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Antioxidant Activity of Curcumin-Loaded Carboxymethyl Cellulose (CMC) Hydrogel

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Abstract
Curcumin is a natural polyphenolic compound that exhibits antioxidant properties; however, its poor bioavailability and rapid degradation limit its therapeutic applications. This study was conducted to produce and evaluate a carboxymethyl cellulose (CMC)-based hydrogel that serves as a drug delivery system. It focuses on the antioxidant activity, as well as the release behaviour from the hydrogel matrix. Cellulose was extracted from oil palm empty fruit bunch (OPEFB) by the process of chemical modification to form CMC. Calcium chloride ($CaCl_2$) acts as a crosslinker to produce a stable three-dimensional hydrogel network. Curcumin was loaded into the hydrogel through a swelling process, with successful incorporation indicated by an increase in weight and a visible yellow coloration. A 24-hour in vitro release study profile has shown an initial burst release. The antioxidant activity was assessed using the DPPH assay, where the curcumin-loaded CMC hydrogel demonstrated significantly lower antioxidant activity due to the limited immediate release and interaction between curcumin and the hydrogel matrix. These findings suggest that curcumin-loaded CMC hydrogel is a promising system for controlled antioxidant delivery with potential application in the biomedical field, such as wound healing.
Keywords: CMC hydrogel; drug delivery system; antioxidant activity; DPPH assay

Introduction

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defence mechanism produced by the body. This accumulation of free radicals can attack normal cells, leading to oxidative damage to vital cellular components, such as lipids, proteins, and DNA (Farías et al., 2017).

Curcumin, derived from the ginger family of Zingiberaceae, is known for its wide range of properties, including anti-inflammatory, antioxidant, antiviral, antibacterial, antifungal, antiparasitic, and anticancer effects (Sahoo et al., 2021). Curcumin can scavenge reactive oxygen species (ROS) by neutralizing free radicals through the donation of hydrogen atoms or electrons in the compound's molecule, which has undergone oxidative stress. This mechanism helps curcumin to protect the cells from oxidative stress, making it an effective biological antioxidant treatment.

Hydrogels exhibit remarkable swelling properties due to their ability to absorb water, which is equivalent to tens to thousands of times their dry weight. These properties of hydrogels enable them to adapt and change in response to various conditions. Additionally, the porous structure and soft surface of hydrogels make them suitable for applications in the field of medicine (Podhorská et al., 2023). The hydrogel can mimic the mechanical characteristics of different parts of body tissue to facilitate interaction with biomolecules, making it a valuable tool in medical applications.

Carboxymethyl cellulose (CMC) is a biodegradable and non-toxic polysaccharide widely used in drug delivery systems (Capanema et al., 2018). It can be crosslinked with hydrogels through physical and chemical methods to form water-soluble materials suitable for encapsulating hydrophobic drugs, such as curcumin (Hanafy et al., 2020). These CMC-based hydrogels help control the release of drugs and enhance the effectiveness of delivery (Zhang et al., 2022). Additionally, their hydrophilic and highly viscous nature makes them easy to process and adaptable for various biomedical applications.

To gain a deeper insight into curcumin's antioxidant properties, this study aims to evaluate the antioxidant activity of curcumin when loaded into a CMC hydrogel. By using the DPPH assay to measure free radical scavenging activity, this study aims to provide a deeper understanding of curcumin's antioxidant potential when loaded onto CMC hydrogel compared to curcumin without CMC hydrogel. The findings may contribute to the development of more effective antioxidant delivery systems using biopolymer-based hydrogels.

The objectives of this study were to determine the drug release kinetics of curcumin-loaded CMC hydrogel derived from oil palm empty fruit bunch (OPEFB) and to evaluate the antioxidant activity of curcumin-loaded CMC hydrogel by using DPPH assay.

Materials and methods

Preparation of CMC hydrogel

Cellulose from oil palm empty fruit bunch (OPEFB) was supplied from Southern Malay Palm Oil Mill. Firstly, cellulose from OPEFB was subjected to mechanical pretreatment, which is grinding. Then, the ground OPEFB was sieved to obtain fine materials of OPEFB. Then, 25 g of OPEFB was washed with distilled water and dried in the oven at 70°C for 24 hours. After that, the dried OPEFB was weighed and then subjected to pretreatment with a 2:1 toluene: ethanol ratio for 6 hours. The OPEFB was dried and weighed. Then, the OPEFB was digested with NaOH for alkaline pretreatment at 80°C for 24 hours. The OPEFB continued to undergo washing with distilled water until it reached a pH of 7, and then it was dried. The dried OPEFB was weighed. Next, the OPEFB was bleached using 1.3% sodium chlorite (NaOCl) in an acidic condition using 10% acetic acid at 80°C for 4 hours. Then, the OPEFB was washed with NaOH and distilled water until the pH reached 7. After that, the OPEFB was dried in an oven at 70°C for 24 hours and weighed.

The cellulose from OPEFB was then converted to carboxymethyl cellulose (CMC) through reaction with NaOH. 5.0 g of cellulose was added to a 250 mL beaker, then 10 mL of 30% (w/v) NaOH solution was added dropwise. After that, 100 mL of isopropyl alcohol (IPA) was added to the mixture and stirred for 1 hour at room temperature. Next, 6.0 g of sodium monochloroacetic (SMCA) was added, and the reaction was continued for 3 hours at 45°C. The mixture was filtered, and the slurry of CMC was soaked in 300 mL of methanol overnight. The slurry of CMC was neutralized with glacial acetic acid, then sieved and washed with 70% ethanol, followed by 99.7% ethanol. The process was repeated three times. Then, the CMC was dried in an oven for 24 hours at 65°C and stored in an airtight container.

The 20% CMC was dissolved in 1% $CaCl_2$ solution, prepared by diluting 10 ml of distilled water with 1% $CaCl_2$. CMC was dissolved in a $CaCl_2$ solution and stirred homogeneously until a paste-like solution was obtained; then, the solution was placed into a petri dish. The paste-like CMC-$CaCl_2$ was left to undergo the cross-linking process for 24 hours at room temperature. Then, the paste-like hydrogel was placed into the cap of the Falcon tube before the freeze-drying process.

Curcumin loading and release on CMC hydrogel

The process of swelling the hydrogel started with 50 mg of hydrogel being swelled in 0.2mg/mL of curcumin solution for 24 hours at room temperature in dark conditions. Then, the hydrogel was removed from the curcumin solution and washed three times with 20mL of water to remove the excess curcumin present on its surface. Then, the hydrogel was dried at room temperature in the dark conditions for 48 hours. The dried hydrogel was weighed. The percentage of loading capacity was calculated using the formula below.

$$\text{Loading Capacity (LC)\%} = \left(\frac{weight\ of\ drug\ loaded\ in\ hydrogel}{weight\ of\ drug{-}loaded\ hydrogel}\right) \times 100\%$$

The curcumin-loaded CMC hydrogel was soaked in 20mL of PBS containing 0.1% DMSO medium at 37°C. Every 2 hours, 500 µL of the solution was pipetted out for analysis using the UV-visible spectrometer at 420nm. 500 µL of fresh medium solution was added to replenish the solution taken out for the absorbance measurement. This process was repeated for 24 hours. After that, the step was repeated for absorbance measurement every 12 hours and 24 hours. The released amount of curcumin

from the CMC hydrogel was determined by calculating the drug concentration in PBS containing 0.1% DMSO, and this value was compared with the standard curve.

Measurement of free radical scavenging capacity

The curcumin-loaded CMC hydrogel was soaked in 20 mL of PBS containing 0.1% DMSO medium at 37°C for 10 hours. The solution was used to conduct the DPPH assay. A 96-well plate was labelled as follows: standard (Row A-D, Column 3), blank (Row E, Column 1-2), curcumin-loaded CMC hydrogel (Row A-D, Column 1) and free curcumin (Row A-D, Column 2). The control group consisted of 100 µL of ethanol mixed with 100 µL of DPPH 0.004% (w/v). The blank group consisted of 100 µL of sample mixed with 100 µL of ethanol, and the sample group consisted of 100 µL of sample mixed with 100 µL of DPPH 0.004% (w/v). then, the sample was incubated for 30 minutes in the dark condition at 37 °C. After that, the incubation solution was measured for the absorbance at 517 nm. The DPPH radical scavenging rate was calculated as shown below.

$$RSC\% = [\frac{As-Ac}{Ao}] \times 100\%$$

where “Ao” represents the absorbance of the blank control group, “As” represents the absorbance of the sample group, and “Ac” represents the absorbance of the sample control group.

Statistical analysis

A one-sample t-test was conducted using quadruplicate samples (n = 4) to assess the antioxidant activity of the curcumin-loaded CMC hydrogel. The analysis revealed that the hydrogel demonstrated statistically significant free radical scavenging activity ($p < 0.001$), indicating its potential antioxidant effect.

Results and discussion

Preparation of CMC hydrogel

To obtain cellulose from oil palm empty fruit bunch (OPEFB), both mechanical and chemical pretreatments were employed to remove hemicellulose, lignin, and other impurities. The raw OPEFB fibre was first ground using a mechanical grinder to reduce the particle size and then sieved to obtain more suitable particles for further processing (Fig. 1). This mechanical treatment enhances the efficiency of chemical pretreatment by increasing the material's surface area. The ground OPEFB was then subjected to chemical treatment for three days to remove lignin, hemicellulose, and waxes. This treatment involved acid hydrolysis, alkaline treatment, and bleaching processes, which gradually transformed the OPEFB from a brown fibrous material into a white substance with a harder texture.

In the acid hydrolysis step, the process was assumed to facilitate the breakdown of the cell wall and initiate the degradation of lignin and hemicellulose at the early stage of treatment (Susi et al., 2022). This was followed by an alkaline pretreatment using sodium hydroxide (NaOH), which further removed lignin and hemicellulose by breaking the ester bonds between them. Lastly, a bleaching process using sodium chlorite under acidic conditions was carried out, resulting in a white-coloured cellulose product. This bleaching step not only enhanced the colour but also increased the purity of the cellulose extracted from OPEFB (Susi et al., 2022). After the bleaching, the sample was neutralized to stabilize the cellulose within a suitable pH range, making it more compatible for subsequent applications such as carboxymethylation and hydrogel formation.

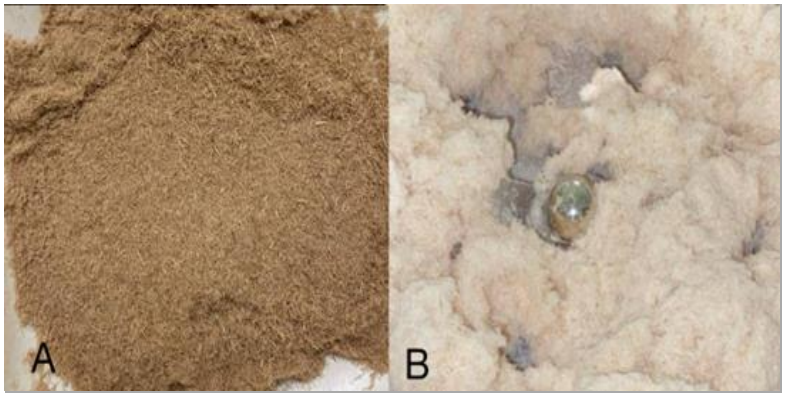

Figure 1 (A) Grounded OPEFB (B) Raw cellulose

The purified cellulose derived from OPEFB was further processed through an etherification reaction to produce carboxymethyl cellulose (CMC) (Mohamood et al., 2021). This chemical modification involved the use of sodium hydroxide (NaOH) and isopropanol (IPA), followed by treatment with monochloroacetic acid. During this step, sodium hydroxide was used to activate the cellulose through a process known as alkalization, where the hydrogen bonds in cellulose were broken, forming reactive alkoxide ions for substitution (Rasid et al., 2021). These activated sites then reacted with sodium monochloroacetate (SMCA) to form CMC. Isopropanol acted as an inert solvent, promoting the swelling of the reaction mixture and minimizing the formation of unwanted glycolate by-products (Rasid et al., 2021).

The carboxymethylation process involved the substitution of hydroxyl groups in cellulose with carboxymethyl groups, introducing carbonyl functionalities into the polymer structure. According to Rahman et al. (2021), this substitution enhanced the functional properties of cellulose, such as water solubility, dispersibility, and chemical reactivity, making it highly suitable for hydrogel applications. Mohamood et al. (2021) further reported that the presence of carboxyl groups in CMC is essential for crosslinking with divalent ions like Ca^{2+} during the hydrogel formation process using $CaCl_2$. After the reaction, the mixture was neutralized with glacial acetic acid to bring the pH to a neutral range, thereby removing excess alkalinity and stabilizing the final CMC product. The success of this carboxymethylation was evident in the production of smooth, white, water-soluble CMC powder from OPEFB-derived cellulose, as shown in Figure 2. The final product showed no signs of degradation or discoloration, indicating its chemical stability during drying and storage.

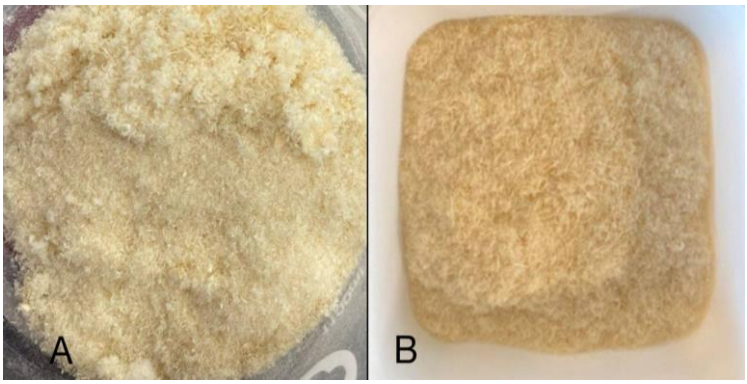

Figure 2 (A) Grounded OPEFB (B) Raw cellulose

The CMC hydrogel was successfully produced through ionic crosslinking using calcium chloride ($CaCl_2$), which served as a divalent crosslinking agent. In this process, calcium ions (Ca^{2+}) interacted with the negatively charged carboxylate groups ($-COO^-$) present on the CMC chains, resulting in the formation of a stable three-dimensional gel network. This interaction transformed the CMC paste into a gel-like material, as illustrated in Figure 3, indicating the successful formation of the hydrogel. According to Mohamood et al. (2021), calcium chloride effectively acts as a crosslinker in the hydrogel formation of CMC derived from OPEFB. This process enhances the structural integrity of the gel and its ability to retain water by allowing divalent cations, such as calcium, to interact with negatively charged groups on the polysaccharide chains. This crosslinking step is crucial in enhancing the mechanical integrity and functional performance of the hydrogel for its further applications.

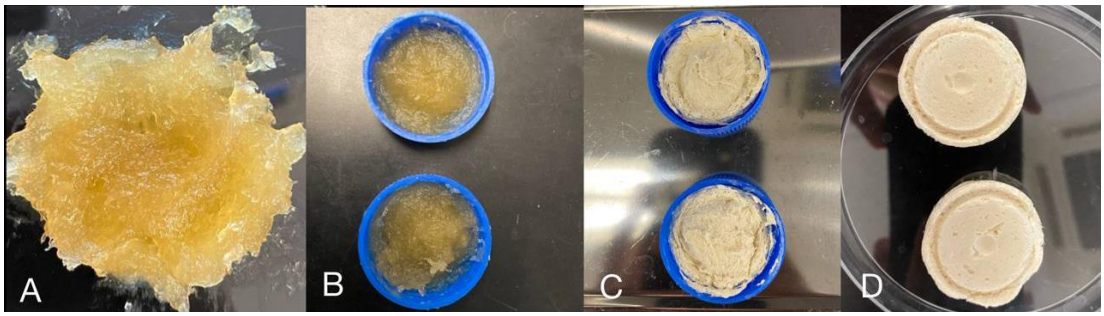

Figure 3 (A) The paste-like CMC-CaCl2 solution (B) The hydrogel inside Falcon tube cap (C) The freeze-dried hydrogel inside Falcon tube cap (D) The freeze-dried hydrogel

The CMC-$CaCl_2$ mixture was placed into a petri dish and left at room temperature for 24 hours to allow sufficient time for gelation and optimal interaction between calcium ions and the CMC chains. This crosslinking step plays a crucial role in forming a cohesive and stable hydrogel matrix, as supported by Che Nan et al. (2019). Then, the hydrogel underwent freeze-drying to preserve its porous structure. The freeze-drying method facilitates the removal of water through sublimation without damaging the cross-linked network of the hydrogel (Sornkamnerd et al., 2017). This method is effective in maintaining the integrity of the hydrogel's porous and interconnected architecture, resulting in a dry, sponge-like material suitable for further applications (Sornkamnerd et al., 2017).

Curcumin loading on CMC hydrogel

The freeze-dried CMC hydrogel with a firm, dry texture was cut into 50 mg pieces, as shown in Figure 4. Each piece was soaked in 20 mL of curcumin solution at a concentration of 0.2 mg/mL and kept in dark conditions for 24 hours to prevent light-induced degradation of curcumin. This concentration was selected to allow sufficient drug absorption while avoiding oversaturation or structural damage. After swelling, the hydrogel was gently washed with distilled water to remove excess surface curcumin and dried at room temperature for 48 hours. A similar approach was used by Gunathilake et al. (2017) to preserve the structure and bioactivity of curcumin-loaded hydrogels.

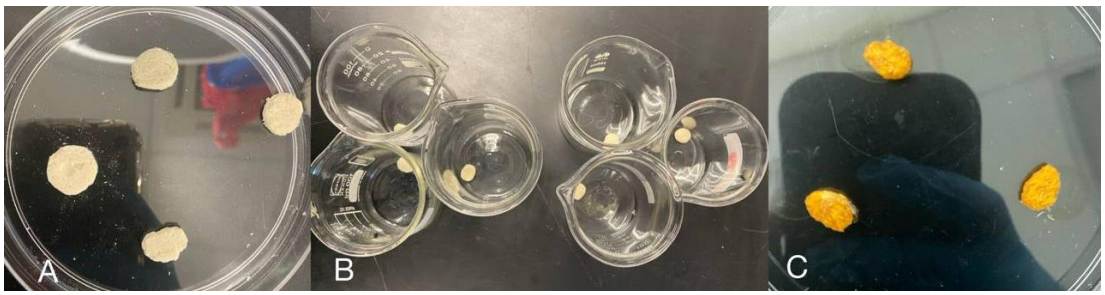

Figure 4 (A) 50 mg of CMC hydrogel (B) 50 mg CMC hydrogel in each beaker (C) Curcumin-loaded CMC hydrogel

After incubation, the hydrogel observed has become swollen with a jelly-like structure and increased in weight from 54.3 mg to 91.3 mg, showing successful curcumin uptake. The loading capacity was calculated to be 4.03%, which falls within the typical range for passive-loading hydrogels, typically ranging between 3% and 6%, as shown in previous studies by Zhou et al. (2021). As shown in Figure 4, the yellowish colour indicates the incorporation of curcumin. The swelling and weight gain indicated that curcumin diffused into the hydrogel matrix. This method aligns with previous studies demonstrating drug loading via swelling mechanisms in hydrophilic polymer systems, such as CMC and chitosan (Gunathilake et al., 2017).

Release study

The in vitro release study of curcumin from the CMC hydrogel was conducted to evaluate its release profile. The dried curcumin-loaded CMC hydrogel was immersed in 20 mL of phosphate-buffered saline (PBS) containing 0.1% DMSO and incubated at 37 °C under dark conditions to protect the curcumin from light degradation. A standard curve, shown in Figure 5(A), was used to establish a linear

relationship between absorbance and curcumin concentration for accurate quantification in the release medium. The linear regression equation obtained was Y = -0.0059 + 0.0021X with an R^2 value of 0.998, demonstrating excellent linearity. This method confirmed a strong correlation between absorbance and curcumin concentration within the range of 0.005–0.025 mg/mL, and the equation was applied to calculate the amount of curcumin released from the hydrogel samples.

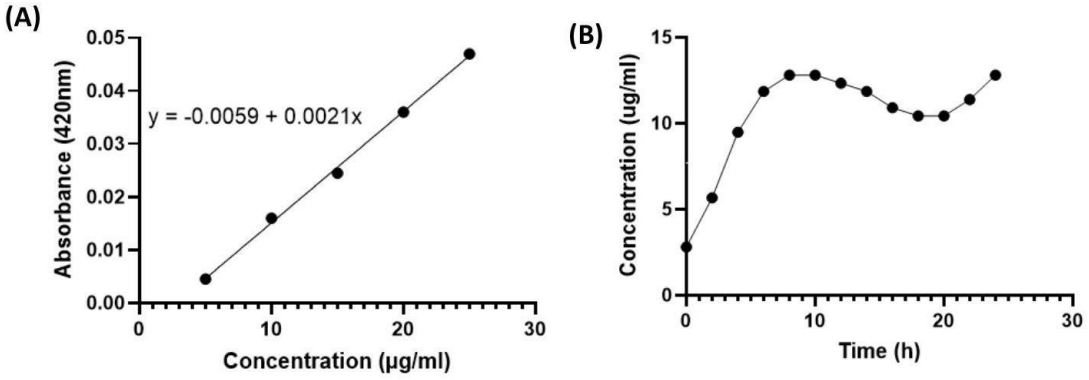

Figure 5 (A) Graph of the standard curve of curcumin (B) Graph of the 2-hour release study of curcumin-loaded CMC hydrogel

The release profile of curcumin, as shown in Figure 5(B), demonstrates a cumulative increase in curcumin release over 24 hours. A rapid release was observed during the initial phase, indicating a burst release effect caused by surface-adsorbed curcumin diffusing quickly into the surrounding medium. This burst release effect is common in hydrogel-based systems because it is typically due to curcumin that is not fully encapsulated within the hydrogel matrix (Wu et al., 2024). The initial burst likely reflects weakly bound curcumin that escapes during early swelling, suggesting that the curcumin-loaded CMC hydrogel is suitable for applications requiring rapid therapeutic effects, such as wound healing. However, the 24-hour observation window represents a limitation, as longer monitoring is needed to evaluate the sustained release behaviour of the hydrogel system fully.

Antioxidant assay

The antioxidant activity of curcumin released from the CMC hydrogel was evaluated and compared with that of free curcumin by using the DPPH assay. The percentage of DPPH radical scavenging activity was determined based on the data presented in Figure 6. Free curcumin exhibited strong antioxidant activity, aligned with findings from Sahu et al. (2015), who reported that the phenolic-OH groups in curcumin can donate hydrogen atoms to neutralize free radicals. This results in increasing its effectiveness as a natural antioxidant. The curcumin-loaded CMC hydrogel (CL-CH) also showed antioxidant activity. However, the scavenging percentage was significantly lower than that of free curcumin. This reduction in activity was attributed to the curcumin partially remaining trapped within the hydrogel matrix, thereby limiting its immediate interaction with DPPH radicals. As reported by Zhang et al. (2022), drug release from hydrogels can be slow and gradual due to the barrier effect of the polymer network. The dense and crosslinked structure of the CMC hydrogel restricts diffusion, resulting in sustained release and a rapid rate that can affect the immediate antioxidant response.

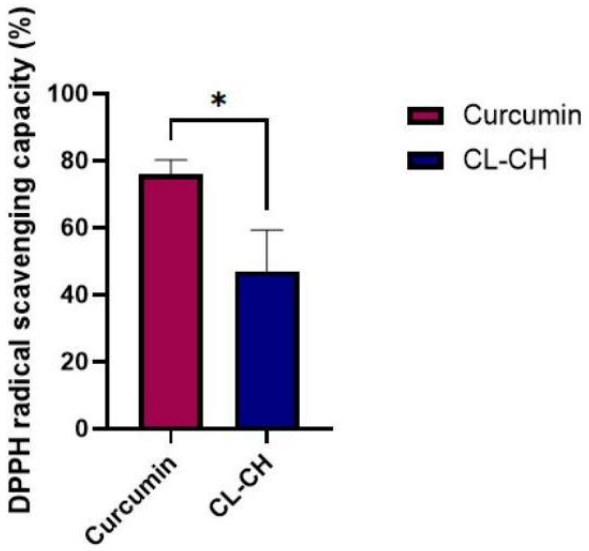

Figure 6 Graph Percentage of DPPH radical scavenging capacity of curcumin and curcumin-loaded CMC hydrogel

The –OH groups of curcumin may interact with functional groups in the hydrogel, potentially affecting its stability and antioxidant activity. Inside the CMC hydrogel matrix, curcumin can form hydrogen bonds or ionic interactions with polymer chains, possibly blocking its active sites and reducing its ability to scavenge free radicals (Li et al., 2022). This interaction limits curcumin's reactivity in the DPPH assay. Additionally, the barrier effect created by the hydrogel's crosslinked network plays a major role in controlling curcumin release. The ionic crosslinking between calcium ions (Ca^{2+}) and the carboxyl groups of CMC helps stabilize the hydrogel's 3D structure, linking polymer chains into a more compact and less soluble matrix. This structural reinforcement restricts the diffusion of curcumin into the surrounding medium. As reported by Duong et al. (2025), such a barrier effect significantly influences release kinetics, reducing the amount of curcumin available for antioxidant activity in short-term assays. Although this results in a lower antioxidant reading compared to free curcumin, the curcumin-loaded CMC hydrogel still exhibits notable antioxidant activity, indicating its potential as a sustained-release antioxidant delivery system.

Conclusion

In conclusion, this study successfully demonstrates that the successful loading of curcumin into the CMC hydrogel indicates the effectiveness of the hydrogel in encapsulating the bioactive compound, specifically curcumin. This effective loading of curcumin highlights the ability of CMC hydrogel to become a drug delivery system to transport certain drugs to a specific area. In addition, the CMC hydrogel also demonstrated the ability to provide continuous release, beginning with an initial burst of curcumin from the CMC hydrogel over time. This controlled release capability is essential for sustaining therapeutic concentrations while reducing dose frequency. Furthermore, this study also showed that CMC hydrogel loaded with curcumin demonstrated significant free radical scavenging capabilities. This finding indicates that the hydrogel has the capacity to offer the well-known antioxidant properties of curcumin, providing defence against oxidative stress. Lastly, these results indicate that CMC hydrogels have substantial potential as efficient and functional drug delivery vehicles for delivering bioactive compounds like curcumin, suitable for applications in areas requiring both controlled release and antioxidant properties.

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