



# Enhancing antibacterial action of gauze by adding gelatin nanoparticles loaded with spectinomycin and chloramphenicol

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**Abstract** Proteins are biocompatible, metabolizable, and susceptible to surface changes and legend attachments for targeted distribution, and are therefore ideal materials for nanoparticle-based drug delivery applications. The production, characterization, and use of gelatin nanoparticles (GNPs) for intracellular administration of weakly cell-penetrating antibiotics (such as spectinomycin and chloramphenicol) to enhance their treatment of bacterial and fungal infections are described in this paper. Gelatin nanoparticles were synthesized using the desolvation method and then loaded with two antibiotics (spectinomycin and chloramphenicol) for addition to cellulosic cotton medical gauze. The concentration of gelatin and a crosslinker were chosen and analyzed among many factors to maximize the particle size of the nanoparticles. Fourier transform infrared spectroscopy, particle size analyzers, and antibacterial activity determination were used to evaluate the medical gauze treated with the nanoparticles that were loaded with antibiotics. The results revealed that gelatin nanoparticles

loaded with the antibiotics and the treated cellulosic cotton gauze exhibit higher antimicrobial activity (than the non-loaded particles and untreated gauze) against the bacteria and fungi. This resulted from the presence of antibiotics and the safety of the nanostructure as its biocompatibility with skin cells.

**Keywords** Gelatin nanoparticles · Drug delivery · Cellulosic cotton · Antimicrobial activity

## Introduction

Nanotechnology is essential in biology and medical research, notably in drug delivery systems aimed at reducing antibiotic cytotoxicity. In the last several decades, the treatment of bacterial infection-related illnesses has received significant attention. Millions of lives have been saved since antibiotics were discovered in the 1940s. However, antibiotic misuse has contributed to the emergence of antibiotic-resistant species such as superbugs, thereby encouraging us to develop an “on-demand” usage of antibiotics. Numerous antibiotic substitutes, including inorganic nanoparticles, photothermal/photodynamic agents, antimicrobial peptides, and cationic polymers, have been found to reduce antibiotic resistance. A new antibiotic nano delivery technology aimed for blind treatment in clinical practice is considered a strong weapon for overcoming antibiotic resistance. (Ibrahim et al. 2019; Li et al. 2014; Mostafa et al. 2022).

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Administration of chloramphenicol, a potent antibacterial antibiotic, is limited by its adverse effects (e.g., aplastic anemia, bone marrow suppression, leukemia, and neurotoxic reactions). Conventional drug delivery of chloramphenicol can lead to severe neurotoxic reactions. Spectinomycin is also limited by its undesirable side effects (e.g., nausea, dizziness, soreness at the injection site, and fever). Based on this information, nanoparticle formulations have been offered as delivery systems of these drugs in order to improve drug efficiency and reduce the side effects of the toxicity associated with these two common antibiotics (Nahar et al. 2008; Naidu and Paulson 2011).

Several attempts have been made over the last decade to lower the toxicity of certain antibiotics by utilizing polymeric nanoparticles, which have the potential to change the distribution profile of medicines in biological systems. (Farag et al. 2021, 2016, 2015; Leo et al. 1997). On their journey to the site of action, many systemically administered medicines must pass through biological barriers. As a result, the primary component might be rendered inactive or dispersed to undesirable locations. Research in recent years has focused considerably on improving medication therapy in terms of achieving a more regulated body distribution (than that currently attainable), thereby reducing adverse effects. To address these issues, many novel drug carrier systems in the micro- to nanoscale size range have been developed. (Balthasar et al. 2005).

Owing to their sustained-release characteristics, sub-cellular size, durability, and ability to target a specific cell or organ, and as indicated by recent advances in polymer nanotechnology, nanoparticles are considered excellent candidates for drug delivery vehicles. Nanoparticle uptake is more efficient than microparticle uptake, with 100 nm particles having a 2.5-fold higher efficiency than 1  $\mu$ m particles and a six-fold higher efficiency than 10  $\mu$ m particles (Ibrahim et al. 2020; Ofokansi et al. 2010; Won and Kim 2008).

Controlled drug delivery of bioactive compounds enhances the stability (to the maximum value), bioactivity, and bioavailability of these compounds. In addition, they protect the incorporated compounds from oxidation or degradation in the gastrointestinal tract (Zou et al. 2012).

Traditional drug delivery methods are successful for most medicines. However, certain drugs are

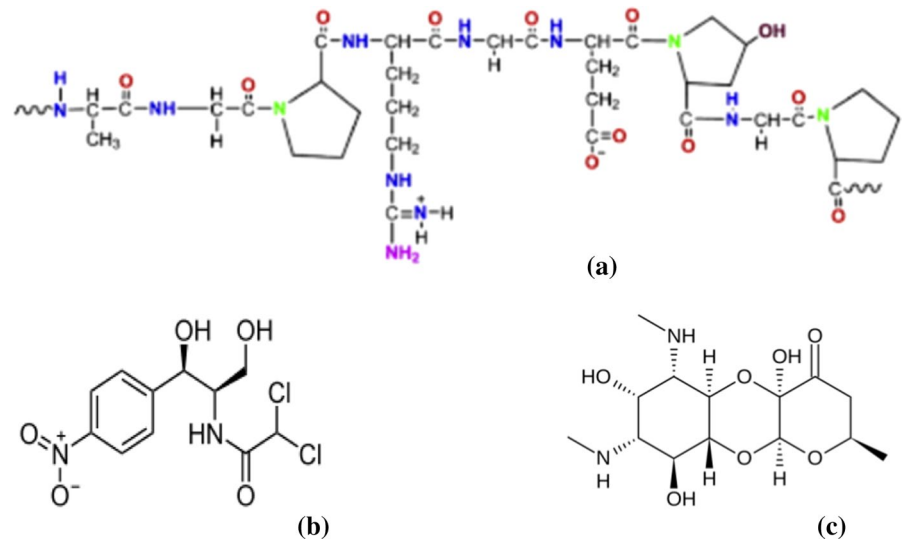
unstable or poisonous, and their therapeutic ranges are limited. The issue of limited solubility is encountered for some medicines. Controlled medication delivery systems were created three decades ago to address these issues. Controlled drug delivery occurs when a medication is delivered at a pace or to a place that is dictated by the body's or disease's demands. The main benefit of this technology is that carrier polymer matrix systems allow for the employment of many less active agents (than those required with other methods) in order to achieve the desired activity (Bajpai and Choubey 2006; Khan and Schneider 2013).

Drug carrier systems are composed of macromolecular materials in which the active principle (drug or biologically active substance) is dissolved, entrapped, encapsulated, and/or adsorbed or linked to the active principle. Polymeric nanoparticles have been identified as possible drug carriers for bioactive components (such as anticancer medicines, vaccines, oligonucleotides, and peptides) due to their appealing physicochemical features (e.g., size, surface potential, and hydrophilic–hydrophobic balance) (Jahanshahi et al. 2008; Li et al. 2014).

In foods like jams, yogurt, cream cheese, and margarine, gelatin is historically employed as a stabilizer, thickening agent, or texturizer. Gelatin is principally employed as a gelling agent, creating translucent elastic thermoreversible gelatins that form the shells of medicinal capsules when cooled below  $\sim 35$  °C. As a result, gelatin microcapsules or nanocarriers for customized medication delivery and flavor release have been created in the pharmaceutical and food processing industries. The use of gelatin for agricultural purposes, such as controlling the release of chemical compounds and pheromones for pest management, has also been investigated (Chen et al. 2010; Khan and Schneider 2013) (Scheme 1).

Starch, chitosan, liposomes, and other biodegradable nanoparticles of natural polymers are widely used as drug carriers in controlled drug delivery technology. The interest in gelatin stems from its low cost, non-toxic nature, non-carcinogenic properties, biocompatibility, biodegradability, low antigenicity, ease of cross-linking and chemical modification, and use in various parenteral formulations. Owing to these attributes, gelatin has been utilized in pharmaceutical and medical applications (Azimi et al. 2014; Bajpai and Choubey 2006; Coester et al. 2000a; Jahanshahi

**Scheme 1** Chemical structure of gelatin (a), chloramphenicol (b), and spectinomycin (c)



et al. 2008; Lee et al. 2011; Leo et al. 1997; Nahar et al. 2008; Vandervoort and Ludwig 2004; Won and Kim 2008).

Previously, gelatin nanoparticles (GNPs) have been used to transport medicines (such as methotrexate, doxorubicin, cycloheximide, paclitaxel, and chloroquine phosphate) as well as genes. GNPs modified with antibodies have also been used to target leukemic cells and primary T lymphocytes (Abrams et al. 2006; Bajpai and Choubey 2006; Nahar et al. 2008).

Several techniques, including nanoencapsulation, water-in-oil emulsion, desolvation, and coacervation-phase separation, have been devised for the manufacture of gelatin particles. These approaches all have benefits and drawbacks. To generate small-sized GNPs via the water-in-oil emulsion method, a considerable quantity of surfactant is necessary, thereby resulting in a complex post-process. The coacervation technique is a phase separation procedure followed by a cross-linking stage. However, this process is prone to non-homogeneous cross-linking and unsatisfactory loading efficiency. Owing to the variability in the molecular weight of the gelatin polymer, GNPs produced through several of these techniques are large and have a high poly dispersity index (PDI). Desolvation, a simpler GNP preparation technique than the aforementioned techniques, allows the synthesis of GNPs with a lower aggregation propensity than that encountered with other methods. The low molecular weight gelatin fractions contained in the supernatant are removed through decanting, while the high

molecular weight gelatin fractions present in the sediment are redissolved after the first desolvation stage. Some researchers have used a two-step desolvation process to generate GNPs containing various bioactive compounds. Furthermore, this technique has been used for the synthesis of protein-loaded GNPs. (Abrams et al. 2006; Azimi et al. 2014; Vandervoort and Ludwig 2004).

In this work, a two-step desolvation approach was used to produce a simpler gelatin nanoparticle preparation process (than the processes typically employed) that allows for the creation of gelatin nanoparticles in a limited size range and a lower tendency for aggregation. The temperature, gelatin concentration, agitation speed, and other important manufacturing factors were discussed. Chloramphenicol and spectinomycin, two popular antibiotics, were then attached to these nanoparticles. In addition, we present data on the controlled release of two antibacterial medicines, chloramphenicol and spectinomycin, from gelatin nanoparticles loaded with these antibiotics.

## Materials and methods

### Materials

Gelatin A (Alfa Aesar Company), glutaraldehyde, acetone, methyl alcohol (Sisco Research Laboratories, India), and all the other chemicals were analytical grade and were used without further purification.

The antibiotics used (Chloramphenicol and spectinomycin) were kindly supplied by Memphis Pharma and Chemical Industry.

EL-Nasr Company for Spinning, Weaving, and Dyeing, El-Mehalla Elkubra, Egypt, provided mill desized, scoured, and bleached 100% cotton gauze textiles for the project. In the lab, the textiles were scoured through washing at 100 °C for 60 min in a solution containing 2 g/L Na<sub>2</sub>CO<sub>3</sub> and 1 g/L Egyptol (non-ionic wetting agent based on ethylene oxide condensate). Afterward, the cloth was repeatedly washed in boiling and cold water and then dried at room temperature.

## Methods

### *Preparation of the gelatin nanoparticles (GNPs)*

Gelatin nanoparticles (GNPs) were prepared based on the desolvation method (Carvalho et al. 2018). Briefly, 1 gm gelatin type (A) was dissolved in 100 mL warm water and stirred for 20 min. Afterward, 100 mL of acetone was added dropwise to the resulting gelatin solution with stirring and then 25 µL glutaraldehyde (4%) (as a crosslinker) was added and left with stirring overnight. The prepared GNPs were separated and collected through centrifugation (5000 rpm for 4 min).

### *Preparation of antibiotic-loaded GNPs*

To obtain a final antibiotic concentration (0.1, 0.2, 0.3, 0.4, and 0.5 g/100 mL), different concentrations of the antibiotic dissolved in distilled water were added to the nano-gelatin solution and stirred for 45 min. The resulting suspension was subjected to ultrasonication for 45 min and then stirred for another 20 min (Ibrahim et al. 2016).

### *Finishing of cellulosic cotton gauze fabrics with antibiotic-loaded GNPs*

Using the pad-dry-cure technique, antibiotic-loaded gelatin nanoparticles were applied to clean cotton gauze textiles. For each treatment, 30×30 cm of textiles were submerged in an antibiotic-gelation poly load solution at concentrations ranging from 0.1 to 0.5 g/mL and then run through a padded mangle with 100% wet pick-up. The textiles were then

dried for 5 min at 80 °C before being thermo-fixed for 3 min at 140 °C. Subsequently, the materials were cleaned and dried prior to being evaluated and characterized.

### *Characterization of antibiotic-CSNP poly load and its treated fabrics*

The size of gelatin nanoparticle batches was determined by means of dynamic light scattering (DLS) with either a Ni comp 380 Particle Sizer (Particle Sizing Systems, Santa Barbara, CA, USA) or a Nanosizer ZS (Malvern Instruments, Worcestershire, UK). Unlike the Malvern software polydispersity index (PDI), the Particle Sizing Systems instrument uses a coefficient of variation to represent the width of the size distribution. The findings of the initial trials were obtained using the Ni comp 380 Particle Sizer, and hence this coefficient was used to assess the quality of the batches. Samples were diluted 10 times in highly filtered water before being measured. All samples were produced and quantified in triplicate.

Fourier transform infrared spectra (FTIR; JASCO FTIR-6100 spectrophotometer) were obtained using the KBr pellet disc technique for transmittance measurements.

A Surpass Electro kinetic Analyzer (Anton Paar GmbH) was used to perform surface charge measurements of the treated gauze samples as a function of pH (pH range: 3.0–9.50). The pH was adjusted with 0.005 M NaOH and HCl with KCl as the electrolyte solution.

- The nitrogen concentration was calculated in accordance with ASTM Method E258-67.

$$\text{Nitrogen content} = \text{Nitrogen\%} \\ \times 100/14(\text{mmole}/100 \text{ g sample})$$

The mechanical characteristics of the treated cotton gauze textiles were determined using H5KS universal tensile testing equipment. The samples were elongated at a steady rate of 30 mm/min. The tensile strength (TS; kg) and elongation (E.) at break were determined by testing three specimens in the warp direction of each treated fabric, and the average value was taken as the fabric breaking load (Lb) (cm). The

values were calculated using ASTM D-1682-94, Standard Test Method (1994).

The whiteness index (WI) and yellowness index (YI) of treated and untreated samples were measured using an Ultra scan Pro. Hunter lab.

The ultraviolet protection factors (UPFs) of untreated fabrics and fabrics treated with gelatin nanoparticles and nanoparticles loaded with antibiotics were measured via ultraviolet visible (UV–Vis) spectroscopy (AATCC 183-2010 spectrophotometer). The control reference was measured as air. The UPFs were calculated from the transmission spectra (range: 290 nm–400 nm) of the fabrics.

The size and shape of the gelatin nanoparticles that were loaded with antibiotics were evaluated via TEM (JEOL). The specimens for TEM were made by placing a drop of colloidal solution over 400 mesh copper grids coated with an amorphous carbon sheet and evaporating the solvent in room temperature air. The average diameter of chitosan nanoparticles was calculated from the diameters of 100 nanoparticles observed in different arbitrarily chosen areas of high-magnification microphotographs. Scanning electron images of the treated fabrics were obtained using a scanning electron probe micro analyzer (type JXA 840A)—Japan. Moreover, surface morphologies were imaged (accelerating voltage: 30 kV) at different magnifications.

Microstructural investigations on fabric samples were performed via scanning electron microscopy (SEM; Philips XL30 equipped with a LaB6 electron gun) and energy-dispersive spectroscopy (EDS; Philips-EDAX/DX4). Scanning electron images were obtained at different magnifications (from 1509× to 30,009×). The fabric samples were fixed with carbon glue and were metalized by gold vapor deposition to allow the recording of images.

#### Evaluation of antibacterial activity

The antibacterial activity of gelatin nanoparticles, their loaded antibiotics, and all treated textiles were quantitatively tested against Gram-positive (*Staphylococcus aureus* (ATCC 25923)) and gram-negative (*Escherichia coli* (ATCC 35218)) microorganisms.

#### Test method

The disc diffusion technique on an agar plate was used to evaluate the antibacterial and antifungal activity of the produced AgNP samples (Ibrahim et al. 2015; Mohamed et al. 2018). After solidification, 1 cm of each fabric sample was cut and placed in 10 mL of nutrient agar, into which 10 L of microbe culture was injected. After a 24-h incubation period at 37 °C, the diameter of the inhibitory zone around the samples was measured and recorded.

FT-IR and SEM tests were performed at the Central unit for analysis and scientific services at the National Research Center.

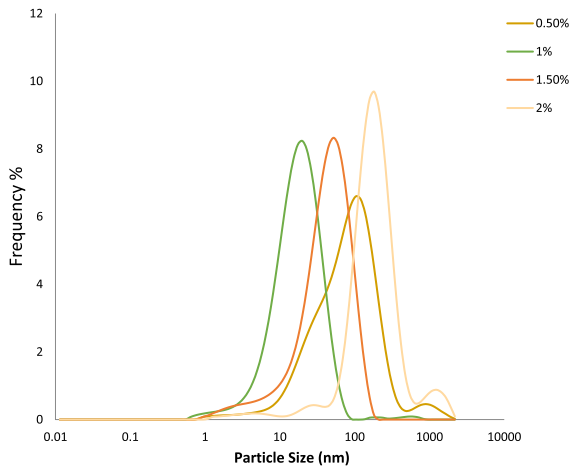
## Results and discussion

#### Preparation of gelatin nanoparticles via desolvation method

Gelatin nanoparticles were prepared via the desolvation process (Coester et al. 2000b; Marty et al. 1978). The nanoparticles were prepared through the addition of a desolvating agent. This addition led to the movement of hydrophobic amino acids into the core of the protein, and the nanoparticles were generated by the resulting precipitation of gelatin under controlled conditions. The size of the nanoparticles may be controlled by the reaction conditions. Reaction parameters such as the concentration of gelatin and the concentration of the cross-linker play an important role in the particle size.

#### *Effect of gelatin concentration on the size of gelatin nanoparticles*

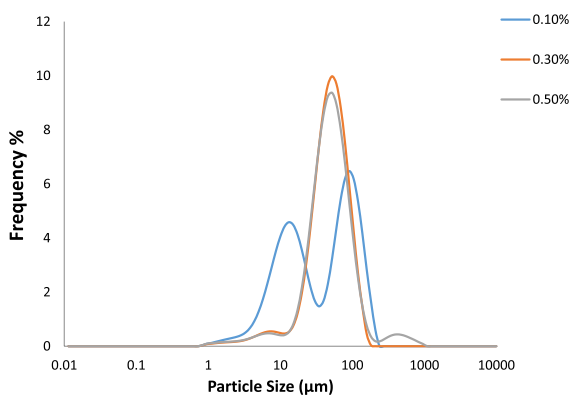
Figure 1 shows the effect of the gelatin concentration (0.5 g/100 mL–2 g/100 mL) on the particle size of the prepared gelatin nanoparticles (the concentration of acetone and glutaraldehyde is fixed). The nanoparticles were formed through the addition of acetone to the viscous gelatine solution. In addition, the particle size of the nanoparticles decreased with increasing gelatine concentration of up to 1.5 g/100 mL and increased thereafter. Therefore, compared with other concentrations, this concentration is more favorable for gelatin nanoparticle formation.



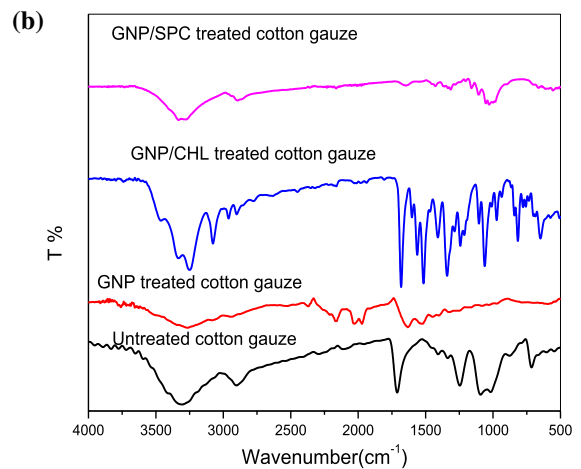
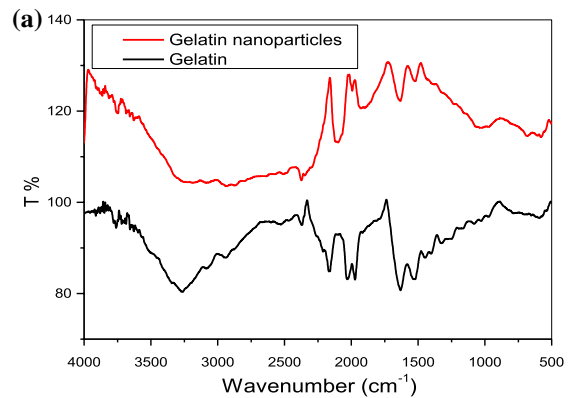
**Fig. 1** Effect of the gelatin concentration on the particle diameter of the prepared gelatin nanoparticles

#### *The effect of cross-linker concentration on the gelatin nanoparticle size*

The effect of glutaraldehyde concentration (0.15, 0.3, and 3.5 g/100 mL) on the particle size of the prepared gelatin nanoparticles was investigated for constant acetone and gelatin (1.5 g/mL) concentrations. Glutaraldehyde was the main cross-linking agent that had a pronounced effect on the properties of the nanoparticle solution (Fig. 2). The particle size of the nanoparticles increased with increasing glutaraldehyde concentration, as shown in Fig. 2. The most favorable nanosize of the particles was obtained at a glutaraldehyde concentration of 0.15 g/mL.



**Fig. 2** Effect of glutaraldehyde concentration on the particle size distribution of the prepared gelatin nanoparticles



**Fig. 3** **a** FTIR spectra of gelatin and gelatin nanoparticles. **b** FTIR spectra of untreated cotton gauze, gelatin nanoparticle (GNP) treated cotton gauze, GNP/CHL (chloramphenicol) treated cotton gauze, and GNP/SPC (spectinomycin) treated cotton gauze

#### *FTIR spectroscopy*

Figure 3a shows the FTIR spectra of gelatin and gelatin nanoparticles. The gelatin peaks occurred at 3263, 2930, 1640, and 1540  $\text{cm}^{-1}$ , which are attributed to  $-\text{NH}_2$  stretching of the primary amide comprising gelatin,  $-\text{CH}_2$  stretching, amide I ( $\text{C}=\text{O}$ ), and amide II ( $\text{N}-\text{H}$ ), respectively. For the nanoparticles, the main peaks occurred at 3259, 2944, 1630, and 1520  $\text{cm}^{-1}$ . These corresponded to  $-\text{NH}_2$ ,  $-\text{OH}$  stretching of the primary amide comprising gelatin and the hydroxyl group,  $-\text{CH}_2$  stretching of aldehyde,  $-\text{C}=\text{O}$  stretching, amide I ( $\text{C}=\text{O}$ ), and amide II ( $\text{N}-\text{H}$ ) with some shifting, respectively. The peaks at 1290  $\text{cm}^{-1}$  corresponded to amide III (Das et al. 2017).



Figure 3b shows the FTIR spectra of cotton gauze treated with chloramphenicol-loaded gelatin nanoparticles. For the spectra of the untreated gauze, the main peaks occurred at 3326, 2912, 1715, 1250, and 1087  $\text{cm}^{-1}$ , which correspond to  $-\text{OH}$  stretching,  $-\text{CH}$  stretching,  $-\text{OH}$  bending of absorbed water,  $-\text{CH}_2$  bending, and  $-\text{C}-\text{O}$  stretching (Trivedi et al. 2015), respectively. The peaks ranging from 3325  $\text{cm}^{-1}$  to 3250  $\text{cm}^{-1}$  were attributed to  $-\text{OH}$  and  $-\text{NH}$  stretching and the peak at 3070  $\text{cm}^{-1}$  corresponded to  $-\text{CH}$  aromatic stretching. The peaks ranging from 1683  $\text{cm}^{-1}$  to 1563  $\text{cm}^{-1}$  resulted from  $-\text{C}=\text{O}$  and  $-\text{C}=\text{C}-$ . Similarly,  $\text{NO}_2$  and  $\text{C}-\text{Cl}$  stretching generated peaks at 1540  $\text{cm}^{-1}$  and 812  $\text{cm}^{-1}$ , respectively; a peak corresponding to  $\text{N}-\text{H}$  bending occurred at 1519  $\text{cm}^{-1}$  (Trivedi et al. 2015). The FTIR spectra of the cotton gauze treated with gelatin nanoparticles and Chloramphenicol-loaded nanoparticles revealed peaks corresponding to the gauze and Chloramphenicol with shifts in peak position and intensity due to physicochemical reactions (Trivedi et al. 2015).

Characterization of the gelatin nanoparticles and the nanoparticle-treated cotton fabrics

#### Zeta potential (IEP)

Table 1 shows the isoelectric point (IEP) of the cotton gauze treated with gelatin nanoparticles loaded with two antibiotics as a function of pH. As shown in the table, the gauze treated with spectinomycin-loaded nanoparticles has a negative charge at pH levels exceeding 3.9.

#### Particle size analyzer

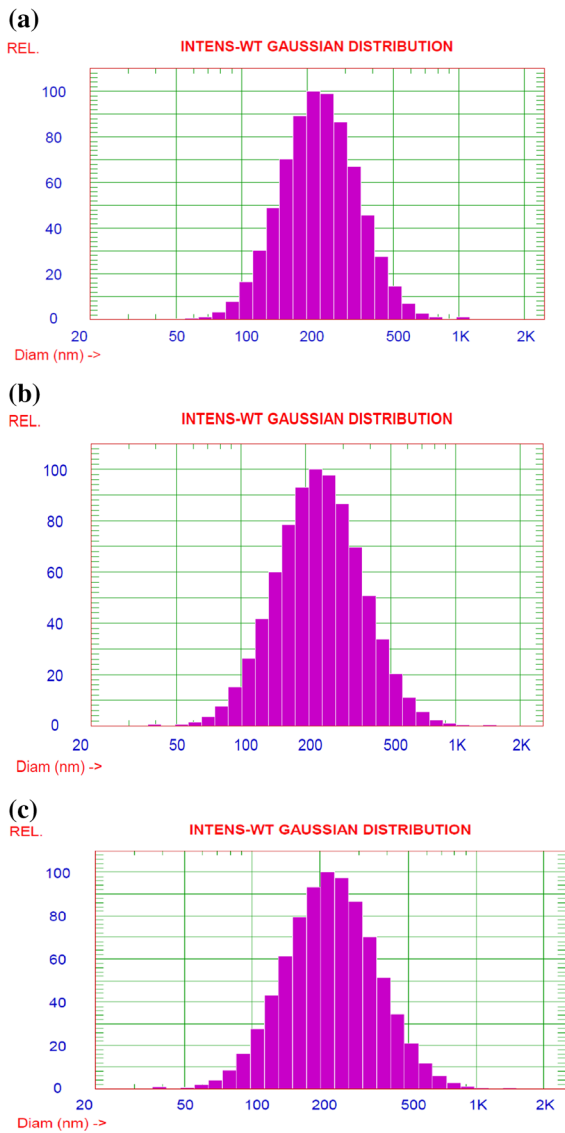
The particle size of the gelatin nanoparticles under optimum conditions is shown in Fig. 4a–c. A main particle size of 247.3 nm (standard deviation: 101.1 nm) was observed for the non-loaded particles. Dominant sizes of 257.7 nm (standard deviation: 118.8 nm) and 250.9 nm (standard deviation: 117.9 nm) were observed for the Chloramphenicol-loaded particles and the spectinomycin-loaded particles, respectively. Therefore, the particle size of the gelatin nanoparticles increased after antibiotic loading of the particles.

#### Physicochemical and mechanical properties of cotton gauze fabrics treated with gelatin nanoparticles loaded with different concentrations of antibiotics

Table 2 shows the changes in some physical and mechanical properties of the cotton gauze fabrics treated with gelatin nanoparticles loaded with different concentrations of different antibiotics. These changes were evaluated by monitoring the tensile strength, elongation at break, nitrogen content, whiteness, and yellowness. The tensile strength of the treated sample with antibiotic-gelatin nanoparticles was higher than that of the untreated sample. This resulted from the penetration of nanoparticles and crosslinking of adjacent fiber molecules by various forces between amino ( $-\text{NH}_2$ ) groups of gelatin and hydroxyl ( $-\text{OH}$ ) groups of cellulose molecules. The nitrogen content N% of treated sample increased with increasing antibiotic concentration and increasing antibacterial activity. The whiteness of the treated sample also increased with increasing

**Table 1** Isoelectric point (IEP) of the cotton gauze treated with gelatin nanoparticles loaded with two antibiotics

Antibiotics concentration	IEP of cotton gauze fabrics treated with Spectinomycin-loaded gelatin nanoparticles	IEP of cotton gauze fabrics treated with Chloramphenicol-loaded gelatin nanoparticles
Cotton gauze/gelatin nanoparticles	7.5	7.5
0.1	8.03	7.74
0.2	7.17	7.4
0.3	7.45	7.8
0.4	7.4	7.41
0.5	8.91	7.13



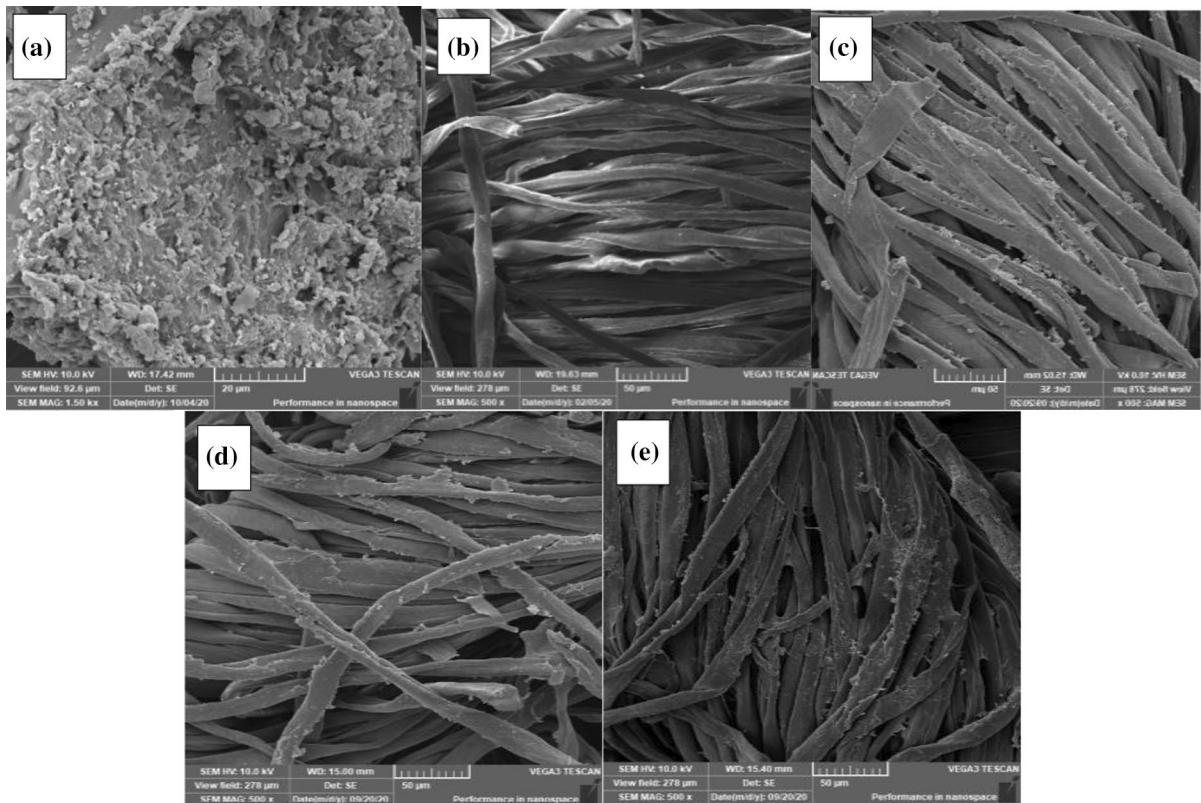
**Fig. 4** **a** Particle size distribution of the gelatin nanoparticles under optimum conditions. **b** Particle size distribution of the gelatin nanoparticles loaded with Chloramphenicol under optimum conditions. **c** Particle size distribution of the gelatin nanoparticles loaded with spectinomycin under optimum conditions

concentration of the antibiotic. Therefore, all the physicochemical properties of the fabrics improved through treatment of the cotton gauze with antibiotic-loaded gelatin nanoparticles.

**Table 2** Physicochemical and mechanical properties of cotton gauze fabrics treated with gelatin nanoparticles loaded with different concentrations of antibiotics

Antibiotic concentration	Cotton gauze treated with Spectinomycin-loaded gelatin nanoparticles					Cotton gauze treated with Chloramphenicol-loaded gelatin nanoparticles				
	N%	Tensile strength (kg)	Elongation at break (mm)	Whiteness (WI)	Yellowness (YI)	N%	Tensile strength (kg)	Elongation at break (mm)	Whiteness (WI)	Yellowness (YI)
Blank	0	29.5	11.2	40.25	3.27	0	29.5	11.2	40.25	3.27
Cotton gauze/ gelatin nano- particles	0.15	29.45	13	40.37	3.75	0.15	29.45	13	40.37	3.75
0.1	0.3	28.04	14	41.96	1.36	0.25	29.02	13.19	40.65	3.49
0.2	0.44	28.5	15	44.23	1.95	0.50	29.25	15	43.54	2.44
0.3	0.56	29.06	14	45.21	1.92	0.65	29.46	14	40.99	3.16
0.4	0.72	29.45	15	44.76	1.45	0.88	29.5	16	43.92	2.08
0.5	0.95	30.08	14	43.41	2.51	0.98	31.2	15	42.91	2.54





**Fig. 5** Scanning electron images of gelatin nanoparticles and cotton gauze Fabrics treated with gelatin nanoparticles loaded with different antibiotics; **a** Gelatin nanoparticles, **b** Cotton

blank, **c** Cotton/gelatin nanoparticles, **d** Cotton/gelatin nanoparticles/Chloramphenicol and **e** Cotton/gelatin nanoparticles/Spectinomycin

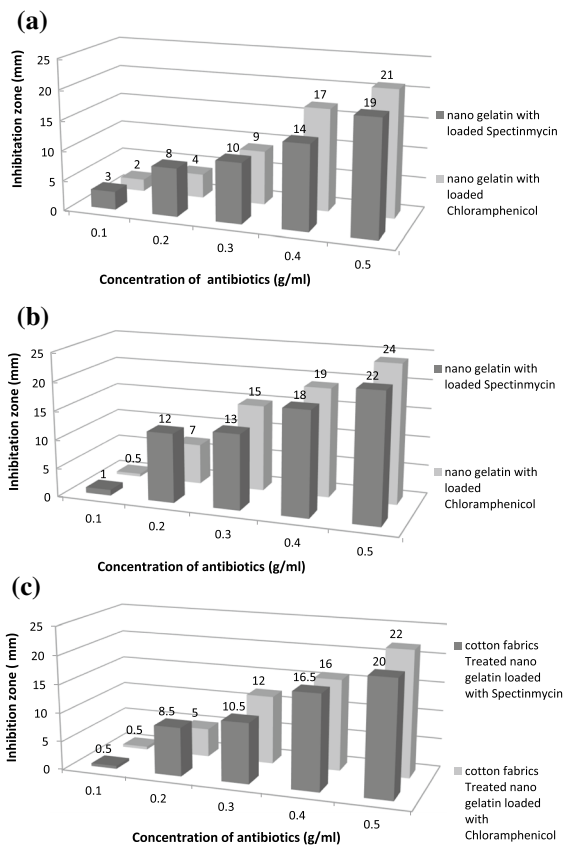
#### *Morphology of untreated cotton gauze and cotton gauze treated with gelatin nanoparticles loaded with different antibiotics*

The surface morphology of untreated cotton gauze and treated cotton gauze was investigated via SEM. Figure 5a shows SEM images of gelatin nanoparticles at magnifications of 1000, 1500, 2000, and 4000 $\times$ . The particles occurred with different sizes and shapes. Figure 5b–e shows SEM images obtained at different magnifications (500, 1000, 2000, 4000, and 8000 $\times$ ) of the (b) control cotton fabrics and cotton treated with (c)–(e) gelatin nanoparticles, Chloramphenicol-loaded gelatin nanoparticles, and Spectinomycin-loaded gelatin nanoparticles, respectively. The surface of the untreated

fabrics is smooth (Fig. 5b). A spherical gelatin nanoparticle precipitate occurred on the surface of the fabrics (Fig. 3c). In addition, a rough thin layer containing chloramphenicol-loaded nanoparticles and Spectinomycin-loaded nanoparticles also formed on the surface of the fabrics (Fig. 3d, e).

#### Antibacterial activity

The antibacterial activity of gelatin nanoparticles, loaded antibiotics, and treated cotton gauzes was tested against Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). For both types of bacteria, the antibacterial activity of Chloramphenicol-loaded nanoparticles, spectinomycin-loaded



**Fig. 6** **a** Dependence of antibacterial activity (against *S. aureus* as Gram-positive bacteria) on the concentration of antibiotics loaded on the gelatin nanoparticles. **b** Dependence of antibacterial activity (against *E. coli* as Gram-negative bacteria) on the concentration of antibiotics loaded on the gelatin nanoparticles. **c** Dependence of the antibacterial activity exhibited by the treated cotton fabrics (against *S. aureus* as Gram-positive bacteria) on the concentration of antibiotics loaded on the gelatin nanoparticles. **d** Dependence of antibacterial activity exhibited by the treated cotton fabrics (against *E. coli* as Gram-negative bacteria) on the concentration of antibiotics loaded on the gelatin nanoparticles

nanoparticles, and cotton gauzes treated with these antibiotics increased with increasing antibiotic dosage (Fig. 6).

For *S. aureus* as gram-positive bacteria and *E. coli* as gram-negative bacteria, the size of the inhibition zone associated with Chloramphenicol-loaded gelatin nanoparticles increased from (i) 1 mm to 22 mm and (ii) 3 mm to 19 mm, respectively (see Fig. s3).

For *S. aureus* as gram-positive bacteria and *E. coli* as gram-negative bacteria, the size of the inhibition zone rose (i) from 0.5 mm to 24 mm and 20 mm,

respectively, with increasing concentration of spectinomycin in spectinomycin-loaded gelatin nanoparticles and (ii) from 0.5 mm to 22 mm and 20 mm, respectively, for cotton gauze treated with Chloramphenicol-loaded gelatin nanoparticles (see Fig. 4).

The inhibition zone of cotton gauze treated with gelatin nanoparticles loaded with spectinomycin for *S. aureus* as gram-positive bacteria and *E. coli* as gram-negative bacteria increased from 0.5 mm to 22 mm and 0 mm to 18 mm, respectively. The size of the zone increased with increasing concentration of spectinomycin. Therefore, gelatin nanoparticles containing various antibiotics are effective against both gram-positive and Gram-negative bacteria (they are more effective against the former than the latter), penetrating cell membranes and disrupting membrane structure, resulting in cell death (Jahani and Shakiba 2015).

## Conclusion

Gelatin nanoparticles (GNPs) have been prepared using a coprecipitation method. These GNPs were loaded with spectinomycin and chloramphenicol to enhance their treatment of bacterial and fungal infections. The concentrations of gelatin and a cross-linker were determined and evaluated in relation to various criteria in order to maximize the particle size of the nanoparticles. We employed FT-IR and particle size analyzers to examine gelatin nanoparticles loaded with antibiotics and medical gauze treated with these particles. Moreover, we performed antimicrobial tests. The results indicate that gelatin nanoparticles loaded with researched antibiotics and the cellulosic cotton gauze treated with these particles exhibit increased antibacterial activity against studied bacteria and fungi. This is attributed to the presence of the medicines as well as the safety of the nanostructure and its biocompatibility with skin cells.

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## Declarations

**Conflict of interest** This work has no conflicts of interest, and all authors have given their approval for publication. The authors state that the study reported was unique and had never been published before. The authors state that they have no known competing financial interests or personal connections that might have influenced the research presented in this publication.

**Human and animal participants** The authors state that they have no known conflicting financial interests or personal connections regarding any possible experiments performed on people or animals.

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