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The study of gelation kinetics and chain-relaxation properties of glutaraldehyde-cross-linked chitosan gel and their effects on microspheres preparation and drug release

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Abstract

The effects of gelation kinetics and chain-relaxation properties of glutaraldehyde-cross-linked chitosan gel on microspheres preparation or drug release were studied. The rate of gelation is zero order corresponding to the chitosan concentration but non-zero order corresponding to the glutaraldehyde concentration. It was suggested that the cross-linking reaction was mainly dominated by the concentration of small molecule reactant, glutaraldehyde. The relaxation of an entangled polymer chain in a gel network as a result of the swelling of cross-linked chitosan hydrogel was investigated by the stress–strain determination. The higher the cross-linking density of chitosan hydrogel, the lower the swelling ability of chitosan hydrogel due to the slower relaxation rate of polymer chain, which then results in the decreased drug-release rate.

Keywords: Chitosan; Glutaraldehyde; Gelation kinetics; Chain-relaxation; Microsphere; Drug-release

1. Introduction

Chitosan, poly[β-(1-4)-linked-2-amino-2-deoxy-o-glucose], is produced from chitin which is naturally abundant in the marine crustacean. Chitosan could be cross-linked using glutaraldehyde (Muzzarelli, Barontini & Rocchetti, 1976). In the recent years, cross-linked chitosan microspheres has been investigated to be used as a drug delivery system for anticancer drug, such as 5-fluorouracil (5-FU), cisplatin (CCDP), oxantrazole (OXZ) etc., to achieve improved drug therapies (Hassan, Parish & Gallo, 1992; Jameela & Jayakrishnan, 1995; Nishioka et al., 1990; Thanoo, Sunny & Jayakrishnan, 1992). It was reported that the drug-release properties of dosages were affected by cross-linked polymeric characteristics (Jameela, Kumary, Lal & Jayakrishnan, 1998).

To prepare chitosan gel beads or microspheres for the use as drug-delivery systems, the cross-linking characteristics of chitosan should be investigated prior, in order to understand which condition dominated the gelation. The gelation properties of the cross-linked microspheres or beads have significant effect on the drug incorporation. In addition, the rate of drug release from chitosan hydrogel was also highly influenced by the chain-relaxation properties of the cross-linked network after being swollen in the dissolution medium. Due to these reasons, it is necessary to identify gelation and chain-relaxation properties of glutaraldehyde cross-linked chitosan gel.

In this study, the gelation kinetics and chain-relaxation characteristics of glutaraldehyde cross-linked chitosan were examined by measuring the viscosity variation or by the strain–stress determination of the chitosan gels. Finally, the preparation and drug-release properties of cross-linked chitosan microspheres were characterized to examine their relation with gelation or the chain-relaxation of chitosan gel.

2. Experimental

2.1. Materials

Chitosan with various molecular weights were obtained from Fluka Co., Switzerland. The molecular weight of chitosan are 70 000 and 2 000 000. Glutaraldehyde (25% in aqueous) was purchased from Merck Co., Germany.

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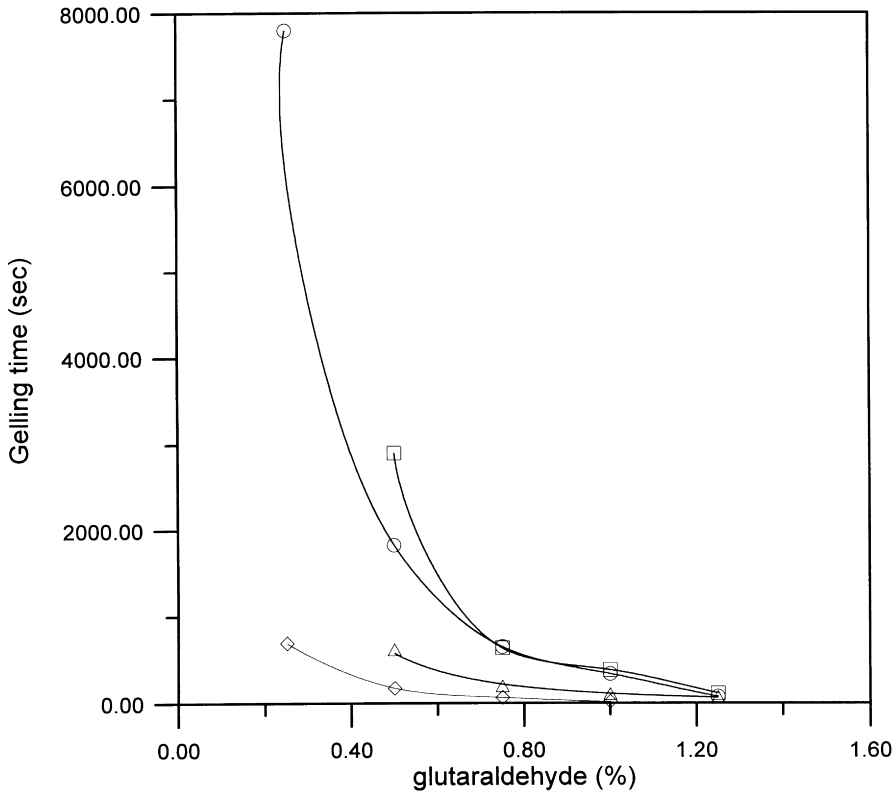

Fig. 1. Gelling time vs. glutaraldehyde concentration. ○: 1.0 wt% of high molecular weight of chitosan (M_w: 2 000 000) at 18°C; □: 1.0 wt% of low molecular weight of chitosan (M_w: 70 000) at 18°C; ◇: 1.0 wt% of high molecular weight of chitosan (M_w: 2 000 000) at 30°C; △: 1.0 wt% of low molecular weight of chitosan (M_w: 70 000) at 30°C.

2.2. Gelation study

The viscosity changes in the chitosan solution were monitored during gelation using a Brookfield viscometer (Model DVII$^+$). The solution was maintained at a constant temperature and the viscosity measured using the number 25 spindle at 60 rpm to give the lowest shear conditions. Every typical run shows an initial constant low-viscosity portion followed by a rapid rise in viscosity. The value obtained by extrapolation of the initial linear portion back to the time axis was taken as a measure of time to the onset of gelation.

2.3. Stress–strain determination

The chitosan solution mixed with glutaraldehyde was cast into the steel tank at room temperature, and then vacuum dried at 40°C for 24 h. The resultant film was peeled from the tank. The membranes (thickness 100–120 μm) were stored in a desiccator at room temperature. The cross-linked chitosan membranes were slid to shape according to the ASTM standard. The stress–strain profiles of the cross-linked chitosan membranes were determined by using a Tensilon (Instron). The various membranes were moistened at room temperature, 80% relative humidity for 3 h to simulate the polymer chain-relaxation behaviour in a wetting environment. The cross-head speed was 5 mm/min. At least five measurements were performed, and the mean was obtained for the films.

2.4. Preparation of cross-linked chitosan microspheres

The anticancer, 6-MP (0.1 mg), was added to a 20 ml of chitosan solution (1.5 wt%) and was mixed well. The suspension was dispersed into 100 ml of soybean oil. The mixture was stirred at 150 rpm with a mechanical stirrer (IKA, RAW 20) for 30 min to form water in oil (w/o) dispersion. Later, glutaraldehyde was added drop-wise slowly into the medium and then further cross-linked for 2 h. The microspheres formed were collected by centrifugation at 3000 rpm for 10 min using a high-speed centrifuge (HERMLE, ZK 365). After the upper layer was removed by decanting, the microspheres were rinsed with acetone and ethyl ether twice and then dried in air for 12 h.

2.5. Dissolution studies

The release of 6-MP from cross-linked chitosan gel beads was measured using the dissolution (Hanson research, Dissoette II) and autosampling (Hanson research, SR6) systems. The dissolution medium was a 500 ml deionized water. The medium was placed in a 1 l round flask fitted with a pump for autosampler to remove the medium and stirred with a mechanical stirrer at a rate of 100 rpm. The

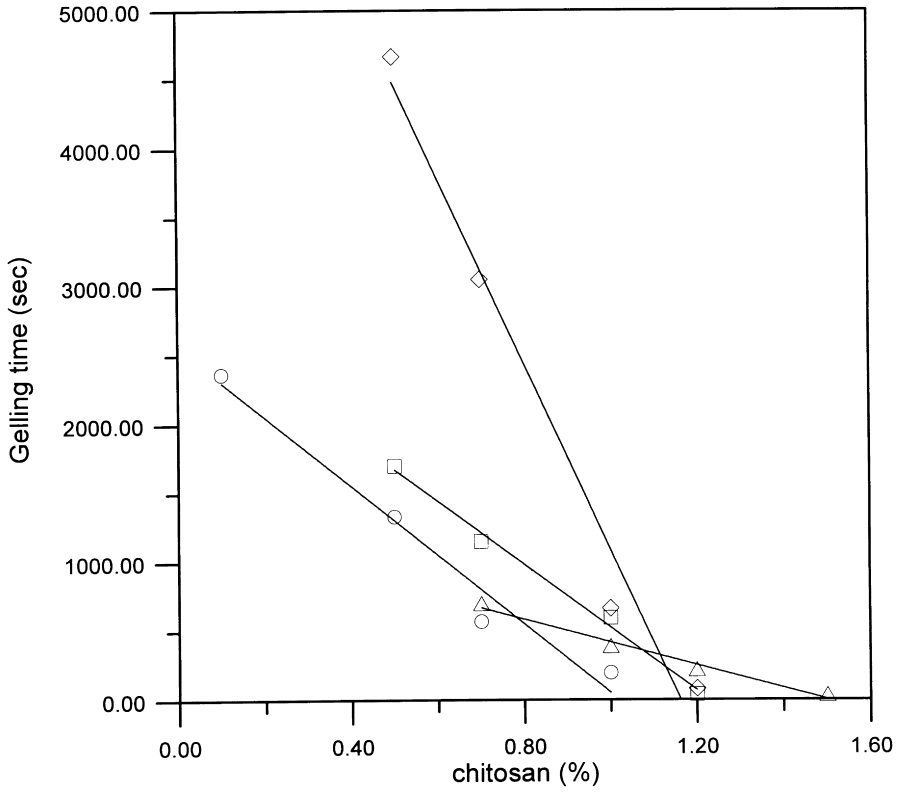

Fig. 2. Gelling time vs. chitosan concentration using 0.5 wt% of glutaraldehyde. ◇: high molecular weight of chitosan (M_w: 2 000 000) at 18°C; △: low molecular weight of chitosan (M_w: 70 000) at 18°C; □: high molecular weight of chitosan (M_w: 2 000 000) at 30°C; ○: low molecular weight of chitosan (M_w: 70 000) at 30°C.

dissolution medium temperature was maintained at 37°C. An equivalent quantity of 100 mg gel beads was dispersed in the dissolution medium. After a predetermined period, 5 ml of the medium was removed and the amount of 6-MP was analyzed spectrophotometrically at 360 nm. In order to maintain the original volume, each time 5 ml of the medium was replaced with fresh water.

2.6. Electron scanning microscopy

The cross-linked chitosan gel beads were gold coated to about 500×10^{-8} cm thickness using an IB-2 coater Hitachi coating unit under a high vacuum, 0.1 Torr; high voltage, 1.2 kV and 50 mA. Coated samples were examined using a Hitachi S-2300 electron scanning microscopy.

3. Result and discussion

3.1. Kinetics and characteristics of gelation

The gelation mechanism of chitosan is significantly dependent on the cross-linking conditions. It was interesting that the time to the onset of gelation decreased non-linearly with the increase of aldehyde, but the time decreased linearly with the increase of chitosan concentration. These results indicate that the kinetics of chitosan cross-linked by glutaraldehyde is zero order with respect to the chitosan concentration while non-zero order with respect to the glutaraldehyde concentration (Figs. 1 and 2). The time to the onset of gelation decreases from 2900 to 1800 s by increasing the molecular weight of chitosan from 70 000 to 2 000 000 for 0.25% glutaraldehyde maintained at 18°C (Table 1). The chitosan with a higher molecular weight and thus larger size should approach the limit faster than the lower molecular weight chitosan because large size units join together by cross-linking. The influence of temperature variation toward the time to the onset of gelation was obvious. The time to the onset of gelation reduced as the temperature increased from 18 to 30°C. Since the rate to the onset of gelation is zero order with respect to the concentration of chitosan, rate constant (k) of the gelation reaction could be calculated by an integrated rate law keeping the concentration of glutaraldehyde at constant but varying the reaction temperature (T). A plot of $\ln(k)$ vs. $1/T$ should enable the activation energy of gelation to be calculated. A linear plot was obtained with a slope of -5977.24 or $-7869.58\ K^{-1}$, given an activation energy of 49.6 or 64.7 kJ mol^{-1} for high molecular weight or low molecular weight of chitosan, respectively.

3.2. Stress–strain determination of mechanical properties

The mechanical properties of the elongation moulded chitosan membrane showed a strong dependence on

Table 1
The effect of variation in chitosan or glutaraldehyde concentration, molecuar weight of chitosan and reaction temperature on the time to onset of gelation

Chitosan		Concentration of glutaraldehyde (wt%)	Temperature (°C)	Gelation time (s)
M_w	Concentration (wt%)			
70 000	0.7	0.5	18	700
70 000	1.0	0.5	18	400
70 000	1.2	0.5	18	230
70 000	1.5	0.5	18	30
2 000 000	0.5	0.5	18	4660
2 000 000	0.7	0.5	18	3050
2 000 000	1.0	0.5	18	610
2 000 000	1.2	0.5	18	80
70 000	0.1	0.5	30	2360
70 000	0.5	0.5	30	1330
70 000	0.7	0.5	30	570
70 000	1.0	0.5	30	195
2 000 000	0.5	0.5	30	1720
2 000 000	0.7	0.5	30	595
2 000 000	1.0	0.5	30	160
70 000	1.0	0.25	18	2900
70 000	1.0	0.5	18	635
70 000	1.0	0.75	18	385
70 000	1.0	1.25	18	115
2 000 000	1.0	0.05	18	7800
2 000 000	1.0	0.25	18	1825
2 000 000	1.0	0.5	18	650
2 000 000	1.0	0.75	18	340
2 000 000	1.0	1.25	18	75
70 000	1.0	0.25	30	620
70 000	1.0	0.5	30	200
70 000	1.0	0.75	30	110
70 000	1.0	1.25	30	70
2 000 000	1.0	0.05	30	690
2 000 000	1.0	0.25	30	175
2 000 000	1.0	0.5	30	65
2 000 000	1.0	0.75	30	18

cross-linking and water containment. The stress–strain profiles of membrane prepared from low (M_w: 70 000) or high (M_w: 2 000 000) molecular weight of chitosan cross-linked with 0.25–1.25% of glutaraldehyde were shown in Fig. 3. Both low and high molecular weight of chitosan membrane display decreased tensile elongations with increase in the concentration of glutaraldehyde, especially for a high molecular weight of chitosan membrane. The cross-linked membrane has a low percentage elongation at break but with a high stress at break (σ_b), exhibiting a brittle character as a result of the increased number of junction zones arising from the increased cross-linking density. The apparent increase in the cross-link:chain ratios significantly decreased the chain-relaxation ability of the polymeric network. The mechanical properties of the membranes swollen in a humidity environment have a significant variation on the stress–strain behaviour (Fig. 4). All membranes swollen in a 80% relative humidity for 3 h have a clear yield point and an extended elongation at break, except for the highest concentration (0.75% of glutaraldehyde) treated chitosan membrane. The results give an evidence to the point that swelling could induce chain relaxation same as the glass–rubber transition, and the increased cross-linking density of membrane would decrease the chain-relaxation ability of the network.

3.3. Characteristics of glutaraldehyde-cross-linked chitosan microspheres related to gelation and mechanical studies

The homogeneous cross-linking of the spherical chitosan microspheres was carried out by the suspension cross-linking method. Parameter studies such as concentration of chitosan or glutaraldehyde and temperature were examined to confirm the investigation of viscosity study for gelation. Table 2 demonstrates the gelation properties of the prepared chitosan microspheres. At the low concentration of glutaraldehye used, the non-gelled chitosan microspheres aggregated as a result of the slow cross-linking rate of chitosan gel (Fig. 5(a) and (b)). While using a low concentration of chitosan, shrunk but non-spherical chitosan microspheres were obtained (Fig. 5(d)). Keeping the concentration of

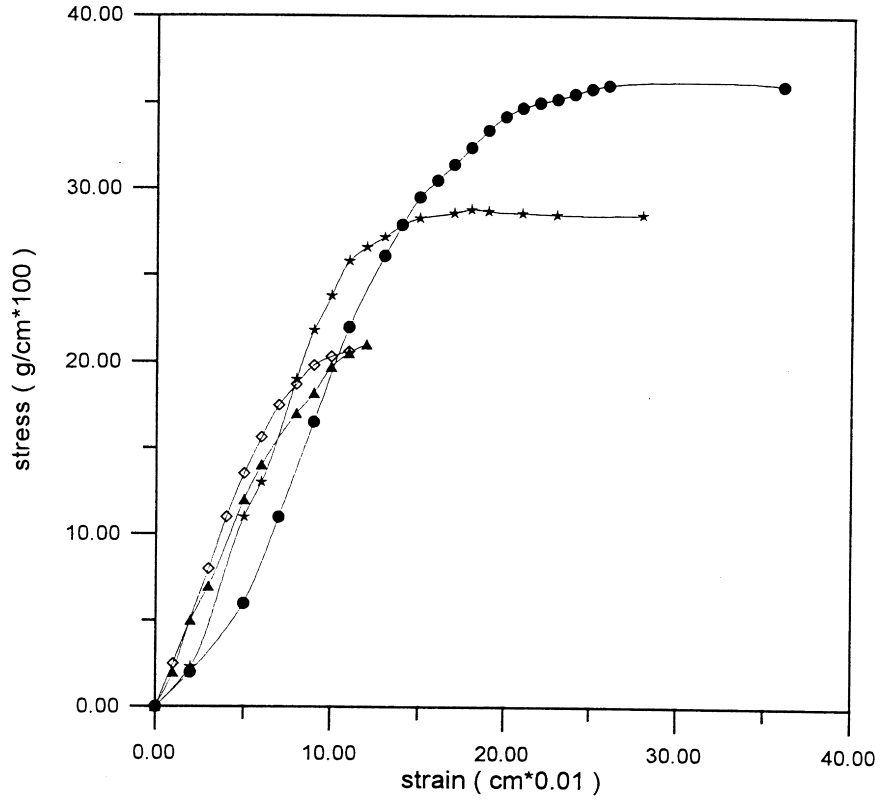

Fig. 3. The effect of glutaraldehyde-cross-linked density on stress–strain profile of non-swollen chitosan membrane. Glutaraldehyde concentration: ●, 0 wt%; ★,0.25 wt%; ◇, 0.5 wt%; ▲, 0.75 wt%.

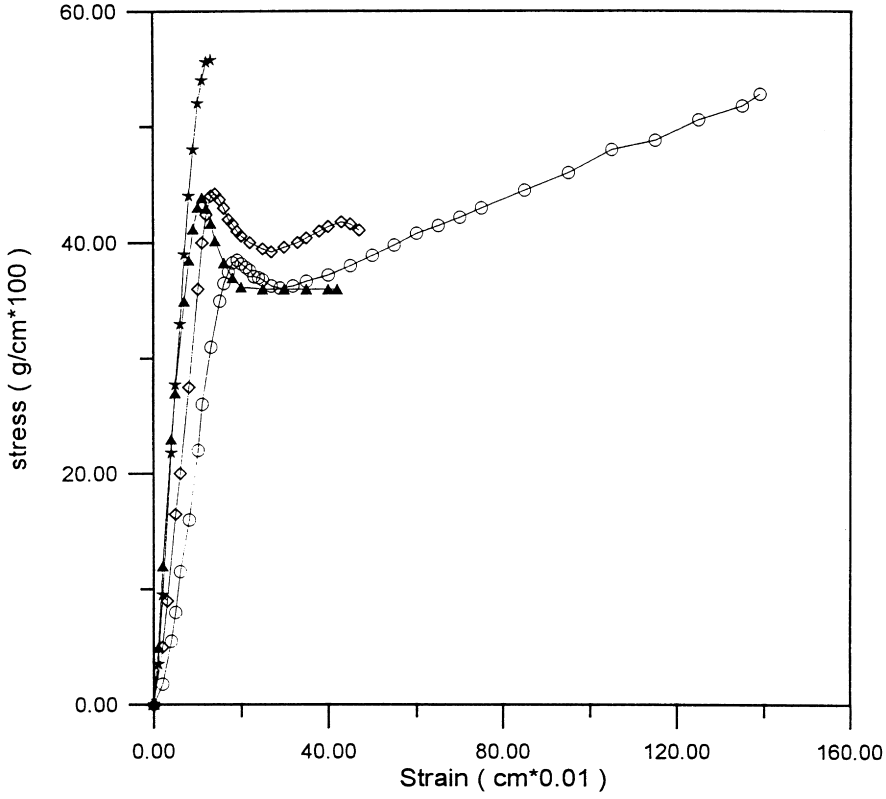

Fig. 4. The effect of glutaraldehyde-cross-linked density on stress–strain profile of swollen chitosan membrane (swollen in a 80% relative humidity for 3 h). Glutaraldehyde concentration: ○, 0 wt%; ◇, 0.25 wt%; ▲, 0.5 wt%, ★, 0.75 wt%.

Table 2
The effect of variation in chitosan or glutaraldehyde concentration, molecuar weight of chitosan and reaction temperature on the morphology of microspheres (at a constant reaction time for 2 h)

Chitosan		Concentration of glutaraldehyde (wt%)	Temperature (°C)	Morphology
M_w	Concentration (wt%)			
70 000	0.1	0.5	30	unformed
70 000	0.5	0.5	30	shrunk
70 000	0.7	0.5	30	shrunk
2 000 000	0.5	0.5	30	shrunk
2 000 000	0.7	0.5	30	shrunk
2 000 000	1.0	0.5	30	spherical
70 000	1.0	0.25	30	aggregated
70 000	1.0	0.5	30	slightly aggregated
70 000	1.0	0.75	30	spherical
70 000	1.0	1.50	30	brittle
2 000 000	1.0	0.05	30	aggregated
2 000 000	1.0	0.25	30	aggregated
2 000 000	1.0	0.75	30	spherical

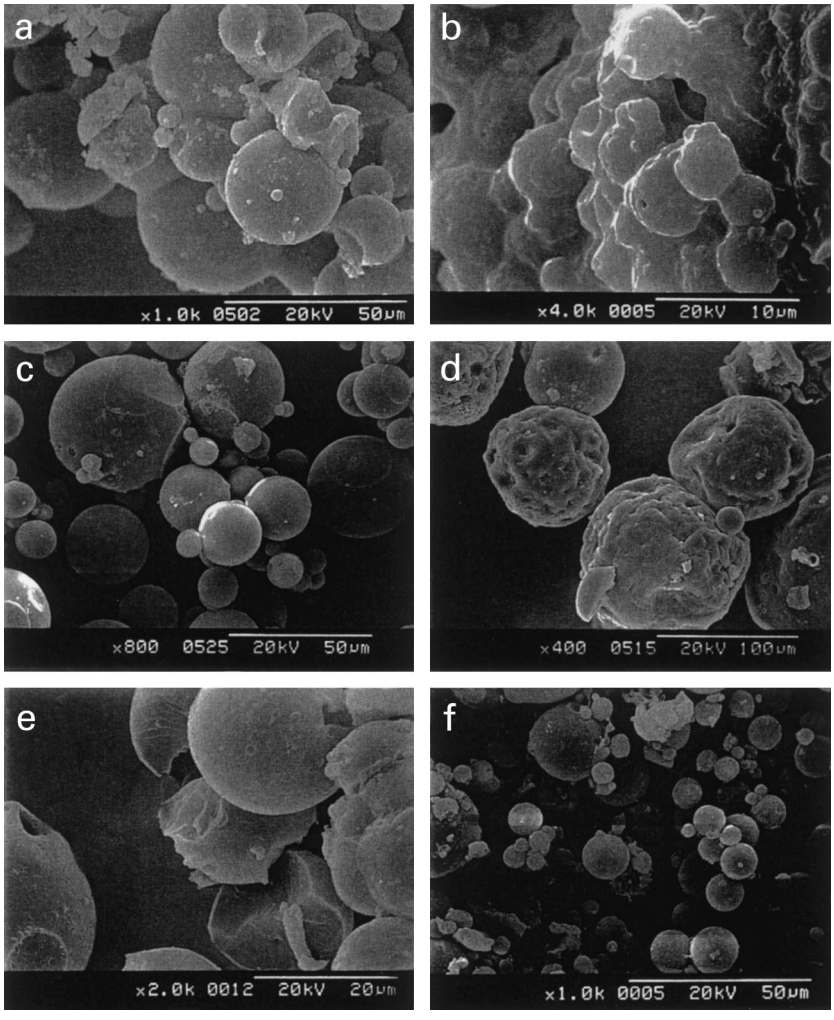

Fig. 5. The effect of gelation properties on the preparation of chitosan microsphere: (a) slightly aggregated chitosan microspheres prepared by 0.5 wt% of glutaraldehyde; (b) aggregated chitosan microspheres prepared by 0.25 wt% of glutaraldehyde; (c) spherical chitosan microspheres prepared by 1.0 wt% of glutaraldehyde using high molecular weight of chitosan (M_w: 2 000 000); (d) shrunk chitosan microspheres prepared by 0.7 wt% of chitosan; (e) brittle chitosan microspheres prepared by 2.0 wt% of glutaraldehyde; and (f) spherical chitosan microspheres prepared by 1.0 wt% of glutaraldehyde using low molecular weight of chitosan (M_w: 70 000).

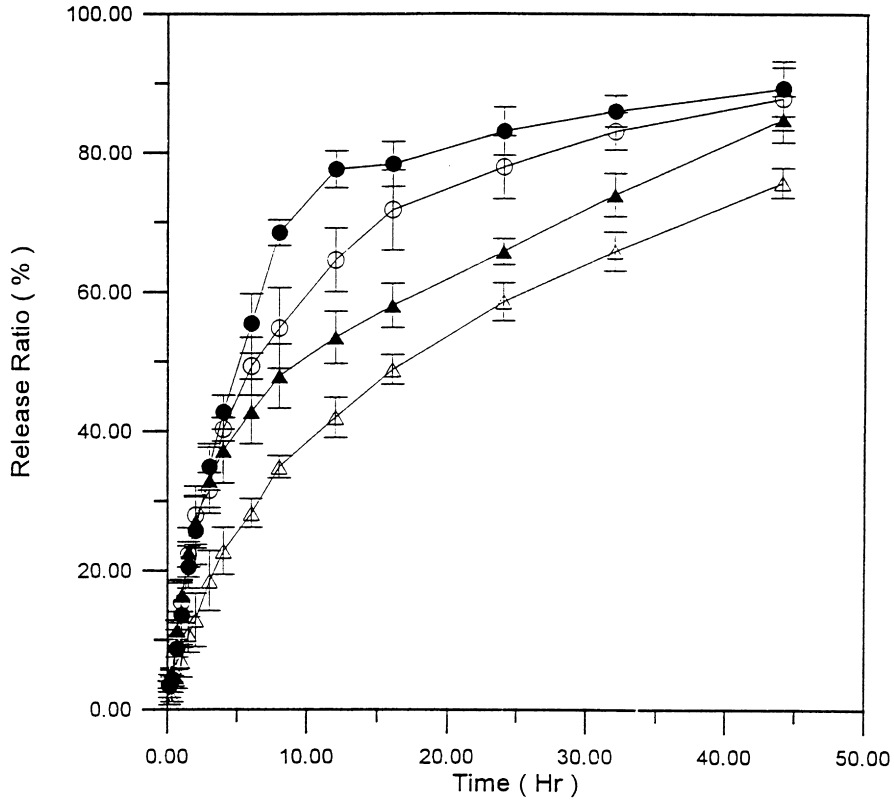

Fig. 6. The effect of glutaraldehyde-cross-linked density on drug-release characteristics of chitosan microsphere. ○, cross-linking for 2 h and △, cross-linking for 24 h at M_w: 2 000 000; ●, cross-linking for 2 h and ▲, cross-linking for 24 h at M_w: 70 000.

chitosan and glutaraldehyde at constant, microsphere prepared from a high molecular weight of chitosan gelled quicker than that prepared from a low molecular weight. This result suggested that the formation rate of glutaraldehyde-cross-linked chitosan microspheres were related to the activation energies of gelation. Chitosan microspheres prepared from a low molecular weight of chitosan have a slow formation rate due to their higher activation energies for gelation.

As shown in SEM pictures, higher cross-linked chitosan beads seem to be easily cracked due to its brittle characteristic (Fig. 5(e)). This result is similar to that of previous studies of stress–strain analysis for cross-linked membrane. Drug release of the slightly cross-linked chitosan gel beads was significantly quicker than that of the highly cross-linked chitosan gel beads due to their higher chain-relaxation ability (Fig. 6). The diffusivity of the penetrant diffusing through the swollen rubbery phase could be of the order 10^3–10^5 higher than that of the penetrant diffusing through the glass region. There are relative mobilities of the penetrating solvent and the drug in the presence of macromolecular relaxation in swollen hydrogel. Thus, a faster drug-release rate was obtained from the highly swollen chitosan microspheres. This result indicated that the higher the cross-linking density of chitosan microspheres, the lower the swelling ability due to the slower relaxation rate of the polymer chain, which results in the decreased drug-release rate.

4. Conclusion

This work determined the gelation kinetics and chain-relaxation properties of glutaraldehyde-cross-linked chitosan gel and their effects on microspheres preparation and drug release. From the results of this study, it could be concluded that gelation reaction was mainly dominated by the concentration of small molecule reactant, glutaraldehyde. The higher the cross-linking density of chitosan hydrogel, the lower the swelling ability of chitosan hydrogel due to the slower relaxation rate of the polymer chain, which results in the decreased drug-release rate.

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