Short Communication:

Prolonged Release of Anti-inflammatory Diclofenac Sodium from *In Situ* Loaded Thermosensitive Chitosan Hydrogels for Localized Applications

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Abstract: *Previous work developed a thermosensitive hydrogel from chitosan (CS) and β-glycerophosphate (GP) loaded with diclofenac sodium (DIC) using a post-loading method. The hydrogel as a wound dressing exhibited burst release of DIC within 5 h, rendering it suitable for immediate anti-inflammatory treatment. For the first time, the* in situ *loading method has been applied to synthesize an injectable CS/GP hydrogel for prolonged DIC release. The optimal synthesis condition is at 1.0 w/v% CS (high molecular weight of 324 kDa) and 4.0 w/v% GP, yielding the hydrogel capable of sol-gel transition at 37 °C. The porous structure of the hydrogels is filled with DIC, ensuring efficient drug entrapment. The hydrogels demonstrate prolonged DIC release over 7 days, achieving a cumulative drug release (CDR) ranging from 62.39 to 80.51%. At an initial DIC loading of 3000 μg/mL, the hydrogel maintains a drug concentration above 10 μg/mL after 6 days of release. DIC release kinetics are temperature-dependent, with a higher CDR at 39 °C (simulating an inflammatory condition) than 37 °C (normal body temperature), and governed by drug diffusion and hydrogel network swelling. This study provides a novel approach for synthesizing an injectable temperature-responsive CS hydrogel for local anti-inflammatory DIC delivery.*

Keywords: *thermosensitive hydrogel; diclofenac sodium;* in situ *loading; characterization; release profile*

■ INTRODUCTION

Drug delivery refers to the process of introducing an active compound into the body to achieve therapeutic effects at a specific site. This field focuses on developing advanced materials and carrier systems that ensure precise control over drug release–delivering the right dose at the right time and to the targeted site–to enhance therapeutic efficacy, reduce toxicity and side effects, and improve patient compliance [1]. Hydrogels, characterized by their distinctive 3D porous network structure, have emerged as a prominent drug delivery material in recent scientific works, utilizing a diverse range of synthetic and natural polymers [2]. Among these, thermosensitive chitosan (CS) hydrogel is highly promising for drug delivery due to its responsive behavior to environmental temperature variations. This hydrogel transitions from a sol to a gel state at physiological body temperature, facilitating the dissolution of active compounds at ambient temperature and their complete entrapment within the hydrogel matrix at body temperature at the target site, while also controlling localized drug release [3]. This capability ensures that the active substances retain their therapeutic potency and are delivered directly to the treatment site, bypassing systemic metabolism associated with conventional administration routes. Thermosensitive CS hydrogels can be fabricated via physical methods by incorporating β-glycerophosphate (GP) as a gelation agent. Moreover, these hydrogels possess essential biomedical properties for drug delivery, including biocompatibility, biodegradability, and non-

toxicity [4]. The active compound can be incorporated into CS/GP hydrogel either through *in situ* loading during the synthesis process [5] or a post-loading method by immersing the dry and porous hydrogel in an active substance medium, allowing the compound to diffuse into the swollen hydrogel matrix [6].

Nowadays, acute and chronic inflammatory diseases significantly impair patients' quality of life [7]. As inflammation worsens, causing damage to healthy tissues or organs, treatment becomes more challenging, leading to increased treatment costs [8]. To enhance healing and mitigate the adverse effects of inflammation, several approaches focus on alleviating inflammation and promoting tissue and cell repair using anti-inflammatory enzyme drugs, steroidal anti-inflammatory drugs, and non-steroidal anti-inflammatory drugs (NSAIDs). Among these, NSAIDs are the most widely used, particularly for chronic inflammation, osteoarthritis, and post-surgical pain [9]. However, previous reports have highlighted the side effects of systemic NSAID use, including nausea, vomiting, abdominal pain, heartburn, dizziness, and headaches, with more severe risks such as heart attack, stroke, gastric ulcers, and gastrointestinal bleeding. Administering NSAIDs through localized delivery offers an alternative to reduce these undesirable side effects [10-11].

Diclofenac sodium (DIC), a water-soluble NSAID with anti-inflammatory activity, is widely used to manage mild to moderate pain and alleviate arthritic symptoms such as osteoarthritis and rheumatoid arthritis. DIC is one of the most extensively investigated NSAIDs for incorporation into thermosensitive CS hydrogel delivery systems [6,12-13]. In particular, DIC was loaded into a thermoresponsive CS hydrogel crosslinked with tricarballylic acid, achieving a DIC release of about 67% over 96 h at pH 5.5 and 37 °C [13]. Qi et al. [12] encapsulated DIC within alginate microspheres, which were subsequently integrated into CS/GP hydrogels for sustained DIC release with 60–80% of DIC released over 5 days. Additionally, a post-loading method was employed to load DIC into CS/GP hydrogels, achieving a maximum drug loading capacity of 8.81 mg/g. The synthesized hydrogels released approximately 87 and 95% of the initial DIC loading after 5 h at 37 and 39 °C, respectively [6].

The previous work successfully developed CS/GP/DIC hydrogel via the post-loading method. Yet, the rapid DIC release of above 90% within 5 h limited its use for localized therapies requiring immediate anti-inflammatory treatment [6]. For managing chronic inflammation, developing a delivery system capable of prolonged DIC release at the target site is essential to reducing the frequency of drug administration and improving patient compliance. For the first time, the *in situ* loading method is employed to synthesize injectable CS/GP/DIC hydrogels for localized anti-inflammatory applications. This study thoroughly investigates the effects of CS, GP, and DIC concentrations on the thermosensitivity, morphology, and drug release profile of the fabricated hydrogel. Consequently, the appropriate synthesis condition is determined to provide a robust delivery system enabling prolonged DIC release. In contrast to the previous work using tricarballylic acid-crosslinked thermosensitive CS for DIC delivery [13], this study is the first to utilize GP-induced thermosensitive CS hydrogels with *in situ* DIC loading, enabling prolonged drug release for up to 7 days.

■ EXPERIMENTAL SECTION

Materials

High molecular weight CS (HMW-CS, 324 kDa) and medium molecular weight CS (MMW-CS, 290 kDa) were obtained from Viet Nam Food Joint Stock Company. Sodium β-glycerophosphate pentahydrate (GP, 97% purity) was purchased from Thermo Fisher Scientific. Phosphate-buffered saline (PBS) at pH 7.4 was prepared from monosodium phosphate dihydrate, disodium phosphate dodecahydrate, and monopotassium phosphate. Lactic acid (85.5–90% purity) for CS dissolution was sourced from Xilong Co Ltd. DIC (99% purity) in pharmacy grade was obtained from Arshine Group Co Ltd.

Instrumentation

The instrumentations used in this study were a scanning electron microscope (SEM, Prisma E) and a

UV-vis spectrometer GENESYS 10S (Thermo Fisher Scientific, USA).

Procedure

Characterization

The thermosensitivity of the CS/GP and CS/GP/DIC hydrogels was evaluated using the test tube inversion method [14]. The experiment was conducted on 2 mL of hydrogel in a "sol" state. After stabilizing the hydrogel at 25 °C for 20 min, the hydrogel's temperature was gradually increased at a rate of 0.5 °C per 10 min until the sol-gel transition occurred, and the gelation temperature was recorded. The sol-gel phase transition graph of the CS/GP and CS/GP/DIC hydrogel was plotted with the vertical axis representing the gelation temperature and the horizontal axis representing the GP concentration. To study the gelation time, 2 mL of hydrogel in a "sol" state was incubated at 37 or 39 °C in a thermostatic bath. Every 1 min, the tube containing the hydrogel was inverted once to evaluate the hydrogel's state. The gelation time was determined from the moment the tube was placed in the thermostatic bath until the hydrogel became non-flowable upon inversion. The morphology of hydrogels was analyzed by SEM.

In vitro *release kinetics of CS/GP/DIC hydrogels*

First, 2 mL of the CS/GP/DIC hydrogel in a "sol" state was completely gelled at 37 °C. Then, 6 mL of PBS at pH 7.4 was added to the tube containing non-flowable CS/GP/DIC hydrogel. The medium temperature was kept at either 37 or 39 °C during the experiment. At predetermined time intervals, the entire medium was collected for analysis and replaced with fresh medium to maintain the volume of the release environment. The withdrawn medium was centrifuged, and the supernatant was utilized to determine the concentration of released DIC at 277 nm using an UV-vis spectrometer. The cumulative drug release (CDR) value was calculated by Eq. (1);

$$\text{CDR}(\%) = \frac{m_{\text{released}}}{m_{\text{loaded}}} \times 100\% \quad (1)$$

where $m_{release}$ (μg) and m_{loaded} (μg) are respectively the amount of DIC released and the initial amount of DIC loaded. Furthermore, the Korsmeyer-Peppas model, as depicted in Eq. (2) [15], was employed to analyze the hydrogels' release kinetics;

$$\frac{M_t}{M_\infty} = k_{KP} \times t^n \quad (2)$$

where $\frac{M_t}{M_\infty}$ represents a portion of DIC released at a defined time point (t). Meanwhile, k_{KP} and n are respectively the coefficient and the exponent of the mathematical model.

Synthesis of thermosensitive CS hydrogels encapsulating DIC

The thermosensitive CS/GP hydrogels containing DIC were synthesized according to the procedure outlined in our previous study [15]. A 2.0 w/v% CS solution and a 40.0 w/v% GP solution were prepared separately in their respective solvents, 0.1 M lactic acid and distilled water. A DIC solution was also prepared in distilled water in advance. All solutions were chilled to 4 °C before hydrogel preparation. The CS and DIC solutions were first combined and stirred for 15 min at 4 °C to ensure proper mixing. Subsequently, the GP solution was introduced dropwise into the CS/DIC mixture while maintaining continuous stirring for another 15 min at 4 °C. After these steps, the CS/GP/DIC hydrogel sol was created and further incubated at 37 °C to induce gelation. Table 1 summarizes the formulations used for hydrogel preparation and evaluates the effects of

Table 1. Experimental design

CS molecular weight (kDa)	CS concentration (w/v%)	GP concentration (w/v%)	DIC concentration (μg/mL)
290	1.0	3.0; 4.0; 5.0; 6.0; 7.0; 8.0; 9.0; 10.0	0
324	1.0	3.0; 4.0; 5.0; 6.0; 7.0; 8.0; 9.0; 10.0	0
324	0.5	3.0; 4.0; 5.0; 6.0; 7.0; 8.0; 9.0; 10.0	0
324	1.0	3.0; 4.0; 5.0; 6.0; 7.0; 8.0; 9.0; 10.0	2000
324	1.0	4.0	1000; 2000; 3000

varying CS molecular weight and final concentrations of CS, GP, and DIC. Each synthesis condition was performed in triplicate to ensure experimental reproducibility.

■ RESULTS AND DISCUSSION

Characteristics of the Synthesized Hydrogels

After synthesis, both CS/GP and CS/GP/DIC hydrogels exhibit a "sol" state at 25 °C and become a non-flowable "gel" with an opaque white appearance when the temperature increases to 37 °C. The sol-gel transition temperature, affected by CS molecular weight, CS concentration, GP concentration, and DIC concentration, is depicted in Fig. 1. At a CS concentration of 1.0 w/v%, the CS/GP hydrogels made from HMW-CS (324 kDa) show a lower gelation temperature (31.5–38.0 °C) compared to those from MMW-CS (290 kDa). This is due to longer hydrophobic chains in HMW-CS, which enhance physical entanglements, enhancing gelation at lower temperatures [16]. Consequently, the HMW-CS is chosen for synthesizing CS/GP and CS/GP/DIC hydrogels in this study.

Fig. 1(a) illustrates the distinct variation in gelation temperature of CS/GP hydrogel at CS concentrations of 0.5 and 1.0 w/v%. It can be seen that at a CS concentration of 0.5 w/v%, increasing the GP concentrations from 3.0 to 10.0 w/v% reduces the gelation temperature from 62.0 to 37.0 °C. The most significant reduction occurs between 3.0 and 5.0 w/v% GP, with a 7.0 °C drop (62.0 to 55.0 °C) from 3.0 to 4.0 w/v% GP, and a 9.0 °C drop (55.0 to 46.0 °C) from 4.0 to 5.0 w/v% GP. The reduction in gelation temperature with increasing GP concentration is attributed to simultaneous changes in the CS solution's pH and ionic strength. As the GP content rises, the pH of the system increases, resulting in reduced protonation of the amino groups in CS. This lowers the overall charge density of the polymer chains, thereby weakening electrostatic repulsion and facilitating intermolecular interactions through hydrophobic interactions and hydrogen bonding. Moreover, elevated GP levels raise the ionic strength of the solution, further promoting the screening of electrostatic repulsion between glucosamine units [17]. These effects favor network formation at lower temperatures, contributing to the observed reduction in the sol-gel transition temperature. The difference in gelation temperature becomes smaller as the GP concentration rises from 5.0 to 10.0 w/v%. The gelation temperature of CS/GP hydrogel at 0.5 w/v% CS reaches the physiological body temperature range (37–38 °C) at extremely high GP concentrations of 9.0 and 10.0 w/v%. Previous study reported that high GP levels (≥ 10%) elevated hydrogel toxicity, as excess GP salts dissolved into tissue fluid, disrupting osmotic balance, impairing cell function, or causing cell death [18].

At a fixed CS concentration of 1.0 w/v%, the sol-gel transition temperature of the hydrogel is significantly lower compared to those of 0.5 w/v% CS (Fig. 1(a)). In particular, the gelation temperature of CS/GP hydrogel within the human physiological temperature range is achievable with low GP concentration of 3.0 and 4.0 w/v%. A notable drop in gelation temperature occurs as GP concentration increases from 3.0 to 5.0 w/v%. Specifically, when GP concentration rises from 3.0 to

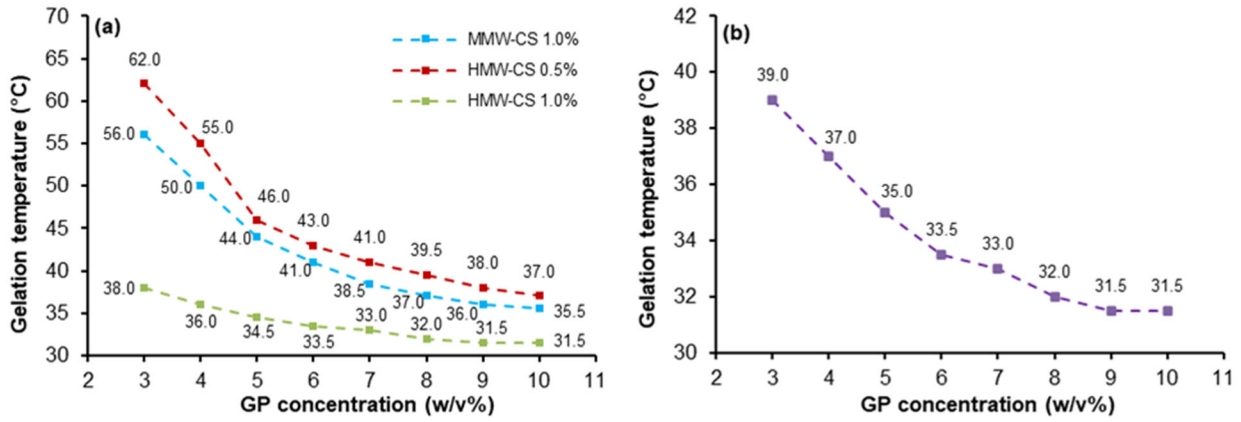

Fig 1. Sol-gel transition graph of (a) CS/GP and (b) CS/GP/DIC hydrogels at DIC concentration of 2000 µg/mL

4.0 w/v%, the gelation temperature decreases by 2.0 °C (from 38.0 to 36.0 °C), and from 4.0 to 5.0 w/v%, it drops by 1.5 °C (from 36.0 to 34.5 °C). However, as GP concentration further increases to 10.0 w/v%, the reduction in gelation temperature is only 0.5–1.0 °C. The effect of increased CS concentration on the gelation temperature of CS/GP hydrogel can be explained by the enhanced physical interactions among the polymer chains. As the CS concentration rises, the denser polymer network facilitates stronger interactions between the associative polymer chains, promoting earlier formation of a physical gel network. These interactions lower the energy barrier for gelation, resulting in a decline in gelation temperature with increasing CS concentration. In this context, the synergistic effect of high CS and GP concentrations leads to a sharp decline in gelation temperature [4]. Since the human body temperature and the temperature during inflammation range from 37–40 °C, the CS/GP hydrogels with 1.0 w/v% CS and 3.0–5.0 w/v% GP are suitable for delivery systems.

The influence of CS molecular weight, CS concentration, and GP concentration on the sol-gel transition time at 37 and 39 °C is examined, with results presented in Tables 2 and 3. Generally, higher GP concentration reduces the gelation time of the hydrogel at both 37 and 39 °C. An increase in GP concentration leads to a decrease in gelation time of the thermosensitive CS hydrogels due to elevated pH and ionic strength, which reduce electrostatic repulsion and enhance intermolecular interactions between polymer chains, accelerating gel network formation [16]. Additionally, a higher environmental temperature of 39 °C promotes gelation of CS/GP hydrogels by enhancing CS-CS interactions. At lower temperatures, strong CS-water associations stabilize the polymer structure and prevent chain aggregation. As the temperature increases, the hydration layer around CS is disrupted, enabling stronger hydrophobic interactions and hydrogen bonding among the CS chains [19]. These temperature-dependent molecular interactions facilitate faster network formation, thereby reducing the time required for complete phase transition. For hydrogels with 1.0 w/v% CS, those made from HMW-CS (324 kDa) exhibit shorter gelation times than those made from MMW-CS (290 kDa). For instance, at 37 °C, the gelation time for a hydrogel containing 1.0 w/v% CS and 5.0 w/v% GP with 324 kDa CS is only 12 min. In contrast, it takes 26 min for a hydrogel with 290 kDa CS (the same CS and GP concentration) to complete the sol-gel transition. At 39 °C, the gelation time for the HMW-CS hydrogel is 3 times shorter than that of the MMW-CS hydrogel. The synthesis condition is set at 1.0 w/v% CS, 4.0 w/v% GP, and 324 kDa CS, yielding a gelation time of 15 min at 37 °C and 9 min at 39 °C. The gelation time of the CS/GP hydrogel at 37 °C in this study is comparable to that of a thermosensitive CS hydrogel encapsulating mesenchymal stem cells for nasal delivery (15 min) [20].

At a CS concentration of 1.0 w/v%, the CS/GP hydrogel is loaded with the anti-inflammatory DIC at a concentration of 2000 μg/mL. Fig. 1(b) shows how GP concentration affects the thermosensitivity of CS/GP/DIC hydrogel. Within the GP concentration range

Table 2. Gelation time of CS/GP hydrogels with CS molecular weight of 324 kDa

CS concentration (w/v%)	0.5	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.0
GP concentration (w/v%)	5.0	6.0	7.0	8.0	9.0	10.0	1.0	2.0	3.0	4.0	5.0
Gelation time at 37 °C (min)	> 30	30	22	19	14	10	> 30	> 30	30	15	12
Gelation time at 39 °C (min)	30	23	16	12	8.0	7.0	> 30	> 30	15	9.0	5.0

Table 3. Gelation time of CS/GP hydrogels with CS molecular weight of 290 kDa

CS concentration (w/v%)	1.0					
GP concentration (w/v%)	5.0	6.0	7.0	8.0	9.0	10.0
Gelation time at 37 °C (min)	26	26	22	16	13	8.0
Gelation time at 39 °C (min)	16	14	13	11	8.0	6.0

of 3.0 to 5.0 w/v%, the gelation temperature of CS/GP/DIC hydrogel is 0.5–1.0 °C lower than that of CS/GP hydrogel. However, at higher GP levels, the gelation temperature of both hydrogels becomes similar. A minor difference confirms that the DIC-loaded CS/GP hydrogel maintains thermosensitivity and undergoes sol-gel transition at human body temperature. The anti-inflammatory drug DIC, with its charged Na^+ and COO^- groups, interacts with the phosphate groups of GP and the amine groups of CS, requiring higher energy to disrupt hydrogen bonds during the phase transition, thus enhancing hydrophobic interactions and physical entanglements to form the "gel" state. The synthesis condition for CS/GP/DIC hydrogel is set at 4.0 w/v% GP, achieving a sol-gel transition at 37 °C, suitable for physiological conditions.

The porous morphology of CS/GP and CS/GP/DIC hydrogel at the same synthesis condition is shown in Fig. 2. The 3D framework forms through hydrophobic interactions among CS chains, with needle-like GP salt particles visible on the CS surfaces. At the same magnification of 250×, the original pores in CS/GP hydrogel are filled when loaded with 2000 µg/mL DIC, indicating effective drug entrapment within the hydrogel matrix. A noticeable reduction in pore size following DIC incorporation suggests that the drug molecules occupy the internal spaces of the hydrogel network, leading to a denser microstructure. This morphological change confirms the successful formation of DIC-encapsulated thermosensitive CS hydrogels, providing a basis for further evaluation of their *in vitro* drug release profile. In the sol state, DIC interacts with the polymer matrix primarily through electrostatic attraction between its negatively charged carboxylate groups and the protonated amino groups of CS. As the system transitions to the gel state with increasing temperature, hydrogen bonding becomes more prominent, specifically between the carboxylate groups of DIC and the amino/hydroxyl groups of CS, as well as between the phosphate groups of GP and the amino/hydroxyl groups of CS [6,12]. These physical interactions, along with the entanglement of polymer chains during gelation, facilitate the incorporation of DIC into the hydrogel matrix, ensuring effective drug loading and prolonged release.

Release Profile of CS/GP/DIC Hydrogels

The effects of GP concentrations (3.0–5.0 w/v%), DIC concentrations (1000–3000 µg/mL) and environmental temperature (37 and 39 °C) on the drug release capability of the CS/GP/DIC hydrogel are investigated, with experimental results shown in Fig. 3. The hydrogel demonstrates prolonged DIC release, with CDR ranging from 62.62 to 81.49% over 9 days, reaching equilibrium after 7 days. Increasing GP concentration from 3.0 to 5.0 w/v% reduces DIC release capacity from 79.30 to 67.44% at a fixed DIC concentration of 2000 µg/mL (Fig. 3(a)). The DIC concentration in the release medium is also highest when the GP concentration is 3.0 w/v% (Fig. 3(b)). This is attributed to the higher GP level increasing the crosslinking density in the hydrogel and reducing pore size, which impedes

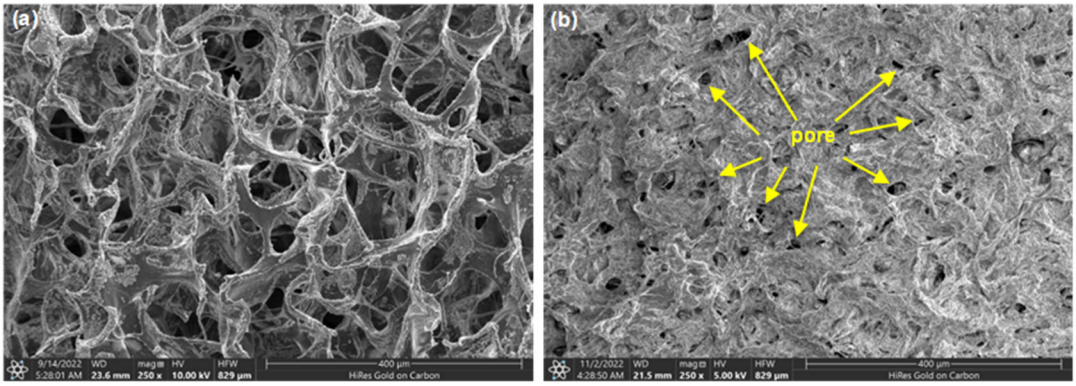

Fig 2. Morphology of the (a) CS/GP and (b) CS/GP/DIC hydrogels containing 2000 µg/mL DIC. The CS and GP concentrations are respectively 1.0 w/v% and 4.0 w/v%

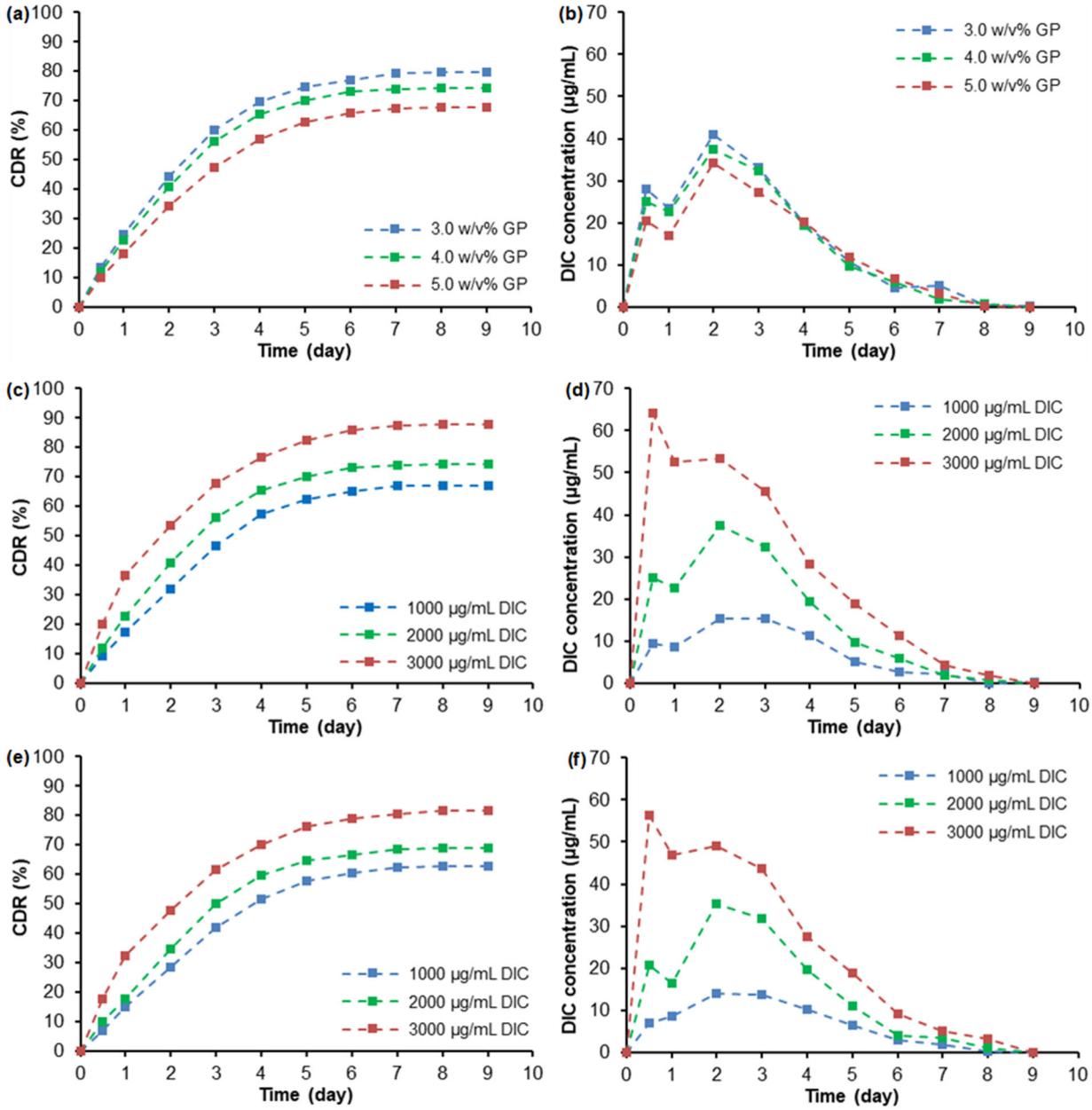

Fig 3. *In vitro* release profile of CS/GP/DIC hydrogels with varying (a, b) GP and (c, d) DIC concentration under simulated inflammatory environment of pH 7.4 and 39 °C and (e, f) physiological environment of pH 7.4 and 37 °C

DIC diffusion into the medium. A similar effect of GP concentration on release capacity was observed for CS/GP hydrogels delivering metronidazole [21] or insulin [22].

The amount of DIC loaded into the hydrogel influences its release efficiency, as illustrated in Fig. 3(c–f). As the DIC concentration increases from 1000 to 3000 µg/mL, the amount of drug released from the hydrogel shows a rising trend, with CDR after 7 days reaching 62.39, 68.53, and 80.51%, respectively (Fig. 3(c)). At a DIC loading of 3000 µg/mL in the CS/GP/DIC hydrogel, the DIC concentration in the medium reaches approximately 65 µg/mL after 30 min and remains above 40 µg/mL after 3 days of release, before gradually decreasing in the following days (Fig. 3(d), 3(f)). Increasing the drug loading within the hydrogel enhances the concentration gradient between

the hydrogel matrix and the release medium, boosting the diffusion dynamics and accelerating the drug release rate. These findings are consistent with the study by Luo et al. on the thermosensitive PEG-PCL-PEG hydrogel for DIC delivery [23].

The impact of environmental temperature on the *in vitro* DIC release from CS/GP/DIC hydrogel is also assessed at 37 °C (normal body temperature) and 39 °C (temperature at inflammatory area). As seen from Fig. 3(c–f), at the same DIC loading in the hydrogel, the drug release is enhanced at 39 °C compared to 37 °C, demonstrating the temperature-responsive ability of the CS/GP/DIC hydrogel. The increased DIC release observed at 39 compared to 37 °C is attributed to greater molecular mobility of DIC and reduced intermolecular interactions within the hydrogel matrix at higher temperatures. The slight elevation in temperature may accelerate polymer chain relaxation and increase diffusivity, thereby promoting drug transport [6].

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The release kinetics of the CS/GP/DIC hydrogels are analyzed using the Korsmeyer-Peppas model, achieving a correlation coefficient ~0.99 (Table 4). The DIC release exhibits anomalous transport ($n > 0.5$), driven by diffusion and hydrogel network swelling, rather than purely Fickian diffusion. Increasing GP concentration reduces the k_{KP} value from 0.306 to 0.270, indicating that DIC release is hindered by the denser crosslinking network. The effects of temperature and DIC loading amount positively correlate with the extent of DIC release. Higher DIC concentrations result in higher k_{KP} values, reflecting increased drug release. Despite all n values exceeding 0.5, the formulation loaded with 3000 μg/mL DIC exhibits the lowest n value, approaching 0.5, which suggests a predominant shift toward Fickian diffusion-controlled release. At elevated DIC loading concentrations, intensified interactions between the drug molecules and the polymeric network enhance entrapment efficiency during release, thereby limiting chain relaxation and promoting a diffusion-dominant release kinetic profile. Interestingly, an atypical release profile is observed in hydrogels loaded with 2000 μg/mL DIC, characterized by an initial decrease in DIC concentration on day 1, followed by a subsequent increase. In contrast, hydrogels containing 1000 μg/mL DIC show a steady rise in release concentration up to day 3, after which a decline is observed. Meanwhile, samples with 3000 μg/mL DIC exhibit an extremely high DIC concentration on day 1, followed by a gradual decrease over time (Fig. 3(d), 3(f)). The biphasic release behavior in the hydrogel with 2000 μg/mL DIC may reflect a Super Case II transport mechanism, as indicated by the n value of approximately 0.9 obtained from the Korsmeyer-Peppas model (Table 4). This pattern suggests an initial lag phase, likely attributed to slow hydrogel swelling, stabilization in PBS, and strong interactions between drug molecules and the

Table 4. Analysis of release kinetics of CS/GP/DIC hydrogels from the Korsmeyer-Peppas model

CS concentration (w/v%)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
GP concentration (w/v%)	3.0	4.0	5.0	4.0	4.0	4.0	4.0	4.0
DIC concentration (μg/mL)	2000	2000	2000	1000	2000	3000	1000	3000
Temperature (°C)	39	39	39	37	37	37	39	39
k_{KP}	0.306	0.303	0.270	0.233	0.265	0.375	0.255	0.391
n	0.857	0.864	0.914	0.978	0.928	0.666	0.895	0.652
R^2	0.999	0.999	0.999	0.999	0.999	0.997	0.999	0.997

polymeric network [24]. These factors may restrict drug diffusion in the early phase, further exacerbated by the medium replacement. As the hydrogels continue to swell and the polymer chains relax, the release rate increases, resulting in higher DIC concentrations at later time points. In comparison, despite a similar n value, the hydrogels with 1000 µg/mL DIC likely exhibit weaker drug-polymer interactions, resulting in lower retention and a sustained release over the initial three days. Furthermore, k_{KP} values for hydrogels with the same DIC concentration are higher at 39 °C than 37 °C), consistent with the experimental data shown in Fig. 3(c–f). The highest k_{KP} value is observed at 39 °C, with the maximum DIC loading of 3000 µg/mL.

■ CONCLUSION

This study marks a significant advancement in the development of thermosensitive CS hydrogels for prolonged DIC release. Unlike previous work, which utilized a post-loading method to incorporate DIC into CS/GP hydrogel, resulting in a burst release within 10 h, suitable only for rapid pain relief, the current research successfully employs an *in situ* loading approach to synthesize injectable CS/GP/DIC hydrogels. By optimizing synthesis conditions at 1.0 w/v% CS (molecular weight of 324 kDa) and 4.0 w/v% GP, the fabricated hydrogels undergo sol-gel transition at a physiological temperature of 37 °C. The *in situ* incorporation of DIC into the porous CS hydrogel structure ensures efficient drug encapsulation, enabling prolonged release over 7 days with a CDR value of 62.39–80.51%. The temperature-responsive release kinetics, with enhanced CDR at 39 °C (*in vitro* inflammatory condition) compared to 37 °C (human body temperature), highlight the potential of CS/GP/DIC hydrogels for targeted therapy at inflamed sites. The Korsmeyer-Peppas model confirms that DIC release is driven by a combination of drug diffusion and hydrogel network swelling. This work provides a novel and effective strategy for synthesizing injectable thermosensitive CS hydrogels for localized delivery of DIC in anti-inflammatory applications.

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■ CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

■ AUTHOR CONTRIBUTIONS

Nga Hoang Nguyen Do performed the investigation and formal analysis, contributed to visualization, and wrote the original draft; Ha Vu Le and Khoa Dang Nguyen contributed to the methodology, and reviewed and edited the manuscript; Anh Cam Ha contributed to the conceptualization, supervised the project, acquired funding, and reviewed and edited the manuscript. All authors agreed to the final version of this manuscript.

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