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ORIGINAL ARTICLE

Chitosan–glycolic acid: a possible matrix for progesterone delivery into skin

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Abstract

Background: Chitosan-EDTA is an interesting matrix for dermal delivery; however, the adhesiveness is too small. Therefore, the purpose of this study was to investigate chitosan-glycolic acid as possible dermal matrix for progesterone in comparison to chitosan-EDTA and carrageenan. *Method:* After preparation of the chitosan-glycolic acid salt and characterization by NMR and FTIR, tensile studies using porcine skin and rheology measurements as well as standard diffusion experiments using dermatomed porcine skin were performed. *Results:* Results showed an improved skin adhesiveness of chitosan-glycolic acid and increased viscosity. Skin diffusion studies indicated the highest cumulative permeation of progesterone after 48 hours from chitosan-glycolic acid followed by carrageenan and chitosan-EDTA. A possible explanation might be a longer residence time on skin caused by the higher adhesiveness and with it higher progesterone skin permeation. *Conclusion:* Chitosan-glycolic acid can be recommended as a suitable polymer for hydrogels and an adhesive matrix for a transdermal application of progesterone exhibiting excellent skin adhesiveness and permeation properties.

Key words: *Carageenan; chitosan–EDTA; chitosan–glycolic acid; progesterone; skin permeation*

Introduction

The topical use of gels based on polymers such as poly(acrylate)- and cellulose ether derivatives is very common. They are widely applied excipients for thickening topical lotions, creams, and ointments. These types of polymers are also used to modify the rheology of water-based systems and to stabilize emulsions and suspensions. One of the interesting gelatinizing agents in terms of topical delivery system is a polymer which has been described earlier[1]. It is a chitosan–EDTA derivative and has been evaluated for topical application with respect to its technical properties. The results demonstrated that this chitosan–EDTA was able to form stable, colorless, completely transparent hydrogels at a polymer concentration above 0.5%. The polymer displayed the comparably lowest incompatibility with multivalent cations as well as ethanol, and exhibited the significantly best swelling properties among several tested polymers. Chitosan–EDTA exhibited additionally high antimicrobial activity compared to other hydrogels. This could be explained by the highest binding affinity of chitosan–EDTA toward magnesium, stabilizing the outer membrane of Gram-negative bacteria[2]. Whereas the antimicrobial action was known to be very good, the adhesive properties of chitosan–EDTA on skin was expected to be rather low. Therefore, a better adhesive chitosan derivative should be designed offering the possibility of extensive hydrogen bonding with parts of the skin membrane.

For this reason the salt of chitosan with glycolic acid was chosen. In this study, chitosan–EDTA and chitosan–glycolic acid would be investigated as possible matrices for progesterone with respect to the skin adhesiveness and suitable viscosity for skin application. These properties would be compared to another interesting polymer carrageenan, a frequently used polymer as food additive with very good skin adhesiveness[3]. Finally, the skin permeation of progesterone would be compared among these matrices.

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The aim of the following study was to find a new chitosan derivative with excellent skin adhesiveness and good skin permeation properties for the model drug progesterone.

Materials and methods

Materials

Progesterone was purchased from Sigma (St. Louis, MO, USA). Carrageenan (Satiagel CT52) was a gift from Degussa (Essen, Germany). Chitosan Pharma TM1658 with a declared deacetylation degree of about 95% and an average molecular mass of 500 kDa was a gift from Syntapharm (Mühlheim, Germany). All other chemicals used were of reagent grade.

Chitosan–EDTA conjugate

The derivative was prepared as previously reported[1]. Briefly to an aqueous mixture of chitosan–HCl and EDTA adjusted to pH 6 a defined amount of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) was added. The reaction mixture was incubated at room temperature (RT) under permanent stirring for 12 hours. The resulting conjugate was isolated by exhaustive dialysis against demineralized water, 0.05 N sodium hydroxide solution, and once more against demineralized water. The purified product was neutralized with sodium hydroxide, lyophilized and stored at RT until use.

Preparation of chitosan–glycolic acid salt

One gram of chitosan was suspended in 90 mL of demineralized water. The pH value of this suspension was kept constant at 3 by continuously adding 1 M HCl (~5 mL) till the polymer was completely dissolved. To this chitosan solution about 3.15 g of glycolic acid was added directly and stirred continuously for 1 hour. Before lyophilization dialysis against distilled water was performed over the time period of 48 hours. Chitosan–glycolic acid was lyophilized and stored at RT until use.

Control chitosan

Control samples for Fourier transform infrared (FTIR) spectroscopy of chitosan (CH) were prepared in the same manner but without glycolic acid.

Methods

Fourier transform infrared (FTIR) spectroscopy

Spectra were recorded on a FTIR spectrometer (model: Bruker Tensor 27, Bruker Optics, Ettlingen, Germany) with a photovoltaic mercury cadmium telluride (MCT) detector using attenuated total reflectance (ATR) technique with a zinc selenide ATR crystal.

The following aqueous solutions were used: (i) chitosan–glycolic acid (CS–GA) with a final concentration of 1% (w/w); (ii) pure glycolic acid (GA) with a final concentration of 0.1% (w/w); and (iii) control chitosan (CS) with a final concentration of 1% (w/w). The FTIR spectra of these solutions were directly recorded on an ATR accessory (model: Bruker Bio-ATR II, Bruker Optics) at 25°C and measured against demineralized water as reference. Each sample amount was 20 µL. Twenty-four scans were averaged at 4 cm^{-1} resolution. All experiments were performed at least three times.

NMR spectroscopy

^1H NMR spectra were recorded at a temperature of 298 K on an Avance DRX 600 NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) at a resonance frequency of 600.13 MHz. The chemical shifts are referenced to the residual solvent peak of monodeuterated water (HDO, $\delta = 4.75$ ppm). The processing was done offline with the software Topspin 2.1 (Bruker BioSpin GmbH, Rheinstetten, Germany) using its integration and deconvolution routines for the quantitative analysis.

Formulations

From chitosan–EDTA, chitosan–glycolic acid, and carrageenan each 2% hydrogels were prepared with demineralized water. These control gels were transparent hydrogels. In each hydrogel 1% (w/w) progesterone was suspended and resulted in white gels with suitable viscosity.

Tensile studies

The adhesive strength of the drug-containing gels was evaluated with a peel adhesion test using a validated adhesive strength measuring device. Abdominal porcine skin was used. First the skin was attached with a strong cyanoacrylate adhesive to a flat glass stopper (30 mm diameter) and mounted on the top of a balance (Mettler PC 4400, Mettler Toledo, Giessen, Germay) which was placed on a moving platform and secured in place with a ring of lead. Demineralized water (100 µL) was applied on the top of the skin. Afterward, about 0.7 g of hydrogel or drug-containing hydrogel was spreaded on the skin. A plug (30 mm diameter) was connected to a force transducer via a nylon thread. The platform was then raised up until the test formulation was attached to the porcine skin. After a contact time of 30 minutes, the platform was lowered at a rate of 2 mm per minute until the test sample pulled clear off the membrane. The strength needed was registered and transferred to a PC for further processing. The total work of adhesion (TWA) representing the area under the force–distance curve was determined using the WINWEDGE software (TAL Technology, Inc., Philadelphia) in combination with Excel 5.0 (Microsoft).

Rheological experiments

In order to obtain flow curves, rheological experiments with following parameters were performed: controlled rate (CR) modus, $\gamma = 1\text{–}100\ s^{-1}$; $100\text{–}1\ s^{-1}$; 21°C ± 1.5°C. CR modus means that a controlled shear rate was applied. For all formulations the used tool was the C35/2Ti. In order to have a good comparison the viscosity values in Pas at a shear rate of $5.5\ s^{-1}$ are shown in Figure 4.

High Pressure Liquid Chromatography (HPLC) analysis

Samples were assayed for progesterone content at a flow rate of 1 mL/minute with an UV detector (series 200 LC, Perkin Elmer, Vienna, Austria) at a detection wavelength of 240 nm. The stationary phase was a Nucleosil 100 5C-18 column (240 mm × 4.6 mm). Any polymer residues were held back on a pre-column (Nucleosil 100-5C-18, 40 mm × 4.0 mm). The mobile phase was methanol:water (70:30). Samples (20 µL) were injected by an auto sampler (ISS-200, Perkin Elmer, Vienna, Austria). The retention time for progesterone was approximately 11 minutes. Calibration curves were calculated on the basis of peak area measurements. The linearity interval established in the receptor phase was for progesterone (0.41–34.6 µg/mL) (r^2: 0.9999).

Skin preparation

Porcine abdominal skin from one pig was shaved and then prepared with a dermatome (GB 228R, Aesculap, Germany) set at 1.0 mm. The skin was stored in a freezer at –20°C until use but not longer than 3 months. Two hours prior to the experiments the samples were thawed.

Diffusion cell preparation

The permeation of progesterone was investigated using Franz-type diffusion cells having a permeation area of $1.13\ cm^2$. The receptor compartment was filled with 2 mL phosphate buffer (0.012 M; pH 7.4) and stirred at 600 rpm. Excised skin was mounted in the cell, stratum corneum uppermost, with the dermal side facing the receptor compartment. The diffusion cells were thermostated to a skin surface temperature of 32°C. At defined time intervals the samples were removed for analysis and replaced with fresh receptor medium for 48 hours. Approximately 1 g of all formulations was applied. The cumulative amount of drug released through the porcine skin, Q/t, at any time, was determined from the following formula: $Q = (C\times V)/A$ where C is the drug concentration in the receiver compartment in mg/mL for the corresponding sample time t, V the volume of fluid in the receptor phase, and A the diffusion area of the cell. For each formulation and all controls three parallel experiments were performed.

Statistical data analysis

The results are expressed as the means of at least three experiments ±SD. Statistical data analysis was performed using the nonparametric Mann–Whitney test with $p < 0.05$ as a minimal level of significance.

Results

On one hand we used the previously characterized chitosan–EDTA[1] and the reported carrageenan[3] and on the other hand the characterization of chitosan–glycolic acid was performed by FTIR as well as by NMR spectroscopy.

Fourier transform infrared spectroscopy

The ATR–FTIR spectra of chitosan (CS), chitosan–glycolic acid (CS–GA), and glycolic acid (GA) are shown in Figure 1. As seen the characteristic absorption bands of chitosan (CS) appear at 1630, 1540, and 1380 cm^{-1}. This is in good agreement with literature data[4].

In the spectrum of glycolic acid (GA) the C=O stretching vibration of the carboxylic group COOH can be observed at $1732 cm^{-1}$.

The spectrum of the CS–GA salt preparation shows a dramatic decrease of the absorption of the carboxylic acid, correlating with the NMR result. A new band at 1585 cm^{-1} appears, which can be assigned to the antisymmetrical stretching of the carboxylate anion COO^-. This broad band may possibly also contain a contribution of $NH3^+$ bending.

1H NMR spectroscopy

The chitosan–glycolic acid salt was characterized by ^1H NMR spectroscopy as a solution of 1 mg in 0.6mL D_2O. Because of the high molecular weight the resonance lines for the chitosan are rather broad (Figure 2). The integral value of the CH_3 group (δ = 2.00 ppm) from the acetyl moiety relative to the intensity of the CH in position 2 of the free glucosamine (δ = 2.80 ppm) reveals a deacetylation degree of 86%. In contrast the signal for the CH_2 group of the glycolic acid can be seen as a sharp line (δ = 3.88 ppm). So its tumbling rate seems not to be interfered by the presence of the chitosan, although a slight upfield shift of about 0.3 ppm can be observed compared to a solution with the same concentration of glycolic acid only in D_2O. The final concentration of glycolic acid in the chitosan salt preparation is 12% w/w as calculated again from the integral values in the ^1H NMR spectrum.

Adhesive properties

In Figure 3 the TWA exhibited by the pure gels based on chitosan–EDTA, chitosan–glycolic acid, and carrageenan are compared. As seen the goal to increase

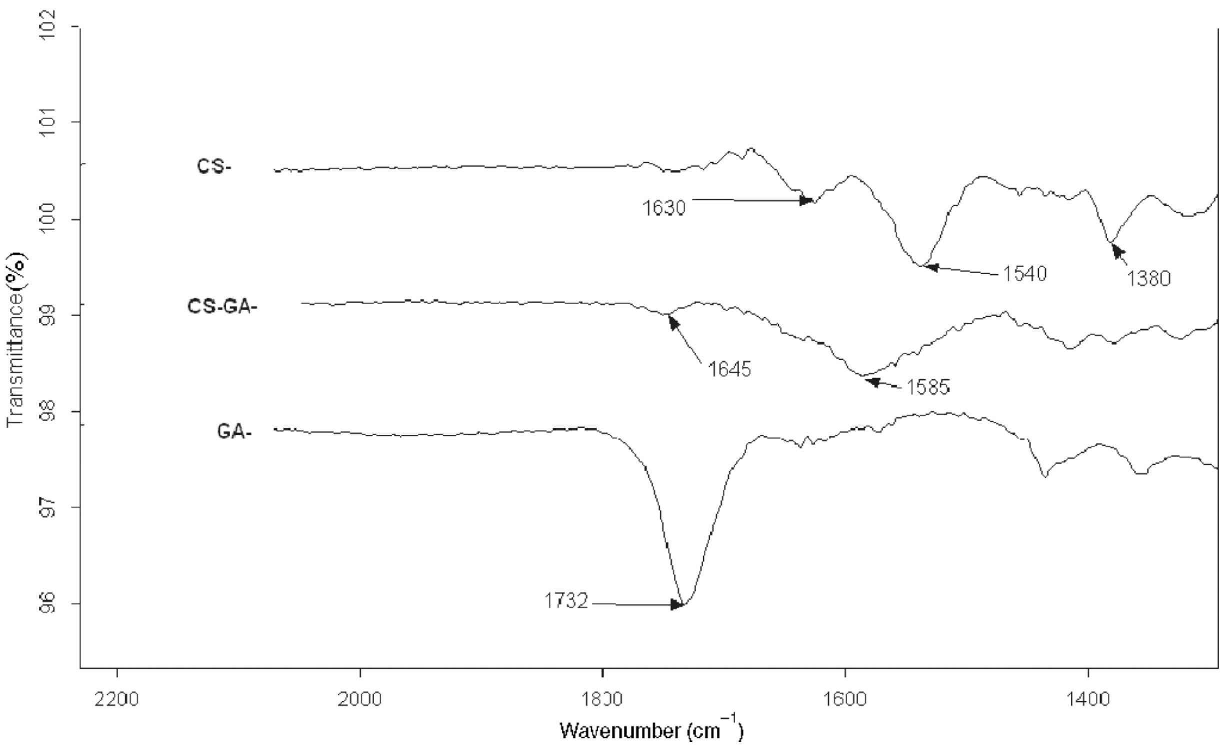

Figure 1. FTIR spectra of the aqueous solutions of control chitosan (CS) with a final concentration of 1% (w/w), chitosan-glycolic acid (CS-GA) with a final concentration of 0.1% (w/w), and glycolic acid (GA) with a final concentration of 0.1% (w/w).

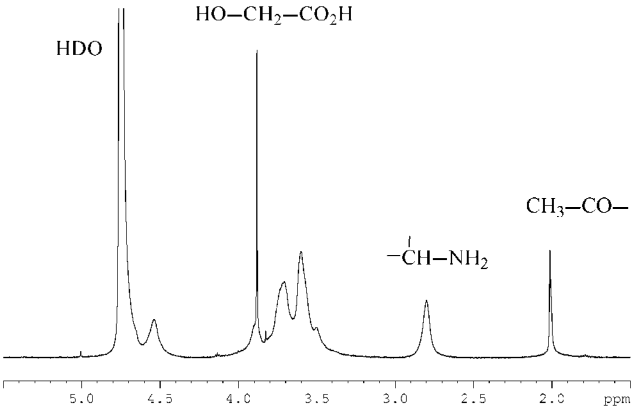

Figure 2. ^{1}H NMR spectrum of chitosan-glycolic acid.

the insufficient skin adhesion properties of chitosan-EDTA was reached. The highest TWA was measured for chitosan-glycolic acid compared to the other tested polymer hydrogels. The TWA of chitosan-glycolic acid in comparison to pure chitosan-EDTA was about 86-fold increased. The incorporation of progesterone decreased the TWA compared to the pure gels for the two chitosan derivatives, whereas the TWA was slightly increased in carrageenan by progesterone.

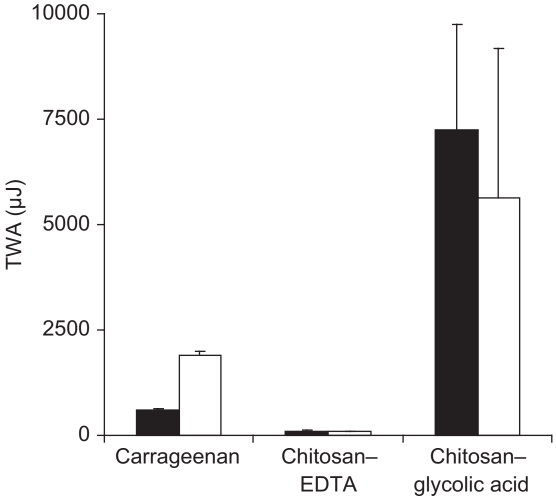

Figure 3. Comparison of the adhesive properties of different hydrogel systems (black bars) including progesterone (white bars). Represented values are the means ±SD (n = 4-6) of the total work of adhesion (TWA) determined in tensile studies with a defined amount of hydrogel.

Viscosity

Another physical parameter is the viscosity. In all gels a pseudo plastic behavior could be observed (Figure 4).

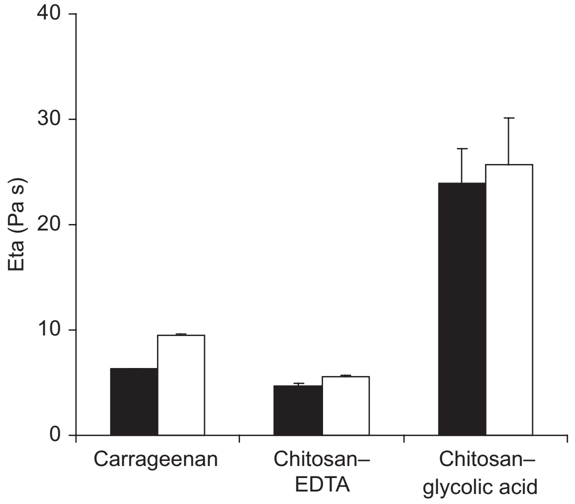

Figure 4. Comparison of the viscosities (eta) at 5.5 s shear rate of the different hydrogel systems (black bars) including progesterone (white bars).

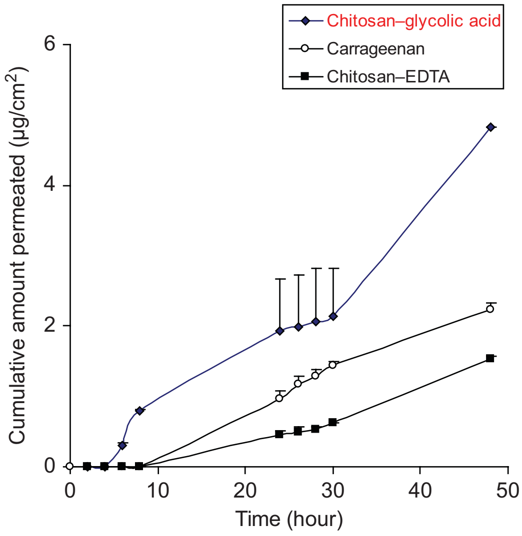

Figure 5. Cumulative amount of progesterone permeated through excised pig skin from different vehicles, $n = 4$.

Progesterone increased the viscosity of the pure gels. In carrageenan gels the viscosity of the pure gel was about 5 Pas. The lowest viscosity could be measured in chitosan–EDTA gels. The highest viscosity was seen in the chitosan–glycolic acid gels with about 24 Pas for the pure gel and about 26 Pas for the progesterone-containing sample.

Skin permeation

Chitosan–EDTA and chitosan–glycolic acid were tested as possible vehicles for progesterone in comparison with carrageenan. Different permeation profiles from the different vehicles can be seen in (Figure 5). The highest cumulative progesterone amount permeated after 48 hours was from chitosan–glycolic acid followed by carrageenan and chitosan–EDTA. The range order is the same as in the adhesion and the viscosity studies.

Discussion

Many polymers are used as basis for dermal and transdermal delivery[5]. At present naturally occurring or slightly modified natural polymers especially from crustaceans and seaweed are in focus. One of these compounds is carrageenan, a polysaccharide from seaweed. It was identified to be a good matrix with excellent releasing properties; however, it is not free of irritation potential. Carrageenan even is used to induce edema[6]. The results of previously studies[3] demonstrated that carrageenan hydrogels display a quite limited compatibility with ethanol compared to chitosan–EDTA[2].

Another interesting natural substance is chitosan, a cationic polysaccharide derived from skeletons of crustaceans and also from fungal cell walls, which is currently extensively investigated in many areas of pharmaceutical preparations[7,8]. Chitosan has valuable properties as a biomaterial because it is considered to be biocompatible, biodegradable, and nontoxic[9,10].

Chitosan and its derivatives are discussed as permeation enhancers also for transdermal delivery[11]. The mode of action is still not fully understood, however, it is widely supposed that chitosan acts through an effect on the barrier properties of the cellular junctions, the tight junctions. But in skin these tight junctions are lying below the stratum corneum, therefore this mechanism on skin is improbable. Another reason for enhanced permeability could be an interaction of chitosan with negative charges in the skin, which is indicated by the results of a recent study, where iontophoresis significantly increased not only the permeation, but also the skin retention of doxorubicin. Despite the fact that iontophoresis of chitosan gels significantly decreased the electro osmotic flow, doxorubicin diffusion throughout the deeper layers of the skin was improved[12]. Pure chitosan cannot be used as a matrix for dermal applications because of the unfavorable solubility behavior. Moreover, the adhesiveness on skin is not high enough. Therefore derivatives were synthesized in order to achieve higher adhesiveness on skin and also practicable as vehicles for the model drug progesterone. An increased skin adhesion is desirable for a longer contact time and with it a higher drug permeation. Therefore chitosan–glycolic acid was used with the aim of a good skin adhesiveness and suitable drug permeation. As expected the glycolic acid had a significant positive impact on the adhesive properties of the polymer[13]. One reason for the excellent skin adhesion could be the building of hydrogen bonds because of the additional OH^-group of glycolic acid. There are several theories of adhesion and underlying forces that appear to act independently[14,15].

Glycolic acid has been demonstrated to induce a decrease in the turnover time of the stratum corneum[16]. On one hand there are studies which show a tumor inhibiting effect of glycolic acid[17] and on the other hand negative effects like increase in the level of skin damage in a dose-dependent manner have been reported[18]. Since glycolic acid is not covalently linked to the chitosan, it might also be possible that the positive effect on skin permeation is because of a small amount of free glycolic acid at physiological pH of the skin. Moreover, the excellent skin compatibility of chitosan is confirmed in numerous studies[19,20].

Conclusion

Chitosan–glycolic acid can be recommended as a suitable polymer for hydrogels and an adhesive matrix for a transdermal application of progesterone exhibiting excellent skin adhesiveness and permeation properties.

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Declaration of interest: The authors report no conflicts of interest.

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