

Evaluation of the physical properties of slow release urea based on irradiated chitosan as a feed supplement

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Abstract. A favorable candidate for innovative feed supplement ingredients that can optimize nutrient utilization and reduce environmental impacts could be a slow-release urea based on irradiated chitosan. Chitosan, a biopolymer derived from chitin, has shown immense potential in various applications due to its unique physicochemical properties and biocompatibility. The findings of this study shed light on the promising prospects of irradiated chitosan as a feed supplement ingredient for slow-release urea formulations. Slow-release gel is composed by starch, acrylamide, polyvinyl alcohol and irradiated chitosan. Copolymer gels are treated by Co-60 gamma-ray with 5 kGy and 10 kGy absorbed doses. The lowest gel viscosity escalation is around four thousand times and the highest is more than seventy thousand times. As time of immersion and acrylamide raise in per vary from dose absorbed, gel swelling capacity also increases, start from 33.13 g/g at 5 minutes rise to 164.71 g/g at 720 minutes. Gel fraction from 5 kGy nearly increases two times from 53.57 g/g to 125 g/g at 10 kGy.

1 Introduction

In the dynamic and ever-evolving domain of livestock nutrition, the quest for innovative and sustainable feed supplementation strategies remains at the forefront. Among the many challenges faced by animal nutritionists and agricultural scientists, optimizing the utilization of urea as a cost-effective source of non-protein nitrogen in animal diets stands out. The potential of incorporating irradiated chitosan as a novel matrix for slow-release urea, aiming

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to improve nutrient utilization in livestock and contribute to the sustainability of modern animal farming practices has been investigated.

The choice of irradiated chitosan as the central component in this investigation stems from its remarkable properties as a biopolymer. Chitosan, derived from chitin, is renowned for its biocompatibility, biodegradability, and non-toxic nature. By subjecting chitosan to controlled irradiation, its physical and chemical properties can be tailored to enhance its functionality as a carrier for urea. This innovative approach not only promises controlled urea release within the digestive tract of animals but also opens doors to environmentally friendly and economically viable solutions for the livestock industry.

Moreover, the need for slow-release urea formulations is underscored by the ecological concerns related to excessive nitrogen excretion from animal waste. Conventional urea supplementation can result in nitrogen losses to the environment, contributing to pollution and ecosystem disruption. In light of these challenges, this study explores the potential of slow-release urea based on irradiated chitosan to mitigate nitrogen waste and reduce the environmental footprint of animal farming, aligning with the investigate slow-released urea substances from physical properties evaluation side.

2 Experimental Procedures

2.1. Chitosan irradiation

Chitosan powder was packed in polyethylene plastics 20x20 cm². Chitosan is irradiated using a Co-60 γ -radiation source with an activity 53.13 kCi and 75 kGy absorbed dose [1] in a natural rubber irradiator (IRKA) at the Research Center for Radiation Process Technology (PRTPR), National Research and Innovation Agency, BRIN, Jakarta, Indonesia.

2.2. Sample preparation

The experimental samples consisted of two types which were sequentially irradiated at absorbed doses of 5 and 10 kGy inside a γ Co-60 irradiator. Each sample was prepared in two variations, each comprising starch, poly vinyl alcohol (PVA), irradiated chitosan, and acrylamide. They were placed in a glass beakers and then 100 ml of distilled water was added, followed by stirring for 30 minutes at 90°C until a thick solution was formed. The solution was allowed to stand at room temperature for 60 minutes. The solution was packed in PE plastics 20 x 20 cm².

Table 1. Synthesis formula of gel copolymer gamma irradiation induced

Sampel	S	Aa	PVA	C	γ
KK-1	5 g	3 g	3 g	5 ml	5 kGy
KK-2	5 g	5 g	3 g	5 ml	5 kGy
KK-3	5 g	3 g	3 g	5 ml	10 kGy
KK-4	5 g	5 g	3 g	5 ml	10 kGy

S (starch), Aa (acrylamide), PVA (poly vinyl alcohol), C (chitosan), γ (absorbed dose)

2.3. Viscosity measurement

The gel copolymer that had been allowed to stand at room temperature and its viscosity was measured using a AMETEK Brookfield™ viscometer with a LV-2 needle type at 6 rpm ((Brookfield Engineering, Middleborough, MA, USA), FINDER Factor (FF) 50 for the gel copolymer before irradiation, and LV-4 needle type at 0.6 rpm, FF 10⁴ for irradiated copolymer. The viscosity values of the gel copolymer before and after irradiation were compared to determine whether a copolymerization process has occurred within the sample.

2.4. Swelling capacity test

A certain amount of gel copolymer was taken and placed in a thin cloth (tea bag) with a known constant weight. Subsequently, each gel was immersed in distilled water [2] at intervals of 5 to 7200 minutes. Weight measurements are taken at 5, 10, 20, 25, 30, and 7200 minutes by separating the unabsorbed distilled water by hanging the bag. Gel swelling capacity was calculated using the following equation.

$$ESV \text{ (Equilibrium Swelling Value)} = \frac{W_t - W_o}{W_o} \times 100\% \quad (1)$$

W_t = weight of the gel sample undergoing swelling at *t* minutes

W_o = weight of the dry gel

2.5. Gel fraction test

Approximately ±3 grams of irradiated gel were packed in a wire mesh and immersed in hot water (±90°C) for 8 hours [3]. They were then dried in an oven at 60°C until a constant weight was achieved. The gel fraction was calculated using the following equation.

$$\text{Gel Fraction} = \frac{W_t}{W_o} \times 100\% \quad (2)$$

W_t = weight of gel before immersion

W_o = weight of dry gel after immersion

3 Results and Discussion

The use of chitosan in agriculture is widely recognized as a growth promotor and antibacterial agent, capable of controlling the release rate of easily lost nutrient elements. Chitosan has a high molecular weight and strong intermolecular and intramolecular hydrogen bonds. Chitosan is derived from the natural polymer chitin, which consists of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose [4][5]. In this study, white to yellowish chitosan powder was used. A sample of 200 grams of chitosan was placed in a 10 x 10 cm² PET container, and then the sample was irradiated using gamma rays from a Co-60 source. The dose rate used for the irradiation of chitosan was 9.3 kGy/hour. The total dose obtained for the irradiation of chitosan was 75 kGy. The purpose of irradiating chitosan was to reduce its molecular weight significantly [6], resulting in what is called oligochitosan [7]. Chitosan degradation is commonly used to transform chitosan into oligochitosan (o-chitosan) with better solubility in water. Chitosan with lower molecular weight and higher water solubility

has several improved biological, chemical, and physical properties compared to regular chitosan [8], such as antibacterial activity [9] antifungal activity [10], and antitumor activity [11]. The irradiation dose of 75 kGy is based on a study conducted by El-sawy et al. in 2010 [12], where chitosan was irradiated with doses ranging from 20 to 200 kGy. The results showed significant changes in the viscosity of chitosan powder as the irradiation dose increased [13]. A substantial decrease in molecular weight occurred at doses between 20 and 120 kGy. The radiation dose of 70-200 kGy resulted in very low degradation rates [14], which is a characteristic of ionizing radiation on biodegradable polysaccharide materials, leading to a decrease in the average molecular weight [15]. The irradiation dose of 20 kGy resulted in an average molecular weight of 7.106 g/mol, 40 kGy resulted in 6.106 g/mol, 80 kGy resulted in 3.106 g/mol, and doses of 75-200 kGy exhibited relatively stable molecular weights ranging around 2.106 g/mol. The irradiated chitosan molecules undergo cleavage of β -(1-4) glycosidic bonds, leading to a reduction in their average molecular weight [16].

3.1. Gel copolymer viscosity

Table 2, showed that all doses of gamma irradiation resulted in a significant increase in viscosity values. Gamma irradiation can influence the viscosity of a starch-acrylamide-PVA gel by promoting cross-linking, which generally increases viscosity [17][18]. The lowest increase was 5234.77 times for sample KK-1 with a radiation dose of 5 kGy, and the highest (measurable) increase is 71596.83 times for sample KK-4 with a radiation dose of 10 kGy.

Table 2. Average viscosity of gels copolymer

Before Co-60 gamma irradiation gel	
Sample	Average viscosity, η ($10^3.50$ cPs)
KK-1	1.23
KK-2	1.31
KK-3	1.94
KK-4	1.84
After Co-60 gamma irradiation gel copolymer	
Sample	Average viscosity, η ($10^3.10^4$ cPs)
KK-1	32.2
KK-2	193.7
KK-3	558.3
KK-4	658.7

Samples KK-1 to KK-4 were copolymer gels formed through irradiation [19], which were not very physically dense and could be used for blending with urea fertilizer. However, despite of KK-1 to KK-4 gel copolymer, the viscosity values after the radiation process were extremely elevated [20], forming highly dense gels [21] that cannot be mixed with urea fertilizer. Radiation doses higher than 10 kGy are too high, resulting in gels with excessively

large cross-linking densities, causing a narrowing of the space between networks for water absorption [22]. Based on the viscosity measurements of the irradiated copolymer gel samples at various doses, it can be concluded that doses of 5 and 10 kGy are the optimum dose for the formation of gel copolymer that can be blended with urea fertilizer. At the lower radiation dose of 2 kGy, the hydrogel was not formed, likely due to the lack of the necessary activation energy required to begin the reaction. Typically, when dealing with an aqueous solution, gamma rays serve to break apart water molecules, forming hydrogen and hydroxyl free radicals that serve as initiators for the reaction [23].

3.2. Swelling capacity of gel copolymer

The development capacity of the synthesized gel copolymer is determined by its ability to absorb water. The swelling ability of gel will decrease as the concentration of ions that dissociate in water increases [24]. When the charge concentration inside the gel is greater than the charge concentration in the external solution, it will cause the gel to expand [25–27]. The results of water absorption capacity for the 5 kGy irradiated gel can be seen in Figure 1a. Swelling capacity of the 5 and 10 kGy irradiated gel can be observed from minute 5 to minute 720. The swelling capacity of gel raised up generally with increasing immersion time and increasing acrylamide concentration [28][29][30]. During the immersion period of 15–720 minutes, sample KK-1 to KK-4 reaches near constant with a swelling capacity at 20–30 minutes. This is because gels were added with 3–5% w/v acrylamide, causing the -NH₂ water-attracting groups, which contain hydrogen bonds, to become saturated at minute 20 due to the low concentration [31][32][33]. Sample KK-1, with the addition of 3% w/v acrylamide, experienced an increase in swelling capacity from minute 5 to minute 30, reaching 121.25 g/g. After being left to stand for 720 minutes, the swelling capacities of KK-1 and KK-2 decreased to 87.87 g/g and 51.07 g/g, respectively. This decrease is possible because during the 720-minute standing period, cross-links between acrylamide molecules and cross-links between acrylamide and polyvinyl alcohol (PVA) molecules broke, causing some acrylamide and PVA molecules to dissolve in water, thereby reducing the swelling capacity [34][35]. During the swelling process, water continuously move into the gel. This process is caused by the difference in osmotic pressure between the gel and water [36][37][38]. The greater the difference in osmotic pressure, the greater the swelling capacity [39].

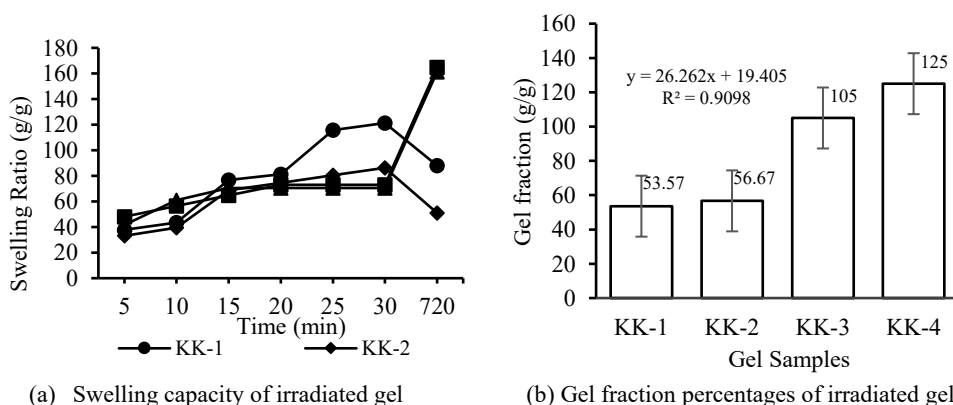


Fig 1. Physical properties evaluation of 5 and 10 kGy gel

3.3. Gel fraction percentages

The fraction of gel values was determined to understand the influence of gamma rays on cross-linked molecules in copolymer gel [40][41]. The gel fraction increases with higher radiation doses and monomer concentrations [42]. A higher monomer concentration increases the likelihood of cross-linking within the gel. In general, the effect of radiation on the solution system makes it difficult to reach 100% gel fraction value. This was because of the presence of oxygen in the water/environment molecules interfered with the polymerization process and the possibility of gel degradation at excessively high radiation doses [43]. The addition of PVA to the gel can increase polymer tacticity [44][45] which means chemical structure of PVA has reactive tertiary protons near the -OH group, making it easier to detach. The gel fraction values for samples KK-1 to KK-4 increase proportionally with the added acrylamide concentration. More acrylamide will increase likelihood of acrylamide and PVA cross-linking, which can improve the physical properties of the copolymer gel [46][47]. Sample KK-4, with a 5% acrylamide concentration, had the highest gel fraction value of 125 g/g, with none of the copolymer gel fraction lost at 90°C for 8 hours. The gel fraction values for samples KK-3 and KK-4 also increased proportionally with the added acrylamide concentration. Sample KK-3, with a 3% w/v acrylamide concentration, had a gel fraction value of 105 g/g, suggesting that there was no gel fraction lost from the gel until after 8 hours. Figure 1b. showed KK-3 and KK-4 had gel fractions exceeding 100 g/g, suggesting that acrylamide and polyvinyl alcohol compounds are strictly cross-linked [46], forming a strong polymer bond that is resistant to high temperatures (the polymer does not easily break down). A high gel fraction value indicates that copolymer gel KK-3 and KK-4 can still absorb water even at high temperatures [48] following 8 hours of immersing.

4 Conclusion

Synthesis of irradiated gel copolymer (slow release urea) based on chitosan as feed supplements ingredients is affected by dose of Co-60 gamma irradiation. Nevertheless, the addition of acrylamide and poly vinyl alcohol also modified water absorption capacity and cross-linking percentage of gels. The physical properties of this gel are qualified as chitosan-based supporting materials which used as a feed supplement ingredient.

The authors acknowledge the HITN (Nuclear Innovation Technology Product) 2023 and IAEA Coordinated Research Project D31031 – 25116 (Nuclear and related techniques to measure the impact of type of feeding and production system on greenhouse gas (GHG) emissions and livestock productivity) for funding source.

5 References

1. D. Darwis, D. S. Pangerteni, B. Abbas, T. Puspitasari, N. Nuryanthi, A. Nurrochmad, and C. M. Airin, *Macromol. Symp.* **391**, (2020)
2. T. Jayaramudu, H. U. Ko, H. C. Kim, J. W. Kim, and J. Kim, *Materials (Basel)*. **12**, (2019)
3. C. Chang, B. Duan, J. Cai, and L. Zhang, *Eur. Polym. J.* **46**, (2010)
4. T. Thirugnanasambandan and S. C. B. Gopinath, *Process Biochem.* **130**, (2023)
5. F. A. Vicente, B. Bradić, U. Novak, and B. Likozar, *Biopolymers* **111**, (2020)

6. D. Xuan Du, B. Xuan Vuong, and H. D. Mai, *Adv. Mater. Sci. Eng.* **2019**, (2019)
7. V. Ngoc Boi, N. Thi My Trang, D. Xuan Cuong, V. Thi Hoan, and L. Hai, *World J. Food Sci. Technol.* **4**, (2020)
8. I. Aranaz, A. R. Alcántara, M. C. Civera, C. Arias, B. Elorza, A. H. Caballero, and N. Acosta, *Polymers (Basel)*. **13**, (2021)
9. F. Zhang, G. Ramachandran, R. A. Mothana, O. M. Noman, W. A. Alobaid, G. Rajivgandhi, and N. Manoharan, *Saudi J. Biol. Sci.* **27**, (2020)
10. P. Poznanski, A. Hameed, and W. Orczyk, *Molecules* **28**, (2023)
11. P. Zou, X. Yang, J. Wang, Y. Li, H. Yu, Y. Zhang, and G. Liu, *Food Chem.* **190**, (2016)
12. N. M. El-Sawy, H. A. Abd El-Rehim, A. M. Elbarbary, and E. S. A. Hegazy, *Carbohydr. Polym.* **79**, (2010)
13. I. Zainol, H. M. Akil, and A. Mastor, *Mater. Sci. Eng. C* **29**, (2009)
14. M. Mahmud, M. I. Naziri, N. Yacob, N. Talip, and Z. Abdullah, in *AIP Conf. Proc.* (2014)
15. K. Wiszumirska, D. Czarnecka-Komorowska, W. Kozak, M. Biegańska, P. Wojciechowska, M. Jarzębski, and K. Pawlak-Lemańska, *Materials (Basel)*. **16**, (2023)
16. M. A. García, N. de la Paz, C. Castro, J. L. Rodríguez, M. Rapado, R. Zuluaga, P. Gañán, and A. Casariego, *J. Radiat. Res. Appl. Sci.* **8**, (2015)
17. J. N. BeMiller, in *Starch Food Struct. Funct. Appl.* (2017)
18. Z. Sui and X. Kong, *Physical Modifications of Starch* (2018)
19. A. W. M. El-Naggar, M. M. Senna, and S. A. Khalil, *J. Appl. Polym. Sci.* **91**, (2004)
20. M. Navaf, K. V. Sunooj, B. Aaliya, C. Sudheesh, P. P. Akhila, S. Sabu, A. Sasidharan, and J. George, *Radiat. Phys. Chem.* **201**, 110459 (2022)
21. Erizal, *Indones. J. Chem.* **12**, (2012)
22. A. Ortega, S. Valencia, E. Rivera, T. Segura, and G. Burillo, *Gels* **9**, (2023)
23. M. M. Bhuyan, M. Islam, and J.-H. Jeong, *Gels* **9**, (2023)
24. L. R. Shivakumara and T. Demappa, *Turkish J. Pharm. Sci.* **16**, 252 (2019)
25. S. Bashir, M. Hina, J. Iqbal, A. H. Rajpar, M. A. Mujtaba, N. A. Alghamdi, S. Wageh, K. Ramesh, and S. Ramesh, *Polymers (Basel)*. **12**, (2020)
26. E. Czarnecka and J. Nowaczyk, *Int. J. Mol. Sci.* **22**, (2021)
27. A. Mahmoud, A. Fahmy, A. Naser, and M. A. Saied, *Sci. Rep.* **12**, 22017 (2022)
28. W. Bai, B. Ji, L. Fan, Q. Peng, Q. Liu, and J. Song, *Polymers (Basel)*. **15**, (2023)
29. S. L. Favaro, F. De Oliveira, A. V. Reis, M. R. Guilherme, E. C. Muniz, and E. B. Tambourgi, *J. Appl. Polym. Sci.* **107**, (2008)
30. S. Noppakundilokrat, N. Pheatcharat, and S. Kiatkamjornwong, *J. Appl. Polym. Sci.* **132**, (2015)
31. F. S. El-banna, M. E. Mahfouz, S. Leporatti, M. El-Kemary, and N. A. N. Hanafy, *Appl. Sci.* **9**, (2019)
32. X. Y. Wu, S. W. Huang, J. T. Zhang, and R. X. Zhuo, *Macromol. Biosci.* **4**, (2004)
33. C. M. Yeng, H. Salmah, and S. Sung Ting, in *Adv. Mater. Res.* (2013)
34. M. Wang, J. Bai, K. Shao, W. Tang, X. Zhao, D. Lin, S. Huang, C. Chen, Z. Ding, and J. Ye, *Int. J. Polym. Sci.* **2021**, 2225426 (2021)
35. J. Yu, S. Gao, D. Hou, P. Wang, and G. Sun, *J. Phys. Chem. B* **124**, 6095 (2020)
36. M. J. Krafcik and K. A. Erk, *Mater. Struct. Constr.* **49**, (2016)
37. J. Wang, S. Gao, J. Tian, F. Cui, and W. Shi, *Water* **12**, (2020)
38. J. L. Holloway, A. M. Lowman, and G. R. Palmese, *Acta Biomater.* **9**, 5013 (2013)

39. S. Saber-Samandari, M. Gazi, and E. Yilmaz, *Polym. Bull.* **68**, (2012)
40. D. R. Barleany, Jayanudin, A. S. Utama, U. Riyupi, H. Alwan, R. S. D. Lestari, A. B. Pitaloka, M. Yulvianti, and Erizal, *Mater. Today Proc.* (2023)
41. E. Erizal and R. C. Rahayu C, *Indones. J. Chem.* **9**, (2010)
42. T. Fekete, J. Borsa, E. Takács, and L. Wojnárovits, *Chem. Cent. J.* **11**, (2017)
43. W. Qiao, G. Zhang, P. Jiang, and H. Pei, *Gels* **9**, (2023)
44. G. W. Oh, I. W. Choi, W. S. Park, C. H. Oh, S. J. Heo, D. H. Kang, and W. K. Jung, *J. Appl. Polym. Sci.* **139**, (2022)
45. G. A. Mahmoud, D. E. Hegazy, and H. Kamal, *Arab J. Nucl. Sci. Appl.* **47**, (2014)
46. A. S. Tarek Mansour Mohamed and G. A. Mahmoud, *Polym. Technol. Mater.* **62**, 866 (2023)
47. M. M. El-Toony, *Egypt. J. Chem.* **60**, (2017)
48. Y. Tang, Z. Yu, L. Tam, A. Zhou, and D. M. Li, *Mater. Today Commun.* **33**, 104834 (2022)