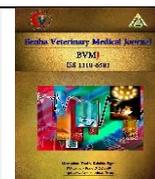




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### Original Paper

## Preparation and characterization of metformin-loaded chitosan nanoparticles for biomedical applications

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### ABSTRACT

Nanotechnology has introduced a novel drug delivery system to improve the stability and bioavailability of drugs. The current work deals with the formulation and characterization of metformin loaded in chitosan nanoparticles (M-CHNPs) for medical purposes. Chitosan nanoparticles (CHNPs) were formulated by ionotropic gelation of chitosan with tripolyphosphate anions. Free chitosan nanoparticles and M-CHNPs were characterized *via* X-ray diffraction (XRD), Raman spectroscopy, dynamic light scattering (DLS), high-resolution transmission electron microscope (HRTEM), zeta sizing, BET surface area, and atomic force microscope (AFM). The drug encapsulation efficiency, drug loading capacity, and *in vitro* release rate were also investigated. The results showed that M-CHNPs were successfully prepared in pure form without undesirable products, as shown in the X-ray diffraction pattern and Raman spectra. Also, the obtained M-CHNPs showed an average hydrodynamic size of 78 nm, zeta potential of -28 mv, and had a drug entrapment efficiency and drug loading capacity up to 91%. *In vitro* release study indicated that metformin release from M-CHNPs was pH-dependent, where it was released with rates of 51%, 40%, and 10% at pH 1.2, 6.8, and 7.4, respectively.

## 1. INTRODUCTION

Metformin hydrochloride (1,1-dimethyl biguanide hydrochloride) is a hypoglycemic drug used to control blood glucose levels in type II diabetes (Bailey, 2017; Bergenstal *et al.*, 2017; Foretz *et al.*, 2019). Repeated metformin administration is typically related to many side effects such as intestinal pain, nausea, and diarrhea that may happen during the medication (Bouchoucha, Uzzan, & Cohen, 2011; Elbere *et al.*, 2018). The risk of vitamin B12 and folate deficiency has also been reported (Aroda *et al.*, 2016). Therefore, the usage of lower dosage frequency may improve patient compliance.

Nano drug delivery is an emerging system that includes drug encapsulation in nanocarriers (Bamrungsapet *et al.*, 2012; Zahin *et al.*, 2020). This novel system provides sustained and controlled drug delivery to improve therapeutic efficiency at lower dosages and reduce side effects (Kadian, 2018; Patra *et al.*, 2018). Generally, polymers have been suggested as the best choice for encapsulating many drugs (Lembo *et al.*, 2013;

Miladi, Sfar, Fessi, & Elaissari, 2016). Polymers are biodegradable and biocompatible while releasing the desired dose via various means, including changes in pH, temperature, surface modification, and conjugation (Ekladius, Colson, & Grinstaff, 2019). Various polymers such as polylactic-co-glycolic acid (PLGA), alginate, albumin, and chitosan have been used in most pharmaceutical industries and research (Ozdil & Aydin, 2014; Schmitt *et al.*, 2020).

Nanoparticle technology has come to the forefront as a viable drug delivery strategy, presenting opportunities for controlled release, protecting active components from enzymatic or environmental degradation, and localized retention (Nagpal *et al.*, 2010). polymeric nanoparticles have gained significant importance as they are biodegradable and biocompatible nanocarriers for different drugs. Chitosan nanoparticles (CHNPs) are particularly appropriate for drug delivery due to their low toxicity, mucoadhesion, and tunable physical properties (Mohammed *et al.*, 2017).

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The present work aimed to formulate and characterize metformin-loaded chitosan nanoparticles as an essential step towards its use as a complementary agent in managing diabetes mellitus. Chitosan nanoparticles and M-CHNPs were characterized using X-ray diffraction (XRD), Raman spectrophotometer, dynamic light scattering (DLS), high-resolution transmission electron microscope (HRTEM), zeta sizing, BET surface area, and atomic force microscope (AFM) to confirm their formation. The drug encapsulation efficiency, drug loading capacity, and *in vitro* release rate were also investigated.

## 2. MATERIAL AND METHODS

Metformin was obtained from El Nassr Co., Egypt. Metformin was obtained as pure powder potency (98%). Chitosan (deacetylation degree 95%, molecular weight 80 kDa) was purchased from Sigma Aldrich. Sodium tripolyphosphate (TPP) was purchased from Merck, Germany).

### 2.1. Synthesis of chitosan nanoparticles

Chitosan nanoparticles were prepared by reaction between chitosan and TPP according to the method described by (Abd-Elhakeem *et al.*, 2016). In brief, chitosan [200 mg/ 100 mL aqueous acetic acid solution (0.05% v/v)] and (TPP, 0.1% (w/v)) solutions were prepared. Chitosan nanoparticles were obtained by gradual mixing of the above solution under constant stirring. The obtained nanoparticles were centrifuged at 35840 g for 30 min, washed with distilled water, and lyophilized.

### 2.2. Synthesis of metformin nanoparticles

The preparation of metformin loaded on CHNPs was performed by the same above method with the addition of either 100, 200, or 400 mg metformin to the chitosan before the dropping (TPP).

### 2.3. Characterization of nanoparticles

Characterization is sorted into three categories, namely, identification, microscopic and physicochemical properties. Identification category was done by XRD (D8 Discovery–Bruker Company) under XRD condition of 40 kV and 30 AM (1200W) at speed scan 0.010 and 2theta range from 5° to 80°C. Raman spectrometer (Lab. RAM-HR Evolution Horiba Co. France) with electrode 1024×256-pixel CCD detector of air-cooled opened and single visible spectrometer was equipped under the condition of Raman. The 532 nm edge laser line with grating 1800 (450-850 nm) and ND filter 25% with acquisition time 2sec, accumulations five without delay, and spike filter and objective were X50-VIS.

The microscopic category was carried out by AFM instrument (5600LS—Agilent Technology Company, USA) in which thin-film samples were prepared using a spin-coated instrument (Spin coating instrument model Laurell-650Sz at the condition of 35xGunder vacuum). M-CHNPs were tested after preparing their thin film in which samples were subjected to ultrasound radiation for one h at the condition of the amplitude of 60% and 0.65 of cycles (up 400s manufactured by Hielscher company, Germany). After sonication, the thin film was prepared using a spin coating instrument. Pico image basics software

was used to analyze the AFM images. AFM images and data were done using 50 nm × 50 nm images for nano-metformin, and 10µm×10µm for bulk-metformin using contact mode, gold tap, 0.5 In/S speed, I. gain 0.33 and P. gain 21. High-resolution transmission electron microscopy (HRTEM) images were taken using EM-2100 at 25X magnification, and 200000 V., Physicochemical properties category, was achieved by surface area and pore size analyzer (quantachrome model Nova touch XL2, USA), which was used for BET surface area measurement. Dynamic light scattering (DLS) and zeta potential were achieved using Entgris instruments model Z3000, USA.

### 2.4. Drug loading and drug entrapment efficiency

Three independent samples of lyophilized chitosan encapsulated metformin were tested (10 mg of formula were tested for each). Each sample was suspended in 10 ml 1N HCl. The mixture was sonicated for 5 minutes then incubated in a shaking incubator for 1 hour at 40 °C to extract metformin. After that, the supernatants were collected by centrifugation at 3500 G for five minutes. Drug loading capacity (LC%) and drug entrapment efficiency (EE%) were determined by analyzing the amount of the metformin in the collected supernatants by measuring absorption at 232 nm by (UV/VIS spectrophotometer, Shimadzu UV1800, Japan). Measurements were carried out three times, then (EE%) and (LC %) were calculated by the following equations:

$$\text{Drug entrapment efficiency} = \frac{\text{The amount of loaded drug} \times 100}{\text{The initial amount of drug}}$$

$$\text{Drug loading capacity} = \frac{\text{The amount of loaded drug} \times 100}{\text{Weight of chitosan}}$$

### 2.5. In vitro release study

*In vitro* release measurements of M-CHNPs were performed 24 hours in phosphate buffer saline (PBS) at pH 1.2, 6.5, and 7.4. Briefly, an aliquot of 40 mg of provided nanoparticles powder sample was suspended in 2 ml of release buffer and added to a dialysis bag (20KD cut off, SpectraPore, Thermo Scientific, USA). Tested release buffers were composed of phosphate buffer (pH 7.4 or 6.8) or HCl pH 1.2. The dialysis bag was appropriately sealed from top and bottom and inserted a 40 ml release buffer in a sealed container. The whole system was incubated at 37 °C and 56 Gin a shaking incubator (Jeio tech SI-300, SEOUL, KOREA). At predetermined sampling points, 1 ml medium sample was withdrawn and immediately replaced with another 1 ml of equally warmed buffer. Samples were measured at 232 nm. The experiment was done in triplicate for each release buffer, averages and standard deviations were calculated.

The cumulative release percentage (CR%) of metformin at each time point was determined using the following equation:

$$\text{Cumulative release percentage (CR\%)} = \frac{\text{Amount of drug released at the time } t \times 100}{\text{The initial amount of encapsulated drug}}$$

### 3. RESULTS

#### 3.1. Characterization of M-CHNPs

XRD pattern of metformin showed eight characteristic peaks at  $2\theta$  12.70, 17.99, 22.38, 25.02, and 37.64. These peaks were also found in the M-CHNPs pattern (Figure 1 A, B). confirming the success of the synthesis method. Also, it was noticed that Raman shift curves of metformin and M-CHNPs had similar Raman shift in peaks position, as illustrated in Figure 2.

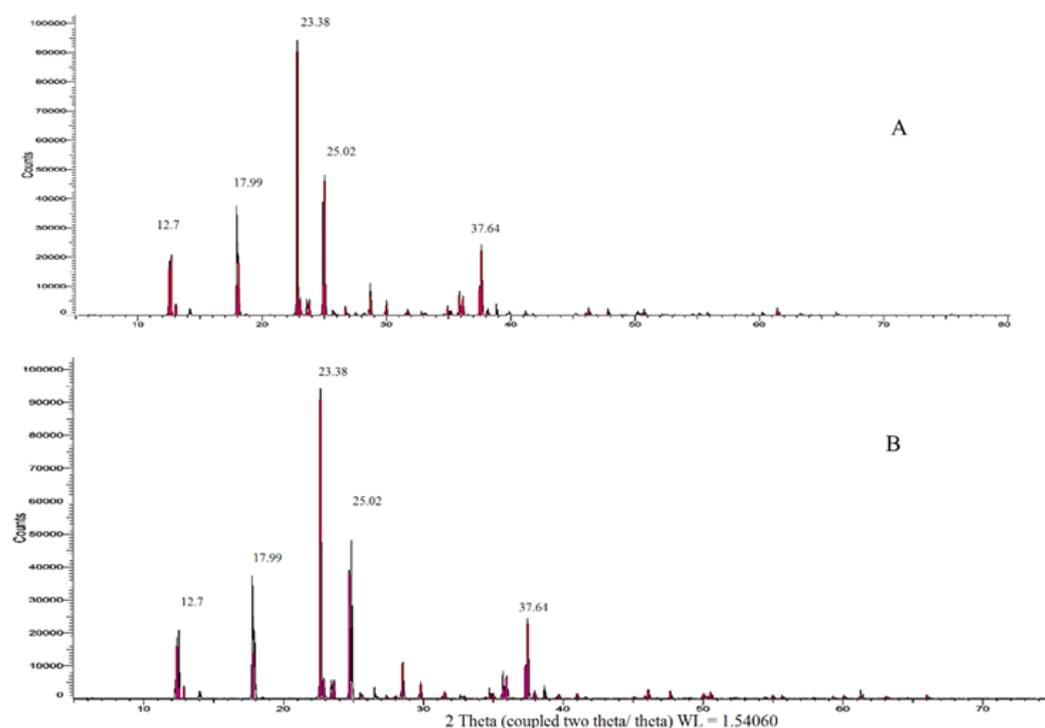


Figure 1 illustrates the XRD curve of A) metformin and B) metformin nanoparticles. The characteristic peaks at  $2\theta = 12.70, 17.99, 22.38, 25.02,$  and  $37.6$  were shown in both metformin and M-CHNPs.

It was observed from the spectra that both of them have 35 Raman shift peaks at 205.41, 291.02, 422.66, 438.12, 475.37, 516.34, 559.95, 609.16, 627.08, 722.64, 738.52, 800.67, 937.13, 1036.38, 1066.53, 1079.73, 1107.39, 1159.98, 1275.51, 1418.48, 1455.30, 1506.88, 1535.91, 1563.37, 1593.74, 1648.10, 2816.07, 2841.05, 2884, 2940.35, 2974.71, 3011.58, 3089.57, 3193.81 and 3383.54  $\text{cm}^{-1}$ . The results of Raman spectra matched and confirmed well with XRD results and proof the success of the synthesis method.

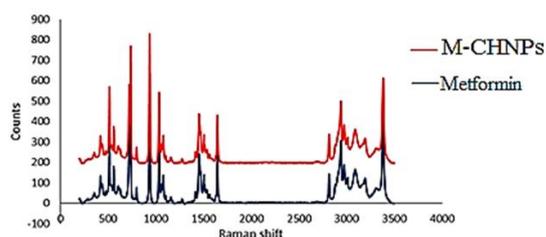


Figure 2. Raman shift curves of nano-metformin and bulk-metformin. illustrate the similarity of them where synthesis method converts to nanosized without any change in chemical composition.

It was observed from the spectra that both of them have 35 Raman shift peaks at 205.41, 291.02, 422.66, 438.12, 475.37, 516.34, 559.95, 609.16, 627.08, 722.64, 738.52, 800.67, 937.13, 1036.38, 1066.53, 1079.73, 1107.39, 1159.98, 1275.51, 1418.48, 1455.30, 1506.88, 1535.91, 1563.37, 1593.74, 1648.10, 2816.07, 2841.05, 2884, 2940.35, 2974.71, 3011.58, 3089.57, 3193.81 and 3383.54  $\text{cm}^{-1}$ . The results of Raman spectra matched and confirmed well with XRD results and proof the success of the synthesis method.

Roughness and surface topography were observed using 3D and 2D AFM images. AFM data and images (2D and 3D) confirmed the formation of M-CHNPs without any agglomeration or concentration of nanoparticles with homogenous size and shape, as shown in Figure 3. Data given in Figure 3 illustrated the spherical shape of M-CHNPs (blue color) with a height (Z direction) of 22 nm.

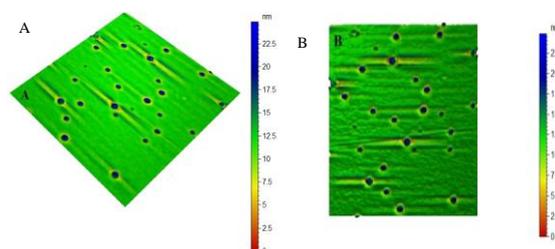


Figure 3. A) 3D AFM image and B) 2D AFM image of M-CHNPs. blue color of both AFM images illustrate M-CHNPs have a spherical shape and in nanosize

HRTEM images (Figures 4) for M-CHNPs illustrated cubic to semi cubic shape without accumulation in a specific area for M-CHNPs with a size of about 20-45 nm.

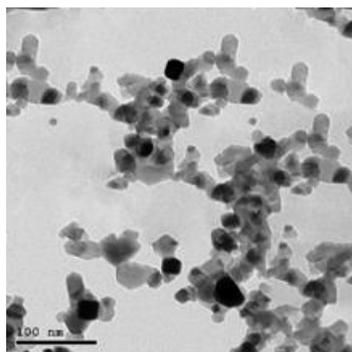


Figure 4. HRTEM image of M-CHNPs. reveals the cubic to semi cubic shape of the composite that ranges in size 20- 45 nm.

DLS illustrated the homogenous particle size distribution of free CHNPs and M-CHNPs, where the DLS patterns showed one peak at 112 and 78 nm (Figure 5 A, B). Zeta potential was found to be -2.65 and -28 mV for free CHNPs and M-CHNPs, respectively (Figure 5 C, D). The high zeta potential value of M-CHNPs revealed a huge surface area of M-CHNPs that indicates the very high chemical activity and its stability in solution.

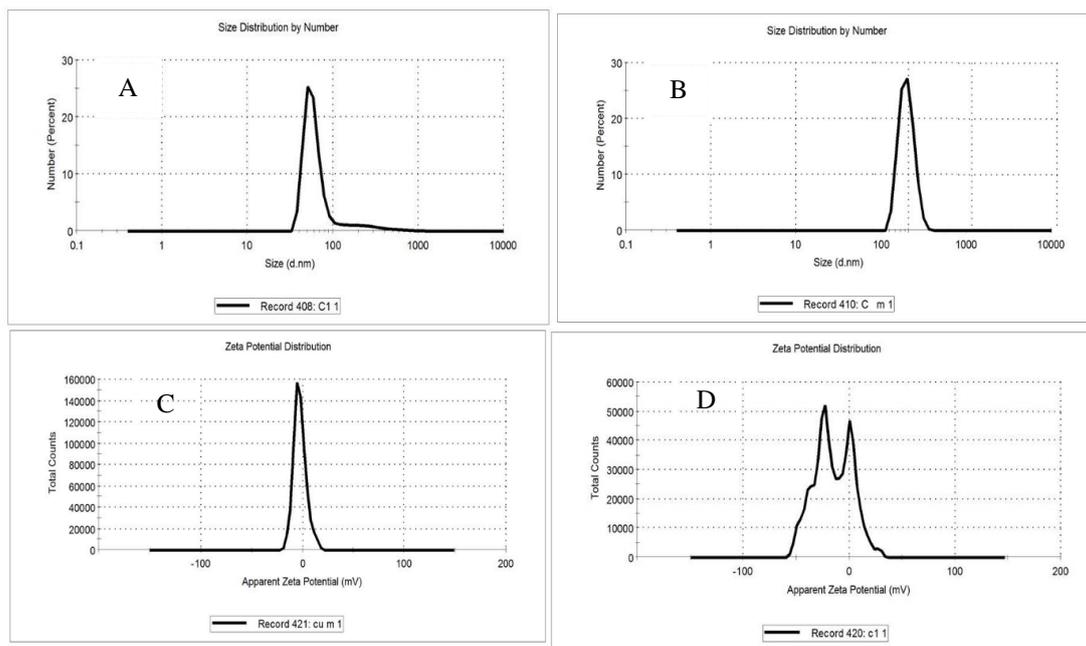


Figure 5. Analysis of composite zeta size and zeta potential. A: CHNPs zeta size (112 nm), B: M-CHNPs zeta (78 nm), C: CHNPs zeta potential (-2.65 mV), and D: M-CHNPs zeta potential (28 mV). The high zeta potential value of M-CHNPs revealed a huge surface area of M-CHNPs that indicates the very high chemical activity and its stability in solution.

Table 1 Drug loading and drug entrapment efficiency of metformin in chitosan nanoparticles

Formula	Amount of metformin	EE%	LC%
1	100 mg	82	41
2	200 mg	91	91
3	400 mg	44	88

3.3. *In vitro* release study

From the previous step, Formula 2 was selected to investigate in vitro release study. A quick release of metformin was seen in the first three h with approximately 51%, 40%, and 10% of the drug released at pH 1.2, 6.8, and 7.4. In general, the amount of drug released at acidic conditions (pH 1.2) was greater than other pH values (Figure 6).

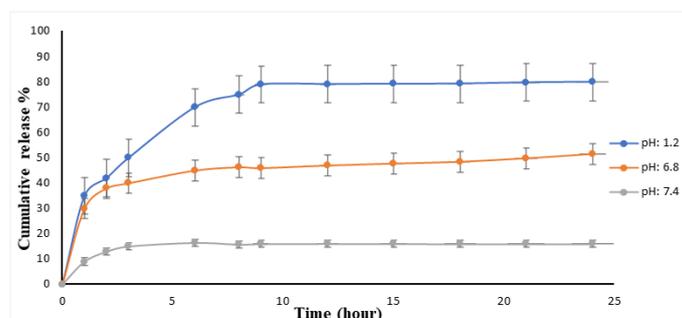


Figure 6. The release profile of metformin from CHNPs at pH 1.2, 6.8, and 7.4. A quick release of metformin was seen in the first 3 hours with approximately 15%, 40% and 50% of the drug was released at pH, 6.8, 7.4, and 1.2 respectively. In general, the amount of drug released at acidic conditions (pH 1.2) was greater than other pH values.

#### 4. DISCUSSION

The clinical application of new drug formulas needs consistent and comprehensive studies to ensure their properties (Hua, 2019). In this study, chitosan was selected as one of the best polymers for pharmaceutical preparation based on their pharmaceutical characters.

As a nanoparticulate delivery system, CHNPs were previously designed to load antibiotics, antitumor drugs, antidiabetic drugs, and others (Kravanja *et al.*, 2019). In the present study, an attempt was made to develop an alternative delivery system that may overcome the severe side effects of existing formulations by reducing the dosage and controlled release.

Particle size is often used to characterize nanoparticles because it facilitates dispersion and aggregation (Zhang *et al.*, 2016). The high Zeta potential value is a vital particle characteristic as it can stabilize particle suspension. Interestingly, the prepared formula showed small particle size and high Zeta potential in agreement with previous reports (Duarah *et al.*, 2015; Kumar *et al.*, 2017)

Furthermore, the similarity of the XRD pattern and Raman spectra of metformin and M-CHNPs illustrate the absence of the chemical interaction that may alter the chemical structure and drug structural integrity between drug and polymer.

The clinical efficacy of the nano-formulated drug is associated with high EE% and LC% and a sustained release profile (Abdel-Hakeem *et al.*, 2021). The drug loading and entrapment efficiency were mainly affected by the polymer and drug ratios. In this work, the excellent EE% and LC% of 91% were shown by a 1:1 ratio indicating metformin's high affinity to the used carrier under experimental conditions. However, some previous reports have shown that EE% increases with chitosan concentration (Xiaofen *et al.*, 2010).

Finally, the *in-vitro* release of drugs from nanoparticles may approximate the drug release profile inside the body. For all used pH values, an initial rapid release occurred for the first three hours and then showed sustained release profiles over 24 h that allowed the release of 80% of the loaded drug under acidic pH. Duarah *et al.* (2015) have similar results in their report.

#### 5. CONCLUSION

Metformin nanoparticles were prepared and characterized using different analytical and spectroscopic techniques. *In vitro* release assessments of M-CHNPs and the cumulative release percentage (CR%) of metformin were studied. The current work has strongly introduced a novel delivery system for metformin. Nanoparticles provide a sustained drug release to ensure a prolonged effect. Thus, encapsulation of metformin in polymeric nanoparticles may cause a marked increase in bioavailability, which may reduce the drug dose and rate of administration. As a result, there may be a significant improvement in the therapeutic activity, a decrease in side effects, and an enhancement of the

patient's response. Therefore, the prepared formula could be a promising alternative to the traditional treatment for type 2 diabetes. So, the optimization of this new formula will be performed via a biological study on the experimental animals.

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