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ORIGINAL RESEARCH PAPER

Synthesis of Chitosan Nanoparticles Loaded with Antibiotics as Drug Carriers and the Study of Antibacterial Activity

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ABSTRACT

In recent years, there is a lot of interest in synthesis of nanostructures as carriers for drug delivery. These structures are considered as a highly effective drug delivery system due to controlling drug release, protecting the pharmaceutical molecule, and environmentally friendly. In this study, the synthesis of chitosan nanoparticles was carried out by chemical method. The nanoparticles size was measured by dynamic light scattering (DLS). Also, it was used from atomic absorption spectrometry (AAS), FTIR and transmission electron microscopy (TEM) to confirm the loading of antibiotic onto nanoparticles and to calculate the percentage of the drug loaded. In addition, it was used for clarithromycin as antibiotic. The antibacterial activity was studied by the disc-diffusion method and the effect of different concentrations of the drug in nano-carriers were investigated and it was determined the optimum antibacterial activity of drug nanocarrier was happened in concentration 0.6 gr/10 ml.

Keywords: *Antibacterial Activity, Chitosan Nanoparticles, Clarithromycin, Nano-Carriers*

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INTRODUCTION

Polymers are the most commonly used in pharmaceutical substances form nanoparticles. The polymer used in controlled release of the drug should be biocompatible and non-toxic and free of leakage impurities. Polymers used to make nanoparticles can be synthetic or natural. Polymer nanoparticles are often biodegradable [1,2]. The advantage of polymer nanoparticles is their high stability and the possibility of making them in large quantities. Polymer nanoparticles include a large number of compositions, including nanocapsules and matrix systems (nanospheres). In nanocapsules, the drug is blocked in the cavity of the polymer, but in the nanospheres, the drug is dispersed in a polymer matrix [3,4].

Chitosan is a derivative of Glucan with repeat units of chitin, which was found by Rogat in 1859 with a known agent of chitin, a natural amino-polysaccharide compound with chemical formula (C8H13NO5) and abundantly found in skeletons of arthropods like shrimp, crabs, and postal plants such as yeast and cuticle insects. Chitin, from the Greek word for Keaton, means hard cover. This compound was first described by Braconunte in 1811. The importance of chitosan in the preparation of chitosan in clinical products is due to biocompatibility with other materials, easy digestibility, non-toxicity, high absorption capacity and availability as a drug carrier [5,6,7]. Chitosan is used to reduce cholesterol and healers of wounds. Due to its positive charge and its ability to connect to negative charge levels, this material is used to transfer the drug and the gene to target cells. The

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use of polymer nanoparticles with antibiotics such as penicillin G, amoxicillin and azithromycin led to antimicrobial effects against gram-negative bacteria and gram-positive bacteria [8, 9]. The polymers used in the nanoparticles are based on hydrophilic and hydrophobic. Hydrophilic nanoparticles, such as chitosan, are a good option for drug delivery systems, because the blood's characteristics are compatible. Chitosan is a biocompatible linear polysaccharide that is obtained from N-acetylation of chitin. The chitosan structure is similar to cellulose. Chitosan has unique chemical and biological properties due to the presence of amine and hydroxyl groups in its structure [10,11].

Clarithromycin is used to treat certain bacterial infections, such as pneumonia (a lung infection), bronchitis (infection of the tubes leading to the lungs), and infections of the ears, sinuses, skin, and throat. It also is used to treat and prevent disseminated *Mycobacterium avium* complex (MAC) infection [a type of lung infection that often affects people with human immunodeficiency virus (HIV)]. It is used in combination with other medications to eliminate *H. pylori*, a bacterium that causes ulcers. Clarithromycin is in a class of medications called macrolide antibiotics. It works by stopping the growth of bacteria.

Antibiotics such as clarithromycin will not work for colds, flu, or other viral infections. Taking antibiotics when they are not needed increases your risk of getting an infection later that resists antibiotic treatment [12].

In this study, the synthesis of chitosan nanoparticles was carried out by chemical method. The nanoparticles size was measured by dynamic light scattering (DLS). Also, it was used from atomic absorption spectrometry (AAS), FTIR and transmission electron microscopy (TEM) to confirm the loading of antibiotic onto nanoparticles and to calculate the percentage of the drug loaded. In addition, it was used for clarithromycin as antibiotic. The antibacterial activity was studied by the disc-diffusion method.

MATERIAL AND METHODS

Materials

Chitosan (Medium molecular weight, Mw 108 kDa) was purchased with the highest purity from Sigma Aldrich Company. Sodium tripolyphosphate (STPP), sodium hydroxide, tween 80, ethyl alcohol, sodium chloride and acetic acid were purchased from Merck Company. All chemical materials were used analytical grade. Antibiotic was used clarithromycin as active pharmaceutical ingredient (API).

Synthesis of chitosan nanoparticles

2 g of chitosan powder was dissolved in 200 ml of acetic acid solution (1% v/v). Then 50 ml of NaCl solution (3 gr/lit) was added to it and leaving it under stirring at 3000 rpm for 30 min. The pH value of solutions was approximately adjusted to 4, 5 and 6 with 0.5 M NaOH for three samples. The solutions were filtered using 0.45 µm filters (Millipore) to remove insoluble chitosan. Then, 20 ml of sodium tripolyphosphate (STPP) solution (3 gr/lit) was added to the samples drop wise under magnetic stirring at 1000 rpm for 5 hr. The above experiment was performed for 3 different temperatures (T=10±2°C, T=25±2°C and T=50±2°C). The solutions were centrifuged at 14000 rpm for 20 minutes. The chitosan sediment was washed and filtered three times using distilled water to eliminate any residue impurities. The product was dried in an oven at 55 °C for 12 hours and finally used for analysis.

Preparation of clarithromycin-loaded chitosan nanoparticles

Antibiotic loading was carried out during the formation of nanoparticles. For this purpose, 3 gr of active pharmaceutical ingredient (API) of clarithromycin were dissolved in 10 ml ethyl alcohol at 80 ° C and added 1 ml of tween 80 to prevent clarithromycin aggregation. Then, this solution was added to chitosan solution with sodium tripolyphosphate to load on chitosan nanoparticles under a magnetic stirrer at 1000 rpm.

Antibacterial activity

It was investigated the antibacterial activities of clarithromycin and drug nanocarriers against Staphylococcus aureus using disc-diffusion method. It was prepared the different concentrations of clarithromycin and drug nanocarrier. Then it was measured the diameter of the zone of inhibition by a millimetre ruler after 24 hr incubation at 37°C.

RESULT AND DISCUSSION

The study of chitosan nanoparticles size by DLS

The DLS analysis was used to measure the size of the chitosan nanoparticles. Fig. 1 shows the size distribution of chitosan nanoparticles by DLS. In this diagram, the X-axis is the size distribution of

particles and the Y-axis is the number of particles. The average size of the chitosan nanoparticles synthesized was obtained about 33 nm by DLS analysis.

The study of chitosan nanoparticles shape by TEM

Transmission electron microscopy (TEM) was used to investigate the morphology of nanoparticles. It was found that the shape of the chitosan nanoparticles synthesized were spherical and pseudo-spherical, and their size were estimated in the range of 15 to 45 nm. The results of DLS analysis were confirmed by TEM (Fig. 2).

The study of temperature and pH effects on chitosan nanoparticles size

According to Figs. 3 to 6 (DLS analysis), it was found the solution temperature of 50 °C and pH 5 was the optimal conditions to synthesize chitosan nanoparticles. The STPP dissolved in deionized water generates OH^- and $P_3O_{10}^{-5}$ ions. At lower temperatures and pH values, OH^- and $P_3O_{10}^{-5}$ ions are in competition for binding to NH_3^- groups. The OH^- ions penetrate easily into chitosan due to their small size and create a sedimentary layer, while acidifying the medium to pH 5 and also increasing the temperature, there is only $P_3O_{10}^{-5}$ ^ ions in the

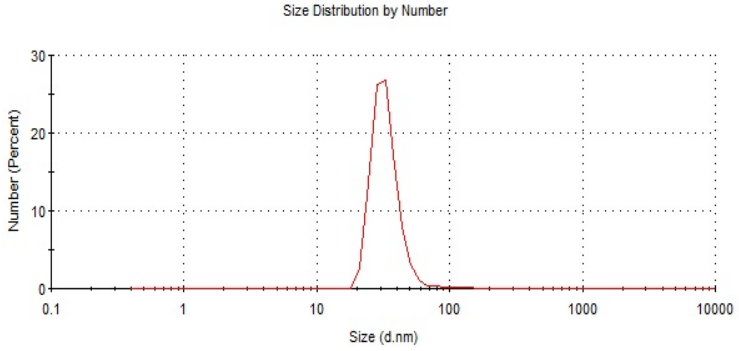

Fig. 1. The size distribution of chitosan nanoparticles

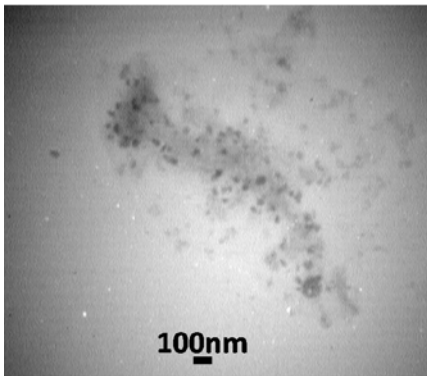

Fig. 2. A TEM Image of Chitosan Nanoparticles

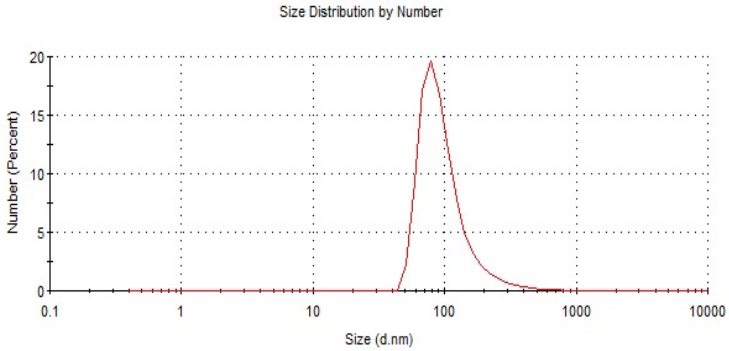

Fig. 3. The size distribution of chitosan nanoparticles at 10 ± 2 ° C and pH = 4

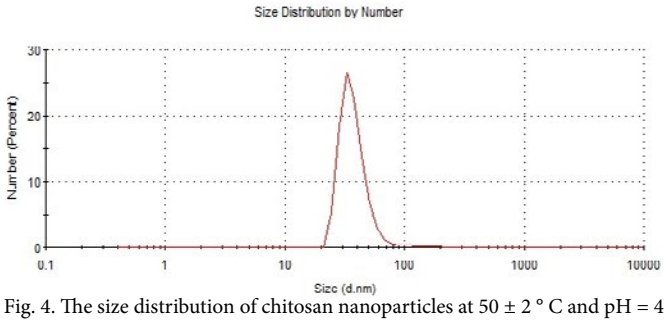

Fig. 4. The size distribution of chitosan nanoparticles at 50 ± 2 ° C and pH = 4

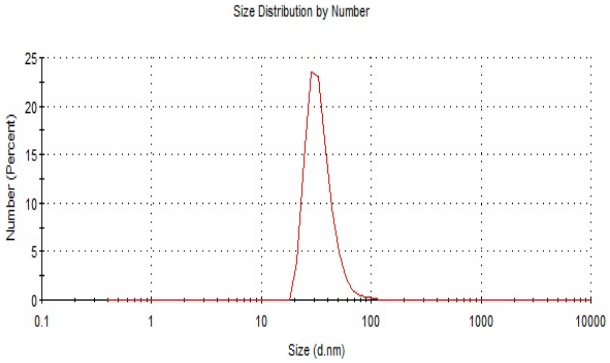

Fig. 5. The size distribution of chitosan nanoparticles at 25 ± 2 ° C and pH = 5

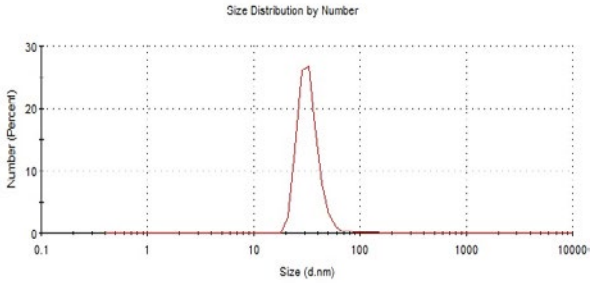

Fig. 6. The size distribution of chitosan nanoparticles at 50 ± 2 ° C and pH = 5

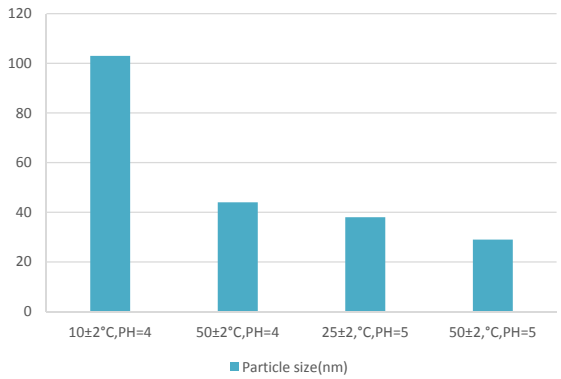

Fig. 7. The comparison of the nanoparticles size at different temperatures and pH values

environment, so chitosan was easily bonded with STPP and create a gel layer with nanometer size. By acidifying the environment to pH 4, the particle size increases as chitosan solubility increase in acetic acid. Fig. 8 shows the comparison of the particles size at different temperatures and pH values.

Studying loading of clarithromycin on chitosan nanoparticles by FT-IR spectrum

Generally, FT-IR is used for the identification of functional groups of various compounds, and its technique is based on the infrared light [11]. FTIR analysis was carried out to identify the presence of substances that might be responsible for their synthesis or stabilization. In this study, FT-IR spectra were performed for three samples. The FT-IR spectra of chitosan showed that absorption peaks at 3100-3600 cm $^{-1}$, 2922 cm $^{-1}$, 5377 cm $^{-1}$ and 1095 cm $^{-1}$ were assigned to –OH, -NH_2 , -CH and –C-O stretching, respectively (Fig. 8). Infrared spectrum showed that -O- stretching vibration absorption peak of clarithromycin was located at 2977 cm^{-1} and -O-C=O at 1734 cm^{-1} (Fig. 9). In FTIR analysis of drug carrier, it was detected the ether functional group (-O-) and -O-C=O that indicating the existence of clarithromycin into drug carriers (Fig. 10).

Studying loading of clarithromycin on chitosan nanoparticles by UV-Vis spectroscopy

UV-Vis spectroscopy is used to measure the amount of antibiotic loading on chitosan nanoparticles. The basis of this technique is the measurement of antibiotic concentration in the sample. It is therefore necessary a correlation between the amount of

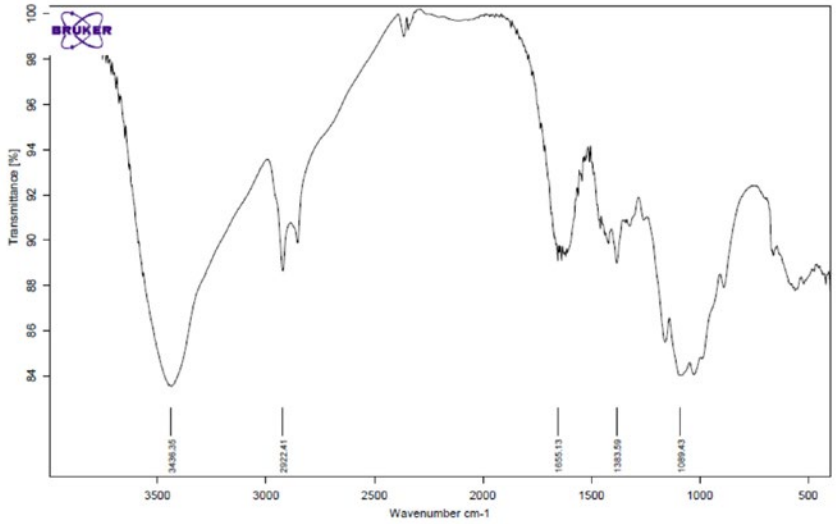

Fig. 8. FT-IR analysis for Nano Chitosan

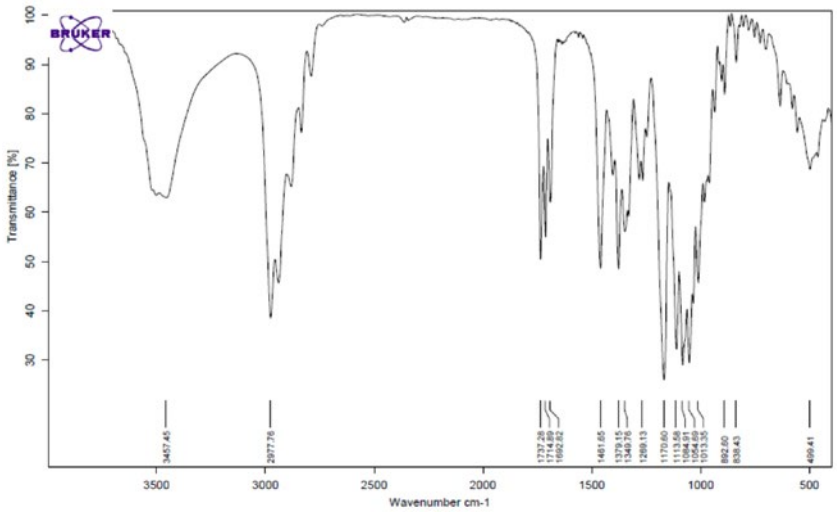

Fig. 9. FT-IR analysis for Clarithromycin

light absorbed by substance and the substance concentration, which is called Beer–Lambert's law. By measuring the amount of antibiotic (clarithromycin) absorption at different concentrations, the calibration curve was plotted. Then, the loading amount of clarithromycin was calculated by determining the amount of drug nanocarrier absorption. Table 1 shows the absorption amount of clarithromycin for six different concentrations and drug nanocarrier. By drawing the calibration curve and measuring the amount of drug nanocarrier absorption by UV-Vis spectroscopy, it was determined unknown concentration (clarithromycin) of drug nanocarrier, which was approximately 0.48 gr / 10 ml.

The study of chitosan nanoparticles size loaded with clarithromycin by DLS

Dynamic light scattering (DLS) analysis was used to measure the size of nanoparticles after loading antibiotic. As can be seen in Fig. 11, the distribution of particle size for drug nanocarrier was about 30 to 65 nm.

Studying loading of clarithromycin on chitosan nanoparticles by TEM

It was used from transmission electron microscopy

Table. 1. The absorption amount of clarithromycin for six different concentrations and drug nanocarrier

Materials	Absorption intensity	Density
Clarithromycin	0/305	0/05
	0/617	0/1
	0/709	0/2
	0/942	0/3
	1/617	0/4
	1/869	0/5
	1/88	0/6
Standard deviation (STDEV)	±0.598	±0.19
Nano carrier	1/704	X

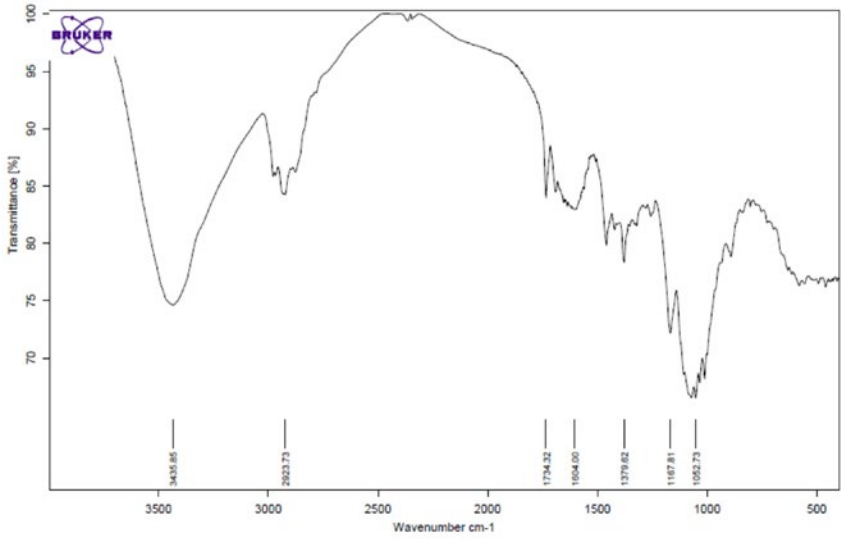

Fig. 10. FT-IR analysis for drug nanocarrier

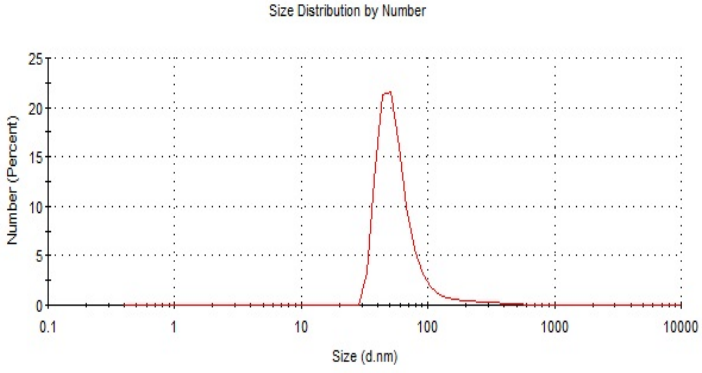

Fig. 11. The size distribution of drug nanocarrier

(TEM) to confirm the loading of antibiotic on chitosan nanoparticles. Fig. 12 shows chitosan nanoparticles and drug nanocarriers (chitosan nanoparticles loaded with clarithromycin).

Antibacterial Activity of clarithromycin and drug nanocarrier

The drug nanocarriers killed S. aureus with zones of inhibition ranging from 18 mm to 42.5 mm (Fig. 13). Table 2 and Fig. 14 shows the antibacterial activity of clarithromycin and drug nanocarriers in different concentrations. It also suggests that the optimum antibacterial activity of drug nanocarrier was happened in concentration 0.6 gr/10 ml. The antibacterial activity of drug nanocarrier with concentrations higher than 0.6 gr/10 ml is approximately same with concentration 0.6 gr/10 ml. Therefore, it was suggested to prepare

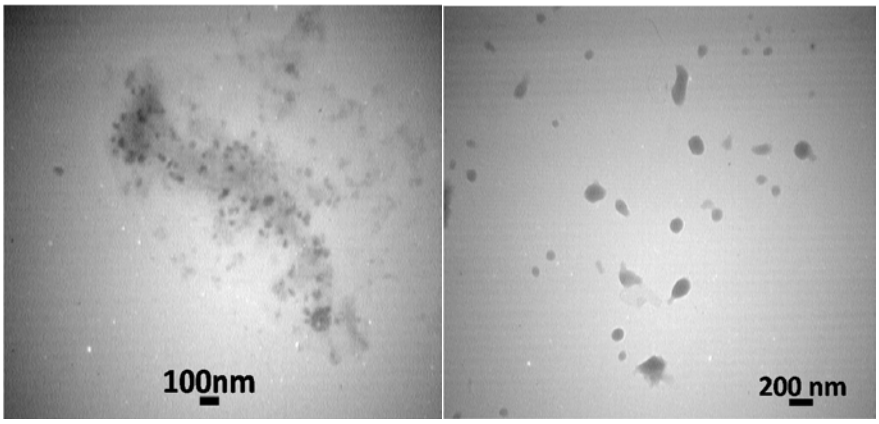

Fig. 12. TEM Images of drug nanocarrier

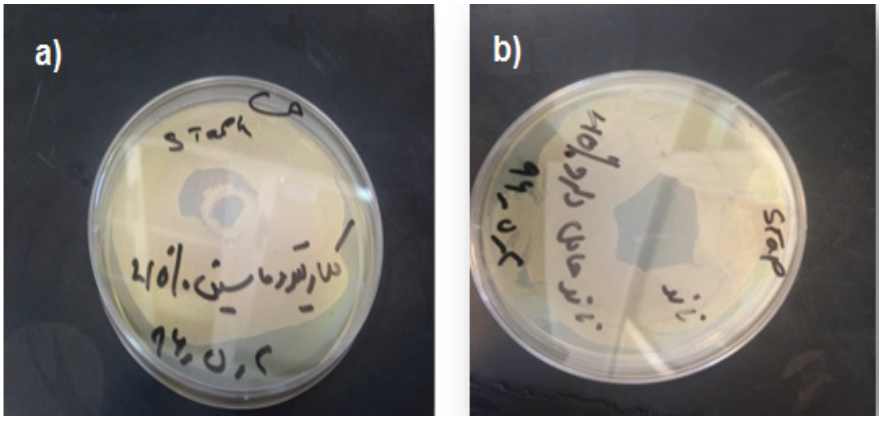

Fig. 13. Zones of inhibition for a) clarithromycin and b) drug nanocarrier

Table. 2. The diameter of inhibition zones for clarithromycin and drug nanocarrier at different concentrations

Size(mm)	Concentration $^{gr}/_{10ml}$	materials
---	---	Reference sample
15	0/3	Clarithromycin
18	0/3	Nano carrier
23	0/4	Clarithromycin
30	0/4	Nano carrier
33	0/5	Clarithromycin
39	0/5	Nano carrier
40	0/6	Clarithromycin
42	0/6	Nano carrier

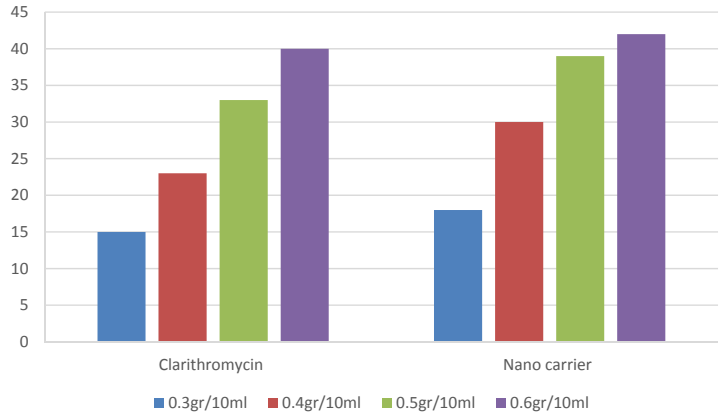

Fig. 14. The diameter of inhibition zones for clarithromycin and drug nanocarrier at different concentrations

drug carrier with concentration 0.6 gr/10 ml with antibacterial activity maximum.

CONCLUSIONS

In this study, chitosan nanoparticles were synthesized by chemical reduction method. It was investigated solution parameters such as concentration, temperature and pH to optimize chitosan nanoparticles synthesis. The optimal conditions for the nanoparticles synthesis were a temperature of 50°C and pH 5. The average size of chitosan nanoparticles was obtained about 33 nm by DLS and confirmed by TEM. Then FTIR analysis was carried out to identify the presence of clarithromycin into drug nanocarrier after loading antibiotic on chitosan nanoparticles. In the resulting charts, the common peaks of clarithromycin were found in the drug nanocarrier, indicating the successful loading of antibiotics on chitosan nanoparticles. TEM analysis was used to ensure loading of clarithromycin on chitosan nanoparticles. Images showed the particle size increased with loading of clarithromycin on chitosan nanoparticles. The drug nanocarrier size was measured about 60 nm by DLS analysis. In addition, the loading amount of clarithromycin was calculated by determining the amount of drug nanocarrier absorption using UV-Vis spectroscopy. Finally, it was studied the antibacterial activity of drug nanocarrier and found concentration 0.6 gr/10 ml of nanocarrier was optimal concentration for antibacterial activity maximum.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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