Preparation characterization and application of Chitosan nanoparticles as drug carrier

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ABSTRACT

To improve the therapeutic efficacy of Mebeverine Hydrochloride (MB.HCl) medication, this study was worked on by reducing the drug dosages while maintaining the level of the drug inside the patients' body. The best treatment results are achieved by loading the drug through a medicated drug carrier that is Chitosan nanoparticle (CSNPs) and preparation was done by Ionic gelation method which including reaction between binding material Sodium tripolyphosphate (STPP) and Chitosan (CS) which was extracted from shrimp shells. The drug was loaded by Chitosan nanoparticles and the proof was done by using several techniques, namely FT-IR and X-ray diffraction spectrum, the size of particles was determined before and after loading using Particle size analyzer, also use the FE-SEM emission electron scanning microscope where the results showed the success of preparing a drug carrier and MB.HCl was loading on Chitosan nanoparticles.

KEYWORDS: Chitosan nanoparticles, ionic gelation, drug carrier, Mebeverine hydrochloride

1. INTRODUCTION

The fish market in the city of Basra is one of the most productive markets for fisheries in Iraq and therefore also produces large quantities of fish waste. Shrimp shells are one of the marine crustal wastes (Jerjees & Al-Rawi, 2018; Niamah, 2012). Chitin is the second natural polymer spread in nature after cellulose. Chitin differs from cellulose in terms of composition in only one location by containing the C2 atom of chitin on the acetyl amide group (NHCOCH3-), Chitosan is a polycation produced by deacetylation of chitin, Also Chitosan is a linear polysaccharide consisted of D-glucosamine and N-acetyl-D-glucosamine units. Chitosan has an amine/acetamide group on C-2 and primary, secondary hydroxyl (OH) groups on C-3, C-6 positions, Chitosan has great adsorption ability with other compounds because of the functional
groups that have it (Varun et al., 2017). Which has been widely employed in many applications in food, chemical industries, drug delivery system, drug controlled release, and drug carriers (Dev et al., 2010; Vasconcellos, Goulart, & Beppu, 2011). Also, it is non-toxic and biodegradable (Kheiri, Jorf, Malhipour, Saremi, & Nikkhah, 2017). Chitosan is used as a drug carrier through the preparation of Chitosan nanoparticles via ionic gelation method, nanoparticles were formed by adding sodium tri-polyphosphate (STPP) as a binder material to Chitosan solution, (STPP) is a polyanion solid inorganic compound (Ma et al., 2020; Naskar, Kuotsu, & Sharma, 2019). The purpose of using chitosan nanoparticles is to increase the therapeutic efficacy of some drugs that are affected by different media within the human body, Ursolic acid (UA) which is used as an anti-tumor due to its low solubility in water, it limits its targeting of the affected tissues and greatly reduces its clinical efficacy after it's loaded by Chitosan nanoparticles succeeded by giving better results and therapeutic efficacy to infected tissues (Jin et al., 2016). In this study, Mebeverine hydrochloride (MB.HCl) was loaded by Chitosan nanoparticles, this medicine belongs to the group of anti-spasmyotic drugs, as it affects the smooth muscles of the digestive system, causing it to relax, and is used to treat a disease Irritable bowel syndrome (IBS) that suffers from most of the world's population (Shanmugam, Ayyappan, & Vetrichelvan, 2019). The drug is available in two types (135mg) tablet and (200mg) capsule (Al Gohary, 1997). The study aimed to prepare chitosan nanoparticles and use them as a drug carrier for MB.HCl drug, this will help many patients who take MB.HCl orally to reduce daily doses of the drug, because most patients who take MB.HCl irregularly to treat colon cramps have been noticed that they do not have the necessary therapeutic efficacy, so MB.HCl was loaded by chitosan nanoparticles and their use as drug carrier for MB.HCl drug, with the controlled drug release system, to reach the best therapeutic efficacy of MB.HCl.

2. Chemicals and reagents & Method

Chemicals and reagents

Prepared Chitosan (CS) was extracted from Shrimp shells, Sodium tripolyphosphate (STPP), Sodium Hydroxide (Fluka), Hydrochloric Acid (Fluka), Mebeverine hydrochloride (RA CHEM PHARMA LIMITED, India), Acetic acid (Fluka), Potassium chlorate (Fluka), Sodium Chloride (Fluka), Carbonate Calcium (Fluka).

Method

Chitosan extraction

The shrimp shells were collected in sufficient quantity and the crusts were cleaned by washing them with tap water for several times later, leaving the crusts under sunlight to dry for a whole day, then they were put into the drying at 70°C temperature (2 h) to ensure complete dryness the shrimp shells are ground and then go through phases until they turn into chitosan (Trung et al., 2020) First deproteinization (1.25 M) of NaOH solution was prepared at (100mL) and added to shrimp shells in (500mL) capacity of the backer. The temperature was set at (80-90)°C and for
(30min) under magnetic stirring. Demineralization Calcium carbonate was removed by used (1.57 M) of HCl solution which prepared at (100mL) and added to shrimp shells at room temperature for (3h) under continues magnetic stirring after filtered from NaOH and washed by ion- free water for many times (Younes & Rinaudo, 2015). The shrimp shells were collected in sufficient quantity and the crusts were cleaned by washing them with tap water for several times later, leaving the crusts under sunlight to dry for a whole day, then they were put into the drying at 70°C temperature (2 h) to ensure complete dryness the shrimp shells are ground and then go through phases until they turn into chitosan (Al-Manhel, Al-Hilphy, & Niamah, 2018). Deacetylation of chitin stated by placed it in a circular beaker and added a solution of sodium hydroxide (NaOH 10M) and the Reflux was continued for (24h) at (120 °C) under magnetic stirring to produce Chitosan (Pires, Vilela, & Airoldi, 2014).

**Preparation of Chitosan nanoparticles**

A solution of (1% v/v) acetic acid / deionized water as prepared. 0.5 g of prepared chitosan was dissolved in (100mL) of the solution of acetic acid using the ultrasonic wave device. The pH of the solution was adjusted at (4.6) by pH meter. Preparation 0.25 % w/v of STPP was achieved by dissolving 0.25 g of STPP in 100 mL of deionized water(Vaezifar et al., 2013). Preparation of chitosan nanoparticles by Ionic gelation was done by withdrawing (3mL) from the previously prepared Chitosan solution which was kept at (4 °C) and added in a beaker with continuous stirring by magnetic stirrer (1mL) of the cross-link substance (STPP) is slowly added where note the formation of a colloidal solution(Ali, Aboelfadl, Selim, Khalil, & Elkady, 2018).

**Chitosan nanoparticles loaded by MB.HCl**

(3 mL) of Chitosan solution was take, then (1mL) of the MB.HCl medicine(10 mg/L) was added and let the solution under continuous stirring for a period of (15min) to the adsorption event between the functional group on drug and the Chitosan polymer chain. (1mL) was added to the binding material (STPP) where we have the chitosan nanoparticles loaded with MB.HCl (Ibrahim, El-Bisi, Taha, & El-Alfy, 2015).

**Characterization of Chitosan nanoparticles and it’s loaded**

Fourier transform infrared (FT-IR) spectrum of prepared chitosan powder and loaded chitosan nanoparticles by MB.HCl was taken after mixed with potassium bromide powder on a spectrometer (Shimadzu8500, Japan) at a wavenumber range of 500–4,000 cm⁻¹. X-Ray Diffractometer of prepared chitosan and loaded chitosan nanoparticles of MB.HCl was obtained using (Shimadzu, XRD-6000 , Japan). The condition of X-ray was the source is Cu radiation (40 kV,30 mA) and the measurement range was 10–90°. Morphological characterization of Chitosan nanoparticles and loaded Chitosan nanoparticles by MB.HCl was performed by Scanning Electron Microscopy (SEM). The samples must by conductive even give a photo thus sample was coated by spraying gold powder on it. Chitosan nanoparticles and loaded Chitosan nanoparticles were suspended for 5 min in water by sonication to obtain a dilute suspension. A
few drops of dilute suspension was deposited onto a slide of glass and allow dry. Thermogravimetric analysis (TGA) was performed using thermogravimeter (Perkin Elmer, TGA4000, USA). The sample was heated from 60 to 360 °C at a heating rate of 2 C/min. To determine the wavelength of the drug Mebeverine hydrochloride (MB.HCl) UV–vis absorption spectrum was obtained using a spectrophotometer (Shimadzu, UV-1800, Japan) in the range of 200–400 nm. The particle size determines the size of Chitosan nanoparticles before and after loaded of drug MB.HCl by using Laser Particle Size Analyzer (Brookhaven, 90 plus, USA). Before analyzing, Chitosan nanoparticles were diluting by water and ultrasonically for 5 min.

3. Results and Discussion

FT-IR Spectral Analysis
The infrared spectrum of the chitosan polymer (Chitosan) shown in Figure(1) This figure shows the appearance of a wide absorption peak in the (3273-3446) cm⁻¹ range due to the stretching vibration of the interfering bonds (O-H) and (N-H). The absorption beam shown in (2881) cm⁻¹ is due to the Bending and stretching vibration of the (C-H) finger. Whereas the peak shown in (1656) cm⁻¹ is attributed to the presence of a stretching vibration of the (N-H) bond. Where the apparent peak at (1155) cm⁻¹ shows the stretching vibration of the cyclotide bond (C-O-C), and the apparent peak (1022) cm⁻¹ shows the vibration of (C-O) bond in the alcohol groups in the polymer (Balkhande & Ratnakar, 2019).

Figure(1). FT-IR spectrum of Chitosan polymer

Mebeverine hydrochloride infrared spectrum loaded on chitosan nanoparticles shows the appearance of peaks in the (3099-3439) cm⁻¹ range, the stretching vibration of the (O-H) hand. The peak shown in (2934) cm⁻¹ is due to the presence of the (C-H) bond. Whereas the peaks apparent in (1658, 1562) cm⁻¹ refer to the (C = O and C = C) bonds. The apparent peak at (1072) cm⁻¹ is from the (C-N) bond, While (P-O) bond appears at 1153 cm⁻¹ all these peaks showed in Figure(2). (Omar, Aldosari, Refai, & Al Gohary, 2007)
Figer(2). FT-IR spectrum of MB.HCl loaded on Chitosan nanoparticles

X-ray Diffraction (XRD) Spectroscopy

X-ray diffraction technology provides detailed information about the chemical composition, crystalline quality, and the minute size of the prepared compounds (Harris & Norman White, 2008). Figer(3) shows X-ray diffraction of the Chitosan polymer and Chitosan nanoparticles loaded by MB.HCl. The broad peak at $2\theta$ of about $20^\circ$ gives the amorphous character of the polymer. Moreover, no additional peaks appear that indicate the prepared Chitosan is posses high purity. Through the Prague Law (Bragg Law), the crystallization of the Chitosan polymer crystal practices reaches 4.5 Å. XRD diffraction spectrum MB.HCl drug loaded with Chitosan nanoparticles shows peaks appearing in $2\theta = 17.9^\circ$ due to MB.HCl drug and in $2\theta = 20^\circ$ broad peak due to Chitosan polymer and this indicates a low crystalline ratio as a result of drug binding with Chitosan while the beam shown in $2\theta = 26.9^\circ$ return to MB.HCl drug and the binding material (STPP). (Qi, Xu, Jiang, Hu, & Zou, 2004)

Figer(3). X-ray diffraction spectrum for Chitosan polymer (CS), binder (STPP), Mebeverine Hydrochloride(MB.HCl), and Chitosan nanoparticles loaded with MB.HCl (CS + MB. HCl + STPP)
Field Emission Scanning Electron Microscopy (FE-SEM)

The image of the scanning electron microscope explains that the binding of Chitosan with sodium triphosphate (STTP) by appearance layers of binder on the surface of the chitosan polymer. This accumulation between the two materials gives the nature of the wave and semi-rugged nature of both materials. Figure (4) illustrates that the size of the nanoparticles ranges between 22-35nm, which is the nanoscale range required to give the prepared material the nanoparticle (Joghataei, Hosseini, & Arab-Tehrany, 2019). Figure (5) shows an image of SEM for Chitosan nanoparticles loaded with (MB.HCl). This image demonstrates asymmetric cumulative aggregations due to the loading of a drug to the surface of Chitosan. The appearance of these groups as a result of using dilution solutions of the mixture. The average volume of Chitosan nanoparticles is 200nm (Noorhamdani, Ramadhan, & Sari, 2019)

![SEM image of chitosan nanoparticle (CSNPs)](image)

Figure(4). SEM image of chitosan nanoparticle (CSNPs)

![SEM image of chitosan nanoparticles loaded with MB.HCl](image)

Figure(5). SEM image of chitosan nanoparticles loaded with MB.HCl

Laser Particle Size Analyzer

A laser analyzer is a technique used to measure the size of particles dispersed in a liquid. So sensitive to some modern systems that it can also be used to measure the size of large particles in a solution. The volumetric distribution of Chitosan nanoparticles (CSNPs) involving the reaction of the Chitosan polymer with the sodium triple polyphosphate binder is shown in Figure(6), where the figure shows that the average diameter of the particles reaches (679.4 nm). Where we note the presence of two sizes of the first nanoparticles in the range (100.8-13.2) nm while the
second particle size in the range (902.5-117.9) nm. This indicates that there is a large difference between the size of the nanosomes of chitosan nanoparticles as a result of the occurrence of accumulation and accumulation of particles, and thus the distribution of particles is irregular and is proven in the examination (FE-SEM).

![Figure 6](image)

**Figure (6).** Particles volume distribution of Chitosan nanoparticles (CSNPs)

Figure (7) shows the volumetric distribution of Chitosan nanoparticles loaded with MB.HCl as the average particle diameter reaches 448.1nm with the appearance of two different sizes of drug
particles in the range of nm (110.7-185.8) and (653.6-200.1) nm due to the accumulation of particles. On top of some and so due to its adsorption and loading of the drug MB.HCl, as well as the result of using an ultrasonically that led to the accumulation of particles and earned it the appearance of a large size (Guan et al., 2011).
Conclusion

- Chitosan was produced from shrimp shells by the deacetylation of chitin.
- the pH of the chitosan solution was adjusted by pH meter at (4.6) and kept it at (4°C).
- Chitosan nanoparticles were prepared by ionic gelation method where use (0.5g/mL) from prepare chitosan solution and (0.25g/mL) from Sodium tripolyphosphate (STPP) and the ratio was 1:3 (STPP: CS)
- Characterization of chitosan nanoparticles loaded by MB.HCl was done by using FT-IR, XRD, and SEM.

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