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IN-VITRO PREPARATION, CHARACTERIZATION AND APPLICATION OF SOLID LIPID NANOPARTICLES AND CHITOSAN NANOPARTICLES EITHER SINGLY OR LOADED WITH CIPROFLOXACIN ANTIBIOTIC AS NANODRUG DELIVERY SYSTEMS

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ABSTRACT

Engineered nanomaterials have been used increasingly in the medecinal field to improve drug delivery efficacy in the healthcare field because of their unique physicochemical characteristics. In the current study, chitosan nanoparticles (CSNPs) were synthesized by polymerization of methacrylic acid in chitosan solution. Meanwhile, solid lipid nanoparticles (SLNPs) were synthesized by using the hot homogenization method. Chitosan nanoparticles and solid lipid nanoparticles either singly or loaded with ciprofloxacin (CIP) antibiotic were characterized using morphological, physical, chemical, electrical and biological methods. Both chitosan nanoparticles and solid lipid nanoparticles had spherical shape meanwhile, the size was range from 50.96 nm to 108.42 nm for chitosan nanoparticles and 107 nm for solid lipid nanoparticles, and retained the properties of the drug after loading. The size of both nanomaterials was increased after loading with ciprofloxacin by 145.5% and 49.66 %, respectively. Both prepared nanomaterials offered controlled drug release after 12 hrs Antibacterial activity of solid lipid nanoparticles and chitosan nanoparticles either singly or loaded with ciprofloxacin was evaluated against *Salmonella enteritidis* as a Gram-ve bacterial strain. The minimum inhibitory concentration (MIC) of each prepared nanodrugs against *Salmonella enteritidis* was determined as 0.0002 mg ml^{-1} and the antibacterial effect of either nanomaterials, antibiotic singly or loaded with antibiotic as nanodrug delivery systems showed significant variable higher increase in inhibition rate of the pathogen. The following sequence of the treatment: CSNPs-CIP; 2.0 mg ml^{-1} > SLNPs-CIP; 2.0 mg ml^{-1} > control (CIP; 2.0 mg ml^{-1}) > SLNPs-CIP; 0.2 mg ml^{-1} > CSNPs-CIP; 0.002 mg ml^{-1} was displayed with respect to percent change inhibition effect on bacterial pathogen. In conclusion, we recommended an intensive work on the biosafety of using nanomaterials as nanodrug delivery systems for treatment of infectious diseases.

Keywords: Antibacterial agent, chitosan nanoparticles, ciprofloxacin, drug delivery, solid lipid nanoparticles

INTRODUCTION

In recent years, improper use of antibiotics has led to the development of multi-drug resistant (MDR) bacteria and the loss of effectiveness of existing antibiotics. Antibiotic-resistant bacteria can be transmitted to human through the food supply chain **(Nair *et al*., 2018)**. The infection with antibiotic-resistant bacteria can cause serious diseases that can not be treated with regular treatment of conventional antibiotics **(Pelyuntha *et al*., 2022)**. *Salmonella* is one of the most commen pathogenic bacteria associated with human. Infection can be associated with the consumption of contaminated food and water. Animals, especially poultry are the primary carriers for these bacterial strain **(Obukhovska, 2013)**.

Currently, *Salmonella* has been reported to be resistant to several antibiotics which can pose a threat to public health and food safety **(Wang *et al*., 2019)**. Ciprofloxacin is a member of the fluoroquinolone drugs which has been proven to be a strong and broad-spectrum antibiotic with high efficacy against a wide range of bacterial infections, especially *Salmonella* infection **(Brunner and Zeiler, 1988**; **Jain and Banerjee, 2008)**. Ciprofloxacin is a poorly water-soluble drug so, its bioavailability can be improved by the nanoformulation of the drug **(Savjani *et al*., 2012).** According to the World Health Organization, antimicrobial resistance considerds one of the greatest threats to global health, and the world needs to develop new strategies and tools to overcome this problem **(Ling *et al*., 2018; WHO, 2021)**. In this context, nanotechnology can offer a real solution to this problem by the development of drug delivery systems which has offered new opportunities to enhance the efficacy of various therapeutic drugs **(Qin *et al*., 2017).**

Recently, there are a great interest in the development of nanotechnology for drug delivery. Because nanotechnology provides a suitable way to deliver both of drugs with small molecular weight and macromolecules like proteins, peptides and genes to several cells and tissues and protect them from enzymatic degradation **(Butowska *et al*., 2022; Helal *et al*., 2022 a)**. Nanoparticles can be used as drug delivery systems because of their unique advantages such as non-toxicity, biodegradability, and sufficent stability to be kept for long periods. One of these developed drug delivery systems, solid lipid nanoparticles (SLNPs) have attacted special attention as promising nanocarriers for controlled drug delivery because they combine between the advantages of polymeric nanoparticles, lipid emulsions, and liposomes simultaneously avoiding some of their negative aspects **(Geszke-Moritz and Moritz, 2016; Helal *et al*., 2022 a, b).**

Solid lipid nanoparticles are colloidal particles with a submicron size of diameter between 50 and 1000 nm **(Ekambaram *et al*., 2012; Helal *et al*., 2022 a)**. They have several advantages of low toxicity, high biocompatibility, high stability, biodegradability and noticeable capacity to bind with both lipophilic and hydrophilic compounds. Additionally, they also can control the release of the incorporated drug and chemically protect the compound and these can be achieved by simple and cheap large-scale production **(Bayón-Cordero *et al*., 2019).**

Chitosan nanoparticles (CSNPs) have been used as a convenient carrier for drugs and genes to improve their transduction in the cells because of their unique properties, including biodegradability, biocompatibility and low toxicity as reported in different studies **(Chopra *et al*., 2014, Csaba and Alonso, 2014, Helal *et al*., 2022 a).** However, the efficiency of the antimicrobial activity of chitosan nanoparticles depends on the type of targeted microorganism and the antimicrobial activity mechanisms are related to the physicochemical properties of chitosan nanoparticles **(Rozman *et al*., 2019)**.

The antimicrobial activities of CSNPs have been extensively investigated in different *in-vitro* and *in-vivo* studies against several microorganisms such as bacteria, fungi, yeasts and algae. In pharmaceutical applications, CSNPs were used as antimicrobial coating to promote wound healing, prevent infections and reduce infectious disease. Besides, CSNPs have also been exhibited significant inhibitory effect on foodborne microorganisms, particularly on fruits and vegetables. It is noteworthy that CSNPs can also be used to deliver antimicrobial drugs, which further enhance the efficiency and stability of the antimicrobial agent **(Rozman *et al*., 2019, Helal *et al*., 2022 a, b)**.

The objective of our study was focused on preparation, characterization and comparing between two different nanoengineered materials; solid lipid nanoparticle and chitosan nanoparticles as nanocarriers for ciprofloxacin antibiotic as an auspicious strategy to overcome or, at least, reduce antibacterial resistance.

MATERIAL AND METHODS

Preparation of CSNPs solution and SLNPs emulsion

Chitosan nanoparticles were prepared according to **DeMoura *et al.* (2008**) and **Hasaneen *et al*. (2014)** method by polymerization of methacrylic acid in chitosan solution. Under magnetic stirring for 12 hours, chitosan powder (0.2 g) was dissolved in aqueous solution of polymethacrylic acid (0.5 cm^3). Potassium persulfate (0.05 g) was then added to the previous solution and then heated to 70 °C while stirring continuously for another one hour until the solution became transparent. The final step is necessary to stop the reaction by cooling the prepared solution in an ice bath.
Meanwhile, solid lipid nanoparticles were prepared *via* hot homogenization method according to **Gazi and Krishnasailaja** (**2018)** method. Firstly, about five grams of glycerol monostearate (GMS) lipid was heated in a glass beaker at 70 ℃ untill completely melting Approximately one gram of lipophilic surfactant such as soya lecithin was added to the previous melted lipid. The mixture was mixed well and put in a water bath at 70 ℃ until complete homogeneity to form the lipid phase. Secondly, 1.5 cm^3 of hydrophilic surfactant such as Tween 80 was added in another beaker, and by using distilled water the volume of the mixture was completed to 100 cm^3 and with continuous stirring, the mixture was heated at 70 ℃ for 15 minutes to form aqueous surfactant phase. The lipid phase mixture was added to the aqueous surfactant drop-wisely. Then, the final mixture was homogenized at 15000 rpm for 5 minutes in a graduate highly speed automatic homogenizer and sonicated for 15 minutes by using automatic high-speed sonicator. The prepared solid lipid nanoparticles (SLNPs) emulsion was stored stably at room temperature until use.

Loading of ciprofloxacin on CSNPs solution and SLNPs emulsion

The loading of ciprofloxacin on both CSNPs solution and SLNPs emulsion surfaces was performed accorging to the method of **Hasaneen *et al.* (2014, 2022)**. Under magnetic stirring for six hours at room temperature, 20 cm^3 of ciprofloxacin suspension (2.0 mg ml^{-1}) was added to 30 cm^3 of each CSNPs solution and SLNPs emulsion.

Characterization of CSNPs and SLNPs either singly or loaded with CIP
Morphological characterization

The size and shape of the prepared CSNPs and SLNPs either singly or loaded with ciprofloxacin antibiotic were detected by using a JEOL 1010 transmission electron microscope at 80 kV (JEOL, EM unit, Mansoura University). All nanomaterial's solutions were sonicated for two minutes to avoid the aggregation of nanoparticles and for better dispersion of the particles on the carbon-coated grid. One drop of the prepared nanosolutions was put the grid, which was then left to dry aat room temperture nd undergoes TEM examination. Both size and shape of the prepared nanoengineered materials were directly determined from the resulting figures by using an Image-ProPlus 4.5 software **(Corradini *et al.*, 2010; Helal *et al*., 2022 a).**

Chemical characterization

According to the method of **Frederick (2009)**, the values of zeta potential of both CSNPs and SLNPs either singly or in-combination with ciprofloxacin antibiotic were measured on zeta-sizer instruments (Malvern Instruments ltd, EM unit, Mansoura University, Mansoura, Egypt). The zeta cell was firstly washed by dist. H_2O, ethanol, and then cleaned with dist. H_2O again and finally dried with an appropriate stream of nitrogen to remove any residual solvents. The cell was then covered to prevent contamination. By using a syringe, about one cm^3 of each nanosolutions was injected carefully into the cell and three runs were performed for each sample.

Fourier transformation infrared spectroscopy (FTIR) analysis.

According to the method of using the method of **Trykowski *et al.* (2010)** and **Hasaneen *et al.* (2017)**, FTIR measurements for the prepared nanomaterials either singly or loaded with ciprofloxacin were performed by using 0.1 g of spectrally pure potassium bromide and approximately three mg of each nanosolution was pressed under vacuum for 10 minutes to form a disc (greyish color) which was analyzed by using a Fourier transform spectrometer (NICOLET IS10 FT-IR instrument) at Faculty of Science, Mansoura University, Mansoura, Egypt.

In-vitro drug release

According to **Kashanian *et al.* (2011)** and **Helal *et al.* (2022a)**, *in-vitro* estimation for ciprofloxacin release from the surface of the prepared nanomaterials were carried out by using the dissolution test. In a beaker containing 50 cm^3 of phosphate buffer solution (pH 6.8), a dialysis bag (redeveloped cellulose membrane; 12–14 KD molecular weight cut-off) containing CIP-based nanomaterials was immersed and the system was maintained under moderate stirring (100 rpm) at 37°C±1. For continuous 12 hours, approximately 5 cm^3 of the release medium was withdrawn every hour and tested for drug release and replaced by 5 cm^3 of preheated fresh phosphate buffer (37°C±1). The cumulative drug levels of ciprofloxacin were calculated by using a UV spectrophotometer at 300 nm *via* comparing the amount of ciprofloxacin in the extract with the blank. The Cumulative drug of ciprofloxacin was calculated based on a pre-made calibration curve.

Evaluation of antibacterial activity of CSNPs and SLNPs either singly or loaded with CIP

Preparation of different concentrations of ciprofloxacin

Different concentrations of ciprofloxacin dissolved in distilled water were added to both SLNPs emulsion and CSNPs solution with stirring for 6 hours to obtain final antibiotic concentrations 2.0, 0.2, 0.02 and 0.002 mg ml^{-1}.

Pre-cultural preparation of *Salmonella enteritidis*

Bacterial growth suspension was prepared at concentration equivalent to 0.8 mg ml^{-1}. About 1-2 colonies of *Salmonella enteritidis* (AC: ON764247) obtained from a culture collection of Microbiology laboratory, Faculty of Veterinary, Mansoura University, were taken by sterile cotton swab to sterilized tube containing 5 cm^3 of sterile distilled water and then shaken.

Determination of minimal inhibition concentration (MIC) and minimal bacterial concentration (MBC)

The Minimum inhibitory concentration test is used for antimicrobial drug (ciprofloxacin) against *Salmonella enteritidis* using a several tubes of sterile MacConkey broth with different dilutions of CIP (2.0 to 0.00625 mg/ml) which were specifically inoculated with 100 µl of standardized inoculum.
The inoculated tubes were then incubated at 37°C for 24 hours. The lowest ciprofloxacin concentration that did not induce visible growth compared to the control tube was recorded as the MIC **(Hassan *et al*., 2009)**. Meanwhile, the minimum bacterial concentration (MBC) was determined by preparing MacConkey solid medium that poured into sterilized plates. After medium solidification, 50 µl of standardized bacterial inoculum was inoculated, wells were created using sterile cork poorer (diameter 1cm) and were filled with 100 µl of different CIP dilutions that were prepared. Dilution that produced no single bacterial colony on a solid medium was taken as MBC **(Kabir *et al*., 2005).**

Determination of the inhibition zone using agar well diffusion assay

According to the method of **Balouiri *et al*. (2016),** agar well diffusion method was used to determine the antibacterial activity of each CIP, CSNPs, SLNPs, CSNPs-CIP and SLNPs-CIP. 15 cm^3 of sterile MacConkey agar medium was poured into sterilized petri dishes and allowed to solidify. Approximately 0.1 cm^3 of the bacterial suspension was poured and uniformly spreaded with sterile cotton swab. After the absorption of inoculum by medium, wells were made by a sterile cork poorer (1 cm diameter) and filled with 100 µl of CIP, CSNPs, SLNPs, CSNPs-CIP and SLNPs-CIP. The dishes were kept at room temperature for 45 minutes to allow proper diffusion of the prepared nano-drugs in the medium and then were incubated at 37°C for 24 hours. Inhibition of bacterial growth was measured as inhibition zone diameters (mm)

RESULTS AND DISCUSSION

Characterization of prepared nanomaterials either singly or loaded with CIP

Physical properties

Morphology and size

This research focuses on the development of experimental stuides to explore nanosystems as new strategies for control delivery of the drug based on nanocarriers that premit to ameliorate and maintain the antibiotic effect in drug-resistant organisms **(Helal *et al*., 2022 a, b)**. To solve this problem, it is necessary to design and develop of new therapeutic systems that can be used as an alternatives to antibiotics or in combination with conventional antimicrobial treatments. Nanotechnology expands the treatment options of current antibiotics by designing drug delivery systems that improve the properties of antibiotics and/or facilitate drug administration **(Kaushik *et al*., 2019; Helal *et al*., 2022 a, b)**.
Nanomaterials in particular have been shown to have broad-spectrum antibacterial activities against both Gram-negative and Gram-positive bacteria, mainly due to their unique physicochemical features, such as very small size, large surface area, high reactivity and functionalized structure **(Hasaneen *et al*., 2014; Wang *et al*.,**

2017). Both SLNPs and CSNPs have a great adsorption capacity due to their large surface area, hydrophilic and hydrophobic nature and strong interactions between these nanomaterials and antibiotic molecules. This may be the reaseon for the reported TEM results **(Shih and Li, 2008).**

Here, it is noteworthy that we succeeded to modify the pervious reported preparation methods and develop a simple synthetic method that allowed us to prepare either chitosan nanoparticles from raw bulk chitosan or solid lipid nanoparticles from tween 80 as the hydrophilic surfactant and soya lecithin as the lipophilic surfactant, easily and inexpensively.

Both prepared nanomaterials either singly or loaded with antibiotic were characterized morphologically by using Transmission electron microscop (TEM). It is clearly observed from the TEM micrographs that both CSNPs and SLNPs had a spherical shape. The minimum diameter of the CSNPs is range from 50.96 nm to 108.42 nm (Figure 1A). Meanwhile, the size of SLNPs was approximately 107 nm (Figure 1C). The size of both CSNPs and SLNPs was increased with the addition of ciprofloxacin as antibacterial agent as showed in Figure 1B and 1D, respectively. The maximum percent ratio of increase in the mean diameter was approximately 302 % and 340.81 % with the addition of CIP to each CSNPs solution and SLNPs emulsion, respectively.

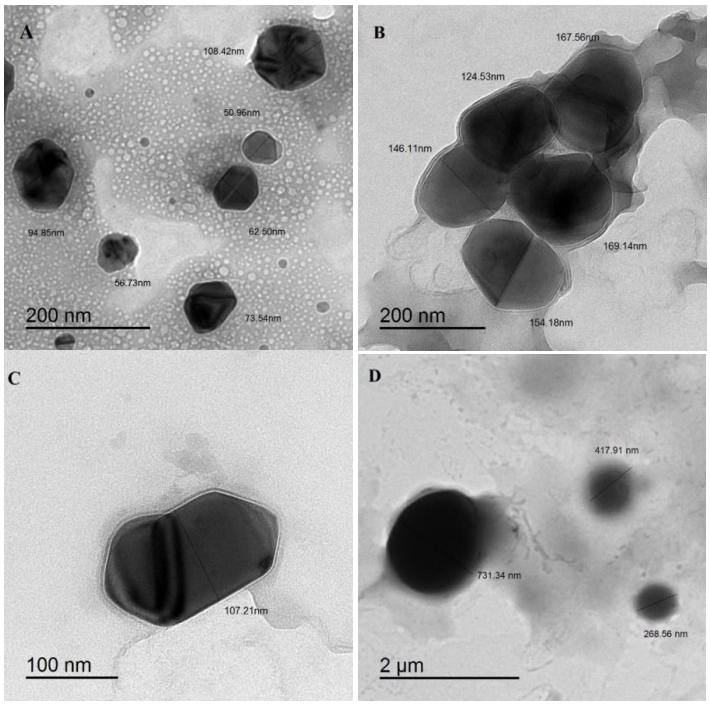


Figure 1 TEM micrograph of **A**; CSNPs, **B**; CSNPs-CIP, **C**; SLNPs and **D**; SLNPs-CIP.

According to the mechanism proposed by **DeVasconcelos *et al*. (2006)** and **Hasaneen *et al*. (2014**), CSNPs were formed *via* the bonds formed inter and intra-molecularly between the NH_4+ of chitosan and the COO^- group of poly methacrylic acid. During the polymerization process of poly methacrylic acid by adding potassium persulfate as a monomer polymerization initiator to chitosan solution, the persulfate anion attacked and cut into the long chain of chitosan at a temperature of 70°C, causing it to become shorter. The reaction between chitosan and potassium persulfate occurred after one hour, and this reaction must be stopped by immersing the solution in cooled ice bath to prevent much more formation of nanoparticles **(Hasaneen *et al*., 2014; Kusrini *et al*., 2015)**.

On the other hand, according to **Ekambaram *et al*. (2012)** and **Helal *et al*. (2022a)**, the preparation of SLNPs can be acheived by the hot homogenization method, at a temperature higher than the melting point of lipid which may be responsible for lowering the size of particles due to the viscosity decreasing of the inner phase. Increasing the number of either cycles or the homogenization pressure often responsible for the increase in particles size due to high kinetic energy of the part. As mentioned above in figure 1 B and 1D, the size of both CSNPs and SLNPs was increased with the addition ciprofloxacin antibiotic. This is due to the presence of much more primary chitosan amino acids and PMAA OH^- groups that combined with either other ions or other molecules through ion exchange or simple chelation creating several chemical interaction with these ions, thus increasing the stability of nanomaterials and prevent aggregation **(Muzzarelli, 2011; Helal *et al*., 2022 a).** In support of the present results, **Corradini *et al*. (2010)** and **Hasaneen *et al*. (2014)** reported that the size of chitosan nanoparticles (CS-PMAA NPs) was enlarged by 53%, 32% and 13% with the addition of phosphorus (60 ppm), nitrogen (400 ppm) and potassium (400 ppm), respectively. Also, **Hasaneen *et al*. (2022)** showed that the diameter of CSNPs loaded with antimicrobial compound was increased approximately 55 upto 100 nm. **Alarifi *et al*. (2020)** recorded an increase in the size of CIP-loaded on SLNPs due to the incorporation of a glyceryl ester lipid with stearic acid depending on the type and ratio of the combined lipid.

Chemical properties

Zeta potential

Zeta potential values are significant factor to the physical stability of nanomaterials as the higher value displays better stability of the dispersion. So, to confirm the stability of the prepared SLNPs and CSNPs either singly or loaded with CIP antibiotic, zeta potential (z p ζ) for were measured. Low potential values means that attractive force are greater than repulsive forces and the solution will agglomerate or coagulate due to Van Der-Waal attraction force between particles. But when zeta potential value is high either negative or positive, the solution is electrically stable (Hanaor *et al*., 2012; Hasaneen *et al*., 2014). Table 1 showed that SLNPs either singly or in combination with CIP had high negative zeta potential values, meanwhile CSNPs and CSNPs-CIP had highly positively values meaning that both CSNPs and SLNPs either singly or loaded with CIP were highly stable. In general, it is thought of interest to mention that SLNPs either singly or loaded with CIP antibiotic were more stable than the prepared CSNPs either singly or loaded with CIP also.

The positive CSNPs zeta potential values either singly or loaded with CIP is due to the cationic characteristics of chitosan. These positive values proved that CSNPs had a positive surface charge, and high values indicating that these nanoparticles were stable. On the other hand, solid lipid nanoparticles exhibited highly negative zeta potential values which indicated that the prepared emulsion whad a negative surface charge and were stable. These negative charge can be attributed to soya lecithin as well as tween 80's hydrogels which can also produce a very small negative charges **(Zardini, et al., 2018)**. The loading of CIP on the surface of both CSNPs and SLNPs change the values of zeta potential due to the loading of additional charges on the surface of both prepared nanomaterials **(Hanaor *et al.*, 2012; Hasaneen *et al*., 2014)**. Zeta potential of nanocarriers signifcanlty improved as a result of CIP loading on the surface of CSNPs and SLNPs (Table 1).

Table 1 Average zeta potential values of the prepared nanomaterials chitosan nanoparticles and solid lipid nanoparticles either singly or loaded with ciprofloxacin.

Nanosuspension	ζ -Potential (mV)
CSNPs	27.10
CSNPs-CIP	28. 00
SLNPs	-35.50
SLNPs-CIP	-36.00

Electrical properties

FTIR analysis

Determination of the prepared nanomaterials structure was carried out by FTIR analysis to confirm the formation of nanoparticles by examining the presence of functional groups of both CSNPs and SLNPs. The FTIR spectra of the nanosolutions investigated in this study was represented in Figure 2, 3, 4 and 5. Depending on the procedures of synthesis, CSNPs and SLNPs may contain various functional groups such as –NH_4, C=O, N–N,–OH and –COOH. Figure 2 shows that the characteristic peaks of chitosan are NH_2 bending vibration at 3571 cm^{-1} indicating the stretching vibration of the –NH_2 and –OH groups and C=O stretching vibration at 1723 cm^{-1} which indicated that chitosan was ionicaly interacted with poly methacrylic acid (PMAA) to form the nanoparticles. Figure 3 confirmed the presence of the distinctive broad peak of ciprofloxacin loaded with chitosan nanoparticles at about 3435 cm^{-1} region that was special overlapping between O–H and N–H stretching vibrations.

SLNPs and CSNPs may encompass different functional groups depending on the manufacturing method such as–OH, N–N, –NH_4, C=O and –COOH which can be added by oxidation or detached by thermal action **(Hasaneen *et al*., 2022)**. The spectrum of CS-PMAA nanoparticles was produced by feeding chitosan and poly methacrylic acid at a molar ratio of 1:1 based on glucosamine unit in chitosan and carboxylic acid group in poly methacrylic acid. The characteristic peaks of the NH_3^+ absorption at 3571 cm^{-1} and COO^- symmetric stretching absorption at 1732 cm^{-1} are found. This confirmed the transfer of a proton from the carboxylic acid group of methacrylic acid to the chitosan amino group resulting in the COO^- and NH_3^+ groups. Subsequently, anionic methacrylic acid and cationic chitosan form polyelectrolyte complex through ionic interaction (**Maw *et al*., 1997; Hasaneen *et al*., 2014; Helal *et al*., 2022 a & b)**.

Meanwhile, SLNPs FTIR spectra were presented in Figure 4 which showed the presence of absorption peak at 3314 cm^{-1}, 2918 cm^{-1} and 2850 cm^{-1} that represents –OH, (–CH_3) and –(CH_2) stretching vibrations, respectively. The peak at approximately 1733 cm^{-1} was the stretching vibration of the COOH group. In Figure 5, new bands not detected in the FTIr specrum of CIP -SLNPs indicated that there were no any chemical reactions between the lipid and drug. In SLNPs FTIR spectrum, the vibration bands of (–OH) at 3314 cm^{-1} and (–CH_3) at 2918cm^{-1} and

– (CH_2) at 2850 cm^{-1} were observed. These results were in full agreement with that obtained from **Sarisuta *et al.* (1999)** and **Helal *et al*. (2022 a, b)** who studied the FTIR spectra of erythromycin loaded on different polymers film and nystatin and fuconazole loaded on CSNPs and SLNPs.

The mothd of membrane diffusion are usually used to study the *in-vitro* release of drug incorporated in colloidal system and in this case, the drug release can follow more than one mechanism. When the drug adsorbed on the surface of SLNPs, it rapidly dissolved up on contact with the release medium. Release profile of ciprofloxacin from the nanoparticles is graphically plotted in Figure 6. Approximately 27.85 % and 23.16 % ciprofloxacin was released in sustained pattarn from CIP-based nanoparticles in the first 6 hours. In addition, about 19.55% and 15 % of ciprofloxacin drug were released from SLNPs from CSNPs at the longest time (12 hours). Due to the nanometer-sized particle size, the high surface area of nanomaterials produces greater drug release **(Reddy *et al*., 2013; Helal *et al*., 2022 a, b)**. When the drug is homogenously distributed in the lipid matrix, slow release of the drug can be achieved and this can be depended on the type and the drug entrapment model of nanomaterials **(Mishra *et al*., 2014)**. Drug release from polymer can be regulated by one of the following mechanisms: (a) surface erosion of the polymer matrix, (b) breaking of polymer bonds at the surface or in the bulk of the matrix, or (c) diffusion of the loaded drug **(Kamaly *et al*., 2016)**.

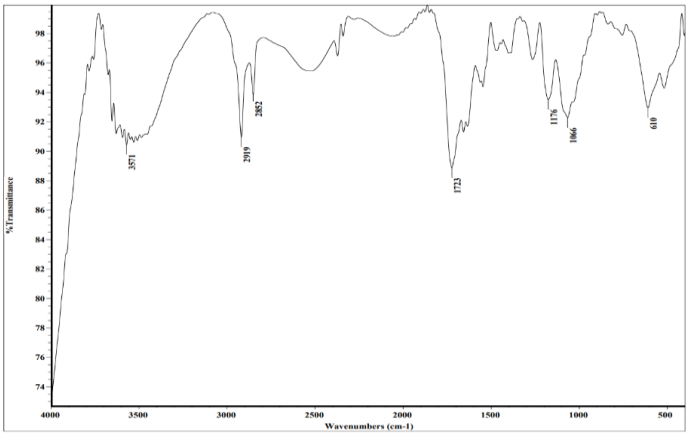


Figure 2 IR spectrum of chitosan nanoparticles (CSNPs).

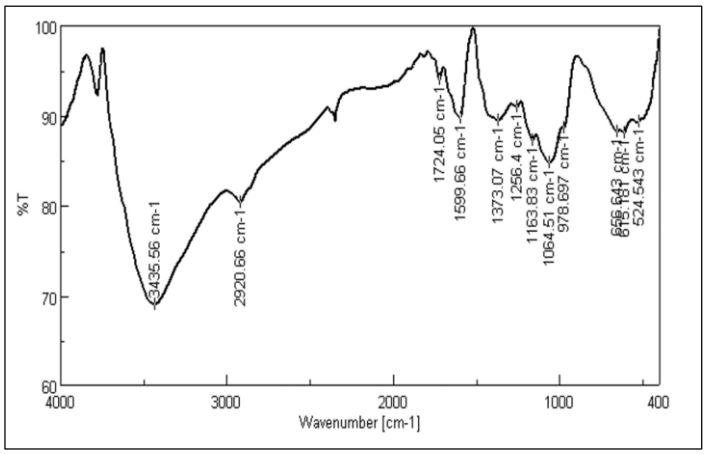


Figure 3 IR spectrum of chitosan nanoparticles loaded with ciprofloxacin.

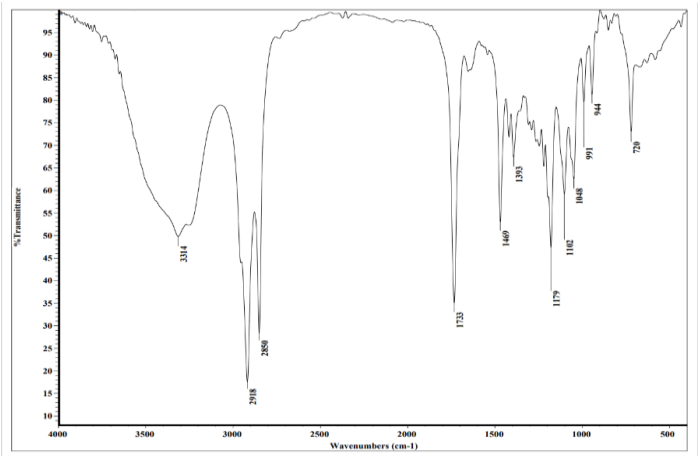


Figure 4 IR spectrum of solid lipid nanoparticles (SLNPs).

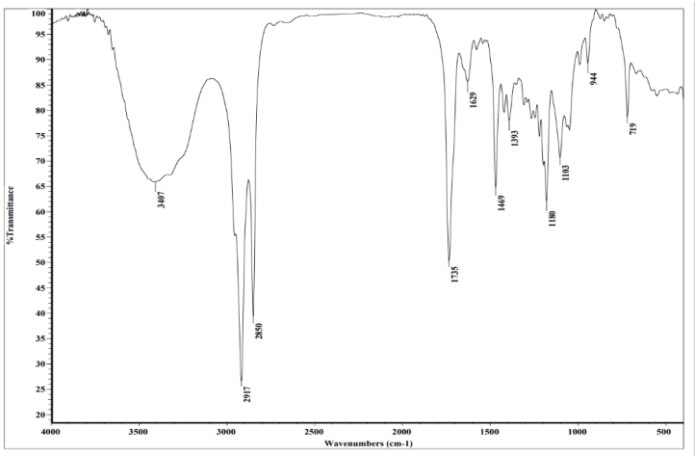


Figure 5 IR spectrum of solid lipid nanoparticles loaded with ciprofloxacin.

***In-vitro* drug release study**

Weng *et al*. (2020) reported that drug can be released by diffusion which involves swelling of matrix due to water penetration into system then the solid lipid could converted into the rubbery matrix and finally the drug could be diffused from the swollen rubbery matrix. In CSNPs, the acidic pH can promote the electrostatic attraction between NH_4^+ and water molecules, dissolution of chitosan polymer causes the erosion of nanoparticles matrix and rapid drug release (**Kulpreechanan and Sorasitthiyanukarn, 2020**). When nanoparticles swelled significantly and even dispersed rapidly, quick drug release can be achieved (**Shu and Zhu, 2002**). The cumulative drugs release from CSNPs might indicate that drug was released faster at lower pH **(Sabra and Billa, 2020).** A promising outcome of the results is the slow and incomplete release of ciprofloxacin. This suggests higher cellular uptake of CSNPs and SLNPs through bacterial cell wall.

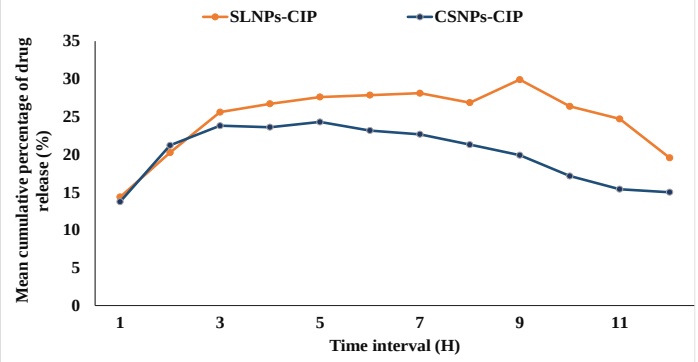


Figure 6 Ciprofloxacin release profile from nanosuspensions.

Biological properties

Determination of MIC and MBC

Ciprofloxacin antibacterial activity was compared with those of ciprofloxacin loaded with both CSNPs and SLNPs against *Salmonella enteritidis*. The different concentrations of CIP showed variable broad activity against the growth of the pathogen. The pathogen was most sensitive at concentration of 0.2 mg ml^{-1}. This concentration showed optimum antibiotic activity. Both MIC and MBC values were recorded as 0.02 and 0.002 mg ml^{-1}, respectively as showed in Figure 7.

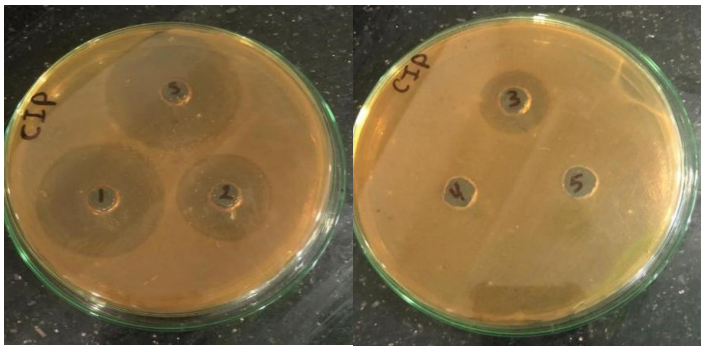


Figure 7 Photographs showing the antibacterial potential of different concentration of ciprofloxacin antibiotic on the growth of *Salmonella enteritidis* (S; 2.0 mg ml^{-1},

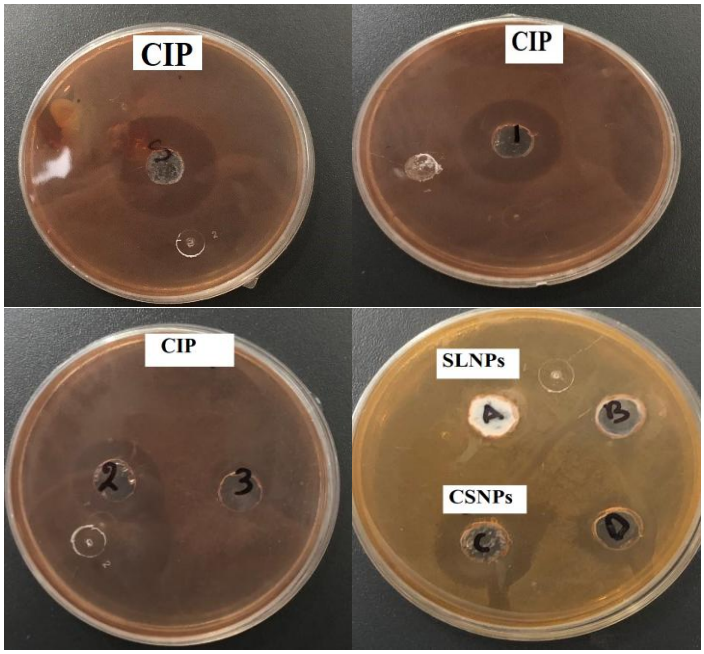
1; 0.2 mg ml^{-1}, 2; 0.02 mg ml^{-1}, 3; 0.002 mg ml^{-1}, 4; 0.0002 mg ml^{-1}, 5; 0.00002 mg ml^{-1}).

Antibacterial activity of CSNPs and SLNPs either singly or loaded with CIP

The antibacterial activity of CSNPs and SLNPs and its loaded form with CIP were evaluated against *Salmonella enteritidis* as Gram-ve bacteria. Administration of ciprofloxacin antibiotic singly or loaded on CSNPs and SLNPs as drug delivery system into the culture media of *Salmonella enteritidis* induced variable inhibition zones of the growth of the pathogen. Figure 8 and Table 2 showed that the antibacterial activity of CSNPs and SLNPs loaded with ciprofloxacin were increased with the increasing of the absorbed dose for Gram-ve bacterium. The results confirmed that the zone of inhibition with both SLNPs and CSNPs which were free of CIP was lower than those of loaded ones. In relation to the antibacterial activity of CIP, CSNPs and SLNPs, the zone of inhibition of chitosan nanoparticles of *Salmonella enteritidis* was enhanced with loading of antibiotic by 13.63% for 2.0 mg ml^{-1} as shown in Table 2 and Figure 8. Meanwhile, the zone of inhibition of SLNPs loaded with ciprofloxacin of *Salmonella enteritidis* was enhanced to 11.5 mm and increased by the increment of the antibiotic concentration by 4.45% for 2.0 mg ml^{-1}.

Table 2 Antibacterial activity of chitosan nanoparticles (CSNPs) and solid lipid nanoparticles (SLNPs) either singly or loaded with ciprofloxacin (CIP) against *Salmonella enteritidis.*

Antibacterial agent	zone of Inhibition (mm)	Percent change (%)
CIP (2.0 mg ml^{-1})	**11.0**	--
CIP (0.2 mg ml^{-1})	9.0	-18.18%
CIP 0.02 mg ml^{-1})	6.5	-40.90%
CIP (0.002 mg ml^{-1})	3.0	-72.72%
SLNPs singly	3.0	-72.72%
CSNPs singly	4.0	-63.63%
CSNPs- CIP (2.0 mg ml^{-1})	**12.5**	**13.63%**
CSNPs- CIP (0.2 mg ml^{-1})	9.5	-13.63%
CSNPs- CIP (0.02 mg ml^{-1})	6.0	- 45.45 %
CSNPs- CIP (0.002 mg ml^{-1})	10.0	-9.09%
SLNPs- CIP (2.0 mg ml^{-1})	**11.5**	**4.45%**
SLNPs- CIP (0.2 mg ml^{-1})	10.5	- 4.45%
SLNPs- CIP (0.02 mg ml^{-1})	7.25	-31.81%
SLNPs-CIP (0.002 mg ml^{-1})	8.5	-22.72%



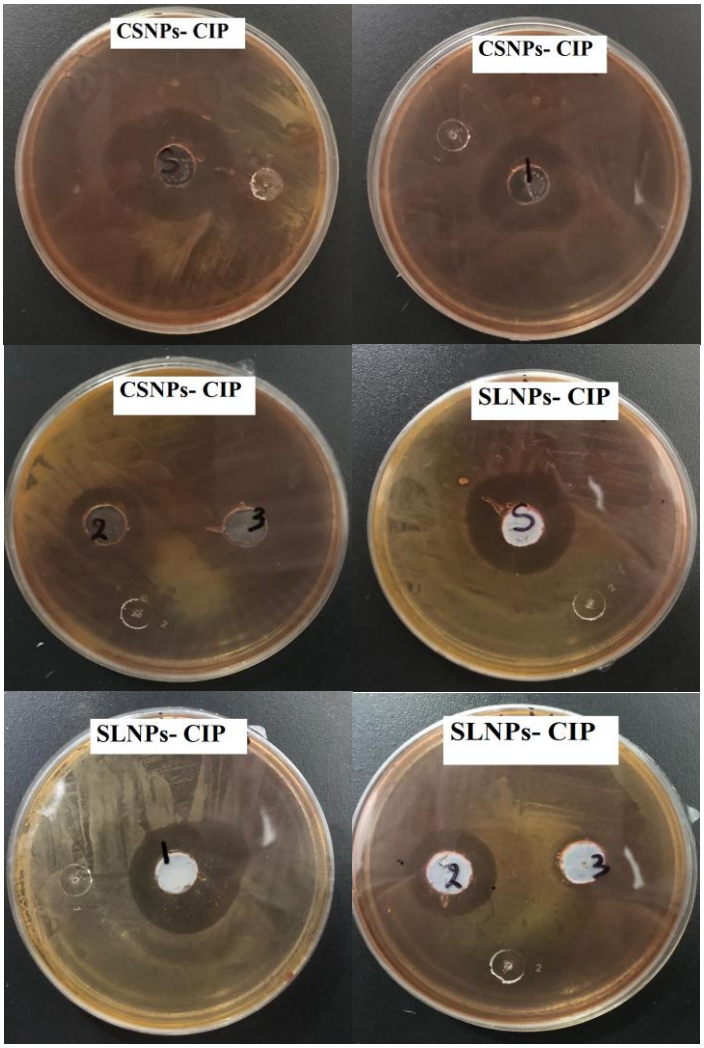


Figure 8 Antibacterial Activity of both chitosan nanoparticles and solid lipid nanoparticles either singly or loaded with ciprofloxacin (1; 0.2 mg ml^{-1}, 2; 0.02 mg ml^{-1}, 3; 0.002 mg ml^{-1}, S; 2.0 mg ml^{-1}, A; SLNPs, B; CSNPs).

The valuable and new data obtained in the present study (Figure 8 and Table 2) showed that the administration of CSNPs and SLNPS loaded with ciprofloxacin antibacterial as nano-drug delivery systems led to variable increases and decreases in its activity by a ratio of 13.63 % and 4.45%, respectively. Thus, CSNPs loaded with CIP is might be considered as an influential nanodrug delivery system for inhibition of the growth of *Salmonella enteritidis* pathogenic bacteria. The decreasing in MIC levels may be attributed to the inhibition of bacterial growth which resulted from more penetration of drug loaded on nanocarriers into the bacterial cell. For this connection, **Hasaneen *et al.* (2022)** reported that nanoformulations can be used as a controlled release system prolonging the half-life of the antibiotic payload, reducing the antibiotics toxicity, improving their pharmacokinetics, reducing the period of administration and increasing the therapeutic index.

The antibacterial activity of CSNPs singly against Gram-ve strain exhibited low activity, this might be due to the inhibitory effect of chitosan nanoparticles at high doses so the increased antibacterial activity of ciprofloxacin loaded on CSNPs, could not be due to the antimicrobial effect of CSNPs alone. The negative charge of the cell wall of *Salmonella* leading to more CSNPs-CIP adsorbed and higher inhibitory effect against the pathogenic bacteria (**Sobhani *et al.*, 2017**). The increased antimicrobial activity of CIP loaded on SLNPs is attributed to the lipophilic nature of soya lecithin which increase the cellular penetration of CIP into the bacterial membrane and the small size of the nanodrug **(Helal *et al.*, 2022 a)**.

Jain and Banerjee (2008) produced five different drug carrier ratios of ciprofloxacin hydrochloride loaded nanoparticles of both chitosan and solid lipid nanoparticles and their results suggests that CSNPs and SLNPs can act as promising carriers for sustained ciprofloxacin release. In this context, CSNPs loaded with ciprofloxacin shows superior antibacterial effect than those loaded on SLNPs. This is due to the polycationic feature of chitosan nanoparticles and highly positive surface charge density which interact with bacteria and strongly absorbed onto the bacterial membrane surface and disrupt the membrane of bacteria, thus kill bacterial cells by the same ratio which overall due to nanoparticle structure **(Avadi *et al.*, 2004; Abou-Zeid *et al.*, 2011)**. Various hypotheses have been proposed to explain the mechanism of antibacterial activity

of CSNPs. The most widely known mechanism of antimicrobial activity is the electrostatic interaction between the bacterial negatively charged cell membranes and the positively charged amino groups of glucosamine of chitosan nanoparticles. This interaction initiates a widespread change on the cell surface and causes a modification in the permeability of bacterial membrane which sequentially induce osmotic imbalance and efflux of intracellular substances that finally result in cell death **(Raafat *et al*., 2008).**

Solid lipid nanoparticles are considered an auspicious nanocarrier for controlled delivery of drug and being a good alternative to liposomes. They have many special properties such as low toxicity, high biodegradability, stability, biocompatibility and the notable potential to combine with both hydrophilic and lipophilic compounds. Furthermore, they can control the release of the incorporated drugs and chemically protects them. This suggests a high cellular uptake of SLNPs through the bacterial cell wall. This approach can lead to a significant reduction in the CIP dose and therefore reduction of all dose-dependent side effects **(Alarifi *et al*., 2020)**.

Sharmeen *et al*. (2018) studied the antibacterial potential of CIP loaded on a nanocomposite composed of MWCNTs, gelatin and chitosan and determined the effect of MWCNTs on the rate of drug release. After one hour, there was a sudden drug release, but the release rate was controlled by a decrease in concentration. The antimicrobial activity of the drug loaded on the nanocomposite was considerably different from those of the drug inserted into gelatin-chitosan nanocomposite without MWCNTs. For all bacteria studied, the antibacterial activity of loaded drug was found to be greater than those of loaded drug on gelatin-chitosan composite without MWCNTs **(Sharmeen *et al*., 2018; Helal *et al*., 2022 a& b).**

CONCLUSION

The promising results of our study: *in-vitro* preparation and characterization of solid lipid nanoparticles and chitosan nanoparticles either singly or loaded with ciprofloxacin antibiotic, optimization of drug, evaluation of the activity of nanodrug delivery systems, could pave the way for developing effective treatment for the diseases induced by *Salmonella enteritidis* and suggest promising useful insights into novel nanodrug delivery systems represented by nanochitosan-ciprofloxacin drug delivery system and nano-solid lipid- ciprofloxacin drug delivery system.

This fact was confirmed by further studies including molecular, biochemical and pathological good need of further studies of application *in-vivo*.

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