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Using chitosan nanoparticles as drug carriers for the development of a silver sulfadiazine wound dressing

Gina S. El-Feky[a,b,*], Samar S. Sharaf[c], Amira El Shafei[c], Aisha A. Hegazy[c]

[a] Pharmaceutical Technology Department, National Research Center, Dokki, Cairo, Egypt
[b] Faculty of Pharmacy, October University for Modern Sciences and Arts, Egypt
[c] Textile Research Division, National Research Center, Dokki, Cairo, Egypt

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ABSTRACT

Burn wounds environment favors the growth of micro-organisms causing delay in wound healing. The traditional treatment with antimicrobial creams offer inaccurate doses. In the present study, a dressing coated with silver sulfadiazine (SSD) loaded chitosan nanoparticles (CSNPs) for the controlled-release of SSD into burn wound to control bacterial growth was investigated. CSNPs were formulated with different concentrations of chitosan and CM-β-CD and loaded with SSD complexed in 1:1 molar ratio with CM-β-CD, CSNPs were assessed for their particle size, polydispersity index, morphology and association efficiency. The formula showing the best characteristics was selected for the preparation of SSD loaded CSNPs wound dressing through a padding process with/without the use of cross-linker. The dressing was characterized for its physical properties, in addition, FTIR, X-ray, SEM and *in vitro* release were used for characterization. The dressing was proven effective for the inhibition of the growth of Gram positive and Gram negative bacteria as well as candida on an infected wound.

1. Introduction

Wounds including those resulting from burns often provide a favorable environment for the colonization of micro-organisms which cause infection (bacterial or fungal) and in turn may delay wound healing. Consequently, in order to improve the opportunity for wound healing, it is important to create conditions that are unfavorable to micro-organisms and favorable for the host repair mechanisms. Treating an infection reduces the wound's bacterial burden, which has favorable effects on the dynamics of oxygen delivery and utilization within the wounds, it also diminishes the chronic inflammatory response and adjusts the tissue's capacity to respond to cell signaling and to develop sustained growth (Kirsner, Orstead, & Wright, 2001). Topical antimicrobial agents are believed to facilitate this process.

In bedsores and burn wounds, the wound stages are often divided into an infectious period, necrosis and agglutination period, proliferation period and epidermis formation period (Shigeyama et al., 2001; Shigeyama, 2004). Generally, formulations are selected based on the disease stage where the application period is a critical factor in the choice of the treatment formulation. Silver Sulfadiazine (SSD) is the drug of choice in the infectious period for combating the threat of bacterial infection and preventing wound sepsis (Salas Campos, Fernandes Mansilla, & Martinez de la Chica, 2005).

SSD has dual antibacterial effects. The free silver can react with both sulfhydryl groups of bacterial enzymes and DNA, and sulfadiazine can stop the synthesis of DNA by interrupting the production of folate (Adhya et al., 2014). However, the antibacterial effect of SSD is badly limited by its poor aqueous solubility (Dellera et al., 2014). Moreover, SSD has been shown to be cytotoxic *in vitro* toward fibroblasts and keratinocytes and consequently to retard wound healing *in vivo* (Rosen et al., 2015).

In addition, SSD is mostly present in a 1% cream. This cream dosage form exhibits a number of general side effects, including their inability to maintain effective drug concentrations for a prolonged period at moist wound surfaces due to their short residence time, their messiness causing inconvenience to patients Dobaria, Badhan, & Mashru, 2009) and according to its manufacturers the SSD cream causes discoloration of the wound bed (Gear et al., 1997), which, after several applications, interferes with judging wound status. Also, it shows low silver release levels which affects the drug's efficacy as the antimicrobial efficiency of silver ions depends directly on its concentration, which should not drop under the limit value required for minimal inhibition.

Over the past few years, there have been a rapid increase in the demand of silver dressings loaded with accurate doses of the drug sufficient to provide controlled and sustained bactericidal action.

* Corresponding author at: Pharmaceutical Technology Department, National Research Center, Dokki, Cairo, Egypt.
E-mail address: gelfeky@hotmail.com (G.S. El-Feky).

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Dressings' advantages include the protection of the wound from physical damage and secondary infection, preventing wound contamination and peripheral channeling into the wound by bacteria, assisting in debridement, thermal insulation and ease of removal without causing any trauma to the wound (Boateng, Matthews, Stevens, & Eccleston, 2008; Wittaya-areekul & Prahsarn, 2006).

However, this is challenging as direct bonding of SSD on textile dressings is practically not possible as silver-based agents are not chemically bonded to textile fibres and in turn no specific drug concentration or release rate could be precisely expected from the dressing to the infected wound. In this case, encapsulation of SSD on a suitable hydrophilic nano-carrier that is then used to coat the dressing might prevent the cytotoxic effect of the drug and enhance the effective controlled application of SSD on the wound as it will lead to enhancing the SSD solubility and enabling uniform dispersion, embedment and controlled release of the drug from the prepared dressing (Agnihotri, Bajaj, Mukherji, & Mukherji, 2015).

During the past decades, chitosan has got great attention and been broadly applied in medical areas with respect to its excellent biological characteristics of bio-compatibility, absorptivity, non-hypersensitivity, biodegradability and wound healing (Jayakumar, Deepthy Menon, Manzoor, & Nair, 2010). Coating the cotton dressing with SSD-loaded chitosan nanoparticles is expected to allow the adherence of the dressing to the moist surfaces of the wound due to the bioadhesive property of chitosan (Dobaria et al., 2009) and thereby prolong contact time and subsequently offer prolonged and controlled SSD release.

In this work, we combine the advantages of silver sulfadiazine as an effective antimicrobial and antifungal drug with the advantages of chitosan nanoparticles as drug carrier systems and effective fabric coating material to present a novel antimicrobial silver sulfadiazine dressing.

2. Materials and methods

2.1. Materials

Chitosan was purchased from Mallinckrodt, USA [M.W = 400,000, degree of deacetylation 95%]. Silver sulfadiazine was a kind gift from El Nasr Pharmaceutical Company, Cairo, Egypt. Glutaraldehyde (25% solution), Sigma-Aldrich, USA. Glyoxal (40% solution), Sigma-Aldrich, USA. Glacial acetic acid 99.8% was obtained from El Nasr Pharmaceutical Chemicals Co., Egypt. CM-β-CD was purchased from Sigma–Aldrich, USA. Other chemicals and solvents were of analytical grade.

2.2. Methods

2.2.1. Formulation of chitosan nanoparticles;

Aqueous solutions of CM-β-CD (1 to 12 mg/ml) were added to acetic acid solutions of CS (pH 4.6) (0.1–0.3% w/v) and stirred for 24 h using a magnetic stirrer. NPs were obtained spontaneously due to ionotropic gelation which involves the interaction between the positively charged amino groups of CS and the negatively charged CM-β-CD (Ammar, El-Nahhas, Ghorab, & Salama, 2012). After the preparation of the NPs, three different systems were identified: clear solution, opalescent dispersion and aggregates. In this step we intended to determine the suitable conditions for the formation of CS NPs using CM-β-CD as cross linking agent. ***Sixty*** CS-CM-β-CD nanoparticle preparations were performed with each repeated thrice to check the results.

2.2.2. Formulation of SSD-CM-β-CD loaded CS NPs

For the association of SSD to the different nanoparticle systems, SSD was dissolved in the CM-β-CD phase in 1:1 molar ratio based on our previous work (Hegazy, Sharaf, El-Feky, & El-Shafei, 2013). Nanoparticles were then prepared as described under 2.2.1.

2.2.3. Quality control of CS NPs by transmission electron microscopy (TEM)

One drop of each sample was deposited on a film- coated 200-mesh copper specimen grid and allowed to stand for 10 min after which any excess fluid was removed with filter paper. The grid was later stained with one drop of 3% phosphotungestic acid and allowed to dry for 5 min before examination. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoparticles droplets.

2.2.4. Determination of CSNPs association efficiency

The nanoparticles were separated from the aqueous suspension medium by ultracentrifugation at 20,000 rpm for 30 min. The amount of SSD loaded into the CSNPs was calculated as the difference between the total amount used to prepare the nanoparticles and the amount found in the supernatant. The SSD association efficiency was calculated as follows:

$$\text{Association efficiency} = \frac{\text{Total amount of SSD - Free SSD}}{\text{Total amount of SSD}} \times 100$$

Drug analysis was carried out using HPLC.

2.2.5. Preparation of the wound dressing

In this study, Egyptian cotton guaze samples were used as the base of the dressing and the best method for coating the gauze dressing with the SSD loaded CSNPs was further studied.

2.2.5.1. Treatment of gauze with SSD loaded CSNPs using padding process. Cotton fabric samples were padded with SSD loaded CSNPs using laboratory padder at 85% wet pick up. The padded fabrics were subjected to drying at 80 °C for 5 min and cured at 150 °C for 3 min. The treated fabric samples were subjected to washing in distilled water, dried and then placed in a desiccators till further study.

2.2.5.2. Treatment of gauze with SSD loaded CSNPs using padding process in presence of cross-linker. The cotton gauze samples were padded in CSNPs solution loaded with SSD using pad-dry-cure process as follows: fabric samples were impregnated for 5 min in pad baths containing a specified amount of SSD loaded CSNPs equivalent to 1% SSD per each $5 \times 5\,cm^2$ wound dressing and 5% owb of two different cross-linkers (glutaraldehyde and glyoxal) with their compatible catalysts. Samples were then padded through laboratory padder. Then drying followed by curing in the thermo-fixation oven at 85 °C, for 5 min and 150 °C for 3 min respectively, were carried out. After curing, the fabric samples were rinsed with warm water for 15 min and dried at room temperature.

2.2.6. Physical characterization of the dressings

2.2.6.1. Weight. The basic weight of the dressing before treatment was measured by averaging the weights of three samples with fixed dimensions. Weight add-ons were determined after conditioning the samples and comparing initial weight (before treatment) and final weight (after treatment).

2.2.6.2. Thickness. The basic thickness of the dressing before treatment was measured by averaging the thickness of ten samples with fixed dimensions. Average thickness was re-determined after conditioning the samples and comparing initial thickness (before treatment) and final thickness (after treatment).

2.2.6.3. Tensile strength (TS). TS (kg) and elongation at break (%) of finished (with/without cross-linker) and untreated fabrics were measured according to the ASTM: D5035-1990 method with initial grip separation of 50 mm and cross head speed of 60 mm/min. TS was calculated by dividing the maximum load for breaking sample by cross-sectional area, and E were determined by dividing sample elongation at rupture by initial gauge length and multiplying by 100%.

2.2.6.4. Nitrogen percentage. After coating the cotton dressing with SSD-loaded CSNPs using three different processes (padding without cross-linker, padding with glutaraldehyde and padding with glyoxal), the cured fabrics were cooled down to room temperature, washed with 1% acetic acid to remove unfixed chitosan nanoparticles, washed thoroughly with cold water until neutral and finally air dried (El-Tahlawy Kh, El-Bendary, El-Hendawy, & Hudson, 2005). The nitrogen content of the treated fabric was estimated as per standard Kjeldhal method (Vogel, 1975) and was used as an indicator of the amount of chitosan nanoparticles loaded onto the cotton dressing.

2.2.6.5. Water absorbency (Aw). Uncoated fabric and Coated (with/without cross-linker) samples were immersed in water until equilibrium was reached. The excess water on the surface of wet samples were allowed to drain for 1 h through a calibrated sieve (diameter = 100 mm, aperture = 4 mm). Aw were obtained as follows:

$$A_w = \frac{m_{wet} - m_{dry}}{m_{dry}} \times 100$$

Where m_{dry} and m_{wet} are the weights of the dry and wet samples, respectively.

2.2.6.6. FTIR. FTIR spectroscopy was applied for evaluating the modification on the chemical structures of the SSD loaded CS NPs coated fabrics with and without cross linker. Infrared spectra of samples were monitored using the KBr disk method (García et al., 2014). Scanning range was 500–4000 cm^{-1} (Shimadzu 435 U-O4 IR spectrophotometer).

2.2.6.7. X-ray diffractometry. X-ray diffractometry was used to detect the changes in the degree of crystallinity after coating by drug loaded CSNPs with/without the aid of cross linker.

2.2.6.8. In vitro *release of SSD from the prepared wound dressing.* The uniformity of SSD distribution among the dressings and within the same dressing was tested thrice to identify the SSD content before the *in vitro* release test (data not shown).

A 2.5 cm × 2.5 cm of cotton dressings coated with SSD-loaded CS NPs prepared using the three different processes (padding without cross-linker, padding with glutaraldehyde and padding with glyoxal) were subjected to drug release study. The release of the drug from the prepared dressings was compared to the release of the drug from 1% SSD marketed cream. Amount of the cream containing drug equivalent to that loaded on a 2.5 cm × 2.5 cm prepared cotton dressing was put in a non-rate controlling clean dialysis bag. The bag was secured with two clamps at each end. All samples were placed into USP dissolution apparatus (USP dissolution tester, apparatus II, Erweka Apparatebau GmbH, model DT-D, Germany) using the paddle method at 60 rpm and 37 °C for 48 h using 500 ml of phosphate-buffered saline, pH 7.4. At appropriate time points, aliquot samples (2 ml) were taken, and replenished immediately after each sampling with the same volume of fresh buffer. The percentage released of SSD was measured using HPLC.

2.2.6.9. Assessment of the antibacterial efficacy of the prepared wound dressing.

2.2.6.9.1. Diffusion disc method. A filter paper sterilized disc saturated with a dressing sample coated with SSD-loaded CSNPs prepared using the three different processes (padding without cross-linker, padding with glutaraldehyde and padding with glyoxal) with specified dimensions was placed on a plate containing solid bacterial medium (nutrient agar broth) which has been heavily seeded with the spore suspension of the tested organism. After incubation, the diameter of the clear zone of inhibition surrounding the sample of the particular test organism was measured.

2.2.6.9.2. Shaking flask method. This standard method is used to measure the reduction rate in the number of colonies formed and provide quantitative data; average colony forming units per milliliter (CFU/ml). The reduction rate in the number of colonies is calculated using the following formula:

$$\text{Reduction rate}\,(\%) = B - A/B \times 100$$

Where,

A = CFU/ml for the flask containing the treated substrate after 6 h contact time

B = CFU/ml for the flask at time zero, prior to the addition of the treated substrate

The dressing sample coated with SSD-loaded CSNPs prepared using the three different processes (padding without cross-linker, padding with glutaraldehyde and padding with glyoxal) with specified dimensions was shaken at 37 °C for 6 h on a rotary shaker with the flask containing specific bacterial/fungal colonies. The number of colonies present in this liquid were determined before and after the addition of the dressing sample and the percentage reduction by the treated material was calculated.

In both tests, the bacteria used were *Bacillus subtilis* and *Staphylococcus aureus* as Gram positive and *Escherichia coli* and *Pseudomonas aeruginosa* as Gram negative.

2.2.7. Assessment of fungicidal activity of the prepared SSD dressing

The fungicidal activity of the SSD treated cotton samples were estimated qualitatively according to the modified DIN 53931 standard method, where synthetic nutrient-poor agar (SNA) (27), consisting of 1 g of KH_2PO_4, 1 g of KNO_3, 0.5 g of $MgSO_4.7H_2O$, 0.5 g of KCl, 0.2 g of glucose, 0.2 g of saccharose and 15 g of technical agar per 1 l distilled water was used instead of the prescribed malt-extract agar (MEA). SNA is a less nutritious cultural medium, allowing a more delicate colony growth and easier evaluation of the antifungal activity of silver. A specific volume of Candida spore-suspension were spread on each SNA plate. The inoculated plates were incubated at 29 °C for 24 h, afterwards, samples of cotton fabrics were placed on the medium and incubated at 29 °C for 7 and 14 days. After incubation, the fungicidal activity were determined in terms of the inhibition zone diameter.

For quantitative analysis, the shaking flask method as described under task **2.2.7.2** was performed using *Candida albicans* and the reduction percentage of the colonies was again calculated.

3. Results and discussion

3.1. Formulation of CSNPs

When the CSNPs were prepared, it was necessary to establish the best ratio of components that enabled their formation. Chitosan has been widely studied for its application in controlled drug delivery (Berger, Reist, Mayer, Felt, & Gurny, 2004).

Nanoparticles (NPs) were prepared using the ionotropic gelation method, this ionotropic gelation takes place when the positively

Table 1
Particle size and polydispersity index of nanoparticles prepared in the concentration range of 4–10 mg/ml CM-β-CD.

CM-β-CD conc.	4 mg/ml	5 mg/ml	6 mg/ml	7 mg/ml	8 mg/ml	9 mg/ml	10 mg/ml
Av. PS (nm)	40.56	41.57	55.33	51.67	54	56.5	87.67
±SD	11.73	9.23	17.57	12.55	16.52	18.41	9.69
PI	0.29	0.22	0.32	0.24	0.31	0.33	0.11

*Av. PS; average particlesize, **SD; Standard Deviation, ***PI; Polydispersity index.

charged amino groups of chitosan interact with the negatively charged polyanions by forming inter- and intramolecular linkages (Calvo, Remunan-Lopez, Vila-Jato, & Alonso, 1997). The negatively charged cyclodextrin derivative, CM-β-CD, was reported for its ability to form strong ionic interactions with CS (Perrin, Coussot, Lefebrve, Perigaud, & Fabre, 2006).

The formation of chitosan-CM-β-CD nanoparticles occurred spontaneously upon the addition of CM-β-CD to the chitosan solution. The results showed that the appearance of the solution changed when a certain amount of CM-β-CD was added to the chitosan solution from a clear to opalescent solution that indicated a change of the physical state of chitosan to form nanoparticles, then microparticles and eventually aggregates.

It was noted that the formation of nanoparticles was strongly influenced by the concentrations of chitosan and CM-β-CD. In general, it is possible to argue that when the amount of polyanions was too low (relative to CS), nanoparticles could not be formed, or that the quantity of the formed nanoparticles was too low (Oyarzun-Ampuero, Brea, Loza, Torres, & Alonso, 2009) However, when the amount of polyanions was too high, it was impossible to isolate the particles, because the nano-systems were not re-suspendable or, in extreme cases, precipitation occurred. In the presence of 1, 2 and 3 mg/ml CM-β-CD, no formation of NPs was observed, whereas, the use of 11 and 12 mg/ml CM-β-CD gave rise to unstable particles with a marked tendency to agglomerate.

Results revealed that amongst the tested concentrations of CS, 0.2% w/v CS concentration corresponded to the highest process yield of nanoparticles with different concentrations of CM-β-CD, thus, it was selected for subsequent studies.

3.2. Quality control of CSNPs using TEM

The size characteristics have been found to affect the biological performance of CSNPs. Therefore, the morphology, particle size and polydispersity index of the selected CSNPs was assessed by means of TEM (Fig. 1 and Table 1).

TEM images (Fig. 1) showed that the prepared CSNPs have a spherical morphology with average particle size ranging from 40.56 nm (4 mg/ml) to 87.67 nm (10 mg/ml). In addition the formed CSNPs exhibited relatively narrow particle size distribution, as indicated by the relatively low (from 0.11 to 0.33) polydispersity index (PDI) values calculated (Table 1).

3.3. Determination of CSNPs association efficiency

After the preparation of SSD-CM-β-CD loaded CSNPs as stated under 2.2.3, the following step was mainly meant to assess the capability of CSNPs to efficiently entrap the hydrophobic drug SSD after the success achieved in our previous work to enhance its solubility through complexation with CM-β-CD (Hegazy et al., 2013), Fig. 1b.

SSD association efficiency (AE) onto CSNPs of different CM-β-CD concentrations was evaluated. The AE of chitosan nanoparticles was found to increase from 60.31 ± 2.65 to 89.71 ± 11.01% on increasing CM-β-CD concentration from 4 mg/ml to 7 mg/ml, $p < 0.05$; on further increasing CM-β-CD concentration to 10 mg/ml, no significant change in AE was recorded.

Table 2
SSD association efficiency and yield of preparation after cross linking 0.2% chitosan with different concentrations of CM-β-CD(4–10 mg/ml) and formation of nanoparticles.

Concentration of CM-β-CD (mg/ml)	Association efficiency[a] (%)	Yield[a] (%)
4	60.31 ± 2.65	90.15 ± 9.37
5	71.57 ± 6.98	82.67 ± 3.24
6	78.07 ± 5.34	69.91 ± 10.11
7	89.71 ± 11.01	53.52 ± 6.92
8	80.43 ± 8.98	44.85 ± 3.02
9	71.54 ± 4.54	36.78 ± 5.34
10	73.40 ± 1.60	21.18 ± 2.43

[a] (mean values ± SD; n = 3).

An inversely proportional relationship was observed between CM-β-CD concentration and the production yield, where the highest yield was obtained with 4 mg/ml CM-β-CD concentration, while, the lowest was with the 10 mg/ml CM-β-CD concentration (Table 2). Results are in agreement with those reported by Krauland and Alonso where they found that higher concentrations of CM-β-CD showed lower production yield and vice versa (Krauland & Alonso, 2007).

Based on the above results, the 7 mg/ml CM-β-CD concentration together with 0.2% w/v CS were selected for the preparation of SSD loaded CSNPs for subsequent study.

3.4. Preparation of the wound dressing

3.4.1. Fabric selection

Fine and wide meshed weaved cotton gauze has been used for many years for debridement of heavily contaminated exudative and necrotic wounds, in addition, cotton fabric contains a certain amount of wax (0.5–1%), which causes softness and pleasant touch.

3.4.2. Treatment of gauze with SSD loaded CSNPs using padding process, with/without cross-linker

For the preparation of the dressing, the cotton gauze was padded with the prepared SSD loaded CSNPs with/without the use of crosslinking agents. Studies indicated that the mechanical properties of chitosan loaded fibres and chitosan fibres could be enhanced using cross-linkers and the cross-linking method (Knaul, Hooper, Chanyi, & Creber, 1998; Knaul, Hudson, & Creber, 1999) as this improves the mechanical properties for chitosan fiber due to the fact that chitosan is held on cotton only by the numerous hydrogen bonds and Vander Walls forces of attraction.

However, in the presence of a cross linker and a catalyst, there is a possibility of acid catalyzed covalent bond formation between the hydroxyl groups of cellulose and the amino groups of chitosan at the high temperature of curing (150 °C) thus giving better fixation in this case.

Dialdehyde compounds, *e.g.* glutaraldehyde and glyoxal, have been extensively used as cross-linking reagents for chitosan (Knaul et al., 1999), cellulose (Yagi et al., 1998) and starch (Ivanov et al., 1992). The mechanism of how these reagents bond to chitosan is still not fully understood. The two cross linkers; glyoxal and glutaraldehyde were used separately. During the drying and curing

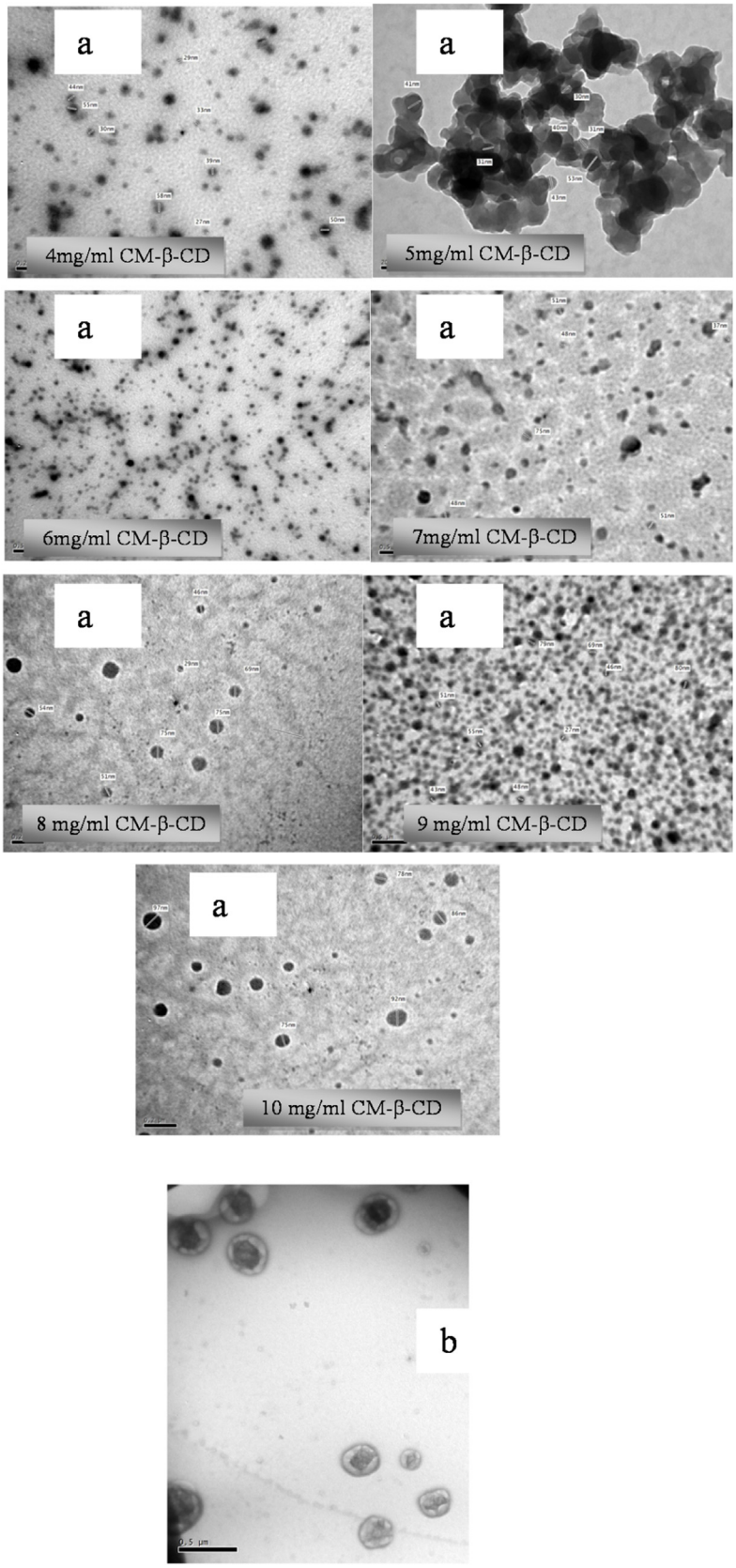

Fig. 1. a, b. TEM photographs identifying the morphology of nanoparticles prepared in the concentration range of 4–10 mg/ml CM-β-CD and TEM photograph showing the SSD loaded chitosan nanoparticle, respectively.

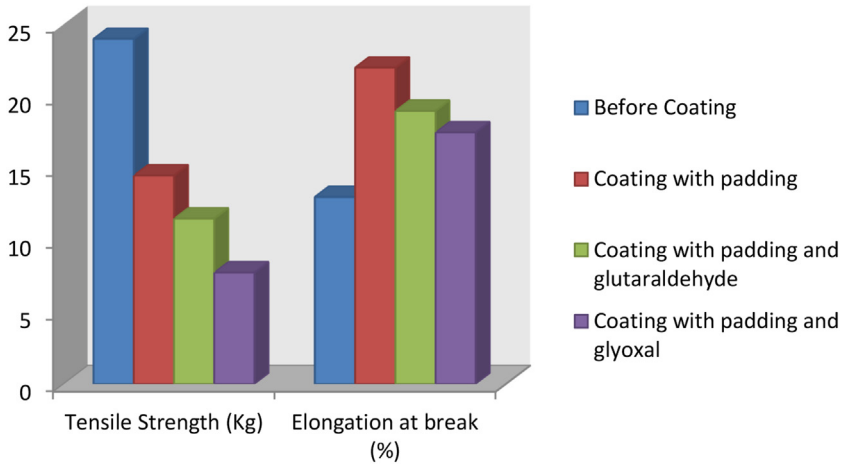

Fig. 2. Tensile strength and elongation at break after using three different methods for coating the dressing.

steps, part of the crosslinking agent was consumed in linking chitosan to the surface of the cotton dressing.

3.5. Physical characterization of the prepared dressing

The dressings obtained after the padding process with/without the help of cross linkers (glyoxal and glutaraldehyde) were characterized from different aspects to pick the one with the best physical properties.

3.5.1. Weight

The SSD loaded CSNPs add-on in cotton gauze was found to be 3.74% when using the padding process without cross linker and to be 3.88% and 3.96% when using glyoxal and glutaraldehyde as cross linkers, respectively. This could be an indication of the amount of SSD loaded CSNPs fixed on the gauze dressing, in other words, it could be hypothesized that using a cross linker especially glutaraldehyde during the padding process might have led to an increase in the amount of CSNPs loaded onto the cotton dressing.

3.5.2. Thickness

Thickness results showed that in absence of cross linker, the padding process investigated bring about finished gauze dressings which exhibit lesser percentage increase in their thickness (5%) compared to the percentage thickness increase obtained with glyoxal (7%) and glutaraldehyde (8.87%), respectively. Thickness results again supports the hypothesis derived from the weight add-on results.

3.5.3. Tensile strength

As is evident (Fig. 2), fabrics TS decreased generally after treatment with drug loaded chitosan nanoparticles inspite of the finishing process used. After treatment, the TS was found to be highest with conventional padding process and lowest with the use of glyoxal as cross linker. Rigidity conferred on the structure of cotton by inclusion of chitosan through various interactions with cotton may account for the decrease in the TS. It could be also attributed to the high molecular degradation of the weak cotton component of the gauze under the effect of finishing conditions and thus, leads to general loss in the TS of the wound dressing.

The elongation at break generally increased after padding. However, it is worth noting that with the use of cross linker the percent elongation at break decreased dramatically. This could be ascribed to the rigidity imposed to the fabric structure due to the use of the cross linker and in turn the increase in amounts of SSD loaded CSNPs fixed onto the cotton dressing.

3.5.4. Nitrogen percentage

The amount of SSD loaded CSNPs bonded to the fabrics was found to be dependent on the process of coating and the type of cross linker used. It was clear that the maximum chitosan fixation was obtained on using glutaraldehyde as crosslinking agent during the padding process (N%; 0.46%) whilst the minimum fixation was obtained when no cross-linker was used (N%; 0.39%).

3.5.5. Water absorbency (Aw)

Water absorbency of gauze dressing treated with the three different techniques indicated that the water absorbing nature of the gauze dressing generally increased with the use of cross-linkers. The highest increase was reported upon using glutaraldehyde as cross-linker (Aw%; 25.15%), followed by the use of glyoxal as cross-linker (Aw%; 19.16%) whilst the lowest increase was reported with padding without cross-linker (Aw%; 2.99%)

Based on the weight add-on, thickness and nitrogen percentage results which were used as indicators of the amount of chitosan nanoparticles fixed onto the cotton dressing and which showed that maximum chitosan fixation was obtained with the use of glutaraldehyde as crosslinking agent. And based as well on the fact that CSNPs possess a strong hydrophilic nature, therefore, it could be expected that the fabrics treated with glutaraldehyde would show high water absorbency due to the hydrophilic nature of the larger

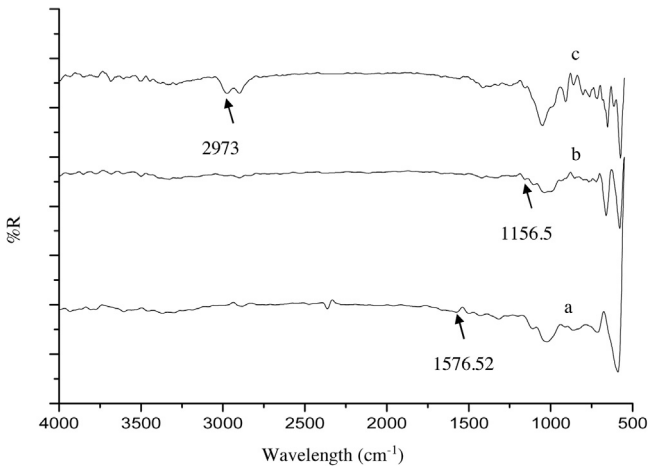

Fig. 3. a–c. FTIR of cotton dressings treated with SSD loaded CS NPs without cross linker, with glyoxal as cross linker and with glutraldhyde as cross linker, respectively.

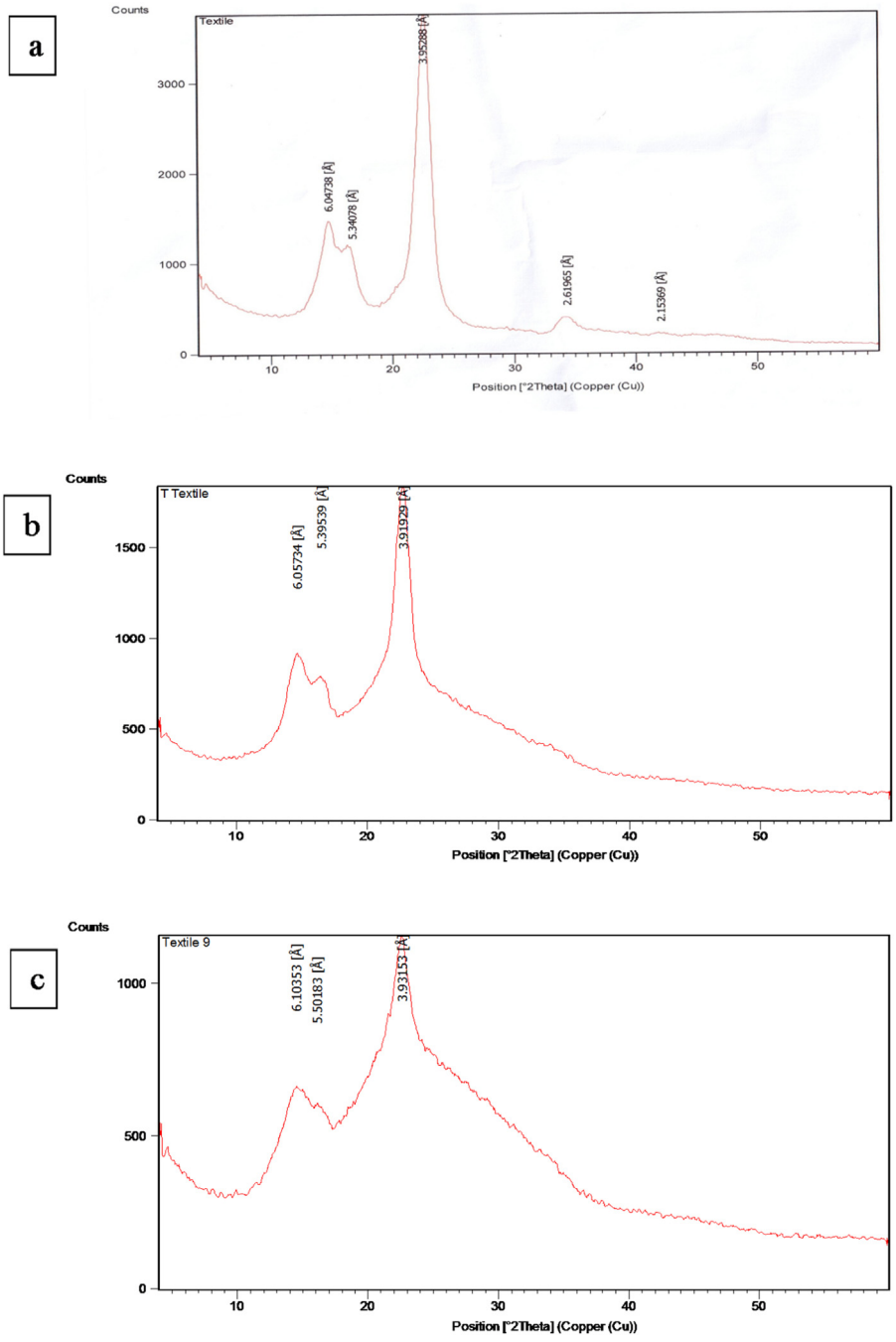

Fig. 4. a–c. XRD of SSD loaded CSNPs treated dressing/without cross-linker, SSD loaded CSNPs treated dressing/glutraldehyde and SSD loaded CSNPs treated dressing/glyoxal, respectively.

amount of chitosan nanoparticles forming thin film-like coating over the cotton dressing.

3.5.6. FTIR

FTIR spectra for chitosan treated dressing without and with crosslinkers were presented in Fig. 3a–c.

Comparing the FTIR spectra of cotton dressings treated with SSD loaded CS NPs without cross linker to those treated with the use of cross linkers, one observed the absence of the 1576.52 cm^{-1} peak that exists when cross linker was not used, The peak at 1576.52 cm^{-1} was assigned to strong vibrations of chitosan's secondary amide. Peaks in the region of 1030–1160 cm^{-1} were assigned to the C—O bonds (Xiao, Zhang, Zhang, & Zhang, 2003; Pranoto, Rakshit, & Salokhe, 2005). Using a cross linker, peak shifting occured due to co-ordination bond between the cross linker and electron rich groups (oxygen/nitrogen). This caused an increase in the bond length, ultimately shifting the frequency.

In case of the dressing treated with glyoxal as cross linker, one could observe that a new peak appeared at 1156.5 cm^{-1} for chitosan fiber after crosslinking reaction with glyoxal indicating the reaction of glyoxal with the hydroxyls of the glucosamine rings due to acetalization which comes in good agreement with Solomons (1980).

An additional peak appeared at 2973 cm^{-1} due to increased C—H stretching vibrations of chitosan with the incorporation of glutaraldehyde.

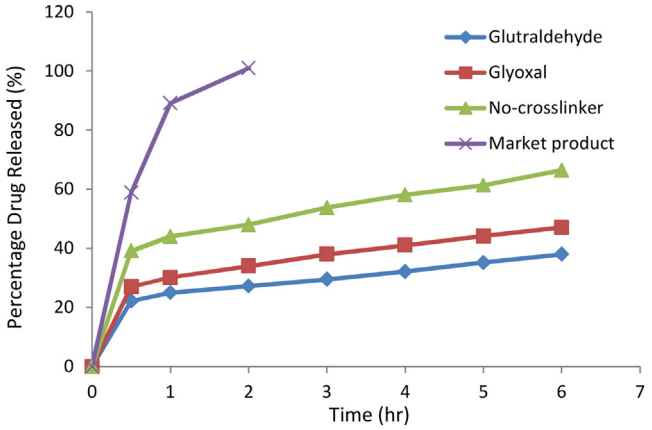

Fig. 5. *In vitro* Drug Released from Different Wound Dressings.

3.5.7. X-ray diffractometry

Fig. 4a–c depicts the X-ray diffractograms. Differences on crystallinity for SSD loaded CSNPs treated dressings without cross linkers and those with glutaraldehyde and glyoxal as cross linkers can be noticed.

Fig. 4b, c shows a reduction in crystallinity by crosslinking with glutaraldehyde and glyoxal. This result is in accordance to Koyama, Taniguchi, Huang, and Blakenship (1986). This can be understood on basis of glutaraldehyde and glyoxal chains being attached and inserted to the previously arranged chitosan chain configuration, inducing chemical modification and in turn producing less organized structures.

It was postulated in previous studies (Saito, Tabeta, & Ogawa, 1987) that water molecules are loosely bond to chitosan chains along (0 1 0) direction. In thermally treated samples as is the case in this study, there is the development of a peak at 15.1° which corresponds to the reflection (1 2 0). In this study, the crosslinking of chitosan with glutaraldehyde and glyoxal induced a degree of attenuation of the peak that depends exactly on the water quantity (0 2 0).

It was previously reported that the dialdehyde in glutaraldehyde alter the species responsible for water bonding (Saito et al., 1987).

3.5.8. In vitro *release study*

Comparing the *in vitro* release of SSD from different dressings in comparison to each other and to the market SSD, it was obvious that all dressings had a slower release rate *versus* the market SSD (Fig. 5).

Drug release investigations from both the SSD loaded CSNPs dressing prepared without cross-linker and that prepared with cross-linker demonstrated a biphasic trend characterized by swift release for the first half an hour followed by a sustained slower release phase reaching almost 30%, 40% and 60% of the initial loaded drug amount after 8 h for SSD loaded CSNPs dressing prepared with glutraldhyde, glyoxal and without cross-linker, respectively. It is apparent that the initial swift release is attributed to drug particles adsorbed on the surface of the dressing whereas, the slower release thereafter can be the outcome of the time taken for wetting the dressing (Azuma et al., 2015) and since the dressing prepared with glutaraldehyde showed the highest water absorbency and in turn swelled most, it had the narrowest free space around the SSD loaded CS powder region, resulting in slower dissolution and sustained release of the drug.

Table 3
Qualitative and quantitative antifungal study of SSD loaded chitosan wound dressing.

	Qualitative Study Zone of inhibition (mm)	Quantitative Study Fungal Reduction (%)
Control	0	0
Chitosan without cross-linker	4.2	20.35
Chitosan/glutraldehyde	13	36.85
Chitosan/glyoxal	11.2	32.91

3.6. Assessment of antibacterial efficacy of prepared SSD dressing

The antibacterial properties of materials were studied by qualitative (diffusion disc method) as well as quantitative test methods (shaking flask method) (Sathianarayanan, Bhat, Kokate, & Walunj, 2010).

Results showed that SSD loaded CSNPs treated dressings have a degree of antibacterial properties to both gram positive and gram negative microorganisms. All tested dressings showed an inhibition zone ranging from 4.9 to 17 mm for gram positive bacteria and from 4 to 14 for gram negative bacteria, it has been also found that the% reduction value to both gram positive and gram negative microorganisms was in the ranges of 3.7–25% and 5.1–18.2%, respectively. The untreated dressing showed bacterial growth under the test specimen and zero% reduction to both types of bacteria.

The zone of inhibition values as well as the percentage reduction values showed that the dressings treated with SSD loaded CSNPs using the padding process in presence of glutaraldehyde as cross linker had the best anti-bacterial activity not only through preventing the growth of bacteria under the fabric but also through the amount drug released which was enough to kill the bacteria. This could indicate that the padding process with the use of cross-linker especially glutaraldehyde has the ability to upload a larger amount of the drug loaded carrier onto the dressing surface and in turn render the dressing more efficient in treating burn wounds.

In the antimicrobial tests, although the growth medium does not resemble the wound exudates, it simulates the worst case scenario where the number of microorganisms is much greater than that possibly present at a wound surface. The culture conditions in the antimicrobial test provide the optimum environment in terms of medium composition, pH, and temperature for the growth of test microorganisms. In these conditions the maximum microbial growth and activity are achieved. Therefore, it would be appropriate to conclude that, if significant antimicrobial activity is observed at these extreme conditions, higher activity is expected in real cases because wound surface and exudates do not exhibit optimum growth conditions for the microorganisms. Additionally, the natural defense mechanism during healing process also helps to eliminate the growth of these microorganisms (Altiok, Altiok, & Tihminlioglu, 2010).

3.7. Assessment of fungicidal activity of the prepared SSD dressing

In the antifungal quantitative and qualitative tests, results showed that only the control specimens had no anti-fungal effect whereas, positive inhibition was observed when SSD loaded CSNPs treated wound dressings were tested. Test specimens showed clear antifungal activity towards *Candida albicans* with SSD loaded CSNPs treated wound dressing using gluteraldhyde as cross-linker showing the best anti fungal potential of all treated dressings due to their capability to efficiently coat the dressing with the largest amount of drug loaded nanocarrier (Table 3).

4. Conclusion

The results indicate that the SSD loaded CSNPs wound dressing prepared using padding with glutaraldehyde may be a very potential wound dressing with antibacterial capability to prevent an injured skin from infections. The dressing formulated in this study showed continuous delivery of SSD that could extend over 24 h compared to two hours release offered by the market product which should help to improve patients' compliance by removing the need for multi- daily drug administration and is expected to offer an efficient treatment due to the longer undisrupted contact time between the drug and the burn wound.

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