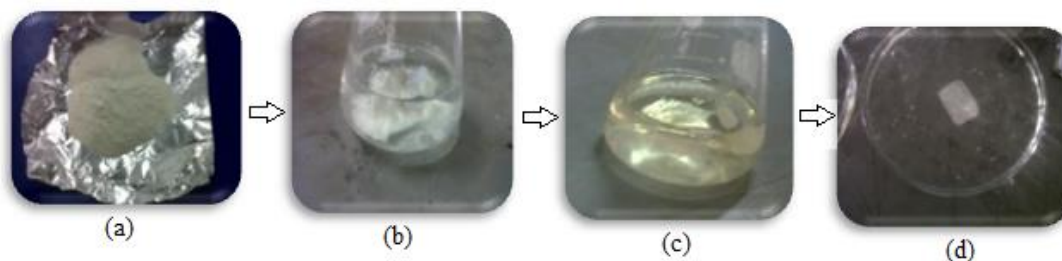


Figure 1. Chitosan membrane synthesis process a) chitosan powder, b) dissolution in acetic acid, c) chitosan solution, d) printed membrane

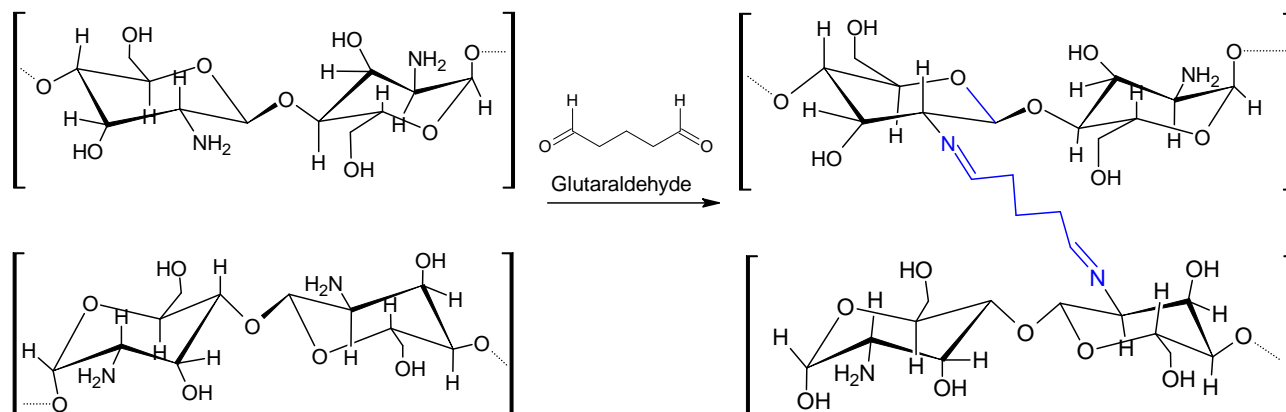


Figure 2. Crosslinking reaction between chitosan and glutaraldehyde

The synthesis of chitosan membrane needs a crosslinker to enhance their physical advantages [13], [15]. Chemical crosslinking mainly forms intermolecular covalent bonds, which give higher stability and crosslinking efficiency than physical crosslinking, which is based on inter-ion interaction. Glutaraldehyde is an extremely widely used crosslinking agent, that can make linear chitosan molecules form a network structure through chemical bonds and improve the mechanical properties of chitosan [25].

Chitosan membranes were fabricated through a crosslinking reaction with glutaraldehyde (GA), as illustrated in Figure 1. Initially, chitosan was dissolved in acetic acid [26], resulting in

protonation [27], which facilitated the subsequent conjugation of chitosan [28]. The addition of GA thereafter induced a crosslinking reaction between the polycations groups of chitosan and the aldehyde groups of GA, forming covalent imine bonds (C=N) [27]. It is postulated that two carbonyl groups in each GA molecule participate in the crosslinking reaction with two chitosan units [29]. Overall, the crosslinking reaction replaced all amino groups (-NH²) on the chitosan nanoparticle surface with amine groups, as depicted in Figure 2. The addition of crosslinking agents aims to improve the mechanical and porous properties of the chitosan membrane [30].

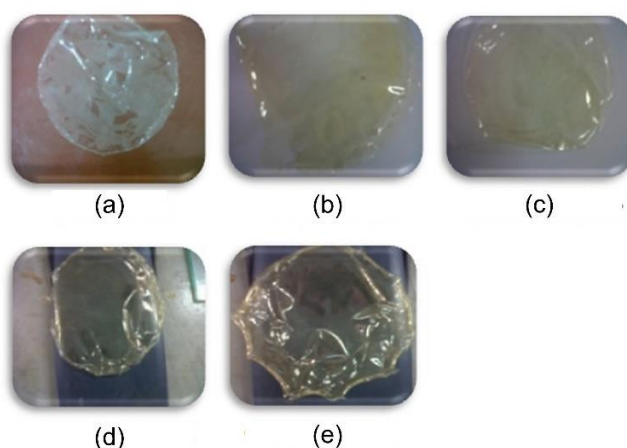


Figure 3. Membrane matrix with variations in glutaraldehyde concentration (a) glutaraldehyde 0% (b) glutaraldehyde 0.4% (c) glutaraldehyde 0.9% (d) glutaraldehyde 1.3% (e) glutaraldehyde 1.8%

In this experiment, the glutaraldehyde-crosslinked chitosan membranes exhibited yellow to brownish colors. This observation aligns with previous findings by Munoz-Tebar, et al (2023) [31], who reported that edible films derived from chitosan typically display transparency or a slight yellowish color with a smooth surface. Notably, the membrane color in this research gradually darkened with increasing glutaraldehyde concentrations (Figure 3). This finding is consistent with Arianita, *et al.* (2019) [32], which demonstrated a darkening of the chitosan solution with higher glutaraldehyde concentrations, indicating the successful cross-linking between these two compounds. Furthermore, Galan, *et al.* (2021) [26] stated that the intensification of the orange-like color in such solutions results from the formation of unsaturated bonds, including C=N (imine), C=C (unsaturated carbon), and C=O (aldehyde).

Chemical Characteristics of Glutaraldehyde-Crosslinked Chitosan Membranes

FTIR Analysis

The functional groups of chitosan-based membranes were characterized using Fourier Transform Infrared (FTIR) spectroscopy. In this technique, samples were subjected to infrared (IR) radiation, inducing atomic vibrations within each molecule due to the absorption of specific energy levels [33]. FTIR analysis confirmed the successful formation of crosslinks between chitosan and glutaraldehyde, as evidenced by distinct IR

spectra observed for each sample. Figures 4 depicts the FTIR spectra of uncrosslinked compared to glutaraldehyde-crosslinked chitosan.

Chitosan is a biopolymer composed of glucosamine monomers, exhibiting a glucose-like structure with an amine group [16]. The FTIR spectrum of uncrosslinked chitosan (0% glutaraldehyde) displayed a broad band in the 3290 cm⁻¹ regions, characteristic of -OH stretching vibrations. Additionally, an amine peak was also observed around 1560 cm⁻¹, consistent with previous research by Wang, *et al.* (2006) [28]. This band is attributed to the bending vibration of the N-H bond in the primary amine group. Furthermore, the polysaccharide structure of chitosan was confirmed by the presence of bands at 2892, 1072, and 1377 cm⁻¹, corresponding to C-H, C-O, and C-N stretching vibrations, respectively [26] and a band at 1152 cm⁻¹ attributed to C-O-C bending vibration [34].

Compared to the FTIR spectrum of uncrosslinked chitosan, distinct spectral differences were observed in glutaraldehyde-crosslinked chitosan samples (Figure 4). Notably, the peak at 1560 cm⁻¹, characteristic of N-H bending vibrations, disappeared in the crosslinked samples, indicating the conversion of amine groups in chitosan to imine groups during the crosslinking reaction [34]. This reaction was further evidenced by the emergence of a new peak at 1650 cm⁻¹, attributed to the stretching vibrations of the newly formed imine (C=N) bonds [27], [29], [35], although the intensity was low.

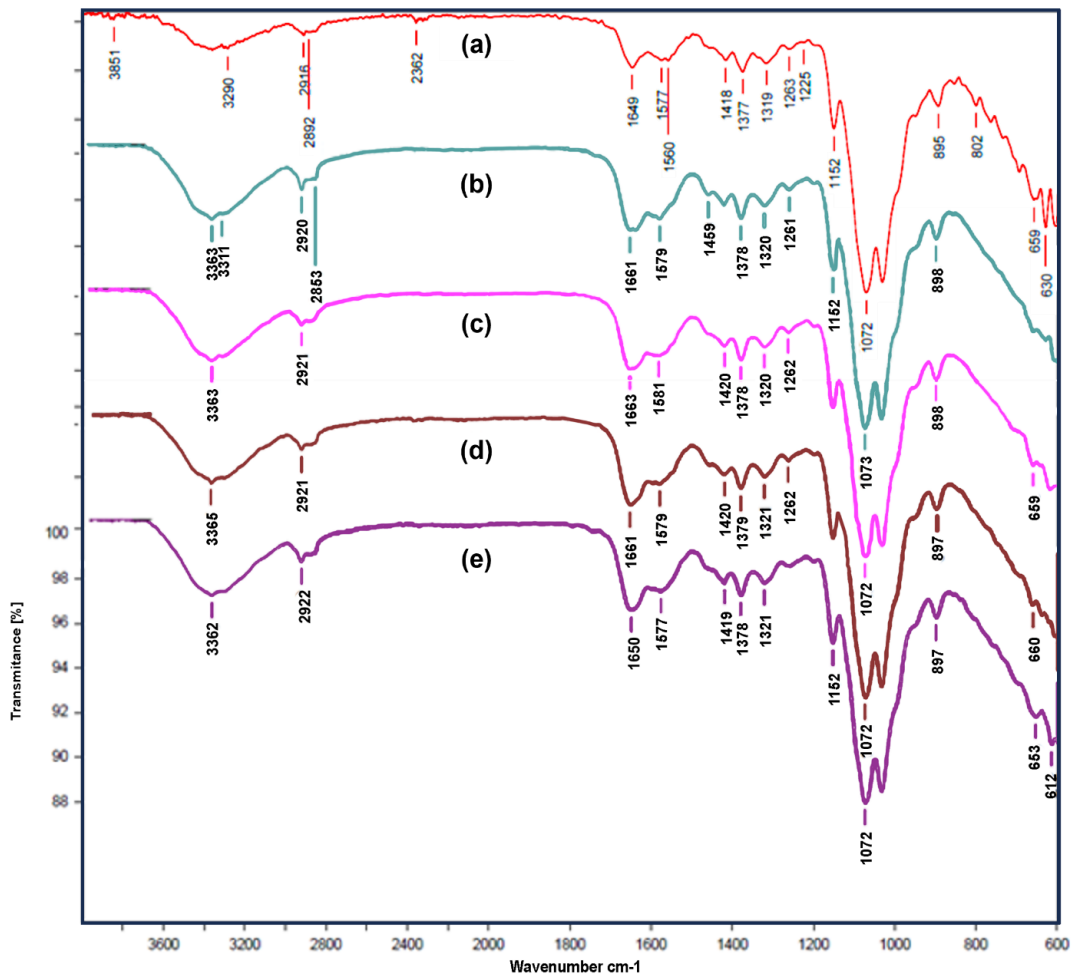


Figure 4. IR spectra of chitosan-glutaraldehyde membrane with glutaraldehyde concentration variations (a) 0%, (b) 0.4%, (c) 0.9%, (d) 1.3%, (e) 1.8%

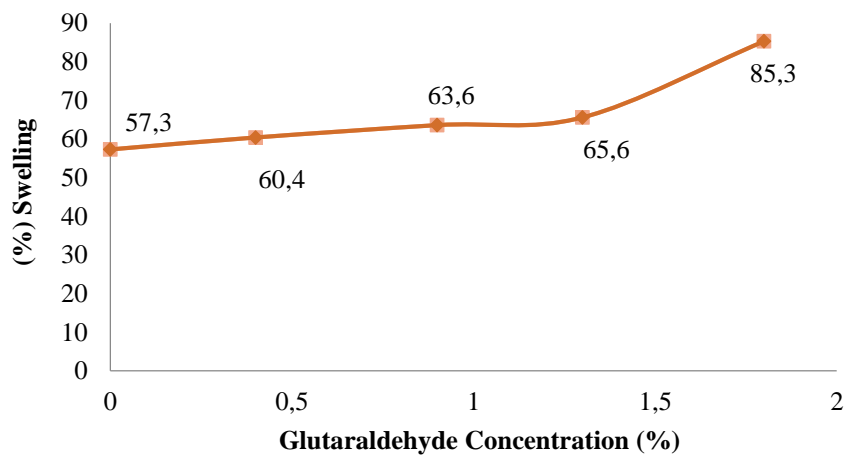


Figure 5. Swelling behavior of chitosan-glutaraldehyde membranes with varying glutaraldehyde concentrations

Physical Characteristics of Glutaraldehyde-Crosslinked Chitosan Membranes

Determination of Water Absorption (Swelling Degree Assay)

The swelling degree of chitosan membranes provides insights into their hydrophilicity [36]. Figure 5 presents the swelling data for uncrosslinked and glutaraldehyde-crosslinked chitosan hydrogels at various crosslinker concentrations. Generally, the swelling degree rises as the glutaraldehyde crosslinker

concentration increases, reaching a maximum level of 85.3% at 1.8% glutaraldehyde concentration. This finding slightly diverges from some previous research. Typically, a swelling percentage will decrease with increasing glutaraldehyde concentrations as the formation of more compact structure through crosslinking interactions can hinder water molecule penetration [8] [37] [38].

In contrast, consistent with our findings, Akakuru and Isiuku

(2017) reported an increase in swelling degree with higher crosslinker glutaraldehyde concentrations, although the underlying mechanism remains less well understood [39]. According to Muzarelli (1997), the excess glutaraldehyde in the chitosan system can form oligomers [40]. Thereafter, the active aldehyde groups (CHO) of these oligomers [40], together with the hydrophilic functional group (amine and hydroxyl) of uncrosslinked chitosan [16], [17] exhibit a strong affinity for water, thus potentially contributing to increased membrane swelling. This finding is further supported by FTIR results, which show a low intensity for peaks associated with C=N, indicating limited crosslinking between glutaraldehyde and chitosan. Hence, this suggests that the formation of glutaraldehyde

oligomers within the system plays a significant role in increasing the degree of swelling.

Urea Release Test

In this experiment, urea release was determined spectrophotometrically using the Nessler method. In this method, the Nessler reagent reacts with ammonium (NH_4^+) ions from the alkaline samples, forming a yellow-to-brown complex. The absorbance of this complex is measured at a wavelength between 400-480 nm [5], [41]. Figure 6 demonstrated that the color intensity produced is directly proportional to the ammonium ion concentration within the samples, as reported previously [41]

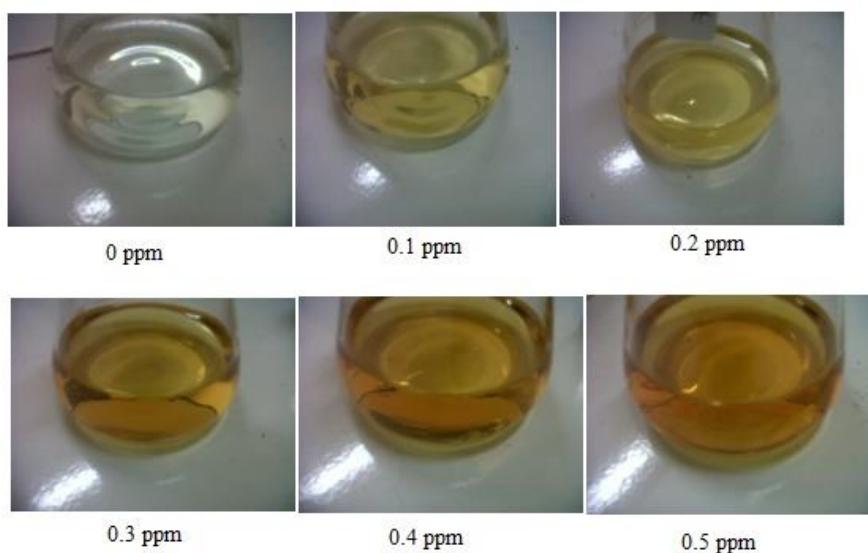


Figure 6. Standard solution of NH_4Cl with a concentration of 0 - 0.5 ppm after adding Nessler's reagent

In this experiment, the results showed that the rate of urea release increased over time along with increasing concentrations of glutaraldehyde (Figure 7). This finding can be caused by a large amount of urea not being adsorbed and attached to the chitosan membrane [42]. This finding contradicts the previous studies, which generally showed a decrease in fertilizer release when using coating agents. The addition of crosslinker in the polymer-based coating agents is known to form more stable complexes, reducing and slowing nutrient release compared to polymer alone [17]. Meanwhile, the increased urea release in this study suggests that glutaraldehyde tends to form oligomers, as supported by the former findings in this research. This phenomenon hinders its ability to form crosslinks, thereby increasing the rate of urea release. Furthermore, Figure 8 shows that the trend in swelling degree is proportional to the trend in urea release. This correlation was expected, as urea is highly soluble in water and may not be effectively absorbed into the matrix [8]. A larger amount of water absorbed in the system may contribute to the increasing measured urea concentration.

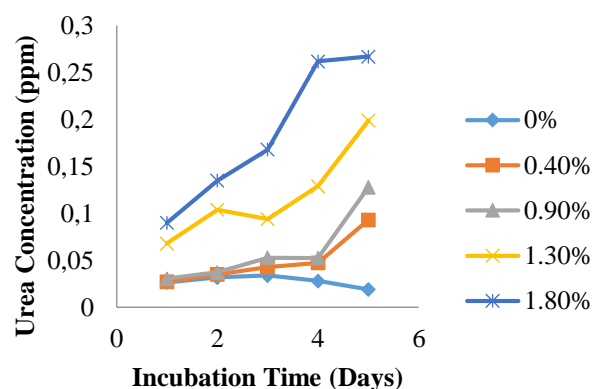


Figure 7. Urea release curve in chitosan-glutaraldehyde membrane matrix, variation of concentration versus time

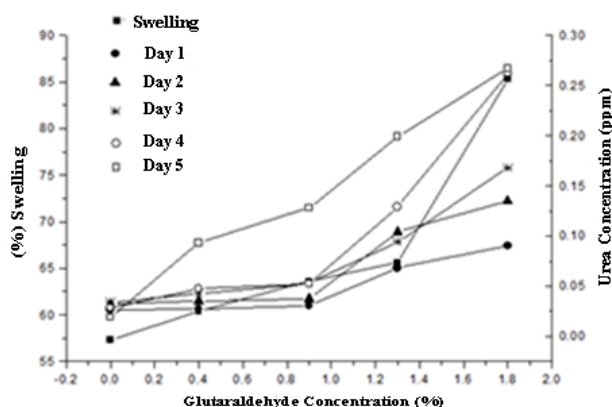


Figure 8. The relationship between glutaraldehyde concentration on swelling degree and urea release

CONCLUSION

This study investigated the potential of chitosan-glutaraldehyde membranes as controlled-release systems for urea fertilizer. Chitosan membranes were crosslinked with varying glutaraldehyde concentrations (0%, 0.4%, 0.9%, 1.3%, and 1.8%) and characterized using FTIR and swelling degree analysis. Despite the successful synthesis of the physical membrane, FTIR analysis revealed limited crosslinking reactions between glutaraldehyde and chitosan, evidenced by low intensity of the C=N stretching vibration. This finding was further supported by the observation of increased swelling and urea release with increasing glutaraldehyde concentrations added. These findings suggest that glutaraldehyde may not be the most suitable crosslinking agent for this system within the studied concentration range. Future research should explore alternative concentration of crosslinking agents to improve the controlled-release performance of chitosan-based fertilizers.

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