ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 14, Issue 1, pp: (1-6), Month: January - March 2026, Available at: www.researchpublish.com

Development of Haemostatic Hydrogel Sheets from Chitosan Extracted from Shrimp Shells

Phonrawin Chirdchim[1], Boonyawee Sujeerakulkrai[2], Piengpor Kanghae[3]

[1]Srinakharinwirot Prasarnmit University, Bangkok, Thailand.

[2]Princess Chulabhorn Science High School, Chonburi, Thailand.

[3]Engineering Science College (Darunsikkhalai School), King Mongkut's University of Technology Thonburi, Bangkok, Thailand

DOI: https://doi.org/10.5281/zenodo.18467924

Published Date: 03-February-2026

Abstract: **Introduction. Hemostasis is essential in first aid and emergency medical care; however, physiological clotting mechanisms are often insufficient for rapid bleeding control in acute or severe injuries. Chitosan, a biopolymer derived from shrimp shell chitin, has demonstrated effective hemostatic properties and represents a low-cost and environmentally sustainable biomaterial. This study aimed to compare the hemostatic efficiency of chitosan–citric acid solutions at different ratios and to develop a chitosan-based hydrogel sheet for potential bleeding-control applications.**

Methods. Chitosan was extracted from shrimp shells through demineralization and deacetylation processes. Chitosan solutions were prepared using citric acid at ratios of 1:12, 1:6, and 1:4. Hemostatic efficiency was evaluated *in vitro* using fresh porcine blood by observing clot formation and measuring the weight of blood clot residue after 10 minutes. Distilled water and citric acid solution served as control groups. Differences among experimental groups were analyzed using the Kruskal–Wallis test. The formulation exhibiting the highest hemostatic performance was subsequently fabricated into a hydrogel sheet using polyvinyl alcohol, polyvinylpyrrolidone, glycerin, and microwave irradiation.

Results. Significant differences in blood clot residue weight were observed among the experimental groups (p = 0.009). The chitosan–citric acid ratio of 1:12 exhibited the highest mean blood clot weight, indicating superior hemostatic performance compared with other formulations and controls. The selected formulation was successfully fabricated into a stable hydrogel sheet with satisfactory physical properties and handling characteristics.

Conclusion. The hemostatic efficiency of chitosan–citric acid systems is strongly dependent on formulation ratio, with the 1:12 ratio providing optimal performance *in vitro*. The developed chitosan-based hydrogel sheet demonstrates potential as an effective, safe, and environmentally sustainable hemostatic material. Further *in vivo* and clinical studies are warranted.

Keywords: **Chitosan; Hemostasis; Hydrogel; Shrimp shells; Biomaterials.**

I. INTRODUCTION

Hemostasis is a critical component of first aid and emergency medical care; however, intrinsic physiological mechanisms are often insufficiently rapid or effective in cases of acute or severe injury. Consequently, uncontrolled hemorrhage remains a leading cause of morbidity and mortality worldwide.[1], [2] This clinical challenge has driven extensive research into advanced hemostatic materials capable of accelerating clot formation and improving bleeding control in both emergency and surgical settings.[3], [4]

Chitosan has emerged as a promising hemostatic biomaterial due to its unique physicochemical and biological properties. Derived from chitin, a naturally occurring polysaccharide abundantly found in marine waste such as shrimp shells, chitosan is biodegradable, biocompatible, and environmentally sustainable.[6] In acidic environments, the amino groups of chitosan

become protonated, conferring a positive charge that enables strong electrostatic interactions with negatively charged blood components, including erythrocytes and platelets. These interactions promote rapid cellular aggregation and clot formation, largely independent of the classical coagulation cascade.[3], [5], [9] This mechanism is particularly advantageous in emergency situations or in patients with coagulopathies, where conventional hemostatic pathways may be compromised.[2], [10]

Previous experimental and clinical studies have demonstrated that chitosan-based dressings can significantly reduce blood loss and improve hemostatic outcomes.[3], [5] In addition to their hemostatic activity, chitosan-based materials exhibit antimicrobial properties and favorable tissue compatibility, further supporting their use in wound-care applications.[4], [11] Nevertheless, the hemostatic performance of chitosan is highly dependent on formulation-related parameters such as polymer concentration, molecular dispersion, solubility, and degree of protonation, all of which influence the accessibility of positively charged functional groups for interaction with blood cells.[6], [9]

Citric acid is commonly used as a solvent for chitosan owing to its mild acidity, biocompatibility, and ability to effectively protonate amino groups without introducing excessive toxicity. Variations in the chitosan-to-citric acid ratio can markedly alter solution viscosity and polymer distribution, thereby affecting hemostatic efficiency. Earlier studies comparing chitosan derived from different marine sources have emphasized that optimal formulation, rather than increased polymer content alone, is critical for achieving effective hemostasis and favorable handling properties.[7] However, systematic investigations focusing specifically on the influence of chitosan-to-citric acid ratio on hemostatic performance remain limited.

Advances in fabrication techniques have further enabled the development of chitosan-based hydrogels with enhanced structural stability and practical applicability. Microwave-assisted synthesis has been reported as an efficient approach for hydrogel preparation, offering reduced processing time and energy consumption while promoting the formation of stable three-dimensional polymer networks.[8] Such hydrogel systems may increase contact between the hemostatic material and the bleeding surface, thereby improving clot formation and retention.[12] Importantly, the effectiveness of these hydrogel-based dressings fundamentally depends on the selection of an optimal chitosan formulation.

Accordingly, this study builds upon previous research on chitosan-based hemostatic materials [7], [8] by systematically evaluating the effect of different chitosan-to-citric acid ratios on hemostatic efficiency. By identifying an optimal ratio and incorporating it into a hydrogel sheet, this work aims to advance both mechanistic understanding and practical development of effective, sustainable, and locally sourced chitosan-based hemostatic materials.

II. MATERIALS AND METHODS

A. A simple method for synthesizing chitosan-based hydrogels using microwave heating was developed.[8]. Page Layout and Font Used

Chitosan/polyvinyl alcohol/polyvinylpyrrolidone (CS/PVA/PVP) hydrogels were fabricated using microwave irradiation as an energy source to induce a three-dimensional network structure for potential application in heavy metal ion adsorption. The optimal CS:PVA:PVP ratio was 0.3:0.6:0.3 (g) with epichlorohydrin employed as a crosslinking agent under microwave heating at 600 W for 3 minutes. The synthesized hydrogel exhibited a high swelling degree (1627.4%) and gel fraction (22.96%), indicating excellent solution absorption capacity and enhanced interaction with heavy metal ions through functional groups within the hydrogel structure. In addition, the reaction mechanism was investigated to predict hydrogel structure and its interactions with metal ions. The resulting hydrogel demonstrated environmental friendliness, biodegradability, and significantly reduced synthesis time and energy consumption due to microwave-assisted processing.

B. Preparation of Chitin and Chitosan (Figure 1)

Shrimp shells, excluding the head, were thoroughly washed, dried in a hot-air oven at 80 °C for approximately 17 hours, and ground into fine powder as the raw material for chitin and chitosan preparation. The processed shrimp shells were treated with 1.0 M HCl at a shell-to-acid ratio of 15:190 (w/v) and stirred at room temperature for 1 hour and 30 minutes to remove minerals. The residue was then filtered, repeatedly washed with deionized water until neutral pH was achieved, and dried at 80 °C to obtain chitin. Chitosan was subsequently produced by deacetylation of chitin through boiling in 50% (w/v) NaOH at a chitin-to-solution ratio of 1:10 (w/v) at 100 °C for 2 hours. The resulting product was thoroughly washed with deionized water, neutralized using 1.0 M HCl while monitoring pH with a pH meter, and finally dried to obtain chitosan.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 14, Issue 1, pp: (1-6), Month: January - March 2026, Available at: www.researchpublish.com

Figure 1. A= Mixing of shrimp shells with hydrochloric acid at a ratio of 15:190 (w/v)., **B**= Shrimp shells treated with hydrochloric acid and subsequently washed with distilled water until neutral pH was reached., **C**= Shrimp shells after drying in a hot-air oven., **D**= Mixing of shrimp shells with sodium hydroxide solution., **E**= Mixing of shrimp shells with sodium hydroxide at 60 °C., **F**= pH adjustment of chitosan., **G**= Moisture removal from chitosan using a desiccator.

C. Comparison of the Hemostatic Efficiency of Chitosan Solutions (Figure 2.)

Five experimental groups were prepared as follows: Group 1: Deionized water, 12 mL, Group 2: 2% (w/v) citric acid solution, 12 mL, Group 3: 1 g chitosan dissolved in 12 mL citric acid (ratio 1:12), Group 4: 2 g chitosan dissolved in 12 mL citric acid (ratio 1:6), Group 5: 3 g chitosan dissolved in 12 mL citric acid (ratio 1:4)

For Groups 3–5, chitosan was dissolved in citric acid and stirred using a magnetic stirrer at room temperature for 3 hours to ensure complete dissolution. Fresh pig blood was dropped onto five separate watch glasses, with three drops per dish. One drop of solution from each experimental group was added to the blood on each watch glass. The samples were left undisturbed for 10 minutes, after which changes in blood clotting behavior were observed. The samples were then filtered through filter paper, and the weight of the remaining blood clot residue on the filter paper was measured to assess hemostatic efficiency.

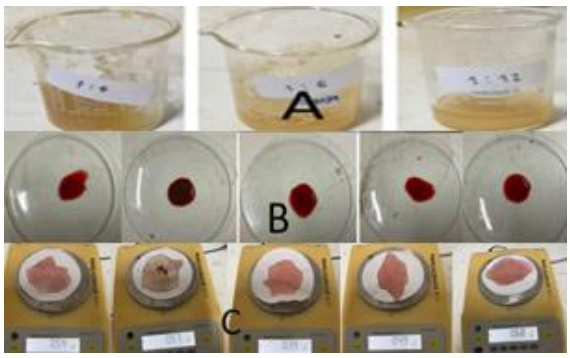


Figure 2. A = Mixing of chitosan with citric acid at ratios of 1:4, 1:6, and 1:12., **B =** Effect of applying samples from five experimental sets onto blood**., C =** Weight of blood clots obtained from experimental sets 1–5.

D. Fabrication of Hydrogel Sheets (Figure 3.)

Polyvinylpyrrolidone (PVP, 2 g), polyvinyl alcohol (PVA, 4 g), and glycerin as a crosslinking agent (8 g) were added to 2 g of the chitosan solution from Group 3. The mixture was stirred thoroughly until homogeneous. The resulting solution was subjected to microwave irradiation at a power of 300 W for 16 minutes to induce hydrogel formation. The product was then dried in a hot-air oven at 60 °C for 24 hours. The formed hydrogel film was immersed in a 0.16 M sodium bicarbonate ($NaHCO_3$) solution for 24 hours to neutralize the pH of the hydrogel. Subsequently, the hydrogel sheet was rinsed again with deionized water to ensure neutral pH and dried at 60 °C for an additional 6 hours.

Figure 3. A = Mixing of PVP, PVA, glycerin, and chitosan., **B** = Samples transferred to a hot-air oven after microwave treatment at 60 °C for 24 hours., **C** = Hydrogel sheets immersed in $NaHCO_3$ solution for pH adjustment., **D** = Samples dried in a hot-air oven at 60 °C for 6 hours after immersion in $NaHCO_3$ solution., **E** = Experimental results of chitosan-based hemostatic hydrogel sheets.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 14, Issue 1, pp: (1-6), Month: January - March 2026, Available at: www.researchpublish.com

E. statistical analysis

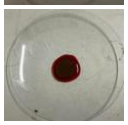
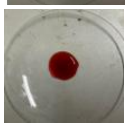
Differences in blood clot weight among the five experimental groups were summarized as mean ± standard deviation (SD) and analyzed using the Kruskal–Wallis test. All statistical analyses were two-tailed, and a p-value < 0.05 was considered statistically significant.

III. RESULTS

This in vitro study systematically evaluated and compared the hemostatic efficiency of chitosan extracts prepared at different concentrations. Chitosan was extracted from shrimp shells and dissolved in citric acid at three chitosan-to-citric acid ratios: 1:12, 1:6, and 1:4. Hemostatic performance was assessed based on blood-clotting behavior, and the experimental outcomes are summarized in **Table 1.**

As illustrated in Fig. 4, the chitosan–citric acid solution at a ratio of 1:12 exhibited the highest mean blood clot residue weight among the five experimental groups. This was followed, in descending order, by the citric acid group, the distilled water group, the chitosan–citric acid solution at a ratio of 1:6, and the chitosan–citric acid solution at a ratio of 1:4.

Table 1. Characteristics of Blood Droplets After 10 Minutes of Standing and the Weight of Blood Clot Residue

Experimental Group	Observation of Blood Droplet		Weight of Blood Clot on Filter Paper (g)			Mean ± SD (g)
			Trial 1	Trial 2	Trial 3	
Distilled water		Blood remained fluid, minimal clotting	0.54	0.53	0.55	0.54 ± 0.01
Citric acid (2% w/v)		Slight clot formation	0.56	0.56	0.56	0.56 ± 0.00
Chitosan–citric acid (1:12)		Dense clot formation	0.70	0.70	0.60	0.67 ± 0.06
Chitosan–citric acid (1:6)		Moderate clot formation	0.49	0.45	0.53	0.49 ± 0.04
Chitosan–citric acid (1:4)		Partial clot formation	0.37	0.34	0.31	0.34 ± 0.03
Overall p-value						0.009*

*Differences among experimental groups were analyzed using the Kruskal–Wallis test. The overall comparison revealed a statistically significant difference (p = 0.009).

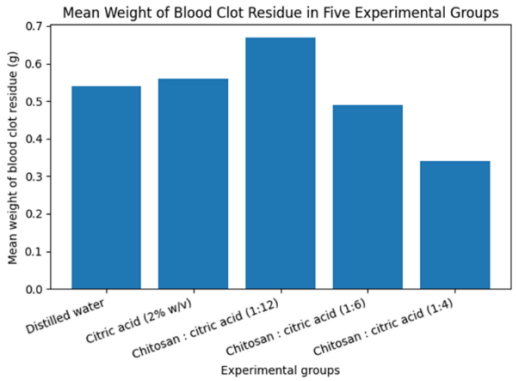


Figure 4. Mean weight of blood clot residue measured from five experimental groups after 10 minutes.

IV. DISCUSSIONS

This study demonstrates that the hemostatic efficiency of chitosan–citric acid systems is strongly dependent on formulation ratio, with the 1:12 chitosan-to-acid ratio exhibiting superior blood-clotting performance compared with higher chitosan concentrations. This observation is consistent with previous findings by Charoenrat (2001), which emphasized that appropriately optimized chitosan formulations derived from shrimp shells can effectively promote hemostasis and wound healing, underscoring the importance of formulation optimization rather than polymer quantity alone.[7]

The enhanced performance of the 1:12 formulation may be attributed to improved solubility and molecular dispersion of chitosan in citric acid, leading to greater exposure of protonated amino groups. These positively charged functional groups facilitate electrostatic interactions with negatively charged erythrocytes and platelets, thereby promoting rapid aggregation and clot formation. This mechanism is well aligned with the hemostatic pathways described for chitosan-based dressings, which act largely independently of the classical coagulation cascade through platelet activation and red blood cell adhesion.[9], [12], [17]

In contrast, higher chitosan concentrations (1:6 and 1:4 ratios) exhibited reduced hemostatic efficiency, likely due to increased solution viscosity and incomplete polymer dissolution, which can hinder effective interaction between chitosan functional groups and blood components. Similar concentration-dependent limitations have been reported in both in vitro and in vivo studies, where excessively dense chitosan networks compromised biological performance despite increased polymer content.[7], [9], [13], [14] These findings collectively reinforce that optimal molecular accessibility, rather than maximal chitosan loading, is a critical determinant of effective hemostasis.

Based on the in vitro findings, the 1:12 chitosan–citric acid formulation was selected for hydrogel fabrication. The successful preparation of a stable hydrogel sheet using microwave-assisted processing is consistent with prior reports demonstrating that microwave irradiation enables rapid formation of three-dimensional chitosan-based hydrogel networks while significantly reducing synthesis time and energy consumption.[8] The incorporation of PVA and PVP further enhanced mechanical stability and handling properties, which are essential for practical wound-care applications and have been similarly reported in recent hydrogel-based wound dressing studies.[14], [17]

The broader translational relevance of chitosan-based hemostatic hydrogels is supported by accumulating clinical and preclinical evidence. Recent reviews and experimental studies have highlighted that chitosan-based dressings provide rapid hemostasis, favorable biocompatibility, and antimicrobial activity, making them suitable for surgical, dental, and emergency applications.[10], [12], [15], [16], [18] Moreover, animal and human studies have confirmed the safety and efficacy of chitosan-based hemostatic sponges and dressings, including use in patients with impaired coagulation or receiving antithrombotic therapy.[11], [16], [19], [20]

Overall, the present findings corroborate existing literature and further demonstrate that careful optimization of chitosan formulation is essential for maximizing hemostatic performance. The chitosan-based hydrogel sheet developed in this study represents a promising, safe, and environmentally sustainable candidate for further preclinical and clinical development as an effective bleeding-control material.

V. CONCLUSION

This study demonstrated that the hemostatic efficiency of chitosan–citric acid solutions is strongly dependent on the formulation ratio. Among the three ratios evaluated (1:4, 1:6, and 1:12), the chitosan–citric acid ratio of 1:12 exhibited the highest blood-clotting performance in vitro, confirming the study hypothesis. The enhanced hemostatic activity at this ratio is likely attributed to improved chitosan solubility and optimal exposure of positively charged functional groups that facilitate interactions with blood components.

The selected 1:12 formulation was successfully fabricated into a stable chitosan-based hydrogel sheet using microwave-assisted processing combined with PVA, PVP, and glycerin. The resulting hydrogel demonstrated satisfactory physical properties and practical handling potential, supporting its suitability as a hemostatic dressing. Overall, this study highlights the importance of formulation optimization in chitosan-based hemostatic materials and supports the potential of shrimp shell–derived chitosan hydrogels as effective, safe, and environmentally sustainable materials for bleeding control. Further studies are warranted to evaluate in vivo performance and clinical applicability.

REFERENCES

[1] Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. *J Trauma*. 2006;60(6 Suppl):S3–S11.

[2] Eastridge BJ, Mabry RL, Seguin P, et al. Death on the battlefield (2001–2011): implications for the future of combat casualty care. *J Trauma Acute Care Surg*. 2012;73(6 Suppl 5):S431–S437.

[3] Pusateri AE, McCarthy SJ, Gregory KW, et al. Effect of a chitosan-based hemostatic dressing on blood loss and survival in a model of severe venous hemorrhage and coagulopathy. *J Trauma*. 2003;54(1):177–182.

[4] Boateng JS, Matthews KH, Stevens HN, Eccleston GM. Wound healing dressings and drug delivery systems: a review. *J Pharm Sci*. 2008;97(8):2892–2923.

[5] Wedmore I, McManus JG, Pusateri AE, Holcomb JB. A special report on the chitosan-based hemostatic dressing: experience in current combat operations. *J Trauma*. 2006;60(3):655–658.

[6] Kumari S, Rath P, Sri Hari Kumar A, Tiwari TN. Extraction and characterization of chitin and chitosan from fishery waste by chemical method. *Environ Technol Innov*. 2015;3:77–85.

[7] Charoenrat M. Comparison of the effectiveness of chitosan derived from squid pens and shrimp shells on hemostasis, wound healing, and safety of use [Internet]. 2001 [cited 2022 Dec 11]. Available from: http://kb.psu.ac.th/psukb/handle/2553/2907

[8] Tatsanapakdi Y. Simple synthesis of chitosan hydrogel using microwave heating [Internet]. 2020 [cited 2022 Dec 12]. Available from: https://app.gs.kku.ac.th/images/img/support/grc2020/pdfabstracts/PMO3.pdf

[9] Wang YW, Liu CC, et al. Biological effects of chitosan-based dressing on hemostasis mechanism. *Polymers*. 2019;11(11):1906.

[10] Cassano R, Perri P, Scarcello E, Piro P, Sole R, Curcio F, Trombino S. Chitosan hemostatic dressings: properties and surgical applications. *Polymers*. 2024;16(13):1770.

[11] Baldrick P. The safety of chitosan as a pharmaceutical excipient. *Regul Toxicol Pharmacol*. 2010;56(3):290–299.

[12] Zhang W, Geng X, Qin S, Xie Z, Li W, Li J. Research progress and application of chitosan dressings in hemostasis: a review. *Int J Biol Macromol*. 2024;282(Pt 1):136421.

[13] Che X, Zhao T, Hu J, Yang K, Ma N, Li A, Sun Q, Ding C, Ding Q. Application of chitosan-based hydrogel in promoting wound healing: a review. *Polymers (Basel)*. 2024;16(3):344.

[14] Zanchetta FC, De Wever P, Morari J, Gaspar RC, Prado TP, De Maeseneer T, Cardinaels R, Araújo EP, Lima MHM, Fardim P. In vitro and in vivo evaluation of chitosan/HPMC/insulin hydrogel for wound healing applications. *Bioengineering*. 2024;11(2):168. doi:10.3390/bioengineering11020168

[15] Yadav VS, Makker K, Haidrus R, Dawar A, Gumber B. Chitosan-based dressing for management of palatal donor site: a randomized clinical trial. *J Periodontal Res*. 2024;59(6):1153–1161. doi:10.1111/jre.13267

[16] Jolly SS, Rattan V. Is chitosan-based dressing more effective than gauze pressure in achieving early hemostasis after dental extractions in patients with deranged coagulation profiles? *Arch Craniofac Surg*. 2025;26(2):65–69. doi:10.7181/acfs.2024.0082

[17] Wang X, Liu C, Liu C, Shi Z, Liu X, Huang F. A chitosan macroporous hydrogel integrating enrichment, adsorption and delivery of blood clotting components for rapid hemostasis. *Int J Biol Macromol*. 2024 Nov;281(Pt 4):136482. doi:10.1016/j.ijbiomac.2024.136482

[18] Agrawal D, Zaheer S, Newaskar V. Efficacy of chitosan dressing as a local haemostatic agent in the management of dental extractions in patients on antiplatelet therapy: a prospective randomized study. *J Oral Biol Craniofac Res*. 2025 Nov-Dec;15(6):1715–1720. doi:10.1016/j.jobcr.2025.10.012

[19] Perri P, Curcio F, De Luca M, Piro P, Trombino S, Cassano R. Evaluation of chitosan-based Axiostat as hemostatic dressing for endovascular procedures in patients with Leriche syndrome on anticoagulant therapy. *Pharmaceuticals (Basel)*. 2025 Apr 16;18(4):584. doi:10.3390/ph18040584.

[20] Yudin AB, Nosov AM, Volkova MV, Demchenko KN, Zhabin AV, Zaychikov DA, Andreev NYu, Kovalevsky YaB. Evaluation of the safety and efficacy of chitosan-based hemostatic sponges in a chronic large-animal model. *Russian Military Medical Academy Reports*. 2025;44(1):19–26. doi:10.17816/rmmar641798.