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Development of a mucoadhesive nanoparticulate drug delivery system for a targeted drug release in the bladder

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ABSTRACT

Purpose: Purpose of the present study was the development of a mucoadhesive nanoparticulate drug delivery system for local use in intravesical therapy of interstitial cystitis, since only a small fraction of drug actually reaches the affected site by conventional treatment of bladder diseases via systemic administration.
Methods: Chitosan-thioglycolic acid (chitosan-TGA) nanoparticles (NP) and unmodified chitosan NP were formed via ionic gelation with tripolyphosphate (TPP). Trimethoprim (TMP) was incorporated during the preparation process of NP. Thereafter, the mucoadhesive properties of NP were determined in porcine urinary bladders and the release of TMP among simulated conditions with artificial urine was evaluated.
Results: The particles size ranged from 183 nm to 266 nm with a positive zeta potential of +7 to +13 mV. Under optimized conditions the encapsulation efficiency of TMP was 37%. The adhesion of prehydrated chitosan-TGA NP on the urinary bladder mucosa under continuous urine voiding was 14-fold higher in comparison to unmodified chitosan NP. Release studies indicated a more sustained TMP release from covalently cross linked particles in comparison to unmodified chitosan-TPP NP over a period of 3 h in artificial urine at 37 °C.
Conclusion: Utilizing the method described here, chitosan-TGA NP might be a useful tool for local intravesical drug delivery in the urinary bladder.

1. Introduction

The development of effective dosage forms for the local administration in the bladder represents for several reasons an important field of research. There are numerous diseases of the bladder, such as cancer, inflammation, infection or incontinence that may be treated by pharmaceutical active compounds. Many of these compounds are currently administered orally. In this case, systemic delivery must be regarded as less effective, since only a small fraction of the drug actually reaches the target side. Therefore, a local application would be more effective in order to ensure maximum delivery of active ingredients on the site of the disease. In addition, intravesical administration would also minimize systemic side effects and avoid the first pass metabolism, whereby a dose reduction would be feasible. In case of intravesical treatment, the drug is simply instilled by solution into the bladder through a catheter. The anatomy of the urinary bladder allows relatively easy access and manipulation by a catheter. Intravesical drug delivery has its inherent limitations as well. The bladder is a very well purged organ so that the periodical voiding of urine washes out the drug and thus reduces the residence time of the drug in the bladder. This requires frequent treatments with repeated catheterizations. Consequently, the potential for infections is increased by repeated catheterization into the bladder. Another hindrance is the very low permeability of the urothelium. In the diseased state the urothelial layer does not allow penetration of the drug into the bladder wall (Lewis, 2000; Au et al., 2002). Accordingly, permeation enhancers would be necessary to improve the transport of the therapeutic agent. To overcome these problems, it would be desirable to provide a mucoadhesive, permeation enhancing and sustained delivery system which is able to deliver the drug over an extended period of time with the device biodegrading or bioeroding time-dependently to be eliminated from the bladder. So far a prolonged residence time of drug delivery systems, however, was strongly limited by an insufficient adhesion provided by state of the art mucoadhesive polymers. These are just capable of non-covalent bond formation such as hydrogen bonds, van der Waal's forces or ionic interactions of polymer chains and mucus. In contrast, thiomers are mucoadhesive basis polymers displaying thiol bearing side chains that are capable of forming covalent bonds with cystein-rich subdomains of the mucus gel layer (Leitner et al., 2003). The bridging structure most commonly utilized in biological systems – namely the disulfide bond – is thereby exploited. So far, however, thiomers have not been utilized in order to improve the residence time of delivery systems on the mucosa

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of the urinary bladder. It is therefore the aim of this study to investigate the mucoadhesive properties of thiomers on the intravesical mucosa in comparison to well-established mucoadhesive polymers in order to prolong the residence time of instilled drugs in urinary bladders. Thiolated chitosan nanoparticles were chosen as intravesical dosage form, because of stability (Bernkop-Schnürch et al., 2006a), comparatively higher mucoadhesion (Bernkop-Schnürch and Steininger, 2000; Grabovac et al., 2005) and controlled drug release (Greimel et al., 2007). In vitro mucoadhesion studies of prehydrated thiolated chitosan NP in comparison to unmodified chitosan NP were carried out in isolated porcine urinary bladders among urinary voiding. Furthermore, the release rate of incorporated TMP in artificial urine was characterized in order to evaluate the suitability of thiolated chitosan NP as intravesical drug delivery system.

2. Materials and methods

2.1. Materials

Chitosan (middle-viscous), thioglycolic acid (TGA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), 5.5-dithiobis (2-nitrobenzoic acid), sodium tripolyphosphate (TPP), fluorescein diacetate (FDA), trimethoprim (TMP), ethyl acetate, trehalose, sodium nitrite, components for artificial urine (Chutipongtanate and Thongboonkerd, 2010) and hydrogen peroxide were purchased from Sigma–Aldrich, Austria. For determining TMP and FDA in samples, chromatography grade methanol (MeOH) and acetonitrile (ACN) were used from Fisher Scientific, Germany. All other chemicals were of analytical grade.

2.2. Preparation of low molecular mass chitosan

Middle-viscous chitosan was dissolved in acetic acid (6% (v/v)) to get a clear solution. Subsequently, sodium nitrite was added to obtain low molecular mass chitosan (Schmitz et al., 2007). After 1 h incubation under continuous stirring, chitosan was precipitated with 5 M sodium hydroxide. The precipitate was filtered and washed by cold acetone. The received low molecular mass chitosan was resolubilised in 200 ml of 0.1 M acidic acid and exhaustively dialyzed against demineralised water. Finally, the product was lyophilized under vacuum at 0.2 mbar and −70 °C (Benchtop 2K, VirTis, NY, USA) and kept in desiccator for further use.

2.3. Synthesis of chitosan-TGA

The covalent attachment of TGA to chitosan was achieved by the formation of amide bonds between primary amino groups of the polymer and the carboxylic acid groups of TGA mediated by EDAC as described by our research group previously (Greindl, 2008).

2.4. Identification of thiol groups and disulfide bonds

The amount of free thiol groups immobilized on the polymer was quantified by Ellman's method using a microplate reader (FluoStar Galaxy, BMG, Offenburg, Germany) (Bernkop-Schnürch et al., 1999). The content of disulfide bonds was measured after reduction with $NaBH_4$ at 37 °C for 2 h and determined with Ellman's reagent. The total amount of thiol moieties is represented by the summation of reduced and oxidized thiol groups in the form of disulfide bonds (Werle and Hoffer, 2006).

2.5. Preparation of FDA loaded NP

The NP were prepared by in situ gelation of unmodified chitosan and chitosan-TGA, respectively, with TPP according to a method described previously (Bernkop-Schnürch et al., 2006b). In brief, unmodified and thiolated low molecular mass chitosan, respectively, were hydrated in 0.05 M acetic acid/sodium acetate buffer solution (pH 6.2) at a final concentration of 0.5% (w/v). Afterwards a 0.5% (w/v) TPP solution in demineralised water pH 5 was added dropwise to the solution to create ionically crosslinked NP. The particle suspensions were partially oxidized by the addition of 0.5% (v/v) H_2O_2 solution and the mixture was incubated for 1 h under continuous stirring at room temperature.

Then a 0.05% (w/v) solution of FDA solved in ACN was added to the NP in a ratio of 1:2. Subsequently, the suspension was incubated under continuously stirring for 1 h. After that, NP were purified by centrifugation for 2 min at 14,000 rpm. In order to avoid aggregation of NP during the centrifugation process, trehalose was added to the samples to obtain a final concentration of 3% (w/v). To enhance the suspendability after the lyophilisation (Benchtop 2K, VirTis, NY, USA), particles were resuspended in a 3% (w/v) trehalose solution immediately after the supernatant was removed. To determine the FDA load of particles, they were resuspended in 2 ml of ACN/water mixture in a ratio of 80:20 (v/v). For complete extraction of FDA the NP were sonicated for 5 min and afterwards centrifuged at a speed of 14,000 rpm for 5 min. Then, 100 μl of the supernatant was removed and 10 μl of the aliquot was injected into the HPLC system for analysis. HPLC analysis was achieved using a nucleosil 100-5 C18 HPLC column, 125 mm × 4.6 mm i.d., 5 μm particle size, 100 Å pore size (Macherey-Nagel, Germany) as stationary phase and a mixture of ACN/water in the ratio 80:20 (v/v) as mobile phase. FDA was monitored at a wavelength of 248 nm with a flow rate of 1 ml/min at a column oven temperature of 40 °C. A calibration curve was established by linear regression from peak areas versus nominal concentrations. The collected data were analyzed with EZ Chrome Elite 3.2.1 version software, taking into account the peak heights of analytes.

2.6. Preparation of TMP NP

For loading of nanoparticles a combination of embedding and diffusion was used. In brief, the polymer solutions were prepared as described above. Afterwards, a 0.5% (w/v) TPP solution in demineralised water pH 5 containing 5 mg/ml TMP was added dropwise into the solution to form NP. After partial oxidation, the NP were further treated with 0.5% (w/v) TMP/MeOH solution for 1 h. Subsequently, the particles were purified and resuspended as described above.

2.7. Particle characterisation

Size distribution and zeta potential of particles were determined by photon correlation spectroscopy using particles sizer (Zeta Potential/Particle Sizer, Nicomp™ 380 ZLS, PSS, Santa Barbara, CA, USA) with laser wavelength of 650 nm and an E-field strength of 2 V/cm. For size measuring particles were suspended in demineralised water at room temperature and the intensity distribution was fit to a Gaussian size distribution curve.

2.8. TMP content determination

For extraction NP were dispersed in 2 ml of ethyl acetate and homogenized for 30 s using a vortex mixer (Vortex Mixer SA7, Stuart). To ensure complete extraction of TMP the NP were sonicated for 15 min and afterwards centrifuged at a speed of 14,000 rpm for 5 min. The supernatant was removed and evaporated under shaking in a thermomixer (Thermomixer Comfort, Eppendorf, Hamburg, Germany) at 80 °C for 25 min. Then the residue was dissolved in 1 ml of mobile phase and 10 μl of the aliquot was injected into the HPLC system for analysis. TMP was quantitatively determined by HPLC

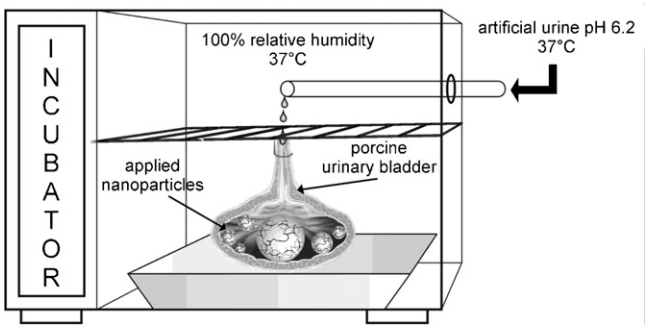

Fig. 1. Experimental setup for mucoadhesion studies in porcine urinary bladders.

according to a method described in literature previously (Biswas et al., 2007). HPLC analysis was achieved using a supelcosil LC-18 HPLC column, 100 mm × 4.6 mm i.d., 5 μm particle size, 120 Å pore size (Sigma–Aldrich, Austria) as stationary phase and a mixture of 50 mM ammonium acetate buffer pH 4.65/acetonitrile in the ratio 73:27 (v/v) as mobile phase. The eluent was monitored at a wavelength of 265 nm with a flow rate of 1 ml/min at a column oven temperature of 40 °C. A calibration curve and the analysis of the collected data were done as described above.

2.9. Determination of thiol groups on the porcine urinary bladder and intestinal mucosa

Mucus from porcine urinary bladder and small intestine was gently scraped off. After lyophilisation 0.5 mg of mucus was dissolved in 500 μl of 0.5 M phosphate buffer pH 8 and 500 μl of Ellman's reagent were added in order to quantify free thiol groups as described above.

2.10. In vitro mucoadhesion studies on porcine urinary bladders

Porcine urinary bladders from the slaughterhouse were used for the evaluation of mucoadhesive properties of thiolated and unmodified NP. An analytical method to determine the amount of remaining fluorescent marker on the mucosa was developed by our research group (Albrecht et al., 2006a). In brief, the porcine urinary bladders were mounted into an incubation chamber with 100% humidity and a temperature of 37 °C, as shown in Fig. 1. Thereafter, bladders were continuously filled with 0.3 M artificial urine pH 6.2. To humidify the mucosa of urinary bladders, an equilibration time of 10 min was allowed before administering the NP. During the whole experiment, the temperature of the artificial urine was kept at 37 °C and every 30 min the bladders were emptied. A constant flow rate of 2 ml/min was provided using a peristaltic pump. After the equilibration period, bladders were emptied and an amount of approximately 8 mg of prehydrated thiolated and unmodified NP were transferred into the bladders. After 60, 120 and 180 min the treated bladders were cut into pieces and incubated in 35 ml of 5 M NaOH for 30 min at 37 °C under regularly stirring to quantitatively hydrolyze FDA to sodium fluorescein (Albrecht et al., 2006b). Then, 1 ml was taken, centrifuged at a speed of 13,400 rpm for 5 min and the fluorescence was measured at an excitation wavelength of 485 nm and an emission wavelength of 520 nm with a microplate reader (FluoStar Galaxy, BMG, Offenburg, Germany). For the calibration curve increasing amounts of sodium fluorescein were used as standard samples.

2.11. Release studies

Drug release tests were carried out on all TMP-loaded NP. Around 8 mg of NP were suspended in 3 ml artificial urine and were incubated in a water bath at 37 °C under continuous shaking. For this experiment, six formulations were prepared at the same time point. After scheduled time intervals of 30, 60, 120 and 180 min the nanoparticle suspension was centrifuged and the supernatant collected. Subsequently, the supernatant was extracted with ethyl acetate and evaporated at 80 °C for 25 min, as described above. Then the residue was dissolved in 1 ml of mobile phase and 10 μl of the aliquot was injected into the HPLC system for analysis.

2.12. Statistical data analysis

Statistical data analyses were performed with Student's *t* test and nonparametric tests for example with Mann–Whitney-test with $p < 0.05$ as the minimal level of significance. Calculations were done using the software GraphPad Prism version 5.01.

3. Results

3.1. Synthesis and characterization of chitosan-TGA

Low molecular mass chitosan was obtained according to a method described previously by our research group (Schmitz et al., 2007). Schmitz et al. examined the molecular mass of low molecular mass chitosan using capillary viscosity measurements. It was found that the recovered chitosan had a mean molecular mass of 10 kDa (Schmitz et al., 2007). Thioglycolic acid was covalently attached to this low molecular mass chitosan by formation of amide bonds. The lyophilized polymer conjugate was white, odorless, of fibrous structure and easily soluble in aqueous solution at pH 6.5 and lower. The resulting amount of free thiol groups immobilized on one gram of chitosan was 1456 ± 136 μmol and approximately 9% of thiol groups were oxidized.

3.2. Nanoparticle preparation and characterization

Chitosan and chitosan-TGA NP were prepared by ionic gelation. According to the results of several experiments, pH 6.2 was identified as ideal for the polymer solution whereas pH 5 appears to be ideal for the TPP solution. A polymer–TPP ratio of 7.5:1 delivered the most favorable NP with a positive zeta potential as illustrated in Table 1. Consequently, this preparation technique generated particles with a size range of 183–266 nm with a narrow size distribution, as shown in Fig. 2. Images in Fig. 3 illustrate particles of spherical shape which could be investigated by scanning electron microscopy. In order to enhance the stability of chitosan-TGA NP against fluctuating pH values in the bladder, inter- and intramolecular disulfide bonds were formed due to the addition of H_2O_2. The resulting decrease in thiol groups within the particles was quantified by Ellman's reagent, to characterize the oxidation process. The more H_2O_2 was added, the more thiol moieties were oxidized. Results of this study are shown in Table 2. In addition, fluorescence labeled NP were prepared by a simple diffusion technique, making use of the solubility of FDA in organic solvents and its insolubility in aqueous solutions. As opposed to this, TMP loaded NP were prepared by a combination of embedding and diffusion. The payload of FDA and TMP loaded NP is illustrated in Fig. 4.

3.3. In vitro studies

Mucoadhesive properties of prehydrated unmodified and thiolated NP on the urinary bladder mucosa were evaluated indirectly using FDA as fluorescence marker. The studies revealed that chitosan-TGA NP display significantly higher adhesion than the corresponding unmodified chitosan particles. When NP were

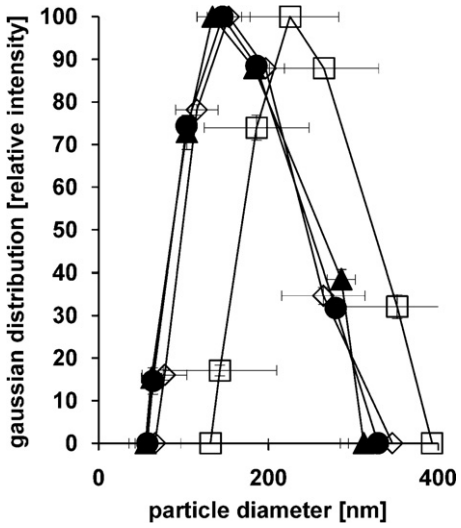

Fig. 2. Size distribution of ionically crosslinked nanoparticles based on chitosan [□] and chitosan-thioglycolic acid [◊], as well as covalently crosslinked nanoparticles based on thiolated chitosan with 426 μmol/g [▲] and 559 μmol/g [●] disulfide bonds. The relative intensity of particles describes the result gained by the measurement based on the principle of the Gaussian distribution analysis. Indicated values are means ± SD of last three experiments.

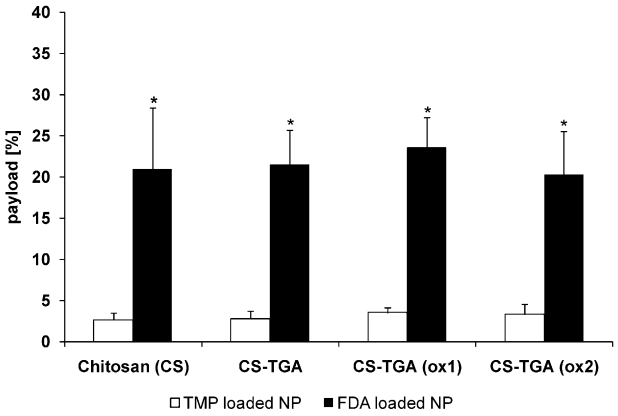

Fig. 4. Payload of trimethoprim [white bars] and fluorescein diacetate [black bars] loaded nanoparticles based on chitosan, chitosan-thioglycolic acid and chitosan-thioglycolic acid with 426 μmol/g (ox1) and 559 μmol/g (ox2) disulfide bonds. Indicated values are means ± SD ($n \geq 3$). *Differs from control, $p < 0.05$.

applied to the mucosa in prehydrated form, 14.2 ± 7.2% of fluorescence marker embedded in chitosan-TGA remained on the mucosal surface, whereas only 1.1 ± 0.1% of FDA incorporated in unmodified chitosan adhered to the mucosa for 3 h and a total of six micturitions. For this study chitosan-TGA particles with 1.13 mmol/g thiol groups were used and the results are shown in Fig. 5.

3.4. Thiol groups on porcine urinary bladder and small intestine

The quantification by Ellman's reagent resulted in 159 μmol of free thiol groups on one gram urinary bladder mucus and 209 μmol of thiol groups on one gram mucus of the intestinal mucosa. The

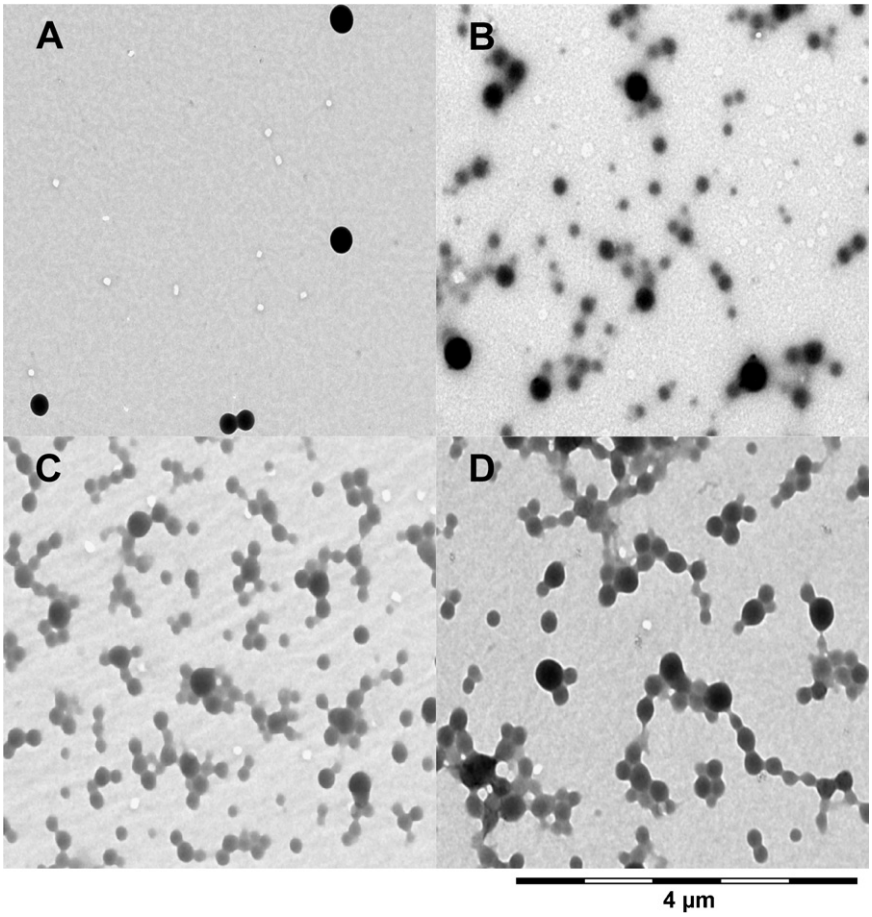

Fig. 3. Transmission electron microscopy images of the spherical shape of nanoparticles based on chitosan [A], chitosan-thioglycolic acid [B], chitosan-thioglycolic acid with 426 μmol/g disulfide bonds [C] and chitosan-thioglycolic acid with 559 μmol/g disulfide bonds [D]. Displayed bar represents 4.0 μm.

Table 1
Mean particle diameter and zeta potential of TMP loaded chitosan-TGA NP obtained by ionic gelation with TPP and followed by different oxidations with H_2O_2, respectively. Indicated values are means ± SD ($n \geq 3$).

Nanoparticles	Mean particle diameter [nm]	Polydispersity index	Zeta potential [mV]
Ionically crosslinked			
Chitosan	266 ± 64	0.44	7 ± 1
Chitosan-TGA	197 ± 24	0.38	7 ± 1
Covalently crosslinked			
Chitosan TGA (ox1)	183 ± 7	0.31	13 ± 3
Chitosan TGA (ox2)	186 ± 6	0.29	12 ± 1

Table 2
Amount of thiol groups and disulfide bonds immobilized on the basic thiomer chitosan-TGA and nanoparticles after ionic gelation with TPP and different degrees of oxidation with H_2O_2, respectively. Indicated values are means ± SD ($n \geq 3$).

	H_2O_2 [μmol]	–SH [μmol/g]	–S–S– [μmol/g]	$\sum$–SH [μmol/g]
Chitosan-TGA	–	1456	136	1728 ± 62
Chitosan-TGA NP	–	1391	178	1747 ± 36
Chitosan-TGA NP (ox1)	10.60	903	426	1753 ± 55
Chitosan-TGA NP (ox2)	21.21	641	559	1758 ± 27

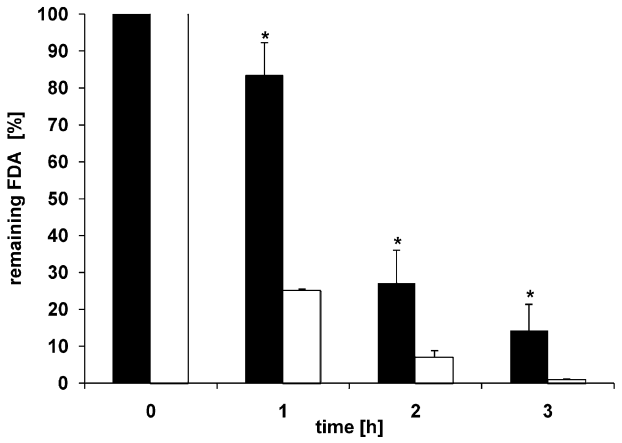

Fig. 5. Percentage of fluorescein diacetate remaining on porcine urinary bladders as a function of time. Studies were carried out with chitosan-thioglycolic acid nanoparticles [black bars] and unmodified chitosan nanoparticles [white bars] as control. Indicated values are means ± SD ($n \geq 3$). *Differs from unmodified chitosan nanoparticles, $p < 0.05$.

total amount of thiol moieties after reduction with $NaBH_4$ was determined to be 402 ± 38 μmol/g on the bladder mucosa and 428 ± 46 μmol/g on the intestinal mucosa. Results of this study are shown in Table 3.

3.5. TMP release studies

The influence of covalent crosslinking on release rate in comparison to ionic crosslinking was also studied. The particles loaded with TMP showed the most significant difference in drug release after 3 h in artificial urine at 37 °C. NP release profiles are shown in Fig. 6: an initial burst release is achieved for the unmodified ionically crosslinked chitosan NP, which is approximately 54% within 30 min. In contrast, the release of TMP from covalently crosslinked particles was comparatively slower and more sustained. The most sustained release profile showed the chitosan-TGA (ox2) NP with 36% of liberated drug within 3 h.

Table 3
The thiol and disulfide bond content on the surface of urinary bladder and small intestine mucosa. Indicated values are means ± SD ($n \geq 3$).

Mucus	–SH [μmol/g]	–S–S– [μmol/g]	$\sum$–SH [μmol/g]
Small intestine	209	109	428 ± 46
Urinary bladder	159	121	402 ± 38

4. Discussion

Numerous strategies for intravesical drug delivery systems have been reported, whereby nanocarriers represent the most widely used technology. They can form a variety of structures and can be formulated from materials such as lipids, synthetic polymers and biopolymers, proteins, metals, inorganic- and organometallic compounds. In this case, mucoadhesive polymers are mostly used to prepare nanoparticulate intravesical drug delivery systems because the uptake of drugs is often limited by the short contact time between the formulation and the absorption membrane and by a rapid wash out. Accordingly, mucoadhesive formulations should be able to fulfill three main criteria for intravesical drug delivery: rapid adhesion to the urothelium after instillation, not obstruct the flow of urine or any of the normal functions of the bladder and be retained on the bladder wall for at least several hours (Tyagi et al., 2006). In previous studies, the emphasis of investigations of drug delivery systems for intravesical administrations was focused on the contact between the formulation and the absorption membrane. Consequently, the detachment force between mucoadhesive polymeric films and the urinary bladder mucosa was evaluated (Kos

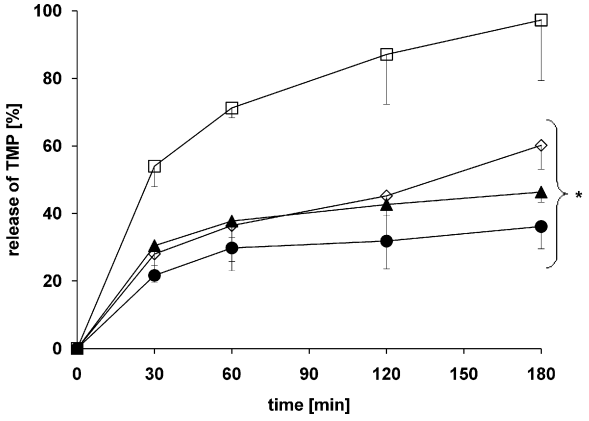

Fig. 6. Release properties of trimethoprim nanoparticles among simulated conditions with artificial urine as a function of crosslinking. Studies were carried out with nanoparticles based on chitosan [□], chitosan-thioglycolic acid [◊], chitosan-thioglycolic acid with 426 μmol/g disulfide bonds [▲] and 559 μmol/g [●] disulfide bonds. Indicated values are means ± SD ($n \geq 3$). *Differs from chitosan nanoparticles, $p < 0.05$.

et al., 1999; Bogataj et al., 1999; Burjak et al., 2001). Nevertheless, investigations of carrier systems as particles and their adhesion on the bladder wall for several hours during urine voiding were neglected and not considered. Accordingly, our approach was to develop an intravesical drug delivery system for local application, which shows sufficient mucoadhesion under so far unobserved natural conditions. Therefore, an appropriate urinary bladder model, under continuous voiding of urine was used. Moreover, the particles were prehydrated in comparison to previous studies in order to allow an instillation. Additionally, the bladder was emptied every half hour to simulate washing out of an oversensitive bladder by inflammation. This study considered these conditions in order to simulate the natural course. The preparation of NP relies on a widely used ionic gelation technique between positively charged chitosan and negatively charged TPP (Yang et al., 2009). Therefore, unmodified chitosan and chitosan-TGA solution was mixed, respectively, with the polyanion TPP. The obtained particles were in a nanosize range, with a narrow size distribution and had a positive zeta potential. Furthermore, transmission electron microscopy images demonstrated particles of spherical shape and visualize the tendency of chain formation of NP among themselves. The more H_2O_2 was added, the greater the chaining of the particles was. For further studies, such as the release and mucoadhesion of the intravesical delivery system, the payload of the therapeutic agent TMP and fluorescent marker FDA were determined. Results of this study indicate that the formation of inter- and intramolecular disulfide bonds, during the preparation process, has no influence on the incorporation of TMP into the NP. In contrast, a significant difference in loading efficiency between TMP and FDA was determined. TMP is a hydrophilic drug and much better soluble in aqueous media in comparison to the fluorescence marker. Accordingly, a lower drug content of TMP was incorporated into the particles during the preparation process. In case of FDA, loaded nanoparticles were prepared via a simple diffusion process making use of the solubility of FDA in organic solvents and its insolubility in aqueous solutions. Consequently, a higher payload was determined in comparison to TMP. This demonstrates that lipophilic drugs do not get lost in the aqueous ambient medium, resulting in improved drug content in nanoparticles (Barichello et al., 1999). For this reason, FDA was used because of its insolubility in aqueous media such as artificial urine. Thereby it cannot be washed out from the particles and remains on the bladder mucosa. Mucoadhesion studies under continuous urine voiding indicated, that chitosan-TGA NP with free thiol groups are sufficient to provide superior mucoadhesion compared to unmodified chitosan NP. The adhesion time of chitosan-TGA NP was around 14-fold increased, while unmodified chitosan NP were washed out after three hours and six micturitions. The improved mucoadhesive properties of chitosan-TGA NP are explained by the formation of covalent bonds between thiol groups of the polymer and cysteine-rich subdomains of glycoproteins on the intestinal mucus layer (Bernkop-Schnürch, 2005). In case of the urinary bladder the mucosa consists of glycosaminoglycans (GAGs) which cover the luminal side of the urothelium. The GAG layer is composed of proteoglycans and glycoproteins. Its function is still not fully elucidated, but GAGs are dynamic structures that play multiple physiologic functions and take part in several cellular processes (Levy and Wight, 1995; Jackson et al., 1991; Soler et al., 2008). Therefore, it was hypothesized that free sulfhydryl groups of the particles interact also with cysteine-rich subdomains of the presented glycoproteins in the bladder mucus. In order to prove this, a quantitative analysis of the mucus layer via Ellman's test was done. Results demonstrated no significant difference between the total amount thiol moieties on the bladder mucosa and on the intestinal mucosa. This outcome supports the expectation of formation of covalent bonds between thiol groups of NP and cysteine-rich subdomains of glycoproteins on the intravesical mucus layer. In consequence, the amount of remaining chitosan-TGA NP was higher in comparison to unmodified particles.

In this study chitosan was used as matrix material for the development of the intravesical drug delivery system. The in vitro release of TMP from the ionically crosslinked chitosan NP was almost complete within three hours. The hydrophilic nature of chitosan resulted in the rapid penetration of the matrix and consequently to a faster diffusion of TMP out of the particles. Furthermore, ionically crosslinked chitosan NP show poor mechanical strength under such conditions, as already mentioned in former studies (Pan et al., 2002; Zhang et al., 2004; Dhawan et al., 2004). In order to avoid the above difficulties, chitosan-TGA NP were used. Utilizing increasing amounts of H_2O_2 the particles were partially oxidized to form disulfide bonds and to ensure their stability. An increase in inter- and intramolecular disulfide bonds affects the release kinetics of the drug. The release profiles of TMP from the covalently crosslinked chitosan-TGA NP were significant different in comparison to the chitosan-TPP NP. Covalent crosslinking hardens the particles and leads to differences in the release rate of TMP. Additionally, it provides increased resistance for the penetration of artificial urine into the NP. Therefore, the diffusivity of TMP through the matrix material is lowered and allows a continuously release (Domb et al., 1990; Warren and Kellaway, 1998).

Finally, the developed mucoadhesive intravesical drug delivery system based on thiolated chitosan offers an adequate release profile besides its mucoadhesive properties in contrast to ionically crosslinked chitosan NP. With the aid of this drug delivery system likely an efficient adhesion to the mucus membrane of the urothelium is feasible even during urine voiding. This drug delivery system allows prolonged residence time in the bladder and a sustained drug delivery of TMP over a longer time span.

5. Conclusion

Direct instillation of therapeutic agents into the urinary bladder is an efficient alternative to systemic delivery. However, this method is limited by the fact that instilled drug solution becomes diluted during filling of the bladder and finally gets washed out during urine voiding. To overcome these shortcomings, the suitability of chitosan-TGA NP as efficient mucoadhesive nanoparticulate drug delivery system was investigated within this study. Due to thiol groups and disulfide bonds chitosan-TGA NP showed greater stability, superior mucoadhesion and more sustained and controlled release than the corresponding unmodified chitosan particles. The described chitosan-TGA intravesical drug delivery system might be a useful tool for a local drug application in the urinary bladder, which increases the residence time of the drug at the target site and enables sustainable delivery for an extended period of time.

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