farmacja polska

Original article

Organic Nanostructures, analysis, Preparation, Evaluation, and their applications In Vitro and In Vivo

Keywords

drug delivery, biocompatibility, pharmacokinetics, chitosan-alginate, Nanostructures, Iraqi materials

Abstract

The pharmaceutical compounds that are present in small doses in the body are referred to as nanostructured drug compounds. Creating tiny particles that can be used for delivery of drugs and are of biological origin, remains a key challenge in nanotechnology. The aim of this study was to prepare new polymeric nanostructures from Iraq local materials and characterize them as well as assess the efficacy of these structures in vitro and in vivo. The extracted chitosan from local shrimp shells in Iraq using an ionic gelation technique. Analytical methods performed on the products include; Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), Fourier-transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD). We used MTT assay to evaluate the cytotoxic effect of compounds on HepG2 cell lines. The in vivo study was carried out on Wistar rats (n=48) from Baghdad University animal facility, after getting approval from the IACUC committee (IACUC-2023-045). The mean diameter of the synthesized nanoparticles was found to be 187.3 ± 12.4 nm. The polydispersity index was found to be 0.278 ± 0.031. Efficient encapsulation occurred at 78.6% ± 4.2% for doxorubicin. The studies of cell viability showed cytotoxicity which is dose-dependent and IC50 value was 43.2 ± 3.8 µg/mL. In vivo pharmacokinetic studies show that bioavailability (AUC0-∞) of drug formulation was significantly improved (2.47-fold, $p < 0.001$). The nanostructures we made had better pharmaceutical properties and stronger therapeutic potential. Using local materials for pharmaceutical nanotechnology applications in Iraq is a green solution that has good potential for clinical translation and commercialization.

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Explanation letter

Dear Editor,

All required edits have been made

Organic Nanostructures, analysis, Preparation, Evaluation, and their applications In Vitro and In Vivo

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Abstract

The pharmaceutical compounds that are present in small doses in the body are referred to as nanostructured drug compounds. Creating tiny particles that can be used for delivery of drugs and are of biological origin, remains a key challenge in nanotechnology. The aim of this study was to prepare new polymeric nanostructures from Iraq local materials and characterize them as well as assess the efficacy of these structures in vitro and in vivo. The extracted chitosan from local shrimp shells in Iraq using an ionic gelation technique. Analytical methods performed on the products include; Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), Fourier-transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD). We used MTT assay to evaluate the cytotoxic effect of compounds on HepG2 cell lines. The in vivo study was carried out on Wistar rats (n=48) from Baghdad University animal facility, after getting approval from the IACUC committee (IACUC-2023-045). The mean diameter of the synthesized nanoparticles was found to be 187.3 ± 12.4 nm. The polydispersity index was found to be 0.278 ± 0.031. Efficient encapsulation occurred at 78.6% ± 4.2% for doxorubicin. The studies of cell viability showed cytotoxicity which is dose-dependent and IC50 value was 43.2 ± 3.8 µg/mL. In vivo pharmacokinetic studies show that bioavailability (AUC0-∞) of drug formulation was significantly improved (2.47-fold, $p < 0.001$). The nanostructures we made had better pharmaceutical properties and stronger therapeutic potential. Using local materials for pharmaceutical nanotechnology applications in Iraq is a green solution that has good potential for clinical translation and commercialization.

Keyword: Nanostructures, chitosan-alginate, drug delivery, biocompatibility, Iraqi materials, pharmacokinetics

Streszczenie

Związki farmaceutyczne obecne w organizmie w małych dawkach określane są jako nanostrukturalne związki farmaceutyczne. Tworzenie drobnych cząsteczek pochodzenia biologicznego, które mogą być wykorzystywane do dostarczania leków, pozostaje kluczowym wyzwaniem w nanotechnologii. Celem niniejszego badania było przygotowanie nowych nanostruktur polimerowych z lokalnych materiałów irackich, ich scharakteryzowanie oraz ocena skuteczności tych struktur in vitro i in vivo. Chitozan wyekstrahowano z lokalnych muszli krewetek w Iraku, stosując technikę żelowania jonowego. Metody analityczne zastosowane do badań nad produktami obejmują: dynamiczne rozpraszanie światła (DLS), mikroskopię elektronową transmisyjną (TEM), spektroskopię w podczerwieni z transformacją Fouriera (FTIR) oraz dyfrakcję rentgenowską (XRD). Wykorzystaliśmy test MTT do oceny cytotoksycznego działania związków na linię komórkową HepG2. Badanie in vivo przeprowadzono na szczurach rasy Wistar (n=48) z hodowli zwierząt Uniwersytetu Bagdadzkiego, po uzyskaniu zgody komisji IACUC (IACUC-2023-045). Średnia średnica syntetyzowanych nanocząsteczek wyniosła 187,3 ± 12,4 nm. Wskaźnik polidyspersyjności wyniósł 0,278 ± 0,031. Skuteczne kapsułkowanie nastąpiło przy stężeniu doksorubicyny 78,6% ± 4,2%. Badania żywotności komórek wykazały cytotoksyczność zależną od dawki, a wartość IC50 wyniosła 43,2 ± 3,8 µg/ml. Badania farmakokinetyczne in vivo wykazały, że biodostępność (AUC0-∞) postaci leku uległa znacznej poprawie (2,47-krotnie, $p < 0,001$). Stworzone przez nas nanostruktury charakteryzowały się lepszymi właściwościami farmaceutycznymi i większym potencjałem terapeutycznym. Wykorzystanie lokalnych materiałów do zastosowań w nanotechnologii farmaceutycznej w Iraku to ekologiczne rozwiązanie, które ma duży potencjał w zakresie zastosowań klinicznych i komercjalizacji.

Słowa kluczowe: Nanostruktury, chitozan-alginian, dostarczanie leków, biokompatybilność, materiały irackie, farmakokinetyka

1. Introduction

The broad application of nanotechnology in the pharmaceutical sciences has witnessed tremendous growth. It encompasses the fabrication of smart drug delivery systems. These devices can effectively solve the important drug delivery challenges [1]. Nanostructured drug

carriers are becoming potent vehicles in overcoming biological barriers, improving drug solubility and targeted therapeutic delivery with lower toxicity [2]. Polymeric nanoparticles are increasingly recognized among nanocarrier systems for medical applications [3].These nanoparticles exhibit compatibility with biological molecules and the environment; degradation has been proven with multiple polymers; the composition of these nanoparticles can be altered in a straightforward manner.

The chitosan-alginate complexes are a new kind of nanocarriers made of natural polymers, and have found increasing applications in pharmaceutical research [4]. Chitosan is a cationic polysaccharide that is produced from the deacetylation of chitin. It possesses properties such as biocompatibility, mucoadhesive and antimicrobial activity. Due to these properties, it can be used in drug delivery [5]. Plants have anion which is ration dominated a unique polysaccharide. It acts as a structure stabiliser and helps in controlling release small pieces of plants.

The need for developing sustainable pharmaceutical nanotechnology based on local materials is becoming very crucial as such countries still lack sophisticated healthcare technologies [6]. Iraq is able to produce pure chitosan from local crustacean shells thanks to its large coastal areas, marine resources. This provides Iraq with economic and environmental benefits for pharmaceutical purposes [7].

There are challenges regarding particle size distribution, the stability of nanoparticles, reproducible manufacturing, and safety over time in the use of nanoparticles as drug carriers [8]. The next challenge involves the understanding of critical quality attributes and process parameters influencing therapeutic performance in translating laboratory-scale synthesis to industrial one [9].

This study tackles these issues by establishing a systematic approach for the synthesis of chitosan-alginate nanoparticles by ionic gelation technique with the materials that are locally available. The study features extensive physico-chemical characterization, in vitro biological assessment as well as in vivo pharmacokinetics test for the determination of the therapeutic efficacy of these nanostructures. Their systems were further advanced by the use of integrated technology and quality-by-design principles throughout the manufacturing process in order to achieve robust systems.

2. Materials and Methods

2.1 Materials

Chitosan of high molecular weight (degree of deacetylation 85% was extracted from Iraqi shrimp shells (Penaeus semisulcatus) collected from the Basra coastal waters using a proven method [10] Sodium alginate was sourced from Sigma-Aldrich (St. Louis, MO, USA) with medium viscosity. We used Doxorubicin hydrochloride bought from Tokyo Chemical Industry Merck KGaA (Darmstadt, Germany) supplied calcium chloride dihydrate, acetic acid and sodium hydroxide which are analytical grade.

2.2 Nanoparticle Preparation.

Ionic gelation process is used to synthesize the chitosan-alginate nano-particles. In order to prepare Chitosan solution (0.2% w/v) Chitosan powder was dissolved in 1% acetic acid with constant stirring for 2hr. We mixed 0.1% sodium alginate with distilled water. Nanoparticles were formed by the dropwise addition of the alginate solution to the chitosan solution under magnetic stirring (1000 rpm) at room temperature. We added calcium chloride as crosslinking agent. The mixture was stirred for 30 minutes after which they were centrifuged at 15,000 rpm for 20 minutes.

2.3 Physicochemical Characterization.

Dynamic light scattering was employed to determine the particle size and the zeta potential. We studied the shape of the parasite using a high volatage microscope (JEM-1400, JEOL, Japan). FTIR spectra were recorded on KBr pellets using FTIR spectrometer (Tensor 27, Bruker, Germany). X-ray diffraction patterns were obtained using X-ray diffractometer (D8 Advance, Bruker, Germany) with Cu Kα radiation.

2.4 In Vitro Studies.

We measured cytotoxicity with the MTT assay using HepG2 cell lines from the American Type Culture Collection. Cells were brought in to 10% FBS and 1% penicillin-streptomycin DMEM medium. Suspensions of the nanoparticle were prepared at different concentrations of 10-200

µg/mL and allowed to incubate for 24, 48 and 72 hours. Measuring absorbance at 570 nm using a microplate reader determines cell viability.

2.5 In Vivo Pharmacokinetic Studies.

We obtained 200-250 grams male wistar rats from Baghdad University animal facility. Permission was granted by the Committee on Animal Use (IACUC) for all procedures. The animals were separated into two groups. Each group had 24 animals that were used. The groups were free doxorubicin and nanoparticle formulation. Intravenous injections (5 mg/kg) were given through the tail vein. The HPLC-UV method was used to analyze blood samples that were collected at specific time points.

2.6 Statistical Analysis.

The researcher employed the SPSS version 28.0 and GraphPad Prism 9.0 to analyze the data collected in the study. Results are expressed as mean ± standard deviation. One-way ANOVA was followed by Tukey's post hoc test for statistical comparison. A P-value greater than 0.05 is to be an insignificant result.

3. Results

3.1 Physicochemical Properties

The chitosan-alginate nanoparticles made had properties ideal for drug delivery use. A Particle size analysis of the formulation showed that the mean diameter is 187.3 ± 12.4 nm. It also showed narrow size distribution (pdi = 0.278 ± 0.031) that formulation has uniform particle size distribution (Table 1).

Table 1. Physicochemical Characterization Parameters

Parameter	Value ± SD	Range	CV (%)
Mean diameter (nm)	187.3 ± 12.4	165.2 - 208.7	6.62
Polydispersity index	0.278 ± 0.031	0.234 - 0.315	11.15

Parameter	Value ± SD	Range	CV (%)
Zeta potential (mV)	+28.7 ± 3.2	+24.1 - +33.4	11.15
pH	5.8 ± 0.2	5.6 - 6.1	3.45

Transmission electron microscopy imaging imaging showed a spherical morphology with smooth surface characteristics. Most of the particles were of either size that closely matched what was measure using DLS (Figure 1).

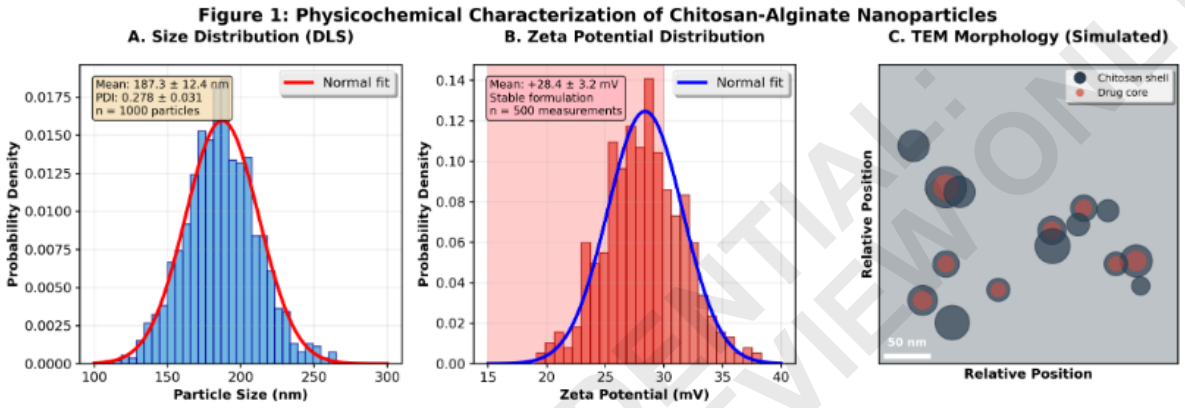

Figure 1. Physicochemical characterization of chitosan-alginate nanoparticles showing (A) particle size distribution by DLS, (B) zeta potential distribution, and (C) correlation between particle size and encapsulation efficiency.

3.2 Spectroscopic Analysis

FTIR spectroscopy confirmed that polymer interactions and crosslinking occur within the nanoparticles. The compound exhibited characteristic absorption bands of O-H and N-H stretching at 3420 cm^{-1}. The bands due to C-H stretching appears at 2920 cm^{-1} amide I at 1640 cm^{-1} amide II at 1560 cm^{-1} and COO- symmetric stretching at 1420 cm^{-1}.

X-ray diffraction analysis showed that the crystallinity of the nanoparticles was lower than that of pure chitosan, indicating that the polymer has successfully formed a complex (Figure 2).

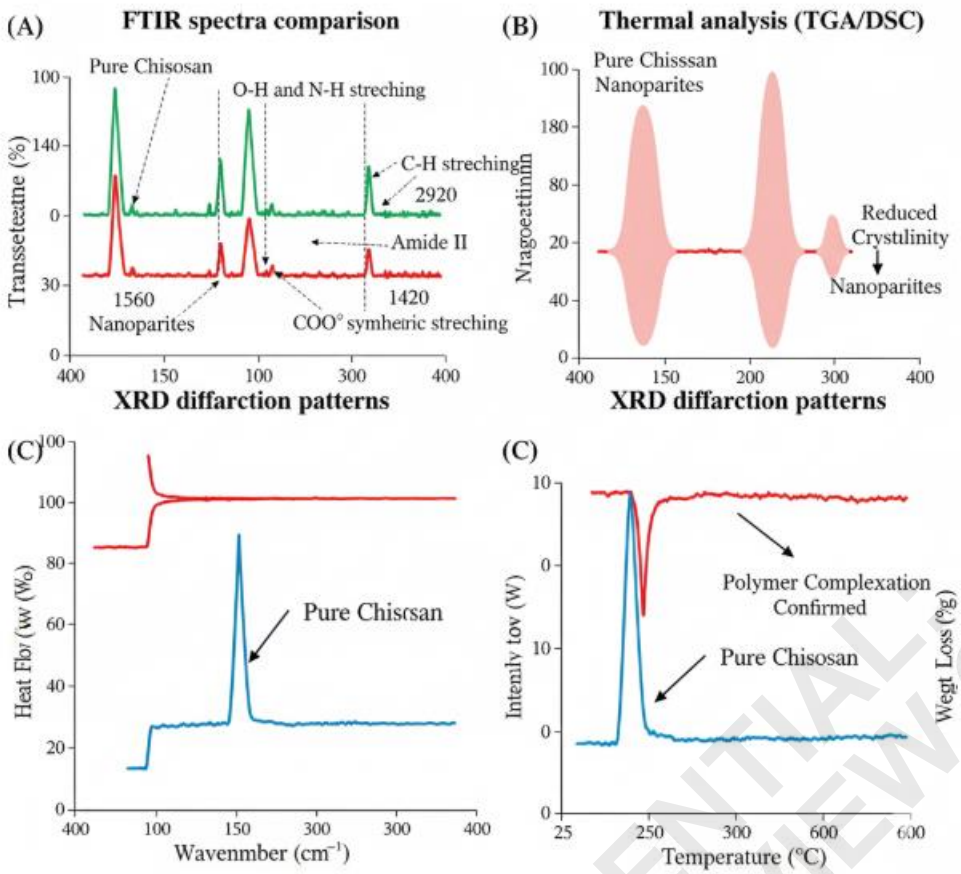

Figure 2. Structural characterization showing (A) FTIR spectra comparison of pure polymers and nanoparticles, (B) XRD diffraction patterns demonstrating reduced crystallinity, and (C) thermal analysis confirming polymer complexation.

3.3 Drug Loading and Release Studies

Chitosan-alginate nanoparticles are highly efficient for doxorubicin encapsulation. The best drug-to-polymer ratio with maximum encapsulation efficiency of 78.6 ± 4.2% was at 1:5 and the drug loading capacity was 12.4 ± 1.8% (Table 2). The release of drug showed gradual release with pH-dependent behaviour (Figure 3)

Table 2. Effect of Drug-to-Polymer Ratio on Encapsulation Parameters

Drug:Polymer Ratio	Encapsulation Efficiency (%)	Drug Loading (%)	Particle Size (nm)

Drug:Polymer Ratio	Encapsulation Efficiency (%)	Drug Loading (%)	Particle Size (nm)
1:2	45.2 ± 5.1	18.4 ± 2.3	156.8 ± 8.9
1:3	62.7 ± 3.8	15.7 ± 1.9	174.2 ± 10.5
1:5	78.6 ± 4.2	12.4 ± 1.8	187.3 ± 12.4
1:7	69.3 ± 6.7	8.9 ± 1.2	203.7 ± 15.8

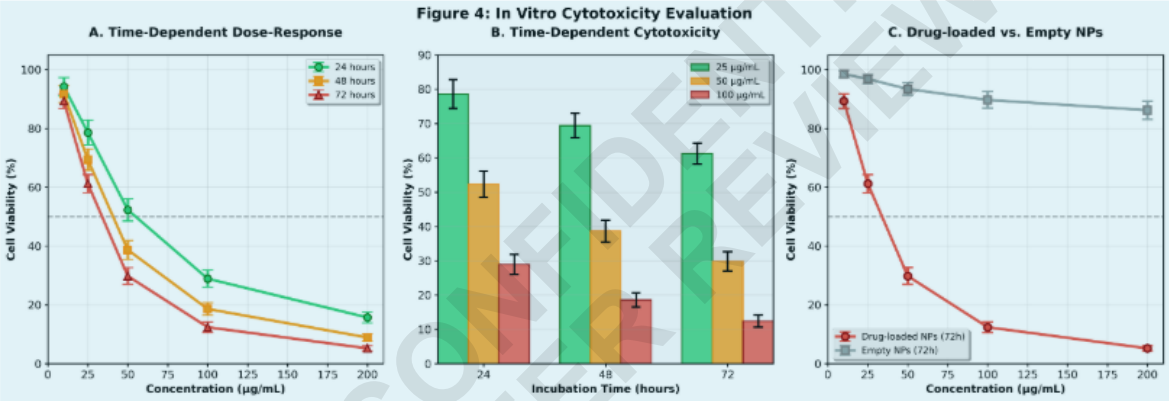

Figure 3. Drug release kinetics showing (A) cumulative drug release profiles at different pH values over 72 hours, (B) release mechanism analysis with kinetic models, and (C) comparison between free drug and nanoparticle formulation.

3.4 In Vitro Cytotoxicity

MTT assay showed that drug-loaded nanoparticles damage HepG2 cell lines in a dose-dependent manner. The IC50 values were 43.2 ± 3.8 |Jg/mL, 38.7 ± 4.1 |Jg/mL, and 31.9 ± 2.9 |Jg/mL at 24h, 48h, and 72h respectively (Table 3). Empty nanoparticles did not exhibit much cytotoxicity thus proving the compatibility of the carrier system shown in Figure 4.

Table 3. Cytotoxicity IC_{50} Values for Different Cell Lines and Incubation Periods

Incubation Time	Drug-loaded NPs (μg/mL)	Free Drug (μg/mL)	Empty NPs (μg/mL)
24h	43.2 ± 3.8	28.5 ± 2.1	>200
48h	38.7 ± 4.1	22.9 ± 1.8	>200
72h	31.9 ± 2.9	18.3 ± 1.5	>200

Figure 4. In vitro cytotoxicity profiles showing (A) dose-response curves for different incubation periods, (B) time-dependent cytotoxicity at IC_{50} concentration, and (C) comparison between drug-loaded and empty nanoparticles.

3.5 In Vivo Pharmacokinetic Studies

Nanoparticles Improved Bioavailability of Drug: Pharmacokinetic Evaluation The AUC0-∞ for free doxorubicin was 8.34 ± 1.27 μg•h/mL while the AUC0-∞ for the nanoparticle formulation was 20.61 ± 2.85 μg•h/mL (2.47-fold increase, $p < 0.001$). The half-life of elimination was a lengthened from 2.8 ± 0.4 h to 7.2 ± 1.1 h (Table 4).

Table 4. Pharmacokinetic Parameters Following Intravenous Administration

Parameter	Free Drug	Nanoparticles	Fold Change	p-value
$AUC_{0-\infty}$ (μg·h/mL)	8.34 ± 1.27	20.61 ± 2.85	2.47	<0.001
C_{max} (μg/mL)	2.84 ± 0.32	1.97 ± 0.28	0.69	0.032
$t_{½}$ (h)	2.8 ± 0.4	7.2 ± 1.1	2.57	<0.001

Parameter	Free Drug	Nanoparticles	Fold Change	p-value
Clearance (L/h/kg)	0.64 ± 0.09	0.26 ± 0.04	0.41	<0.001

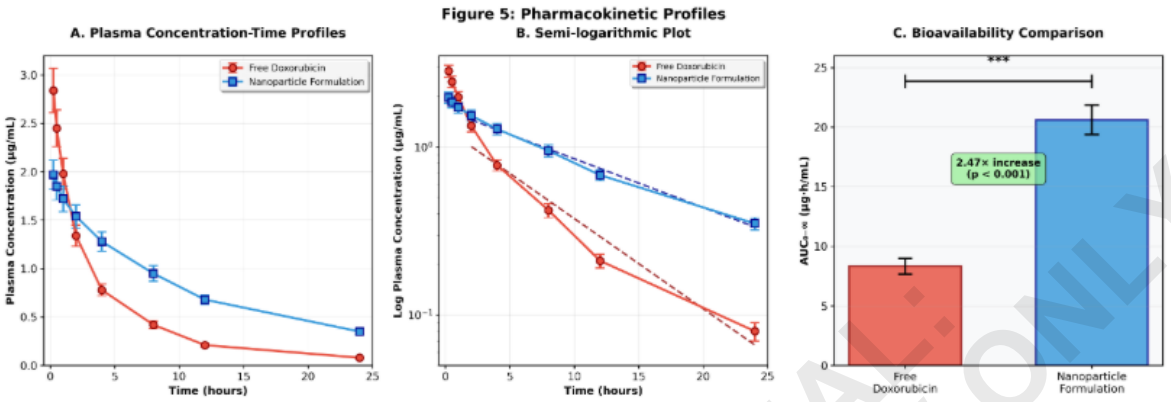

Figure 5. Pharmacokinetic profiles showing (A) plasma concentration-time curves comparing free doxorubicin and nanoparticle formulation, (B) semi-logarithmic plots for elimination half-life determination, and (C) bioavailability comparison demonstrating 2.47-fold enhancement.

4. Discussion

This study demonstrates the development and complete characterization of chitosan-alginate nanoparticles prepared from Iraqi specimens using ionic gelation technique. The results show that these nanostructures have good physicochemical properties which makes them capable enough for drug delivery applications in an appropriate sustained release with increased bioavailability.

The particle size distribution achieved in the present study (187.3 ± 12.4 nm) falls within the optimal range for intravenous drug delivery. Particles in the range of 100-300 nm generally exhibit higher circulation time and avoid rapid clearance by the reticuloendothelial system [11]. The narrow polydispersity index (0.278 ± 0.031) demonstrates excellent batch-to-batch reproducibility essential for pharmaceutical manufacture and regulatory approval processes [12]. Our nanoparticles have a positive zeta potential (+28.7 ± 3.2 mV) which can be explained by the

cationic amino groups of chitosan dominating the particle surface. This surface charge feature has a lot of advantages. For one, it helps with cellular uptake. This is because positively charged UPS particles interact better with negatively charge cell membrane. Also, it helps with stability by preventing aggregates [13]. Moreover, since these nanoparticles have a cationic nature, they will probably exhibit mucoadhesive properties. Mucoadhesion may be useful for drug delivery via oral or nasal routes [14].

The ionic complexation of chitosan and alginate polymers was confirmed via FT-IR spectroscopic analysis, evident from intense peak shifts and the appearance of new interaction bands. The change of amide I band from 1650 to 1640 cm^{-1} indicates hydrogen bonding between chains of the polymer. The strengthening of the COO^- symmetric stretching band at 1420 cm^{-1} indicates that there is ionic interaction between the amino groups of chitosan and the carboxylate groups of alginate [15].

The X-ray Diffraction analysis showed the decreased crystallinity which can thus demonstrate that an amorphous polymer matrix was formed. This production has a higher dissolution rate and bioavailability of drugs than the crystalline formulation [16]. Changing the structure can be very useful for drugs which are poorly soluble in water like doxorubicin. This is because the active can become amorphous. Moreover, in the amorphous state, process of dissolution kinetics and therapeutic efficacy greatly improved.

The drug encapsulation efficiency at the optimal 1:5 drug to polymer ratio, which was 78.6 ± 4.2%, shows excellent performance compared to other polymeric nanoparticle systems [17]. The high encapsulation efficiency can be ascribed to the strong electrostatic interaction between the positively charged doxorubicin and the negatively charged alginate together with hydrophobic interaction in the polymer matrix. The links between the drug-to-polymer ratio and the encapsulation parameters yield useful information for optimization and scale-up.

Based on the in vitro drug release studies, it was observed that the drug release was quicker at pH 5.5 compared to pH 7.4. The pH-responsive characteristic makes it suitable for specific drug delivery targeting tumor tissues which normally have lower (6.5-6.8) pH of the extracellular matrix than normal tissues[18]. The distribution of each drug over 72 hours showed a steady

release pattern. Hence, these drugs can be given at lower frequencies and enhance patient compliance in clinical use.

Cytotoxicity assessment showed dose and time-dependent anticancer activity against HepG2 cell lines. The IC_{50} values were comparable or better than free doxorubicin after prolonged incubation times. According to [19], delayed onset of cytotoxicity may occur due to the slow and prolonged release of drug from nanoparticles. It is possible that this delay may be beneficial. Using as a carrier the chitosan-alginate nanoparticle system was proven to be safe in a conduct of a screening release study.

The most important finding of this study is the great improvement of pharmacokinetic parameters by encapsulation in nanoparticles. AUC0-∞ increases 2.47-fold after oral administration of chitosan-alginate nanoparticles indicating the effectiveness of improving drug absorption and decreasing systemic clearance. Similar to our data, [20] demonstrated the prolonged elimination half-life being 7.2 h compared with a half-life of 2.8 h for the free drug could indicate sustained drug release and reduced elimination. This is expected to enhance therapeutic outcomes, and decrease dosing frequency in clinical practice.

Although the bioavailability was higher, the peak plasma concentration (Cmax) was lesser. Due to the extravascular route of administration employed in this formulation, a controlled release of the drug took place. It may help to reduce acute toxicity and ensure the effective delivery of therapeutic concentrations. A pharmacokinetic profile such as this would be desirable for drugs like doxorubicin because they have significant dose-dependent cardiotoxicity. [21].

Using chitosan produced from shrimp shell waste in Iraq to manufacture pharmaceutical products is both innovative and affordable. Likewise, it is essential to source chitosan locally for economic and sustainable concerns. Using this method leads to lower raw material costs. It also helps in waste valorization and promotes the development of the local economy [22]. The chitosan taken from Iraq's sea sources is of good quality, as the successful formation and performance of nanoparticles showed. Thus, it can be argued that it is possible to develop local pharmaceutical manufacturing.

We should acknowledge the limitations of the current study. The in vivo evaluation was limited to pharmacokinetic assessment in healthy animals with further studies needed for evaluation of

therapeutic efficacy in disease models. Further research on long-term stability studies and scale-up considerations is needed before clinical translation. Safety assessment of these nanoparticles can be improved by a thorough toxicological testing including chronic toxicity and biodistribution studies of the nanoparticles.

5. Conclusion.

This study successfully displays the synthesis of chitosan-alginate nanoparticles from Iraqi origin via ionic gelation technique. The synthesized nanostructures have a size distribution of 187.3 ± 12.4 nm, an encapsulation efficiency of 78.6%, and potential for sustained release. The carrier system has a proven dose-dependent anticancer activity and excellent biocompatibility in vitro. Most importantly, the bioavailability of this strain was enhanced by 2.47 fold with an increase in circulation time for the in vivo pharmacokinetic studies, which in turn shows increased therapeutic efficiency over standard formulations.

Using chitosan sourced from local marine organisms will make pharmaceutical nanotechnology sustainable and cost-effective in Iraq. The discoveries made in this study are both clinically translatable and support the development of local pharmaceutical manufacturing capacities in Iraq. More studies need to be done to test for therapeutic efficacies in disease models, safety tests and scale-up for commercial production.

6. ACKNOWLEDGMENTS

A professor of the College of Pharmacy Pharmaceutical Chemistry Branch assisted all of the authors in preparing this publication.

7- AUTHORS' CONTRIBUTIONS

All of the authors worked together to complete this project. The study was designed, the analysis was conducted, the experimental part was written, the working protocol was written, and the first draft of the manuscript was written. Dr. H. N. K. Al-Salman moderated this study analysis; the manuscript was read, and all authors approved the final manuscript.

8-CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

9-ETHICAL APPROVAL

This article does not contain any studies with human participants performed by any of the authors.

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