Drug Development and Industrial Pharmacy, 31:885–893, 2005

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040590405002791985

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Viscoelastic Properties of a New in situ Gelling Thiolated Chitosan Conjugate

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ABSTRACT The aim of this study was the synthesis of a new thiolated chitosan conjugate and the evaluation of its viscoelastic properties in vitro. The modification of chitosan was achieved by covalent attachment of isopropyl-S-acetylthioacetimidate to chitosan. The resulting conjugate (chitosan-TEA; chitosan-thioethylamidine) exhibited 300.7±27.4 μmol thiol groups per gram polymer and no disulfide bond. For rheological studies, the pH of 0.5% and 1% polymer solutions was adjusted to 6.5 in order to simulate a physiological pH-level. Both, 0.5% and 1% chitosan-TEA solutions showed the transition from sol to gel within 30 min. Within 6 h of incubation, the storage modulus of 0.5% and 1% chitosan-TEA increased 3354-fold and 6199-fold, whereas the loss modulus increased 11-fold and 38-fold, respectively. Frequency sweep measurements demonstrated an increase in crosslinking of the thiolated polymer as a function of time. The formation of inter- and/or intramolecular disulfide bonds was monitored indirectly via determining the decrease of thiol groups. Unmodified chitosan did not exhibit in situ gelling properties. The release of a fluorescent marker being incorporated in a 0.5% chitosan-TEA solution was significantly ($p<0.001$) slower, when the formulation was preincubated for one hour and consequently already highly crosslinked. The polymer generated within this study represents a promising novel tool for various drug delivery systems, where in situ gelling properties are advantageous.

KEYWORDS Thiomer, Chitosan, In situ gelation, Controlled release

INTRODUCTION

Liquid formulations for nasal, ocular, and vaginal drug delivery have the disadvantage of being rapidly cleared from the site of drug absorption. This rapid elimination results in a short duration of the therapeutic effect and, consequently, in a frequent dosing regimen. Gels, in contrast, are known to show a prolonged residence time at various mucosae and the amount of absorbed drug is increased. Unfortunately, gels are not as easy to apply as liquid formulations limiting patient compliance.

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Consequently, drug delivery systems that combine both advantages, residing at the site of drug absorption and being simple to administer—namely in situ gelling formulations—have gained increasing interest in the last years. The drug is administered in liquid form, but after application the formulation shifts to a gel. When timolol, for instance, is embedded in the in situ gelling polymer deacetylated gellan gum, a once daily ocular application of this system leads to the same efficacy as a twice daily administered timolol solution (Hommer et al., 1995; Shedden et al., 2001). Such an in situ gelling system is in form of Timoptic XE® eye drops on the market [Timoptic XE® is a trademark of Merck and Co. Inc. (Whitehouse Station, N.J., USA)]. The in situ gelation can be achieved by a change of the pH (Gurny et al., 1985), an increase (Edsman et al., 1998) or decrease (Jeong et al., 1997) of the temperature, or an increase in the ionic strength (Grasdalen & Smidsrød, 1987). The prolonged residence time of in situ gelling formulations in comparison to simple liquid formulations can be further improved by the incorporation of mucoadhesive polymers like polyacrylates (Chang et al., 2002a; Park et al., 2002). The addition of mucoadhesive polymers was shown not to hinder the in situ gelation process (Chang et al., 2002a; Park et al., 2002). The combination of the in situ gelling polymer Pluronic with poly(acrylic acid), for instance, led to a significantly prolonged residence time of the formulation on the nasal mucosa compared to Pluronic or poly(acrylic acid) alone (Bromberg, 2001).

Polymers featuring both in situ gelling and mucoadhesive properties are thiolated polymers or so-called thiomers, hydrophilic polymers derivatized by the covalent attachment of thiol group bearing moieties (Bernkop-Schnürch et al., 1999). Their in situ gelation mechanism is explained to be based on the formation of inter- and/or intramolecular disulfide bonds at pH-levels above 5 (Marschütz & Bernkop-Schnürch, 2002). By the immobilization of cysteine to deacetylated gellan gum, a thiolated polymer was formed, gelling also in the absence of mono- and divalent cations without leakage of its original gelling mechanism (Krauland et al., 2003). The thiolation of chitosan with thioglycolic acid yielded a chitosan-thioglycolic acid conjugate (chitosan-TGA) with in situ gelling features (Hornof et al., 2003). Due to the immobilization of thiol groups on this polymer, its mucoadhesive properties were 10.3-fold improved (Kast & Bernkop-Schnürch, 2001). Another thiolated chitosan conjugate, chitosan-4-thiobutylamidine (chitosan-TBA), showed a strong increase in dynamic viscosity within 6 h (Bernkop-Schnürch et al., 2003). Additionally, chitosan-TBA is considered to be the most mucoadhesive thiolated chitosan conjugate (Bernkop-Schnürch et al., 2003, 2004). Unfortunately, 2-iminothiolane modified amines displayed insufficient stability due to undesired side-reactions (Singh et al., 1996). A new thiolated chitosan conjugate, namely chitosan-thioethylamidine (chitosan-TEA) depicted in Fig. 1, was therefore developed for which side-reactions as known for chitosan-TBA can be excluded (Kafedjiiski et al., 2004). Chitosan-TEA displays promising mucoadhesive properties and provides a controlled drug release as solid carrier matrix (Kafedjiiski et al., 2004).

The aim of this study was to evaluate the elastic and viscous properties of chitosan-TEA and to verify its in situ gelling properties at physiological pH levels. Additionally, the formation of disulfide bonds within the conjugate was monitored and the release behavior of a fluorescent marker out of chitosan-TEA gels was determined.

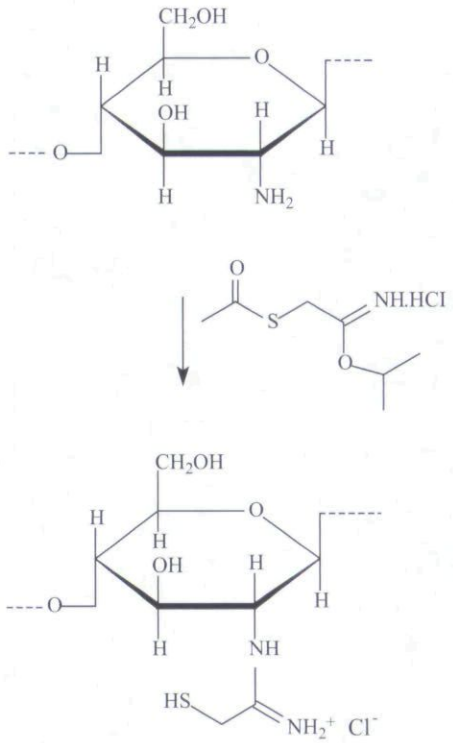

FIGURE 1 **Synthetic Pathway of the Chitosan-Thioethylamidine (TEA) Conjugate.**

MATERIALS AND METHODS

Synthesis and Purification of the Chitosan-TEA Conjugate

Initially, 500 mg of chitosan (Chito-Clear®, deacetylation degree 95%, average molecular weight approx. 450 kDa, Primex BioChemicals AS, Haugesund, Norway) were dissolved in 50 mL of 1% acetic acid. After adjusting the pH to 6.5 with 1 M NaOH, 500 mg isopropyl-S-acetylthioacetimidate (Muco Biomer GmbH, Leobendorf, Austria) were added in five aliquots over 1.5 h under continuous stirring. The reaction mixture was stirred at room temperature for one hour. Deacetylation of the mercapto group occurred spontaneously in the course of the reaction as already observed for S-acetylmercaptoalkylthioimidates (Delprino et al., 1993). The addition of 0.5 M NH_2OH solution at pH 6–7 as a common method for deprotection of S-acetyl groups led to no further increase of absorbance, thus deprotection was assumed as being quantitative (Delprino et al., 1993; Duncan et al., 1983).

The resulting polymer conjugate was dialyzed against 5 mM HCl, two times against 5 mM HCl containing 1% NaCl, two times against 5 mM HCl, and finally against 2 mM HCl. As control, a chitosan HCl solution was prepared. Finally, the aqueous polymer solutions (chitosan-TEA and unmodified chitosan) were lyophilized at −30°C and 0.01 mbar (Christ Beta 1-8k; Osterode am Harz, Germany). The lyophilized polymers were stored in air tight containers at 4°C until further use.

Determination of the Thiol Group and Disulfide Bond Content

The amount of free thiol groups on the chitosan-TEA conjugate was determined via Ellman's reagent [5,5′-dithiobis(nitrobenzoic acid)] as described previously (Krauland et al., 2004). Disulfide content was measured after reduction with $NaBH_4$ and addition of 5,5′-dithiobis(nitrobenzoic acid) as described by Habeeb (1973).

Rheological Studies—— Stress Sweep and Frequency Sweep

The viscoelastic properties of the polymers were determined with a cone-plate viscometer (RotoVisco RT20, Haake GmbH, Karlsruhe, Germany) with a thermostatically controlled cone/plate system (35 mm in diameter and 2° angle). All rheological measurements were shear stress controlled. The chitosan-TEA conjugate was hydrated in demineralized water under continuous stirring. After full hydration of the polymer, 100 mM HEPES-buffer pH 7.8 (N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]; Sigma; St. Louis, MO), pre-equilibrated to 37°C, was added to a final polymer concentration of 1.0% (w/w). For 0.5% (w/w) polymer solutions, a 100 mM HEPES-buffer pH 7.5 (37°C) was used. Polymer solutions of 1.0% required a buffer system of higher pH than 0.5% polymer solutions, as the buffer capacity of the more concentrated polymer solution was stronger than that of 0.5% polymer solutions. As control, unmodified chitosan solutions were prepared in the same way. Additionally, 11.4 mg 1 M HCl/10 mg chitosan were added to 0.5% unmodified chitosan solutions and 5 mg 1 M HCl/10 mg chitosan were added to 1% unmodified chitosan solutions to prevent precipitation of chitosan during the experiment. Final percentage levels of unmodified chitosan solutions were also 0.5% (w/w) and 1% (w/w). The final pH-value of all polymer solutions was 6.5 and was kept throughout the experiment with a deviation of maximum ±0.1. All polymer solutions gave clear solutions and showed no precipitation within the time period of the experiment. Immediately, dynamic oscillatory tests, stress sweep, and frequency sweep were performed of 0.3 mL aliquots at 37°C. Thus, initial rheological results were regarded as time point zero. The samples were incubated in an oscillating waterbath at 37°C. After 30 min, 1 h, and consecutively in 1 h intervals, 0.3 mL aliquots were withdrawn and dynamic oscillatory tests, stress sweep, and frequency sweep were performed. The parameters obtained thereby were the phase shift angle (δ) and the shear deformation (γ). The storage modulus (G') and the loss modulus (G'') were calculated by the following equations:

$$G' = (\tau/\gamma) \cdot \cos\delta$$

$$G'' = (\tau/\gamma) \cdot \sin\delta$$

where τ is the shear stress. Loss tangent ($\tan\delta$), a parameter that represents the ratio between the loss modulus (G'') and storage modulus (G') was also calculated. For the stress sweep, angular frequency

(ω) was kept constant at 6.283 rad/s and the shear stress increased from 0 to 100 Pa, and for the frequency sweep vice versa, where ω varied from 0.628 to 137.8 rad/s and the shear stress was kept constant at 30 Pa. The angular frequency is related to the oscillatory frequency ν by the relationship $\omega=2\ \pi\ \nu$ (Mezger, 2002).

Decrease of Thiol Groups

In parallel to the viscosity studies, the degree of the disulfide bond formation was monitored: 1% and 0.5% chitosan-TEA solutions were prepared as described above. At predetermined time points aliquots were withdrawn. To quench any further reaction, 1 M HCl was added to the aliquots and they were stored at −20°C. The decrease of free thiol groups was determined with Ellman's reagent (Krauland et al., 2004).

Release Studies

For release studies, fluorescent labelled dextran [fluorescein isothiocyanate-dextran (FD_4) av. mol wt 4,000; Sigma; St. Louis, MO] was used. FD_4 represents a non-charged molecule, that should not interact with the polymer matrices. Additionally, FD_4 should show a release behaviour comparable to that of protein drugs. The chitosan-TEA conjugate was hydrated in demineralized water under continuous stirring. After full hydration of the polymer, 100 mM HEPES-buffer pH 7.4 pre-equilibrated to 37°C containing 0.5 mg FD_4 was added to a final polymer concentration of 0.5% (w/w). Samples of 0.5 mL 0.5% polymer solutions were preincubated in closed vessels on an oscillating waterbath at 37°C for 0.5, 1, and 2 h, respectively, in order to obtain gels with different crosslinking degree. Thereafter, 2 mL of 0.2 M HCl pre-equilibrated to 37°C were added carefully to the surface area of the gels and samples were closed to prevent evaporation. The samples were incubated on an oscillating waterbath at 37°C for 3 h. Within the two-phase-system, the chitosan-TEA gel below and the liquid acidic release medium above, FD_4 was released to the supernatant fluid. HCl was chosen as release medium in order to stop the disulfide bond formation within the gels. The two-phase-system remained stable throughout the experiment. At predetermined time points, aliquots of 200 µL were withdrawn from the liquid phase and replaced by an equal volume of release medium pre-equilibrated to temperature. Finally, 100 µL 1 M NaOH were added to the withdrawn aliquots to initiate fluorescence of released FD_4. The intensity of fluorescence was determined by measuring the aliquots with a fluorimeter (SLT, Spectra Fluor, Tecan, Austria). Concentrations were calculated by interpolation from a standard curve. Sink conditions were maintained throughout the whole study.

Statistical Data Analysis

Statistical data analysis was performed using the student *t*-test with $p<0.05$ as the minimal level of significance. Calculations were done using the software Xlstat version 5.0 (b8.3) (Addinsoft, Paris, France).

RESULTS

Characterization of the Chitosan-TEA Conjugate

Isopropyl-S-acetylthioacetimidate was covalently attached to chitosan via an amidine bond between the carboxylic group of the reagent and a free primary amino group of the polymer. The purified chitosan-TEA conjugate exhibited 300.7±27.4 µmol thiol groups per gram polymer (mean±S.D. $n=4$). No disulfide bond could be detected. The obtained polymer was white, odorless, and showed a fibrous structure. For all experiments, the fibrous structured lyophilizate was used and no pulverization of the product was carried out.

Rheological Studies

Stress Sweep

G′, G″, and tan δ were determined at a constant angular frequency of 6.283 rad/s (=1 Hz) and were plotted as a function of time.

The initial storage moduli of 0.5% and 1% chitosan-TEA and the accordant controls did not show a significant difference, whereas after 30 min G′ of 0.5% chitosan-TEA was more than 900-fold increased and after 6 hours even more than 3350-fold increased compared to the initial storage modulus (Fig. 2). G′ of the 1% chitosan-TEA solution was after 30 min more than 1180-fold and at the end of the experiment, after 6 h, almost 6200-fold higher than at

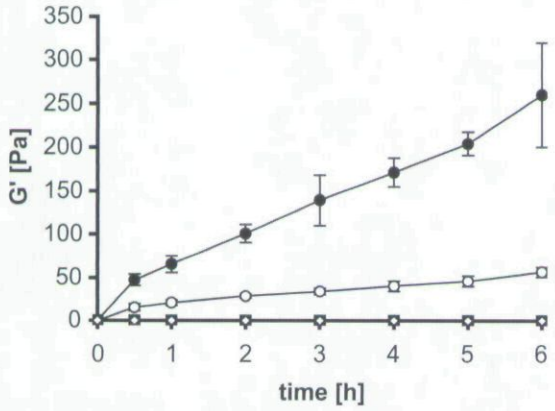

FIGURE 2 Increase in the Storage Modulus G′ of 0.5% (○) and 1.0% (●) Chitosan-TEA Conjugate and 0.5% (◇) and 1% (■) Unmodified Chitosan in Aqueous Solutions was Determined at pH 6.5 and 37°C by Oscillatory Measurements, Stress Sweep, at 6.283 rad/s (=1 Hz). The Means (n=3) and Standard Deviation Bars are Shown.

time point zero. In comparison to 1% unmodified chitosan, the 1% chitosan-TEA conjugate gel represented an approximately 15315-fold higher elasticity after 6 h of incubation.

The loss modulus of 0.5% and 1% chitosan-TEA displayed a strong increase during the first 30 min (Fig. 3). As G″ of the 1% gel rose also during the following 5.5 h, the loss modulus for the 0.5% chitosan-TEA formulation had a maximum after 30 min. A reason for the decrease of G″ in the following 5.5 h could be seen in the low polymer concentration of 0.5%, where more energy is lost during the

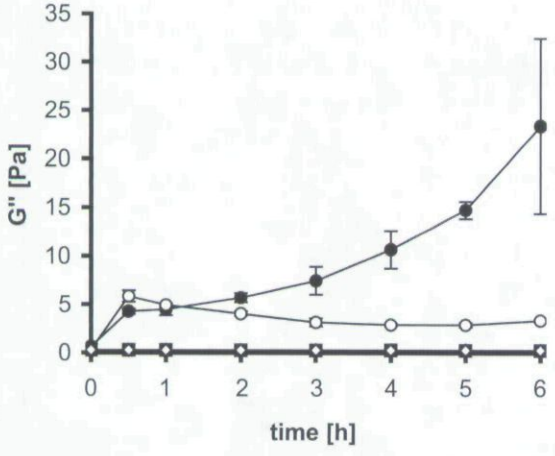

FIGURE 3 Increase in the Loss Modulus G″ of a 0.5% (○) and 1.0% (●) Chitosan-TEA Conjugate and 0.5% (◇) and 1% (■) Unmodified Chitosan in Aqueous Solutions were Determined at pH 6.5 and 37°C by Oscillatory Measurements, Stress Sweep, at 6.283 rad/s (=1 Hz). The means (n=3) and Standard Deviation Bars are Shown.

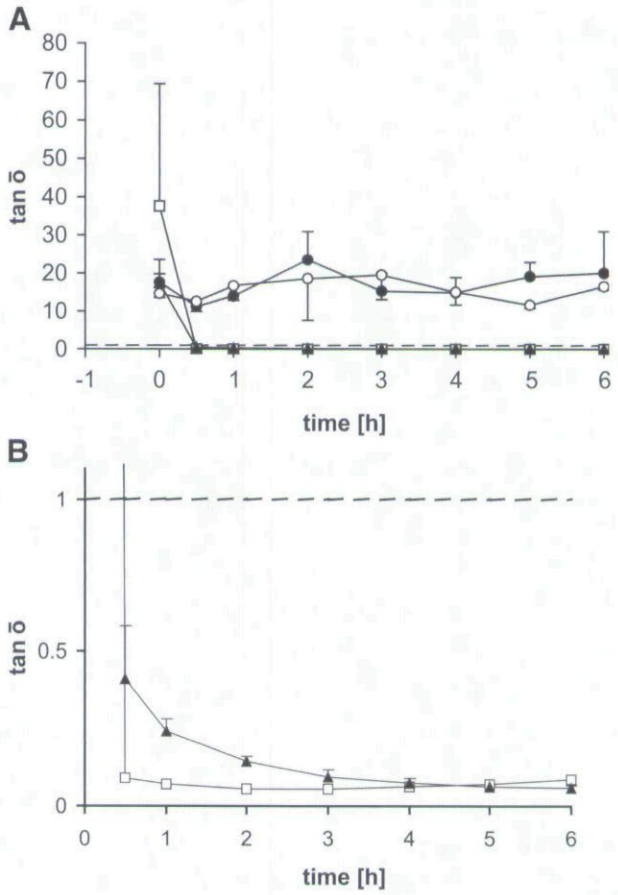

FIGURE 4 Effect of Oxidation on the Loss Tangent tan δ of 0.5% (▲) and 1.0% (□) Chitosan-TEA Conjugate and 0.5% (○) and 1% (●) Unmodified Chitosan in Aqueous Solutions (A) were Determined at pH 6.5 and 37°C. In (B), tan δ Curves of 0.5% (▲) and 1.0% (□) Chitosan-TEA Conjugate Solutions are Displayed More in Detail. Oscillatory Measurements, Stress Sweep, Have Been Worked out at 6.283 rad/s (=1 Hz) and tan δ is Plotted as a Function of Time. Indicated Values are the Mean Results of 3 Experiments ± S.D.

measurement than for the 1% chitosan-TEA. Unmodified chitosan in contrast, exhibited neither for the storage modulus nor for the loss modulus an increase.

The loss tangent tan δ, the ratio between the lost and the saved deformation energy, was calculated by G″/G′. If tan δ is greater than 1, the substance is a sol, whereas if tan δ is smaller than 1, the substance represents a gel. When G″ is equal to G′ at the crossover point, the polymer has as many elastic as viscous constituents. According to this definition, chitosan-TEA 0.5% and 1% started as a fluid to turn to a gel within 30 min getting more and more solid (Fig. 4A and B). At the same time, the control was far away from the crossover point in the sol status with a strongly fluctuating tan δ.

Frequency Sweep

For the frequency sweep, the shear stress was kept constant, whereas the angular frequency ω varied from 0.628 to 137.8 rad/s. G′ and ω of chitosan-TEA were presented in a semilogarithmic diagram (Fig. 5). While the slope of the curves in the G′/ω diagram for the linear non-crosslinked chitosan changed marginally within 6 h with a calculated average slope of the curve after 6 h with 46.6°±7.5° (mean±S.D. n=14) (data not shown), the results for the 1% chitosan-TEA conjugate are totally different. At the beginning of the experiment, before disulfide bond formation, the curve of G′ showed the same characteristics as that of the control. The resulting curve in the G′/ω diagram exhibited a calculated average slope of 43.7°±5.9° (mean±S.D. n=14). After 30 min, however, when the sol-gel transition was over and chitosan-TEA represented a strong gel, the G′ curve in the G′/ω diagram exhibited an almost horizontal line that became more horizontal the longer the experiment lasted (Fig. 5). Since in theory a highly crosslinked polymer displays a horizontal straight line in the G′/ω diagram, because the storage modulus is independent from the used frequencies, the frequency sweep-diagram of the thiolated polymer provides additional evidence for the high inter- and intramolecular crosslinking of chitosan-TEA. Frequency sweep measurements of 0.5% chitosan-TEA and 0.5% chitosan resulted in comparable curves in the G′/ω diagram as achieved for the corresponding 1% polymer solutions (data not shown). Systems that show a more liquid-like behavior exhibit a strong angular dependence of the storage modulus on the frequency as seen for the control solutions.

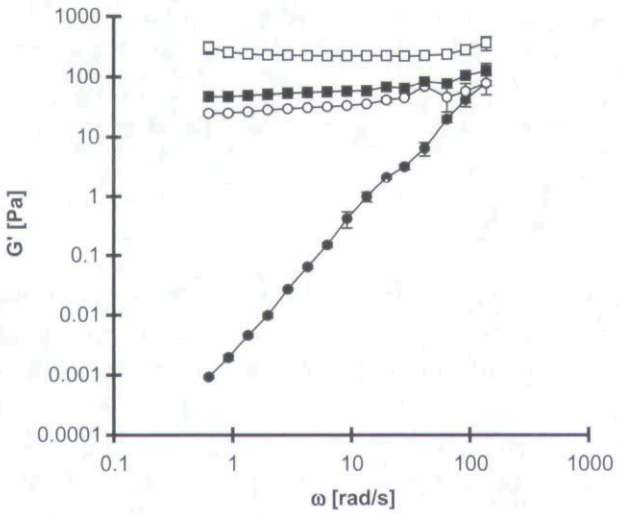

FIGURE 5 In Situ Crosslinking Properties of Chitosan-TEA. Crosslinked Polymers Show a Storage Modulus Independent from the Frequency, Whereas Not Crosslinked Polymers Display a Strong Dependence of the Storage Modulus on Frequency. The Storage Modulus G′ of Chitosan-TEA Conjugate in 1.0% Polymer-Solutions was Determined at pH 6.5 and 37°C with an Angular Frequency Range From 0.628 to 137.8 rad/s and a Fixed Shear Stress of 30 Pa. Oscillatory Measurements, Frequency Sweep, were Made at Time=0 (●), After 0.5 h (○), after 1 h (■), and After 6 h (□) Incubation. Means (n=3) and Standard Deviation Bars are shown.

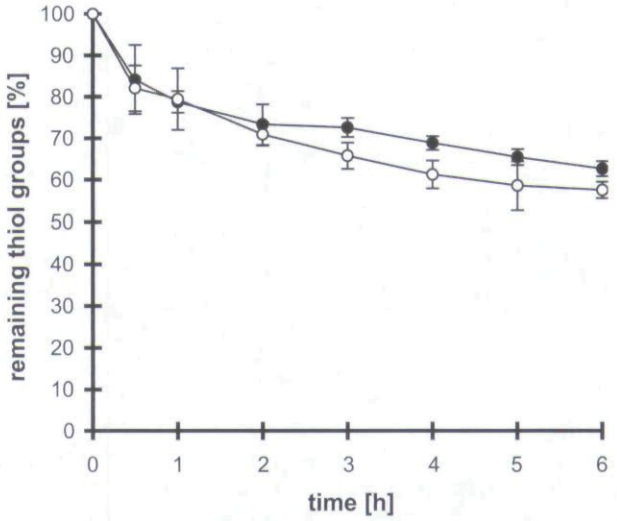

FIGURE 6 The Decrease in Thiol Groups of Chitosan-TEA was Determined of 0.5% (○) and 1% (●) Polymer-Solutions at pH 6.5 and 37°C. Indicated Values are Means±S.D. of at least Three Experiments.

Decrease of Thiol Groups

As indirect evidence for the formation of inter- and/or intramolecular disulfide bonds, the decrease of reduced thiol goups was determined within this study. The driving force for the oxidation of thiol groups are thiolate anions S^- that are present in a sufficient amount at physiological pH-levels. Within the first 30 min, the fastest oxidation of thiol groups can be seen. More than 15% thiol groups were oxidized within this time period. After 6 h the 0.5% chitosan-TEA gel still contained 57.6% reduced thiol groups and the 1% chitosan-TEA gel 62.7%. Results of this study are shown in Fig. 6.

In vitro Release

The release rate of FD_4 from 0.5% chitosan-TEA gels is shown in Fig. 7. To avoid the formation of

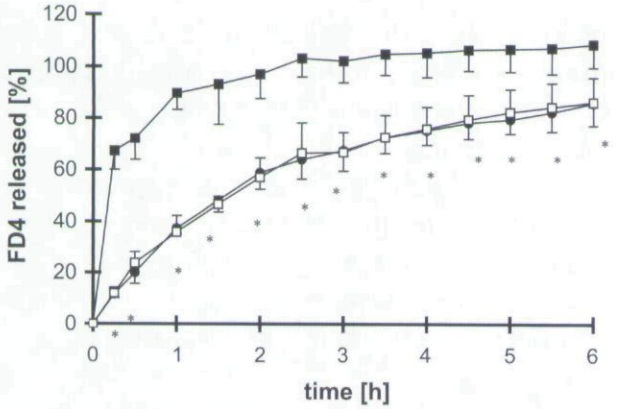

FIGURE 7 Release Profile of Fluorescent Labelled Dextran (FD_4) from 0.5% Chitosan-TEA Gels After Incubation at pH 6.5 and 37°C for 0.5 h (■), 1 h (●), and 2 h (□). The Release Study was Performed in 0.2 M HCl at 37°C. The means ($n=3$) and Standard Deviation Bars are Shown. *Formulation After 1 h Incubation Differs from Formulation with 30 min of Incubation, $p< 0.001$.

additional disulfide bonds during the release experiment, HCl was chosen as release medium. Within this acidic environment, the concentration of thiolate anions should be minimized. During preincubation, polymer solutions showed a sol-gel transition. Chitosan-TEA preincubated for 30 min displayed the fastest release. Samples preincubated for 1 h and 2 h showed a more sustained release with approximately 86% drug released within 6 h indicating the formation of a more crosslinked gel. The difference of the release rate of 1 h and 2 h preincubated polymer solutions was marginal. The preincubation of samples for more than 1 h did not decelerate the release of FD_4 any further.

DISCUSSION

Within this study, a new thiolated chitosan conjugate was synthesized and its viscoelastic properties were determined. By the immobilization of thiol groups to chitosan an in situ gelling polymer was generated. Both 0.5% and 1% chitosan-TEA solutions exhibited a sol-gel transition at physiological pH-levels within 30 min. In comparison to chitosan-TGA and chitosan-TBA conjugates, representing two other in situ gelling thiolated chitosan derivatives, the increase in elasticity within 6 h was highest for chitosan-TEA formed gels with a 6200-fold increase in elasticity for 1% polymer solutions (Bernkop-Schnürch et al., 2003; Hornof et al., 2003). The viscoelastic properties of these three thiolated chitosans, however, are difficult to compare, as studies with chitosan-TGA and chitosan-TBA were performed with 1.5% polymer solutions at pH 5.5. At this pH-level, the amount of thiolate anions presenting the active form for oxidation is comparatively lower than at pH 6.5 as used in the present study.

Besides the concepts of in situ gelation triggered by a change in pH, temperature, or ionic strength, thiomers represent another gelling mechanism. At physiological pH-levels, thiolated polymers are forming inter- and/or intramolecular disulfide bonds. Evidence for this crosslinking process can be given by rheological measurements. As unmodified chitosan displayed no change within its viscoelastic properties, chitosan-TEA solutions changed to gel status with a tan $\delta<1$. Additionally, frequency sweep measurements for chitosan-TEA demonstrated indirectly the internal crosslinking with an almost horizontal straight line in the G'/ω diagram as described for crosslinked polymers. At the beginning of this experiment, in contrast, chitosan-TEA showed the characteristics of a chitosan solution. Furthermore, disulfide bond formation was indirectly evidenced via the loss of reduced thiol groups. After a time period of 6 h, approximately 60% of reduced thiol groups were still available on chitosan-TEA. Probably thiol groups which are close to each other form disulfide bonds resulting in increased viscoelasticity, whereas sterically isolated thiol groups remain stable. The decrease of thiol groups is in good agreement to the increase in viscoelastic properties and decrease of the loss tangent, as in the first 30 min the decrease of thiol groups and the increase in viscoelastic properties are relatively fastest.

The combination of in situ gelling and mucoadhesive properties within the chitosan-TEA conjugate renders this novel polymer a useful excipient for various drug delivery systems. The residence time of the drug delivery system at the site of drug absorption and, consequently, the bioavailability of incorporated drugs should be increased compared to non-gelling liquid formulations. Furthermore, the frequency of administration of the drug can be reduced (Hommer et al., 1995; Shedden et al., 2001) increasing patient compliance. The efficacy of thiolated polymer based gel formulations has already been demonstrated in vivo. The nasal administration of a polycarbophil-cysteine/glutathione/human growth hormone gel formulation to rats resulted in a significantly increased and prolonged human growth hormone plasma

concentration-time profile versus unmodified polycarbophil gel and physiological saline, both containing the same amount of human growth hormone (Leitner et al., 2004).

Because of its excellent viscoelastic features, chitosan-TEA can be used, for instance, in ocular and nasal drug delivery systems. Once easily applied in liquid form, the formulation undergoes the sol-gel transition at physiological pH-levels. Especially ocular and nasal drug delivery systems are often fastly eliminated via tear flow, blinking reflex, and mucociliary clearance, respectively. By the use of chitosan-TEA as ocular drug delivery system, the efficacy of antiglaucoma drugs could be sustained. Nasal drug delivery systems based on chitosan-TEA could increase the bioavailability of peptides or proteins such as human growth hormone, calcitonin, or insulin. The in situ gelation at nasal mucosae should prevent the rapid elimination of the drug delivery system and therefore increase the efficacy of the administered drugs.

Additionally, chitosan-TEA could be used as vaginal drug delivery system, as it was shown that thiolated chitosans as a drug delivery system for clotrimazole showed prolonged mucoadhesion on vaginal mucosa compared to unmodified chitosan formulations (Kast et al., 2002). The therapeutic efficacy of clotrimazole in treatment of candidasis, for instance, could be significantly improved by utilizing poloxamer as in situ gelling excipient (Chang et al., 2002b). At least similar improvements can be expected by the use of the novel polymer described here.

Chitosan-TEA can be generated via a simple synthesis process. Chitosan itself has GRAS status and was shown to be biocompatible and biodegradable (Chenite et al., 2000). Rat nasal mucosa exposed to a chitosan solution did not show significant changes in morphology in comparison to the untreated nostril (Illum et al., 1994). Cytotoxicity of the thiolated chitosan derivative chitosan-TBA is in the same range as that of unmodified chitosan (Guggi et al., 2004). As chitosan-TEA differs in only two CH_2 groups from chitosan-TBA, its toxicological profile should be similar.

CONCLUSION

Due to the introduction of thiol groups to chitosan, a new thiolated chitosan derivative was synthesized. This conjugate exhibited excellent in situ gelling features because of internal crosslinking via inter- and/or intramolecular disulfide bonds. The release of a fluorescent dextran could be controlled by the degree of this crosslinking process. The combination of mucoadhesive and in situ gelling properties renders this thiolated chitosan derivative a promising excipient for drug delivery systems designed to provide a prolonged residence time at the site of drug absorption.

ACKNOWLEDGMENTS

This work was supported by Grant No. P15373-MOB from the Fonds zur Förderung der wissenschaftlichen Forschung (FWF) to A. Bernkop-Schnürch and by Grant Impulsprojekt E 71 from the Fonds zu Förderung der wissenschaftlichen Forschung (FWF) to M. Hoffer and MucoBiomer GmbH.

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