Jurnal Rekayasa Kimia & Lingkungan
(Journal of Chemical Engineering and Environment)
Vol. 20, No. 1, Page 46 – 56, June 2025
ISSN 1412-5064, e-ISSN 2356-1661
https://doi.org/10.23955/rkl.v20i1.40112

Synthesis of Microgel Sulfated Carboxymethyl Cellulose (SCMC) by Double Crosslinked Alginate from Lindur Fruit (*Bruguiera gymnorrhiza*) for Diabetic Ulcer Healing

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Article History
Received: 18 July 2024; Received in Revision: 22 February 2025; Accepted: 23 February 2025

Abstract

Microgels have been recognized as promising scaffolds for diabetic wound care due to their favorable properties in promoting wound healing. This study aims to synthesize a microgel using sulfated carboxymethyl cellulose (SCMC) derived from Lindur fruit (*Bruguiera gymnorrhiza*) for potential application in diabetic ulcer treatment. The synthesis was conducted through a double-crosslinking reaction, and the resulting microgel was characterized using infrared (IR) spectroscopy, scanning electron microscopy (SEM), and in vivo analysis in male mice (*Mus musculus*). The results demonstrated that the yield of Ca-alginate isolate ranged from 18.67% to 19.46%, with a purity of 19.5%–33.1%, viscosity of 31.19–31.46 cP, and stable pH at 7. The Ca-alginate isolate exhibited antioxidant activity at 50 ppm with an inhibition percentage of 22.3%. The modification of SCMC was successfully achieved, as confirmed by the presence of characteristic absorption peaks at 1220 cm^{-1} (asymmetric S–O stretching) and 870 cm^{-1} (symmetric C–O–S stretching vibrations), indicating successful sulfate group incorporation. The microgel swelling ratio was recorded at 660% for microgel A, 700% for microgel B, and 720% for microgel C, with microgel C exhibiting the highest swelling capacity. In vivo wound healing assessment revealed that microgel C facilitated more rapid wound closure, with wound sizes reducing progressively from 0.9 cm on day 3 to 0.5 cm on day 5, 0.3 cm on day 7, and 0.1 cm on day 14. These findings suggest that SCMC-based microgel with alginate derived from Lindur fruit holds significant potential as an innovative formulation for diabetic wound management.

Keywords: Diabetes, double crosslinking, Lindur fruits, microgel, SCMC, ulcer healing

1. Introduction

Diabetes is a severe and long-term condition with a significant impact on the lives and well-being of human life (Furqon et al., 2025). The International Diabetes Federation (IDF) reports that diabetes mellitus is the fastest-growing emergency health problem of the 21st century, estimated to be at least 463 million (9.3%) people aged 20-79 years suffering from diabetes in 2019 worldwide. It is expected to increase to 578 million people by 2030 and 700 million people by 2045 (Saeedi et al., 2019). Diabetics, in general, will experience serious injuries, especially ulcers occuring due to loss of protective sensation and poor blood circulation so that blood cannot flow smoothly to the feet properly (Yan et al., 2022). Diabetic ulcers are a serious complication associated with a high rate of infection, amputation or loss of limbs, resulting in a high healthcare burden and poor quality of patient life. It’s a very serious complication caused by a combination of poor circulation, susceptibility to infection and nerve damage from high blood sugar levels (Yachmaneni et al., 2023).

Ulcers of diabetics have a risk of amputation 154.5 times compared to ulcers in general, but it can also have an impact on death. The mortality rate after a year of amputation was 14.3%, and mortality in three years after amputation was 37%. Therefore, diabetic

To cite this article: Hutapea, J.A., Zaki, N.D., Syahrum, S.F., Putra, S.A., Marbun, S.A., Tarigan, I.L. (2025) Synthesis of Microgel Sulfated Carboxymethyl Cellulose (SCMC) by Double Crosslinked Alginate from Lindur Fruit *(Bruguiera gymnorrhiza)* for Diabetic Ulcer Healing, *Jurnal Rekayasa Kimia & Lingkungan* 20 (1): 46 - 56 https://doi.org/10.23955/rkl.v20i1.40112

ulcers must be given good and effective wound management to speed up the healing process of diabetic ulcer wounds (Everett and Mathioudakis, 2018).

Microgel is considered as an ideal scaffold for diabetic wound care due to provide good results in healing diabetic wounds (Da Silva *et al*., 2019). Microgel is successfully applied as a platform for individual cell cultures and drug carriers. Microgel assembly can create macroscopic scaffolds as tissue substitutes in *vivo* (Feng et al., 2022). Nowadays, many techniques for synthesising microgels have been reported based on the driving force to maximize them. These techniques are categorized into four: chemical reactions, physical reactions, cell interactions, and external driving forces. The microgel facilitates cell infiltration and vascularization without waiting for hydrogel degradation. According to its characteristics, flexible microgels will have better tunability, thus making them adapt to various wound conditions (Yan *et al*., 2022).

The cross-linking synthesis process has successfully produced durable hydrogels with superior tissue affinity and improved wound-healing properties. This process involves the formation of chemical bonds between polymer chains, thereby reinforcing the structural stability of the hydrogel (Khan et al., 2024). Hydrogels exhibit excellent water retention capacity, microbial resistance, and flexibility, making them well-suited for biomedical applications. Their ability to expand in aqueous environments while maintaining their original shape is attributed to the presence of functional groups such as –OH, –COOH, –$CONH_2$, and –SO_3H, which enhance surface wettability, biocompatibility, and hydrophilicity (Ho et al., 2022). These properties enable hydrogels to retain moisture, creating an optimal environment for wound healing.

Additionally, the incorporation of microgels within hydrogels enhances their tunability and adaptability to different wound conditions. Microgels optimized with sulfated carboxymethyl cellulose (SCMC) have demonstrated improved wound regeneration capabilities in diabetic wound models, as confirmed through in vivo studies (Yan et al., 2022).

This study aims to develop a microgel incorporating *Bruguiera gymnorrhiza* (Lindur) plant extract, which has the potential to serve as a potent antioxidant agent that mitigates oxidative stress associated with diabetes and enhances insulin sensitivity. The extract from this plant may contribute to a novel therapeutic formulation with combined effects for diabetes management and its related complications, particularly in improving cognitive function and addressing dermatological manifestations such as diabetic dermopathy, xerosis, and acanthosis nigricans. Phytochemical analysis of *B. gymnorrhiza* extract revealed moderate α-glucosidase inhibitory activity and relatively low α-amylase inhibitory activity, suggesting its potential role in modulating postprandial glucose levels. Given these properties, *B. gymnorrhiza* extract may offer therapeutic benefits beyond a placebo effect and represents a promising strategy for intervening in the progression of diabetes mellitus (Sadeer *et al*., 2022).

In addition, this research synthesis and characterize of microgels derived from the cross-linking of SCMC and evaluates their physicochemical and morphological properties. Furthermore, this study aims to assess the biocompatibility and biological response of the microgels through *in vivo* tests on male mice (*Mus musculus*) via intraperitoneal administration. It is hoped that the findings of this research will provide valuable insights into the potential of microgels as biocompatible materials for various biomedical applications.

2. Methodology

2.1. Materials and Instrumentation

Lindur fruit (*Bruguiera gymnorrhiza*) samples were collected from East Tanjung Jabung, Jambi Province, Indonesia. The chemicals used in this study included carboxymethyl cellulose (CMC), sulfuric acid (H_2SO_4), hyaluronic acid (A-HA), 1% calcium chloride ($CaCl_2$) solution, D-glucono-δ-lactone (GDL), hydrochloric acid (HCl), alloxan, sodium hydroxide (NaOH), distilled water (aquadest), zinc chloride ($ZnCl_2$), sodium bisulfite ($NaHSO_3$), diabetic wound treatment (Binasol), and sodium nitrite ($NaNO_2$). All chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

The experimental model involved *Mus musculus* (mice) as test subjects, with ethical clearance granted by the Faculty of Medicine and Health Sciences, Jambi University. The analytical instruments utilized in this study included a UV-Vis spectrophotometer, Fourier-transform infrared (FT-IR) spectroscopy, and scanning electron microscopy (SEM) (Thermo Fisher Scientific, USA).

2.2. Lindur Fruit Sample Preparation

Samples of *B. gymnorrhiza* fruits were washed, cleaned, and dried at room temperature by aerating. The dried lindur fruit was grouded using a Hammer Mill (AGR-HMR20) to obtain simplicia powder. The isolation process of Lindur fruit was carried out following previous studies with some modifications using the calcium-alginate (Ca-alginate) method. The simplicia (150 g) was soaked in 1000 ml distilled water, immersed in a 1% HCl solution for three days, and filtered and washed with distilled water. The residue is soaked in a 2% solution of Na_2CO_3 for five days and filtered. The filtrate from the filtrate is added with a 14% $CaCl_2$ solution and left for several days with a time variation of 3, 5, and 7 days, then blanched with the addition of 5% $Ca(ClO)_2$ for two days. The precipitated calcium alginate was filtered, washed with distilled water, and dried in an oven. The yield was then recorded. The obtained alginate was subsequently subjected to various physicochemical and functional analyses, including pH measurement, purity assessment, viscosity evaluation, moisture content determination, ash content analysis, protein content analysis, fat content analysis, carbohydrate content analysis, antioxidant activity assessment, and functional group characterization using Fourier-transform infrared (FT-IR) spectroscopy.

2.3. Synthesis of Sulfated Carboxymethyl Cellulose

Synthesis of sulfated carboxymethyl cellulose was carried out by following previous studies with some modifications (Fan et al., 2014). Sodium Bisulfate was dissolved in 40 mL of distilled water, then Sodium Nitrite previously dissolved in distilled water (10 ml) was added dropwise to the beaker with a stirrer magnet at a temperature of 90°C and then reacted for 1,5 h.

Hereafter, a solution of sulfate agent ($N(SO_3Na)_3$). Carboxymethyl cellulose (5 g) is added to the solution of the sulfate agent and then stirred using a stirrer magnet with the same temperature and time. Then, the SCMC that has been obtained is dried at 40 ◦C for 2 h. Then SCMC analyzed its functional groups with FT-IR.

2.4. Synthesis of Microgel (Zn^{2+}-SCMC-AHA)

The modified SCMC is synthesized using Hyaluronic Acid (AHA). Hyaluronic acid (1 g, 100 mL) was added with $NaIO_4$ (500 mg) for 2 h and dialyzed with water (3500), then lyophilized to obtain A-HA. A-HA (50 mg) was incubated with SCMC (200 mg) in 5 mL of water at 37°C for 2 h, after which $ZnCl_2$ (300 mg, 0.5 mL) was added; the solution became opaque and turned light yellow. The mixture was stirred overnight at 37°C. Then, the centrifuge was used for 5 min at 6000 rpm/minute, and the results were obtained in the form of gel-like sediment taken and washed with water three times. The obtained microgels (Zn^{2+}-CMCS-A-HA) were dispersed in water (5 mL) for further treatment (Yan *et al.*, 2022).

2.5. Crosslinked and Double Crosslinked

The reaction was carried out with several conditions and concentrations of sodium alginate (1%, 2 mL) mixed with microgel (0.5 g, 1 g, and 2 g), then added fresh saturated GDL solution (0.1g, 2mL). Microgels were cross-linked with alginates through electrostatic interactions between sulfated carboxymethyl cellulose (SCMC) on their morphological surfaces, facilitated by D-glucono-δ-lactone (GDL). These electrostatic attractions between oppositely charged groups within the microgel structure play a crucial role in maintaining the gel's stability and mechanical strength. GDL will be hydrolyzed and released pro-short into a double-crosslinked alginate-based microgel with modified SCMC developed by combining two physical crosslinks and ionic alginate crosslinking using divalent cations (Ca^{2+}) as well as electrostatic interaction between amino group cations in CMC and carboxyl group anions in alginate.

The pre-gelled solution is first kept at room temperature for 10 min, each of which is dosed differently and marked with labels A, B, and C. Microgel A was treated by crosslinking microgel as much as 0.5 g and alginate soaked for three days, In Hydrogel B was treated by crosslinking microgel as much as 1 g and alginate soaked for five days, In hydrogel C was treated by crosslinking microgel as much as 2 g and alginate soaked for seven days (Feyissa et al., 2023).

To establish double crosslinking, hydrogels A, B, and C were immersed in 1% $CaCl_2$ (10 mL) overnight, after which the aqueous solution was removed, and the gel was lyophilized and tested using a Scanning Electronic Microscope (SEM) and Fourier-transform infrared spectroscopy (FTIR) (Chen, Bolognesi, and Vladisavljević 2021; Feyissa *et al.,* 2023).

2.6. Swelling Test

Lyophilized microgel (50 mg) is immersed in distilled water (50 mL) at room temperature for 1 day. The gel is removed from the water at a certain time and weighed.

Swelling ratio (%) = $(W_t-W_0)/W_0 \times 100\%$ (1)

W_0 is the sample weight and Wt represents the weight of the swelling test result. The experiment was repeated three times (Urich and Denton, 2016).

2.7. Gel Stability Test

Gel Stability Testing The implementation of physical stability tests on microgel as a diabetic wound medicine carried out is organoleptic, homogeneity, syneresis, and pH (Urich and Denton, 2016).

2.8. Diabetic Ulcers Healing

Testing was carried out to determine the activity of microgel in closing diabetic wounds by following previous research. We used mice (*Mus musculus*) male Wistar strain and divided them into five groups (5 mice per Group). Mice in the positive control group (K+) were given microgel and diabetic wound medicine (Binasol). Mice in the negative control group (K-), without microgel. Mice in Group I (K1) were given microgel A. Mice in Group II (K2) were given microgel B. Mice in Group III (K3), C. mice were injected intraperitoneally with alloxan (168 mg/kg) for three consecutive days, and fed normal food for three days. The mice with blood glucose levels greater than 10.5 mmol/L were selected as the overall model of cortical defects. Selected mice were excised and carried out in the middle of the back. Microgel was applied consecutively for 14 days and observed activity (visual) differences in wound closure speed while tracking the process of epidermal re-epithelialization during diabetic wound healing (Yan *et al*., 2022).

3. Results and Discussion

3.1. Physico-chemical Properties of Calcium Alginate (Ca-A)

Lindur is a type of mangrove plant of the species *B. gymnorrhiza* (Wilujeng et al., 2020). Lindur fruit is known to contain 31.56% amylose and 26.17% amylopectin. Physicochemical properties determine the physical and chemical properties of Alginate. The results of Calcium Alginate extraction from Lindur fruit have physico-chemical characteristics present in Table 1.

Table 1. The physico-chemical properties of Ca-A

Parameters	Variation of precipitation time with $CaCl_2$ solution 14%		
	ALG-3	ALG-5	ALG-7
Yield (%)	19.46	19.26	18.67
Purity (%)	19.50	20.10	331
Viscosity (CP)	31.45	31.46	31,19
Acidity Degree	7.00	7.00	7.00
Water Content (%)	11.20	10.74	11.28
Ash Content (%)	19.00	21.2	25.8
Protein (%)	0.70	0.78	0.87
Fat (%)	2.77	3.06	3.20
Carbohydrates (%)	66.32	64.21	58.84

Based on the research result, it is concluded that cream-coloured Ca-Alginate isolate has a yield between 18.67-19.46% with a purity of 19.5-33.1%, viscosity between 31.19-31.46 Cp, and acidity (pH) of 7.00, water content between 10.74-11.28%, ash content between 19-25.8%, protein content between 0.7003-0.8754%, fat content between 2.77-3.2%, and carbohydrate content between 58.84-66.32%.

Ca-alginate is the sodium salt of alginate, an anionic polymer produced from brown algae. Alginates have polymer chains composed of alpha-L-guluronate and beta-D-mannuronic sugar units. The carboxylate group present in these sugars can serve as a binding site for sodium ions (Ca^{2+}), allowing gel formation through the cross-linking process. The cross-linking process occurs when calcium ions (Ca^{2+}) interact with carboxylate groups in alginate. Sodium forms ionic bonds with carboxylate groups, causing alginate polymers to bond together to form a three-dimensional structure similar to a network or gel (Merakchi *et al*., 2019). The yield and purity mentioned earlier can affect the number and quality of carboxylate groups available to interact with calcium ions during the crosslinking process. High yield and good purity will improve crosslinking efficiency, resulting in a stronger and more stable gel (Shang *et al*., 2023).

3.2. Antioxidant Activities of Ca-alginate

Antioxidant activity refers to the capability of compounds within the microgel to neutralize free radicals, commonly assessed through the percentage of DPPH inhibition or alternative analytical methods. Alginate possess diverse biological activities, sucs as anticancer, antibacterial, and antioxidant activities. The total antioxidant activity exhibits in % DPPH Inhibition (Table 2).

Table 2. The antioxidant activities

Abs. of Blank	Abs. of Sample	Average Abs. of Sample	% Inhibition
0.322	0.236 0.232 0.282	0.250	22.3 %

The antioxidant activity test used ALG-7 Ca-Alginate isolate with a concentration of 50 ppm seems due to ALG-7 has a higher purity. The higher the purity, the better the quality of the Ca-alginate isolate obtained. At a concentration of 50 ppm, the isolate exhibits antioxidant activity with an inhibition value of 22.3% against DPPH free radicals. This activity is likely attributed to the presence of functional groups such as hydroxyl (-OH) and carboxylate ($-COO^-$), which donate electrons to neutralize free radicals. Although this inhibition value is relatively low compared to potent antioxidants like ascorbic acid, it still indicates the antioxidant potential of Ca-alginate, which may improve with increased purity.

3.3. IR Spectrum of Calcium Alginate

Isolated calcium alginate powder was carried out functional group analysis using an FTIR (Fourier Transform Infra-Red) spectrophotometer to prove that the resulting product was calcium alginate. The IR spectra of calcium alginate is presented in Figure 1.

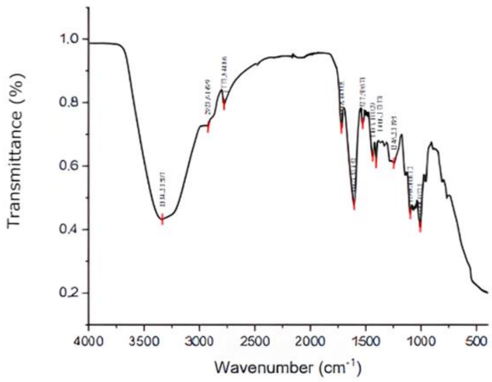


Figure 1. IR-Spectrum of calcium alginate

Functional group analysis is essential for understanding the chemical properties of calcium alginate powder, providing a foundation for its applications in pharmaceutical, food, and biomedical fields. FTIR analysis revealed characteristic absorption peaks corresponding to specific functional groups: hydroxyl (-OH) at 3334.24 cm^{-1}, carbonyl (C=O) at 1605.52 cm^{-1}, carboxyl (COO^-) at 1246.23 cm^{-1}, C-OH bonding at 1437.17 cm^{-1}, C-O-C bonding at 1008.07 cm^{-1}, and alkane (C-H) at 2923.61 cm^{-1}. These findings confirm the presence of key structural components that influence the physicochemical properties of calcium alginate.

3.4. IR Spectrum of SCMC

The hydroxyl group in cellulose can be converted into a cellulose derivative through chemical reactions with certain compounds. Some compounds that can be used to change the hydroxyl group in cellulose include acetic acid, sulfuric acid, nitric acid, and acetic anhydride. The process of modifying cellulose into cellulose derivatives involves several stages, such as the replacement of the hydroxyl group on cellulose with another substituent group, such as a sulfate group.

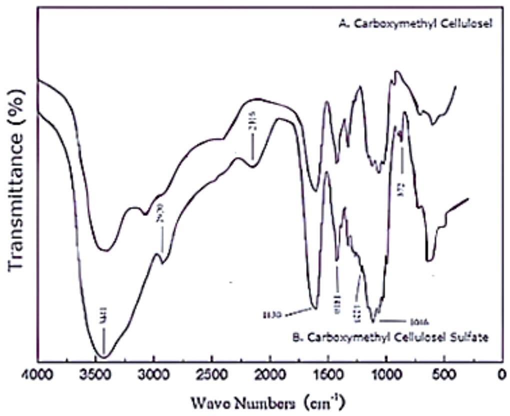


Figure 2. Spectrum of carboxymethyl cellulose (A), carboxymethyl cellulose sulfate (B)

In the spectrum, the appearance of wavenumber 3441 cm^{-1} is the OH group which is characteristic of cellulose (Figure 2). After that, the appearance of vibration at wavenumber 2920 is a C-H cluster. At wavenumber 1410 indicates the presence of a $-CH_2$ group. and at the wavelength of 1630 indicates the presence of carboxyl groups. At wavenumber 1066 shows the presence of ether formed, namely the C-O-C group. Meanwhile, carboxymethyl cellulose sulfate shows two typical absorption bands, with one located at 1220 cm^{-1} associated with asymmetric S-O stretching while the other peak is at 870 cm^{-1} associated with symmetrical stretching vibration C-O-S associated with the $C-O-SO_3$ group. However, in the carboxymethyl cellulose spectrum, these two peaks of uptake are not visible, therefore this indicates that the sulfate group has been successfully incorporated into the carboxymethyl cellulose.

The modification of sulfated carboxymethyl cellulose (SCMC) enhances its hydrophilic properties, enabling it to retain optimal moisture levels within the wound environment, thereby facilitating the wound healing process (Bhutada et al., 2021). Furthermore, spectroscopic analysis reveals the presence of asymmetric and symmetric stretching vibrations of the carboxylate group ($-COO^-$), confirming that the core structural framework of carboxymethyl cellulose (CMC) remains unaltered following the sulfonation process. This structural integrity ensures the preservation of the material's mechanical properties and biocompatibility, which are essential for maintaining the stability and functionality of the wound dressing throughout the healing period.

3.5. Synthesis Microgel Zn^{2+}-SCMC-A-HA

3.5.1. Microgel A, B, and C

The results obtained after the orientation process indicate that Microgel A exhibits a more transparent visual appearance compared to Microgel B and Microgel C, which appear slightly brown. The synthesized microgel samples are presented in Figure 3.

The cross-linking process is a chemical reaction that occurs between a donor molecule, which provides the functional group to be modified, and a recipient molecule, which facilitates the formation of a stable and interconnected network (Khunmanee et al., 2017). In this study, the cross-linking process involves interactions between carboxymethyl cellulose (CMC) as the donor, a sulfate agent as the recipient, Zn^{2+} ions, and A-HA, leading to the formation of a robust hydrogel network (Pinpru and Woramongkolchai, 2020).

SCMC-based microgels possess a distinctive chemical structure characterized by ion hydrogen sulfite ($-SO_3H$) and carboxymethyl ($-COO^-$) functional groups, which play a crucial role in enhancing water retention and adhesion to wound tissue (Fan et al., 2014). The electrostatic interactions between Zn^{2+} ions in the microgel matrix and the negatively charged groups in SCMC and alginate contribute to the formation of a robust and highly elastic hydrogel network. This structure offers superior mechanical stability compared to other natural hydrogels, such as chitosan or pectin.

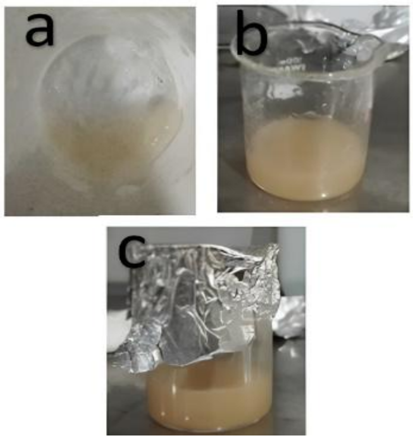


Figure 3. Synthesized microgel A (a), microgel B (b), and microgel C (c)

In biomedical use, combining SCMC and alginate offers benefits like better oxygen flow, lower inflammation, and improved cell growth and blood vessel formation, all important for wound healing.

Table 3. Comparison of different hydrogel polymers for wound healing

Polymer Types	Chemical Structure	Mechanism of Action	Wound Healing Effectiveness	Reference
SCMC-Alginate Microgel	Sulfate groups ($-SO_3H$), carboxymethyl groups (-COO-), and Zn^{2+} ions	Provides a moist environment, enhances fibroblast proliferation and angiogenesis, exhibits antibacterial properties	Accelerated healing (significant reduction in wound diameter within 14 days)	This research
Chitosan Hydrogel	Amino groups (-NH2), hydroxyl groups (-OH)	Intrinsic antibacterial activity, promotes extracellular matrix formation	Effective for infectious wounds, though less elastic compared to SCMC	Khunmanee *et al*., 2017
Pectin Hydrogel	Carboxyl groups (-COO-), methoxyl groups (-OCH3)	Forms a physical barrier, retains moisture	Effective for burn wounds with high swelling capacity and good tensile strength	Tarmidzi *et al*., 2020
Conventional Alginate Hydrogel	Carboxyl groups (-COO-), uronate groups	Absorbs exudate, maintains moisture	Suitable for highly exudative wounds, but does not promote angiogenesis	Merakchi *et al*., 2019

Table 4. Physical stability of microgels

Parameters	Microgels A	Microgels B	Microgels C
Organoleptic			
a. Color	Transparent brown	Light brown	Dark brown
b. Smell	Typical Extracts Bland	Typical Extracts Bland	Typical Extracts Bland
c. Taste	Viscous (non-flowing)	Viscous (non-flowing)	Viscous (non-flowing)
d. Shape	Soft	Soft	Soft
e. Sensibility			
Homogeneity	Homogenous	Homogenous	Homogenous
Sinersis	-	-	-
pH	7	7	7

Additionally, Zn^{2+} ions within the microgel play a vital role in promoting antibacterial activity and stimulating collagen synthesis, both of which are essential for accelerating diabetic wound repair (Yan *et al*., 2022). To provide a comprehensive evaluation of the advantages of SCMC-based microgels over existing wound care therapies, the following section presents a detailed comparative analysis from both chemical and therapeutic perspectives.

The SCMC-based microgels exhibit a superior combination of properties compared to hydrogels derived from chitosan, pectin, or conventional alginate (Table 3). The antibacterial activity conferred by Zn^{2+} ions, coupled with the high swelling capacity, makes SCMC-based microgels a promising candidate for accelerating diabetic wound healing.

3.5.2. Physical Stability Test of Microgels

The Physical stability of gel preparations is carried out by conducting organoleptic, homogeneity, syneresis, and pH value (Table 4). Organoleptic Test refers to the observation and assessment of gel preparations using the five human senses such as sight, smell, and taste.

The microgel formulations exhibited consistent organoleptic properties, including uniform color, a viscous and non-flowing gel texture, tastelessness, and an absence of pungent odor. These characteristics indicate that the gel maintains physical stability and is suitable for biomedical applications, particularly in diabetic wound treatment.

3.5.3. Swelling Test (*In vitro* Swelling Assay)

The swelling ratio is defined as the percentage increase in the volume or weight of a microgel after fluid absorption relative to its initial state. This parameter is a critical indicator of a hydrogel's fluid absorption and retention capacity, making it essential for applications such as wound dressings and drug delivery systems. The swelling properties of the hydrogel hold a key role in the absorption of extrusion in diabetic wounds (Mude et al., 2021). The percentage of swelling was finally 660% for microgel A, increased to 700% for microgel B, and reached 720% for microgel C (Table 5).

Table 5. Swelling ratio of microgels

Microgels	Time (min)	Swelling ratio *(%)*
Microgel A	15	470
	30	520
	45	550
	60	600
	75	660
Microgel B	15	496
	30	540
	45	604
	60	670
	75	700
Microgel C	15	500
	30	560
	45	636
	60	680
	75	720

The swelling ratio remained stable despite an increase in the cross-linking rate. Notably, Microgel C exhibited a significantly higher degree of swelling compared to Microgel B and A. This phenomenon can be attributed to electrostatic interactions, which play a dominant role in microgel formation. These findings are consistent with previous study that the swelling ratio of hydrogels remains unaffected by increased cross-linking density (Yan *et al*., 2022). Among the tested formulations, Microgel C exhibited the highest swelling capacity, reaching 720% at 75 min, followed by Microgel B and A.

These observation underscores the pivotal role of electrostatic interactions in microgel formation and their significant influence on water absorption capacity. Moreover, the pronounced swelling behavior can be attributed to the presence of highly hydrophilic polysaccharides (CMCS and A-HA), which facilitate water retention within the hydrogel network. Consequently, this characteristic counteracts the anticipated reduction in swelling typically associated with higher cross-linking densities, thereby maintaining an enhanced swelling performance.

3.5.4. The IR Fingerprint and Morphological of Microgels

FT-IR spectra of microgels A, B, and C were recorded and compared (Figure 4). The main peaks do not show significant variation. Vibrational stretching of the O-H bond is observed at 3000-3600 cm^{-1}.

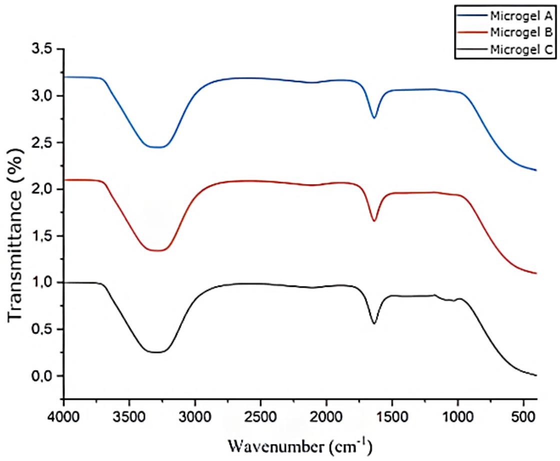


Figure 4. FT-IR Spectrum microgels

The stretching vibration of the aliphatic C-H was observed at 2920-2850 cm^{-1}. The peaks are around 1650 and 1450 cm^{-1}. associated with asymmetrical and symmetrical stretching vibrations of carboxylate salts indicating the formation of cation crosslinked alginate networks.

The SEM analysis was performed to assess the surface morphology of each microgel formulation (Figure 5). Microgels A and B exhibited a relatively smooth and compact surface, suggesting a uniform structural arrangement. In contrast, microgel C displayed a distinctive honeycomb-like pattern with visible pores and a hollow core, which may contribute to its superior swelling ability.

Measurements indicated that microgel C had an average particle size of 25 µm, while microgel B measured approximately 5 µm, and microgel A remained below 5 µm. These structural differences are attributed to variations in crosslinking density, which increased significantly with higher microgel concentrations. The porous structure of microgel C is particularly noteworthy, as it enhances water absorption and diffusion, potentially improving its effectiveness in wound healing applications.

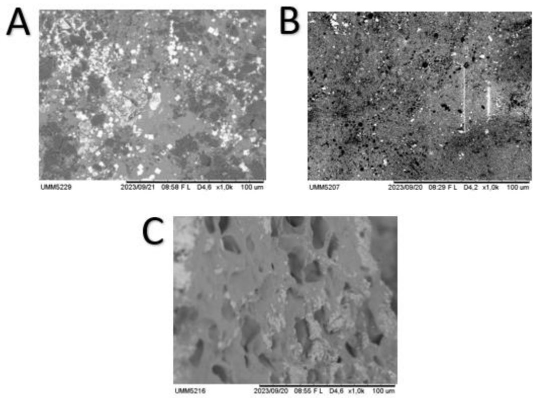


Figure 5. SEM analysis results of microgel A (a), microgel B (b) and microgel (c)

3.5.5. Microgels Wound Healing Activity

Mice that had been injected using alloxan and with blood glucose levels greater than 10.5 mmol/L were selected to be the entire model of cortical defects. The wound healing rate for each mouse was evaluated by determining the uncovered wound area as a function of time (Table 6 and Table 7).

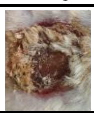
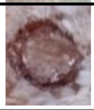
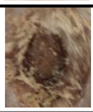
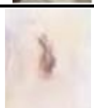
Table 6. Wound healing

Groups	Wound Diameter (mm)/Time (Day)			
	3	5	7	14
K+	0.9	0.8	0.6	0.3
K-	1	0.9	0.8	0.6
K1	0.9	0.8	0.6	0.4
K2	0.9	0.7	0.4	0.2
K3	0.9	0.5	0.3	0.1

Wound closure was observed by measuring the wound diameter over time. The observation was carried out from 3rd day to the 14th day (Table 7). It was found that K2 and K3 have better wound closure activity than K1 and K+. This exhibits on days 5th to 14th. On day 14th, the wound diameters in Groups K2 and K3 were 0.2 mm and 0.1 mm, respectively, while K+ was 0.3 mm. It shows that the wound is getting smaller with the increase in the percentage of wound healing every day.

The increase in wound closure activity was caused by a longer double-crosslinked process and a more significant number of microgels. (Triadi Nugroho et al., 2022). The amount of polymer accelerates the wound closure process due to the increase of crosslinking concentration, the healing efficiency and support the healing process by providing a suitable environment for tissue regeneration (Zhang et al., 2024).

Table 7. Picture of wound observation

Groups	Wound Diameter/Time (Day)			
	3	5	7	14
K+				
K-				
K1				
K2				
K3				

4. Conclusion

The study concludes is that the characterization of alginate shows the yield of Ca-alginate isolate (18.67-19.46%) with a purity of 19.5-33.1%, viscosity 31.19-31.46 Cp, and stable pH at 7. Ca-alginate isolate at 50 ppm showed antioxidant activity of 22.3%. FTIR analysis on calcium alginate powder confirmed the success of isolation. The synthesis of A, B, and C microgels results in different visual appearances, with C microgels showing higher swelling. The physical stability test of the microgel showed good consistency. SEM analysis showed the surface morphology of microgels A and B was flat and smooth, while microgel C had a honeycomb-like structure. Microgel activity tests on mouse wounds showed varying healing potential, with microgel C showing faster healing effectiveness.

Author Contributions

J.A.H.: data collection, analysis, writing–original draft, funding acquisition, N.D.Z.: data collection, analysis, writing–original draft, review, validation, S.F.S.: analysis, validation, S.A.P.: analysis, S.A.M: Project administration, ILT.: conceptualization, supervision, validation, writing-review and editing.

Data Availability Statement

Available on request to the corresponding author.

Declaration of Competing Interest

The author declares that there is no conflict of interest regarding the publication of this manuscript.

Acknowledgment

Thanks to the Directorate General of Belmawa DIKTI for Funding the Research PKM 2023, also Laboratory Faculty of Science and Technology Universitas Jambi for reseach facilities.

References

Bhutada, S.S., Sriram, M., Katti, D.S. (2021) Sulfated carboxymethylcellulose conjugated electrospun fibers as a growth factor presenting system for tissue engineering. *Carbohydrate Polymer.* 268, 118256. https://doi.org/https://doi.org/10.1016/j.carbpol.2021.118256

Chen, M., Bolognesi, G., Vladisavljević, G.T. (2021) Crosslinking strategies for the microfluidic production of microgels. *Molecules* 26. https://doi.org/10.3390/molecules26123752

Da Silva, L.P., Reis, R.L., Correlo, V.M., Marques, A.P. (2019) Hydrogel-Based Strategies to Advance Therapies for Chronic Skin Wounds. *Annual Review of Biomedicial Engineering.* 21, 145–169. https://doi.org/10.1146/annurev-bioeng-060418-052422

Everett, E., Mathioudakis, N. (2018) Update on management of diabetic foot ulcers. *Annals of The New York Academy of Sciences.* 1411, 153–165. https://doi.org/10.1111/nyas.13569

Fan, L., Zhou, X., Wu, P., Xie, W., Zheng, H., Tan, W., Liu, S., Li, Q. (2014) Preparation of carboxymethyl cellulose sulfates and its application as anticoagulant and wound dressing. *International Journal of Biological Macromolecules.* 66, 245–253. https://doi.org/10.1016/j.ijbiomac.2014.02.040

Feng, Q., Li, D., Li, Q., Cao, X., Dong, H. (2022) Microgel assembly: Fabrication, characteristics and application in tissue engineering and regenerative medicine. *Bioactive Materials.* 9, 105–119. https://doi.org/10.1016/j.bioactmat.2021.07.020

Feyissa, Z., Edossa, G.D., Gupta, N.K., Negera, D. (2023) Development of double crosslinked sodium alginate/chitosan based hydrogels for controlled release of metronidazole and its antibacterial activity. *Heliyon* 9, e20144. https://doi.org/10.1016/j.heliyon.2023.e20144

Furqon, M., Saputra, N., Nabila, A., Maulida, F., Syafiq, M.H., Tarigan, I.L. (2025). Synthesis and Characterization of Insulin-MOF-5 Loaded into Cellulose Nanocrystalline-Based Dissolving Microneedle as Anti- Diabetic Delivery System. *Journal of Medicinal and Chemical Sciences.* 8, 177–189. https://doi.org/10.26655/JMCHEMSCI.2025.2.5

Ho, T.C., Chang, C.C., Chan, H.P., Chung, T.W., Shu, C.W., Chuang, K.P., Duh, T.H., Yang, M.H., Tyan, Y.C. (2022) Hydrogels: Properties and Applications in Biomedicine. *Molecules* 27, 1–29. https://doi.org/10.3390/molecules27092902

Khan, M.U.A., Aslam, M.A., Abdullah, M.F. Bin, Al-Arjan, W.S., Stojanovic, G.M., Hasan, A. (2024) Hydrogels: Classifications, fundamental properties, applications, and scopes in recent advances in tissue engineering and regenerative medicine – A comprehensive review. *Arabian Journal of Chemistry*. 17, 105968. https://doi.org/https://doi.org/10.1016/j.arabjc.2024.105968

Khunmanee, S., Jeong, Y., Park, H. (2017) Crosslinking method of hyaluronic-based hydrogel for biomedical applications. *Journal Tissue Engineering.* 8. https://doi.org/10.1177/2041731417726464

Merakchi, A., Bettayeb, S., Drouiche, N., Adour, L., Lounici, H., (2019) Cross-linking and modification of sodium alginate biopolymer for dye removal in aqueous solution. *Polymer Bulletin.* 76, 3535–3554. https://doi.org/10.1007/s00289-018-2557-x

Mude, L., Sanapalli, B.K.R., Anoop Narayanan, V., Singh, S.K., Karri, V.V.S.R. (2021). Overview of in situ gelling injectable hydrogels for diabetic wounds. *Drug Development Research.* 82, 503–522. https://doi.org/10.1002/ddr.21788

Pinpru, N., Woramongkolchai, S. (2020) Crosslinking effects on alginate/carboxymethyl cellulose packaging film properties. *Chiang Mai Journal of Science*. 47, 712–722.

Sadeer, N.B., Sinan, K.I., Cziáky, Z., Jekő, J., Zengin, G., Jeewon, R., Abdallah, H.H., Aldhaheri, Y., Eid, A.H., Mahomoodally, M.F. (2022) Towards the Pharmacological Validation and Phytochemical Profiling of the Decoction and Maceration of *Bruguiera gymnorhiza* (L.) Lam.—A Traditionally Used Medicinal Halophyte. *Molecules* 27. https://doi.org/10.3390/molecules27062000

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A.A., Ogurtsova, K., Shaw, J.E., Bright, D., Williams, R. (2019) Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*. 157, 107843. https://doi.org/10.1016/j.diabres.2019.107843

Shang, M., Jiang, H., Li, J., Ji, N., Li, M., Dai, L., He, J., Qin, Y., 2023. A dual physical crosslinking starch-based hydrogel exhibiting high strength, fatigue resistance, excellent biocompatibility, and biodegradability. *Food Chemistry.* X 18. https://doi.org/10.1016/j.fochx.2023.100728

Triadi Nugroho, R., Saputra, G., Aini, A.N., Dewi, A.A.I., Tarigan, I.L. (2022) Ointment Formulation from Collagen Extract of Tilapia Fish Skin (Oreochromis niloticus) for Healing Burns in Mus musculus. *Pharmaceutical Journal of Indonesia.* 8, 9–15. https://doi.org/10.21776/ub.pji.2022.008.01.2

Urich, M., Denton, A.R., (2016). Swelling, structure, and phase stability of compressible microgels. *Soft Matter* 12, 9086–9094. https://doi.org/10.1039/c6sm02056a

Wilujeng, S., Kristianto, S., Chamidah, D., Laksitarahmi, P. (2020) Environmental observation and potency of lindur fruit (*Bruguiera gymnorrhiza*) as alternative food substance, in: *Proceedings of the International Conference on Industrial Engineering and Operations Management.* pp. 470–479.

Yachmaneni, A., Jajoo, S., Mahakalkar, C., Kshirsagar, S., Dhole, S. (2023) A Comprehensive Review of the Vascular Consequences of Diabetes in the Lower Extremities: Current Approaches to Management and Evaluation of Clinical Outcomes. *Cureus* 15. https://doi.org/10.7759/cureus.47525

Yan, Y., Ren, P., Wu, Q., Zhang, T. (2022) Precise Design of Alginate Hydrogels Crosslinked with Microgels for Diabetic Wound Healing. *Biomolecules* 12. https://doi.org/10.3390/biom12111582

Zhang, H., Lin, X., Cao, X., Wang, Y., Wang, J., Zhao, Y. (2024) Developing natural polymers for skin wound healing. *Bioactive Materials.* 33, 355–376. https://doi.org/10.1016/j.bioactmat.2023.11.012