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Research Article

The Effect of Polymer Molecular Weight on Citrate Crosslinked Chitosan Films for Site-Specific Delivery of a Non-Polar Drug

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Abstract

Purpose: *To develop citrate crosslinked chitosan films using chitosan of different molecular weights (MW) in order to achieve site-specific delivery of a model non-polar drug, indomethacin.*
Methods: *Films prepared with different molecular weights of chitosan and incorporating indomethacin as a non-polar model drug were obtained by a casting/solvent evaporation method. The chitosan films were crosslinked by dipping in varying concentrations of sodium citrate solution and for different crosslinking times. The films were assessed by, amongst others, scanning electron microscopy (SEM), dissolution studies and differential scanning calorimetry (DSC) for surface morphology, drug release and ingredient compatibility, respectively.*
Results: *Crosslinking time and concentration of crosslinking agent significantly ($p < 0.05$) influenced the in vitro release of indomethacin as well as swelling of the films. Also, the higher the molecular weight (MW) of chitosan the lower the drug release rate ($p < 0.05$). Furthermore, film swelling index rose as chitosan MW decreased ($p < 0.05$). The practical absence of the sharp endothermic peak characteristic of indomethacin in the films suggests that crosslinking may have transformed the drug from the crystalline to the amorphous state.*
Conclusion: *The citrate-crosslinked chitosan films can be modulated to vary swelling and drug release at pH 3.5 and 6.2; this feature makes them useful tools for designing site-specific delivery systems.*

Keywords*: Chitosan film, Sodium citrate, Site-specific delivery, Crosslinking, Indomethacin*

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INTRODUCTION

A major feature of medicines development today is the design of suitable vehicles for drug delivery. Delivery vehicles for therapeutic compounds must be biocompatible and nontoxic [1]. Many of these systems are pH/thermo-sensitive or osmotically driven [2]. Thin polymer films have also been employed to control drug release for various routes of administration, such as transdermal, colonic, localized, and ophthalmic. Mucoadhesive polymeric film formulations can circumvent limitations caused by conventional vaginal formulations such as tablets and creams [3]. For example, the release of paclitaxel from PVP-g-PLGA film was found to be continuous and not preceded by initial drug burst [4].

Time- or pH-dependent polymers are often used to target delivery of therapeutic substances to sites of action [5]. Several studies have associated drug release kinetics with the type of drug and device geometry. For instance, Klose *et al* studied these factors in PLGA-based drug delivery systems [6] while results from other studies indicate that polymer films are promising carriers for ophthalmic, localized and transdermal drug delivery systems [7]. Various polymethacrylate films have been evaluated for insulin colonic drug delivery [8].

Chitosan [α (1-4) 2-amino-2-deoxy-beta-D-Glucan] is the deacetylated derivative of chitin. It has recently attracted much interest in the biomedical industry because of its biodegradability, biocompatibility, antimicrobial activity, and accelerated wound healing properties [9]. Due to its unique polymeric cationic character as well as gel and film forming properties, chitosan has been extensively examined for various pharmaceutical applications. Several drug delivery formulations based on chitosan, such as film, beads, microspheres, etc, have been prepared using chemical cross-linking methods. For example, Emmanuel *et al* demonstrated the *in vitro* and *in vivo* zero-order drug release properties and biocompatibility of a chitosan-based implantable film and its potential usefulness in the sustained and local delivery of anti-neoplastic agents such as paclitaxel [10].

When chitosan is dissolved in dilute acetic acid solutions, the amino groups become protonated and associated with acetate counter-ions, making the charged polymer soluble [11]. Chitosan films have been usually prepared by chemical crosslinking via electrostatic interaction between multivalent phosphates and chitosan in the formulation [12]. Chitosan crosslinked films swell under acidic conditions due to the ionization of amino groups but remains in a shrunken state under neutral conditions. Chitosan itself has intragastric-floating characteristics and can cause prolonged retention of the dosage form in the stomach [13].

Controlled release of ciprofloxacin hydrochloride from chitosan/polyethylene glycol blend films produced by the casting-solvent evaporation method has been evaluated [14]. It has also been shown that due to electrostatic interaction between sodium citrate and chitosan, citrate/chitosan film may be useful in drug delivery such as site–specific controlled release in stomach and also as epidermal sheets for humans, rats, and rabbits [15]. Polymer molecular weight has a major effect on chitosan properties [16], and it has also been shown that the thermo-sensitive characteristics, appearance and structure of the hydrogel as well as drug release from it are affected by chitosan MW and degree of deacetylation [17]. In this paper, we sought to prepare citrate-crosslinked chitosan films using three different molecular weights (MWs) of chitosan and investigated whether or not chitosan MW affects some properties of the indomethacin-containing films.

EXPERIMENTAL

Materials

Indomethacin (1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid), produced by Medichem, Spain, was used as a model hydrophobic drug. Low, medium and high molecular weight grades of chitosan (85 % deacetylation) were purchased from Fluka AG, Switzerland while sodium citrate (analytical grade) and other reagents were purchased locally and used as received.

Turbidimetric titration

The interaction between sodium citrate and chitosan was investigated by turbidimetric titration [18]. Briefly, a solution of 0.2 g/L sodium citrate and 0.2 g/L chitosan at pH 1.0 was prepared. The titrant (0.2M NaOH) was delivered with a microburette into 50 ml of the citrate/chitosan solution with constant stirring at 25 ± 0.5 °C and with the pH monitored by a digital pH meter (Metrohm 780, Switzerland). Changes in turbidity were monitored at 420 nm with an UV-Vis spectrophotometer (Genesys TM2, USA and reported as 100 % transmittance.

Preparation of crosslinked chitosan films

Chitosan solution (4 %w/v), was prepared by dissolving chitosan in 4 %w/v acetic acid and then indomethacin (1 %w/v) was dispersed in the solution. This was done for each MW grade of chitosan. The mixture (100 ml) was left to stand until trapped air bubbles disappeared and then was poured on a glass plate. The poured solution was allowed to dry in a hot air oven (Pars Azma 1597) at 37 °C to constant weight. The resulting dry films were cut into 4 cm diameter disks and crosslinked soaking in 100 ml aqueous solution of sodium citrate 4 °C. Other crosslinking conditions were: 0.5 - 10 %w/v sodium citrate; solution pH of 5 - 7; and crosslinking time of 0.5 - 4.0 h. The crosslinked films were then rinsed in 20 ml of distilled water, transferred to a glass plate and oven-dried at 37 °C to a constant weight. Drug loss during the crosslinking process was determined spectrophotometrically at 318 nm.

Measurement of film thickness

The thickness of the dried films was measured with a micrometer (model 2050-08, Mitutoyo, Japan). Five measurements were taken at randomly selected points on each specimen and the mean thickness taken.

Morphological studies

The surface morphology of the crosslinked films was assessed by scanning electron microscopy (model 2360, Leo Oxford, England) after the films were first coated with gold.

Evaluation of swelling ratio

Blank citrate/chitosan films were suspended in glass bottles containing 250 ml of medium (either phosphate buffer, pH 6.2, or acetate buffer, pH 3.5) and placed in a shaker water-bath (at 37 °C, 50 rpm). At 30 min intervals, the films were taken out, the excess water blotted out carefully with filter paper from the film surface and then weighed immediately. The swelling ratio (W_t/W_0) was determined (where W_t was the film weight at time t and W_0 was the initial film weight).

Release studies

Indomethacin release from the films was evaluated by USP dissolution method V (paddle over disk method) in 750 ml phosphate buffer (pH 6.2) or acetate buffer (pH 3.5) at 37 °C and at a stirring rate of 50 rpm, using Erweka dissolution tester (model DT 80). Samples (5 ml) were withdrawn from the medium at various time intervals and the medium replenished immediately with the same volume of fresh dissolution medium. The amount of dissolved indomethacin in the sample taken was measured (after filtration) spectrophotometrically at 318 nm.

Indomethacin concentration in each sample was calculated according to Eq 1

$$Cn = Cnmass + \frac{V}{Vt}\sum_{s=1}^{n-1} Cmass \quad \ldots\ldots\ldots\ldots\ldots (1)$$

where Cn = true concentration of sample, Cnmass = apparent concentration of sample, V = volume of sample, and Vt = total volume of dissolution fluid.

Differential scanning calorimetry (DSC)

Differential scanning calorimetric studies were conducted in a Perkin Elmer DSC facility,using a sample size of approx. 5 mg in a loosely covered aluminum pan and heated in nitrogen atmosphere from 50 to 250 ℃ at a rate of 10℃/min. An empty loosely covered aluminum pan was used as the reference.

Statistical analysis

Experimental results were expressed as mean ± standard deviation (SD). Student's *t-test* and one-way analysis of variance (ANOVA) were applied to check significant differences in drug release, and swelling studies for different film formulations. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS

Film thickness and drug content

Table 1 shows the mean thickness and drug content of the films prepared at varying combinations of crosslinking concentration and time. The results show that there was no significant difference ($p > 0.05$) between the films in terms of film thickness or drug content.

Citrate – chitosan interaction

The turbidimetric titration curve of sodium citrate/chitosan (not shown) indicated that in acidic conditions (pH 1 - 4), the solution was clear; thereafter, it became turbid as the pH further rose but reverted to a clear solution (i.e., no interaction) at pH ≥ 7.

Table 1: Mean thickness and drug content of crosslinked chitosan films

Film code	C_t (h)	C_c (%)	Film thickness (µm, n=5)	Drug content (%)
Low MW chitosan				
F1	-	-	36.3±1.2	100.0
F2	0.5	0.5	40.0±0.8	100.0
F3	0.5	10	36.7±0.5	98.5
F4	4.0	0.5	39.3±1.7	98.5
F5	4.0	10	33.3±1.2	98.6
Medium MW chitosan				
F6	-	-	38.3±1.7	100.3
F7	0.5	0.5	33.3±1.2	100.0
F8	0.5	10	43.3±1.2	99.1
F9	4.0	0.5	41.7±1.7	99.0
F10	4.0	10	34.7±1.7	99.4
High MW chitosan				
F11	-	-	34.7±1.2	98.7
F12	0.5	0.5	35.0±1.6	99.3
F13	0.5	10	39.7±0.9	98.4
F14	4.0	0.5	37.0±1.4	98.1
F15	4.0	10	38.3±0.9	98.6

Film appearance

SEM photomicrographs (not shown) indicate that the inner surface of the films (which interfaced with the glass plate) was smooth while the outer surface was relatively rough. Crosslinking hardly had any noticeable effect on the surface morphology of the films. However, all the films were yellowish cream in colour, with the colour deepening and film texture becoming more tender with increase in crosslinking time.

Swelling ratio

The swelling data are shown in Fig 1. They indicate that at pH 6.2, film swelling for all three chitosan MW grades was significantly ($p < 0.05$) affected by crosslinking time. All the MW grades showed higher swelling in acid conditions (pH 3.5) than at pH 6.2. Furthermore, at pH of 3.5, swelling decreased as MW of chitosan rose.

Drug release studies:

Fig 2 shows that indomethacin was released rapidly from the film matrix at pH 3.5. Drug release was independent of either crosslinking time or concentration ($p > 0.05$). The results also show that drug release rate decreased significantly at pH 3.5 ($p < 0.05$) as chitosan MW increased. On the other hand, at pH 6.2, as Fig 3 indicates, drug release was significantly decreased by cross linking time and/or concentration of crosslinking agent ($p < 0.05$). The greater the crosslinking time, the lower was the drug release rate

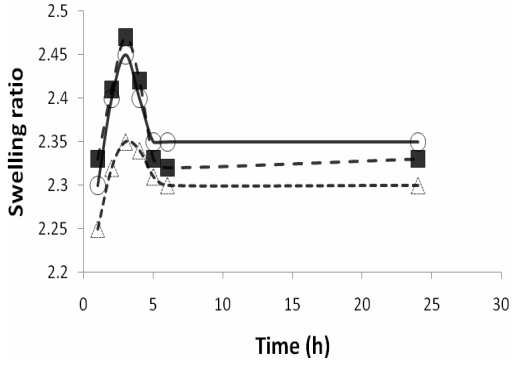

Fig 1: Swelling ratio of chitosan films (○ = LMW, ■ = MMW, ∆ = HMW) in pH 3.5 medium

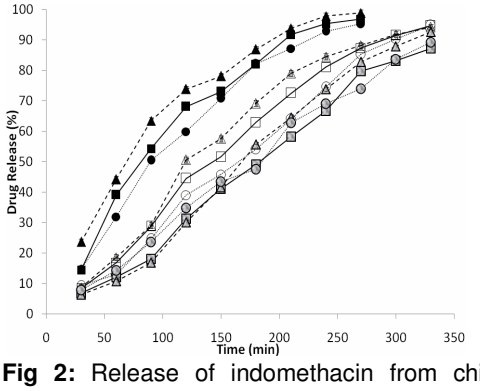

Fig 2: Release of indomethacin from chitosan films in pH 3.5 medium: ■ = F1; □ = F3; ■ = F5; ▲ = F6, ∆ F8, ▲ F10, ● F11, ○ F13, ● F15 (See Table 1 code key)

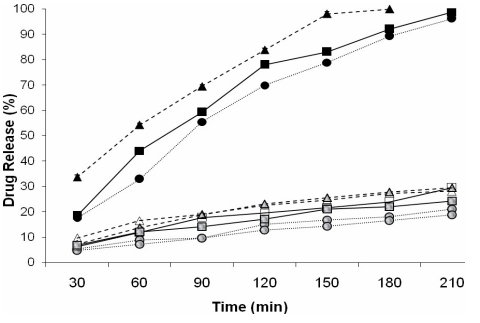

Fig 3: Release of indomethacin from chitosan films in pH 6.2 medium (■ = F1; □ = F3; ■ = F5; ▲= F6; ∆ = F8; ▲= F10; ● = F11; ○ = F13; ● = F15 (Code key as in Table 1)

There was no significant difference between low and medium MW grades of chitosan in terms of the drug release rate of the films ($p > 0.05$). However, high MW chitosan film showed a slight decrease in the rate of drug release in acid conditions.

Thermal characteristics

The DSC thermograms of pure indomethacin and some of the chitosan films are shown in Fig 4. The thermogram of.indomethacin (A) showed a sharp endothermic peak at 165 ℃ which indicates the melting transition of the drug. The thermogram of F6 (B), which was similar to those of F1, F5, F10, F11 and F15 (not shown), manifested a broad endothermic peak. A combination of the highest crosslinking concentration (10 % sodium citrate) and longest cross-linking time (4 h) for the different MW grades of chitosan caused a depression of the endothermic peak in the film thermograms by approximately 57, 12 and 5 ℃ for F1, F6 and F11, respectively.

DISCUSSION

Citrate is an anion with three carboxylic groups and chitosan is polybasic with cations. The charge densities of citrate and chitosan are mainly controlled by solution pH 13-14].

Under neutral and weakly acidic conditions, the degree of ionization of sodium citrate significantly decreased owing to the weak acid characteristics of citric acid. In contrast, chitosan, being a weak polybase, showed a marked decrease in the ionization of its amine groups when solution pH increased to over 6.0 (chitosan pKa = 6.3)[19].

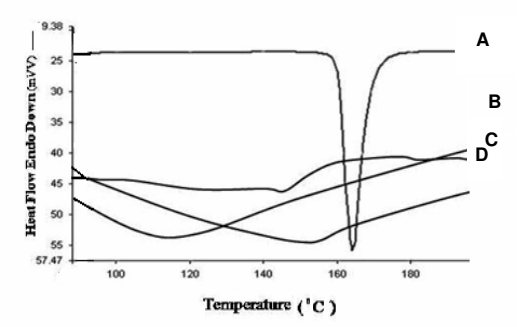

Fig 4: DSC thermograms of pure indomethacin (A) and chitosan films (B = F6, C = F11, and D = F1) **(**Code key as in Table 1)

Film swelling

The pH of the medium had a major effect on chitosan film swelling due to the ionization of both the sodium citrate and chitosan. Swelling ratio was lowest at pH of 5.5 and 6.5, owing to electrostatic attraction between citrate and chitosan. Reduction in pH weakened the salt bonds and, therefore, facilitated chitosan film swelling. However, increase in pH above 6.5 would probably also have weakened the salt-bonds and produced a higher swelling ratio.

Drug release

Drug release from the films was sensitive to pH because electrostatic interaction between the citrate anion and chitosan amine groups were influenced by solution pH. Decrease in pH weakened the salt bonds and therefore, facilitated film swelling, thereby making it more porous and accelerating drug release. pH also has been reported to have a slight effect on the solubility of indomethacin [20]. A lower pH leads to improved solubility of drug and this in turn results in higher drug release rate. However, compared to the strong influence of pH on the film matrix, pH effects on drug solubility was negligible. At low pH (1.0 - 3.5), sodium citrate and chitosan would be in a dissociated state and hence drug release from the films was rapid. As the results show the greater the crosslinking time, the lower was the drug release rate, This may be attributed to a higher degree of crosslinking in the matrix, leading to delay in the diffusional release of the drug. A slight decrease in the rate of drug release in acid conditions for high MW chitosan film maybe related to its higher viscosity compared with low and medium MWs.

Although the protonation constant (pKa) of chitosan slightly decreases (from 6.51 to 6.39) with decrease in its MW [19], in acid conditions (pH 3.5), a slight decrease in pKa appeared not to have played a major role in drug release rate due to high dissociation at all chitosan MWs.

Thermal characteristics

The absence of a sharp endothermic melting peak for indomethacin in the thermograms of these films may be due to the conversion of the incorporated indomethacin from the crystalline to the more soluble amorphous state.

CONCLUSION

The variations in citrate-crosslinked chitosan film swelling and as well as in drug release rate in medium pH of 3.5 and 6.2 suggest that this type of films can be modulated to achieve site-specific delivery in the stomach.

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REFERENCES

1. *Tsukagoshi T, Kondo Y, Yoshino N. Preparation of thin polymer films with controlled drug release. Colloids Surfaces B 2007; 57: 219-225.*
2. *Guo BL, Yuan JF, Gan QY. Preparation and characterization of temperature and pH-sensetive chitosan material and its controlled release on coenzyme A. Colloids Surfaces B 2007; 58: 151-156.*
3. *Yoo JW, Deharmala K, Lee CH. The physicodynamic properties of mucoadhesive polymeric films developed as female controlled drug delivery system. Int J Pharm 2006; 309: 139-145.*
4. *Westedt U, Wittmar M, Hellwing M, Hanefeld P, Greiner A, Schaper AK, Kissel T. Paclitaxel releasing films consisting of poly(vinyl alcohol)-graft-poly(lactide-co-glycolide) and their potential as biodegradable stent coatings. J Control Release 2006; 11: 235-246.*
5. *Tuovinen L, Peltonen S, Liikola M, Hotakainen M, Lahtela-Kaaaonen M, Poso A, Jarvinen K. Drug release from starch-acetate microparticles and films with and without incorporated α- amylase. Biomaterials 2004; 25: 4355-4362.*
6. *Klose D, Siepmann F, Elkharraz K, Siepmann J. PLGA-based drug delivery system: Importance of the type of drug and device geometry. Int J Pharm 2008; 354: 95-103.*
7. *Wang Y, Challa P, Epstein DL, Yuan F. Controlled release of ethacrynic acid from poly(lactide-co-glycolide) films for glaucoma treatment. Biomaterials 2004; 25: 4279-4285.*
8. *Padula C, Nicoli S, Colombo P, Santi P. Single-layer trnsdermal film containing lidocaine: Modulation of drug release. Eur J Pharm Biopharm 2008; 66: 422-428.*
9. *Akhgari A, Farahmand F, Afrasiabi H, Sadeghi F, Vandamme TF. Permeability and swelling studies on free films containing insulin in combination with different polymethacrylates aimed for colonic drug delivery. Eur J pharm sci 2006; 28: 307-314.*
10. *Muzzarelli RAA. Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. Carbohydrate Polymers 2009; 76: 167-182.*
11. *Ho EA, Vassileva V, Allen C, Piquette-Miller M. In vitro and in vivo characterization of novel biocompatible polymer-lipid implant system for the sustained delivery of paclitaxel. J Control Release. 2005; 104: 181-191.*
12. *Chen S, Liu M, Jin S, Wang B. Preparation of ionic-crosslinked chitosan-based gel beads and effect of reaction conditions on drug release behaviors. Intl J Pharm 2008; 349: 180-187.*
13. *Shu XZ, Zhu KJ. The influence of multivalent phosphate structure on the properties of ionically cross-linked chitosan films for controlled drug release. Eur J Pharm Biopharm 2002; 54: 235-243.*
14. *Shu XZ, Zhu KJ. A novel approach to prepare tripolyphosphate/chitosan complex beads for controlled release drug delivery. Int J Pharm 2000; 201: 51-58.*
15. *Wang Q, Dong Z, Du Y and Kennedy J. Controlled release of ciprofloxacin hydrochloride from chitosan/polyethylene glycol blend films. Carbohyd Polym 2007; 69: 336-343.*
16. *Rana V, Babita K, Goyal D and Tiwary A. Sodium citrate cross-linked chitosan films: optimization as substitude for human/rat/rabbit epidermal sheets. J Pharm Pharm Sci 2005; 8: 10-17.*
17. *Zhou HY, Chen XG, Kong M, Liu CS, Cha DS, Kennedy JF. Effect of molecular weight and degree of chitosan deacetylation on the preparation and characteristics of chitosan thermosensitive hydrogel as a delivery system. Carbohyd Polym 2008; 73: 265-273.*
18. *Shu XZ, Zhu KJ, Song W. Novel pH-sensitive citrate cross-linked chitosan film for drug controlled release. Int J Pharm 2001; 212: 19-28.*
19. *Wang QZ, Chen XG, Liu N, Wang SX, Liu CS, Meng XH, Liu CG. Protonation constants of chitosan with different molecular weight and degree of deacetylation. Carbohyd Polym 2006; 65: 194-201.*
20. *Nokhodchi A, Javadzadeh Y, Siahi M, Barzegar Jalali M. The effect of type and concentration of vehicles on the dissolution rate of a poorly soluble drug (indomethacin) from liquisolid compact. J Pharm Pharmaceut Sci 2005; 8: 18-28,*