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Exploring Swelling Behaviour of Chitosan-Based Hydrogels in Diverse Environmental Conditions

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Hydrogels, versatile materials with diverse applications in biomedicine and beyond, have garnered significant attention due to their unique properties. Among these, chitosan-based hydrogels have emerged as promising candidates owing to their biocompatibility, biodegradability, and antimicrobial properties. The hydrogels were synthesized through cross-linking of chitosan graft copolymers with N-vinylpyrrolidone and other vinyl monomers, as well as through thermal methods *in situ*. The impact of cross-linking agent concentration on gel fraction yield and cross-linking efficiency was examined. The results indicate that higher cross-linking densities lead to reduced swelling capacity as a result of a denser network structure. The swelling behaviour of chitosan-based hydrogels under various environmental conditions, including pH, temperature and ionic strength were also investigated. It is found that swelling behaviour varied among different hydrogel formulations, with natural and vinyl graft copolymers exhibiting increased swelling in acidic environments. Semi-synthetic copolymers containing hydrophobic groups showed relatively lower swelling across pH ranges. Additionally, swelling kinetics were studied, revealing equilibrium reached at different time intervals depending on temperature and hydrogel composition. Furthermore, the effect of glucose concentration on swelling behaviour was explored, demonstrating decreased swelling with increasing glucose concentration. Overall, this comprehensive investigation provides valuable insights into the swelling behaviour of chitosan-based hydrogels, essential for optimizing their performance in biomedical applications.

Keywords: Polymers, Drug delivery, Chitosan, Arabinogalactan, Hydrogel, Swelling.

INTRODUCTION

Hydrogels, three-dimensional networks of hydrophilic polymers capable of absorbing and retaining large amounts of water, have emerged as versatile materials with applications spanning drug delivery, tissue engineering, and biotechnology [1-4]. Among the various polymers used in hydrogel fabrication, chitosan, a natural polysaccharide derived from chitin, has gained prominence due to its biocompatibility, biodegradability and antimicrobial properties. Chitosan-based hydrogels offer a promising platform for a wide range of biomedical applications owing to their tunable properties and potential for the controlled release of therapeutics [5,6].

The swelling behaviour of hydrogels, a fundamental characteristic dictating their performance in practical applications, is influenced by several factors including polymer composition, crosslinking density and environmental conditions [7,8]. Understanding how hydrogels respond to changes in environmental parameters such as pH, temperature, and ionic strength is essential for tailoring their properties to specific applications. Moreover, the ability of hydrogels to swell or shrink in response to external stimuli is of particular interest for designing smart materials with stimuli-responsive behaviour [9,10].

Despite the growing interest in chitosan-based hydrogels, there remains a need for comprehensive studies investigating their swelling behaviour under diverse environmental conditions. Such studies are crucial for elucidating the mechanisms governing swelling kinetics, equilibrium swelling ratios and mechanical properties of these hydrogels, thus facilitating their rational design and optimization for targeted applications [11, 12].

In this study, we aim to explore the swelling behaviour of chitosan based hydrogels across a range of environmental conditions. By systematically varying parameters such as pH, ionic

strength and temperature, the impact of these factors on the swelling kinetics and equilibrium swelling ratios of the hydrogels can be elucidated. Additionally, the stimuli-responsive behaviour of chitosan-based hydrogels, shedding light on their potential for applications requiring on-demand drug release or control were also investigated. Through this exploration, we aspire to contribute the novel insights into the swelling behaviour of chitosan-based hydrogels and pave the way for their broader utilization in biomedicine and beyond. By elucidating the relationship between the environmental conditions and swelling behaviour, we can develop chitosan-based hydrogel systems with improved performance and the utility for specific applications.

EXPERIMENTAL

The crosslinking of chitosan graft copolymer with *N*-vinylpyrrolidone was carried out using a continuous ultraviolet irradiation method [13,14]. The reaction was conducted for 4 h under controlled conditions to ensure efficient crosslinking while minimizing undesired side reactions. Simultaneously, the synthesis of arabinogalactan (AQ), gummyarabic (QA), *N*,*N*-diethyl methyl (DEM), benzyl (B) and *N*-methyl-*N*-benzyl (MB) chitosan copolymers was achieved *via* a thermal method in situ. In brief, chitosan was dispersed in an appropriate solvent and vinyl monomers (acrylamide and quaternized acrylamide) were added to the reaction mixture. The reaction was then initiated by heating to the desired temperature, facilitating the grafting and cross-linking processes simultaneously. The effect of varying the amount of crosslinking agent, *N*,*N*′-methylene-bisacrylamide (MBAA), on the crosslinking efficiency and gel fraction yield was investigated. It was observed that increasing the MBAA concentration led to higher yields of gel fraction and improved crosslinking efficiency, indicating the importance of optimizing the crosslinker concentration for desired hydrogel properties.

RESULTS AND DISCUSSION

As shown in Table-1, the semi-synthetic copolymers (*N*,*N*-diethyl methyl (DEM), benzyl (B) and *N*-methyl-*N*-benzyl (MB) chitosan), the decrease in swelling ratio with increasing MBAA composition suggests that higher crosslinking densities are achieved as more *N*,*N*′-methylene-bisacrylamide (MBAA) is incorporated into the hydrogel network. This denser network restricts the uptake of water molecules, resulting in the reduced swelling capacity [15,16]. The presence of MBAA facilitates the formation of additional crosslinks between polymer chains, leading to a more compact and rigid hydrogel structure. Although, the natural and vinyl graft copolymers (chitosan-graft-QA, chitosan-graft-AQ and chitosan-graft-VPr) initially show an increase in swelling ratio with certain compositions of MBAA before reaching a peak and then decreasing. This behaviour may be attributed to the balance between crosslinking density and polymer flexibility. At lower MBAA concentrations, the presence of graft copolymers allows for more efficient crosslinking, resulting in increased swelling due to the formation of a loosely interconnected network with sufficient flexibility to absorb water. However, as the MBAA concentration continues to increase, the network becomes increasingly rigid, leading to a decrease in swelling capacity. Additionally, the observed differences in swelling behaviour highlight the importance of considering the composition and structure of both the polymer and crosslinking agent when designing hydrogels with tailored properties [17,18]. Understanding these relationships is essential for optimizing hydrogel performance for specific applications in areas such as drug delivery, tissue engineering and biomedical implants.

Effect of pH on swelling ratio of hydrogels: The pH of the environment plays a pivotal role in the transportation of medicinal drugs, necessitating a thorough examination of the swelling behaviour of the synthesized hydrogel samples under varying pH conditions. The swelling capacities of hydrogels produced by crosslinking chitosan with *N*-vinylpyrrolidone (VPr) alone, as well as the graft copolymer containing arabinogalactan (AQ) and gummyarabic (QA), were thoroughly investigated and are presented in Fig. 1.

Fig. 1 reveals that hydrogels formed from natural and vinyl monomer copolymers of chitosan exhibit substantial swelling in acidic environments (pH = 1-3). The increased swelling in acidic medium is caused by the high concentration of chitosan molecules, which make up 80 to 90% of the chain containing basic -NH_2 groups. These functional groups readily ionized in acidic mediums, with the hydration of carbonyl groups in the pyrrolidone cycle of the VPr molecule further contributing to this phenomenon [19]. Furthermore, graft copolymer-based hydrogels of chitosan with AQ and QA, rich in ionizable -OH, -COOH, -NH_2 and -CH_2OH groups, demonstrate increased swelling. Hydrogels crosslinked with 5% mass of AQ or QA exhibit significant higher swelling (240-265%) compared to those with vinyl monomer counterparts around pH 6. This is due to the presence of AQ and QA fragments that contain -COOH

TABLE-1
SWELLING RATIOS OF CHITOSAN DERIVATIVE HYDROGELS WITH VARYING MBAA COMPOSITION (T = 20 °C, V = 10 mL, t = 24 h)

Gels	MBAA (%)						
	1	3	5	10	15	20	30
Chitosan-graft-VPr	175	197	151	138	103	74	48
Chitosan-graft-QA	187	203	227	135	109	86	59
Chitosan-graft-AQ	201	213	249	154	125	108	74
Chitosan-graft-DEM	178	159	117	82	56	35	19
Chitosan-graft-MB	146	119	80	69	52	38	21
Chitosan-graft-B	106	83	71	58	37	23	12

VPr = *N*-vinylpyrrolidone; AQ = arabinogalactan; QA = gummyarabic; DEM = N,N-diethyl methyl; B = benzyl; MB = N-methyl N-benzyl

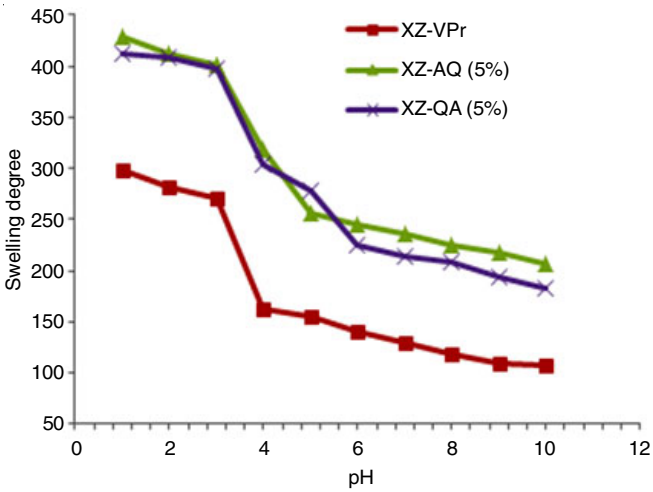

Fig. 1. Swelling ratios of chitosan derivative (QA, AQ and VPr) hydrogels with varying pH (T = 24 °C, V_{buffer} = 10 mL, t = 24 h)

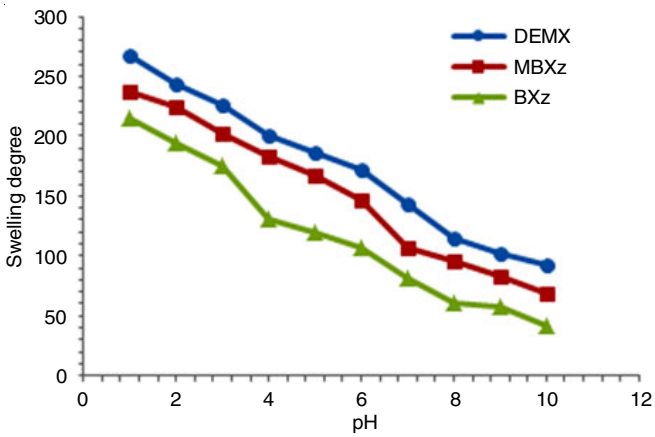

Fig. 2. Swelling ratios of N,N-diethyl, *N*-methyl *N*-benzyl and N-benzyl chitosan-based hydrogels with varying pH (T = 24 °C, V_{buffer} = 10 mL, t = 24 h)

groups in the structure of chitosan. Generally, all the hydrogel samples exhibit a swelling degree of 120-250% within the pH range of 6-8, rendering them suitable for transporting biologically active substances that exhibit biological activity within these pH ranges. Similar trends are observed in hydrogels derived from the graft copolymer synthesized with chitosan and VPr. Notably, the swelling degree of the gel remains consistent at pH values of 1-3, attributed to the incorporation of a weakly acidic pyrrolidone ring into the chitosan chain. This augmentation of hydrophilicity facilitates increased water molecule penetration and enhances the polarizability of functional groups within the gel [20].

The swelling behaviour of the semi-synthetic copolymers hydrogels as depicted in Fig. 2. In these samples, the presence of ethyl, methyl, and benzyl groups in the composition imparts hydrophobic properties, preventing their ionization and resulting in relatively lower swelling compared to other hydrogel formulations. Additionally, chitosan contains amino groups which can undergo ionization, with some of these groups being substituted by alkyl or benzyl groups *via* the Schiff reaction. Therefore, the protonation of nitrogen atoms by H^+ ions in acidic environments is spatially hindered due to the protection provided by methyl, ethyl and benzyl groups. The observed maximum swelling degree at 1% mass of the crosslinking agent is attributed to the presence of unsubstituted amine groups and free hydroxyl groups [21].

Fig. 2 illustrates the swelling behaviour of hydrogels obtained from crosslinking alkyl and benzyl derivatives of chitosan, which decreases consistently with the pH of the environment. This suggests a homogeneous structure composition primarily comprising amino and hydroxyl groups that ionized depending on the medium's acidity. Despite this, the hydrogel samples exhibit significant swelling across a wide pH range, making them suitable carriers for the separation process of medicinal substances not only in acidic environments but also in pH = 5-7 [22]. The release of drugs immobilized within the hydrogels into the medium is directly influenced by the structural properties of gel, the nature of medium and the kinetics of swelling under the studied conditions.

Kinetic study of swelling process: In Fig. 3a-b, the swelling behaviour of chitosan-graft-DEM and chitosan-graft-MB hydrogels reaches equilibrium after 12 h at 24 ºC and 9 h at 37 ºC, whereas chitosan-graft-B hydrogel achieves the equilibrium after 6 h at both temperatures. A linear relationship was observed within the first 6 h at 37 ºC across all hydrogel samples. Increasing the temperature to 37 ºC diminishes the extent of swelling in all the cases [23]. This phenomenon may be attributed to enhanced interaction between the gel's functional groups and water molecules due to increased macromolecular mobility.

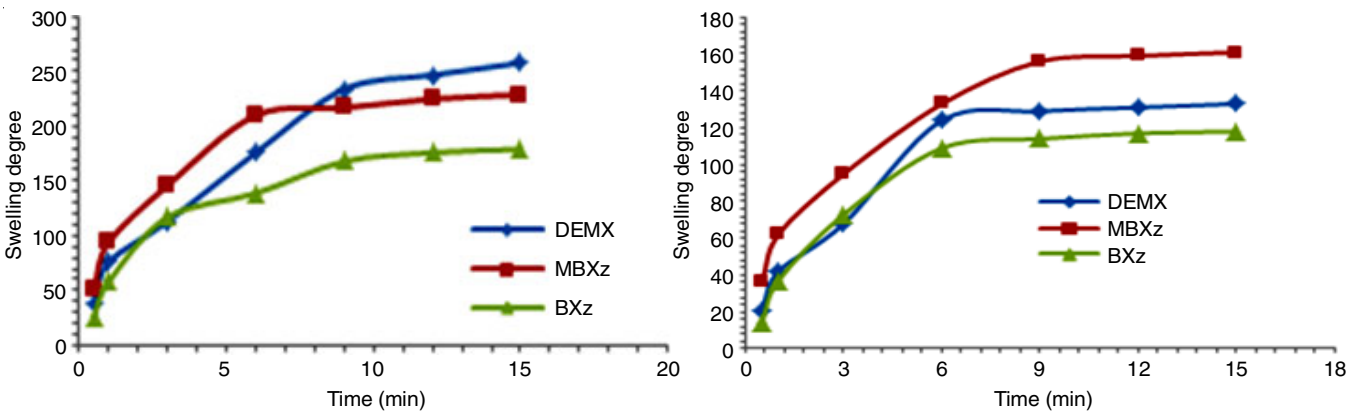

Fig. 3. Chitosan-based polymer hydrogel swelling kinetics under pH = 7 conditions, at both 24 and 37 °C, with a buffering volume of 10 mL

Moreover, the ionization of amine groups at 37 ºC results in the excess desorption of protons, thereby reducing electrostatic interactions within the gel pores.

At 24 ºC, the swelling rates gradually escalate over time, which is advantageous for drug delivery systems. However, excessively high swelling rates can lead to an undesirably rapid release of immobilized drugs initially [24]. On the other hand, a slight decrease in swelling rates to around 1.2-1.4 times at 37 ºC is considered acceptable for these systems. These findings underscore the sensitivity of the synthesized hydrogels to both pH and temperature variations, rendering them promising candidates for the drug carrier applications.

Fig. 4 illustrates the swelling behaviour of hydrogel samples, including chitosan-graft-VPr, chitosan-graft-QA and chitosan-graft-AQ, at pH 7 and two temperatures (24 ºC and 37 ºC), exhibiting the contrasting results compared to chitosan-graft, chitosan-graft-MB and chitosan-graft-B hydrogels. The presence of polar functional groups in the structure of chitosan is responsible for this variation. In terms of swelling kinetics, both chitosan-graft-QA and chitosan-graft-AQ hydrogels achieve swelling equilibrium within the initial 3 h. It was found that at pH 7, the ionizable functional groups within chitosan-based hydrogels exert the limited polarization compared to the acidic conditions, primarily due to the abundance of hydrogen bonds among amino groups, which hinder their ionization [25]. However, an elevation in temperature weakens these hydrogen bonds, facilitating the release of functional groups. Moreover, this phenomenon leads to a rapid and extensive penetration of ions or their hydrate forms into the internal pores of gel, particularly within the pH 7 medium.

For the chitosan-graft-VPr hydrogel, swelling equilibrium is reached after 1 h at 24 ºC, exhibiting a swelling degree of 170-180%. Conversely, at 37 °C, an equilibrium is achieved after 3 h, accompanied by a greater swelling degree of 240-250%. This discrepancy can be attributed to the increased freedom of macromolecular chains due to the high temperature, resulting in a higher concentration of polar functional groups. Thus, the molecules or their ionized forms can more readily form hydrogen bonds and electrostatic interactions with the functional groups of gel, thereby enhancing the degree of swelling [6]. The enhanced swelling behaviour plays a significant role in regulating the release of immobilized therapeutic compounds, depending on environmental inputs. From this perspective, the synthesized gel samples hold promise as matrices for the transport of various medicinal substances as well as the pH and temperature conditions of the surrounding environment where the immobilized drug exhibits activity.

Effect of glucose concentration: The effect of glucose on the swelling behaviour of chitosan-based hydrogel polymers in varying environments, particularly physiological saline (0.9% NaCl) and glucose solutions of different concentrations, over a 24-h period at both room temperature and body temperature (37°C) were conducted [26]. This investigation help to understand the influence of solution composition and temperature on the swelling characteristics of the synthesized hydrogel, which is depicted in Table-2. The results indicated that in the presence of physiological saline, there was a significant increase in the degree of swelling compared to distilled water, attributed to the enhanced polarity of functional groups induced by the addition of NaCl. Furthermore, an increased

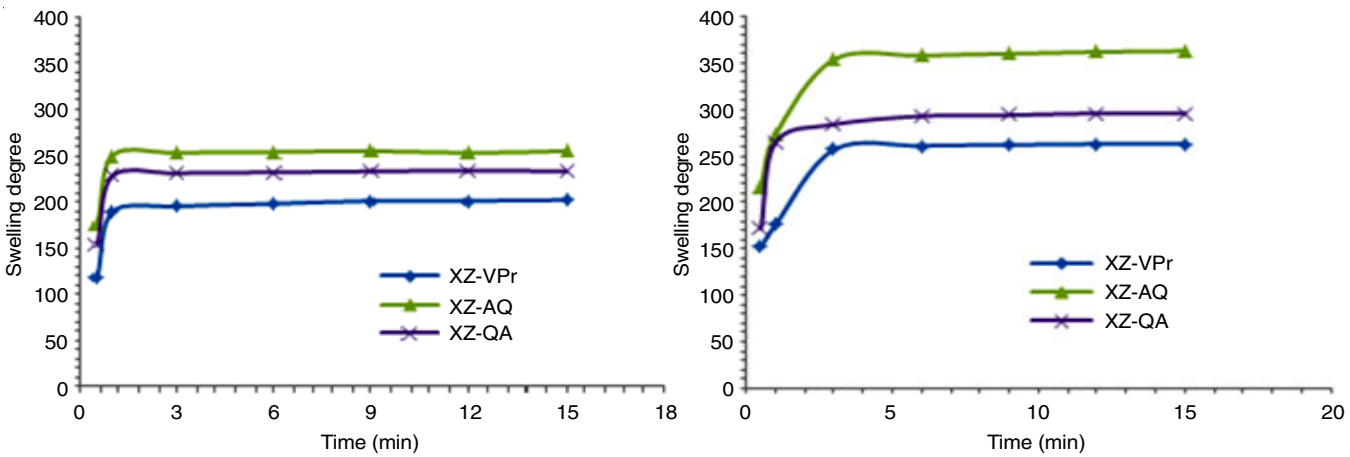

Fig. 4. Chitosan-based polymer hydrogel swelling kinetics under pH = 7 conditions, at both 24 and 37 °C, with a buffering volume of 10 mL

TABLE-2
SWELLING BEHAVIOUR IN CHITOSAN-BASED HYDROGEL POLYMERS EXPOSED TO PHYSIOLOGICAL AND GLUCOSE SOLUTIONS OF VARIOUS CONCENTRATIONS FOR 24 h, UNDER pH 7 CONDITIONS, WITH A VOLUME OF 15 mL

Gel samples	0.9% NaCl		0.1% $C_6H_{12}O_6$		1% $C_6H_{12}O_6$		10% $C_6H_{12}O_6$	
	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C	20 °C	37 °C
Chitosan-graft-VPr, 3% MBAA	216	238	154	172	134	167	78	113
Chitosan-graft-AQ,5% MBAA	263	291	225	251	204	238	137	158
Chitosan-graft-QA 5% MBAA	237	258	196	234	165	188	112	146
Chitosan-graft-DEM, 1% MBAA	186	208	151	172	139	152	83	117
Chitosan-graft-MB, 1% MBAA	163	193	135	152	117	141	71	98

VPr = *N*-vinylpyrrolidone; AQ = arabinogalactan; QA = gummyarabic; DEM = N,N-diethyl methyl; MB = N-methyl N-benzyl

in the temperature is correlated with the increased swelling, facilitated by the greater flexibility of polymer chains and enhanced penetration of ions into the gel pores, along with intensified hydrogen bonding between water molecules and functional groups [27]. Conversely, in glucose solutions of varied concentrations, the degree of swelling decreased with increasing density of the non-electrolyte. Glucose, unlike NaCl, does not polarize the functional groups of the gel, resulting in the swelling primarily driven by water molecule penetration into internal gel pores. Despite this, a modest increase in the swelling occurred with higher temperatures, attributed to the increased polymer chain relaxation.

Conclusion

In conclusion, this study elucidates the swelling behaviour of several chitosan-based hydrogels under diverse environmental conditions, shedding light on the important factors influencing their performance. The results in this study explore the importance of considering polymer composition, cross-linking density and environmental parameters in the design and optimization of hydrogel systems for specific applications. The observed swelling behaviour variation among different hydrogel formulations highlights the need for tailored approaches to meet desired properties. Moreover, the study reveals the potential of chitosan-based hydrogels for stimuli-responsive applications, offering opportunities for on-demand drug release and control. Thus, by enhancing the understanding of the relationship between environmental conditions and swelling behaviour, this research paves the way for the development of advanced hydrogel systems with enhanced functionality and performance in biomedicine and related fields.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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