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SYNTHESIS AND CHARACTERIZATION OF DRUG DELIVERY SYSTEM FOR LOADING SPIRULINA USING CHITOSAN NANOPARTICLES

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ABSTRACT

Chitosan, a chitin-derived biopolymer, is garnering significant academic interest due to its distinctive properties and diverse applications. Spirulina-loaded chitosan nanoparticles demonstrate enhanced antioxidant, antimicrobial, and wound-healing capabilities, providing a natural and biocompatible delivery system for pharmaceutical applications, tissue engineering, and dermatological formulations with superior bioactivity and environmental sustainability. The ionic gelation method prepares these nanoparticles for efficient material encapsulation, ensuring optimal loading capacity and controlled release characteristics. The obtained nanoparticles were characterized using different techniques (particle size, UV, zeta potential, X-ray diffraction, FTIR (Fourier transform infrared spectroscopy) analysis, field emission scanning electron microscopy, and hemolytic activity). Chitosan with vitamin ETPGS reduced nanoparticle size to 93.2 nm and improved stability, dispersibility, zeta potential, and morphology. Spirulina-based nanoparticles were safe with no blood-related side effects. Hemolytic anemia was induced in all rats except the negative control, using intraperitoneal injections of PHZ (20 mg/kg) for two consecutive days. Forty male albino rats, aged 60 days and weighing 200 ± 15 g, were randomly assigned to four groups (n=10 per group): Control (CNG), PHZ-induced anemic group (PHZ-G), spirulina extract-treated group (SPG) 100 mg/kg of spirulina, and spirulina-loaded chitosan nanoparticle-treated group (SP-NPS) 100 mg/kg of spirulina. Treatments were administered daily for eight weeks. An *in vivo* study noted a clear improvement in spirulina-loaded chitosan in many parameters, such as a significant increase in serum vitamin B12 levels and serum ferritin in the group treated with spirulina-loaded chitosan nanoparticles compared to the anemic group. Additionally, phenylhydrazine-induced animals had significantly reduced haptoglobin, which normalized after treatment with spirulina and its nanoparticle form.

Keywords: Chitosan, Nanotechnology, FSEM, zeta potential, spirulina.

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INTRODUCTION

Nanotechnology has significantly improved the pharmaceutical industry by enabling the production of small-sized drugs with improved efficacy and lower toxicity. Nanoparticles can enhance drug pharmacokinetics by increasing solubility, stability, and bioavailability (Malik *et al*., 2023). They can also target specific tissues and cells, reduce side effects and increase their viability. This approach reduces drug demand, improves solubility and stability, and increases bioavailability, allowing for lower dosages and reduced toxicity (Jalloob *et al*., 2022). Therapeutic nanorobots are being developed for antitumor reactions and remote surgeries against various diseases (Suhail *et al*., 2022). Neamah *et al.* (2024) reported that chitosan, a chitin biopolymer, is earning academic interest because of its unique properties and applications. Its biocompatibility, biodegradability, anti-microbial properties, film-forming ability, and adsorption ability make it suitable for use in biomedicine, food science, environmental engineering, and cosmetics. Academics are studying its applications in drug delivery systems, wound healing, water purification, and food preservation (Kou *et al.*, 2021). Spirulina may reduce hypocholesterolemia, regulate blood sugar, boost immunity, and have antioxidant activity (Ahmad *et al*., 2023). Clinical studies show a positive correlation between spirulina supplem-entation and improved nutritional parameters, leading to reduced anemic conditions (Balkrishna *et a*l., 2023). Affordable, high-nutrient products like spirulina could be a tool against malnutrition (Kumar *et al.,* 2023; Jasim *et al.,* 2019). Pharmaceuticals, gadgets, and materials science may all be totally changed by it. Medicated carriers favor utilizing nanomaterials because of their capacity to keep maintained conveyance (Selvakumar *et al*., 2025). Anemia is a widespread global health concern, particularly affecting women and children, leading to fatigue, weakness, and impaired cognitive function. Effective treatment is essential to restore hemoglobin levels and improve overall health (Al-Yasari *et al*., 2024). A recent study done on chitosan/Spirulina nanoformulation showed a protective effect against cyclophosphamide-induced ovarian toxicity by modulating oxidative stress and inflammation. This beneficial effect was achieved through activation of PPAR-γ/Nrf-2/HO-1 and inhibition of NF-κB/TNF-α signaling pathways (Almukainzi *et al*., 2024). The therapeutic application of SPNP-gel to skin surfaces shows significant promise in promoting healing of surgical wounds, with its primary mechanism involving the suppression of HGMB-1 protein expression (Refai *et al*., 2023). The aim of this study was the synthesis of a new model of drug delivery to increase the efficacy of spirulina and ameliorate phenylhydrazine-induced anemia in male rats, focusing on some parameters such as B12, serum ferritin, and haptoglobin biomarkers.

MATERIALS AND METHODS

Drugs and Chemicals

Extra-pure chitosan was obtained from Beijing company China, Sodium tripolyphosphate (85%) was obtained from Lanxess Company, India. Acetic acid (96%) was obtained from BDH, England and sodium hydroxide (NaOH), Tween 80, and TGGS from Adooq (USA). Phenylhydrazine was obtained from BDH, England. Spirulina 90% was obtained from Alta pharma, Germany.

Preparing of chitosan nanoparticles (CNP)

The concentrations were prepared from a solution of chitosan provided by (Beijing) company according to the modulating method ion gelation methods (Pires *et al.,* 2014), where the concentrations of 4 mg/ml of chitosan solution were prepared by adding (200 mg) of chitosan powder to (50ml) deionized distilled water (contains 1% acetic acid) for each and left for 24 hours at room temperature. Then, by continuous movement during stirring by a

magnetic bar in a hotplate stirrer for 30 minutes at 900 rpm, which leads to the formation of semi-colloidal solution. The pH was set at 4.6 by a pH meter by adding NaOH (0.1N), and exposure to sonication with a probe sonicator for 3 minutes, after which the solution was filtered with filter paper (400-800) (Salman *et al.,* 2022)

Loading of spirulina on Chitosan nanoparticles (SCNPs)

According to Ibrahim *et al*. (2014) and Ali *et al*. (2018), with some modification, 200 mg of spirulina powder was dissolved in 1 ml of distilled water and then slowly added to 50 ml of chitosan solution (4 mg/ml) while stirring at 900 rpm for 30 minutes to load the spirulina extract into the chitosan nanoparticles; afterward, the solution was sonicated for 5 minutes. The solution was returned to continuous stirring, then 10 ml of TPP (0.25%) was added with a ratio of 5:1 of solution by slow distillation and left to stir for 30 minutes to allow the spirulina extract particles to adsorb on the surface of the chitosan particles. Then the solution is returned to sonication for 5 minutes and then filtered with filter paper in order to get rid of non-bound particles. The solution was placed at a centrifugation force of 10,000 rpm for 15 minutes. The sediment nanoparticles were taken, and the supernatant was discarded to get spirulina extract loaded on chitosan nanoparticles.

Characterization of SCNPs

Particle Size Analyzer:

Measured using laser diffraction from Malvern Panalytical (UK) (0.5–50000 nm, 90 s), with results shown via software as curves and tables (Almukhtar and Karam, 2020).

Zeta potential analysis:

The purpose of zeta potential analysis is to know the surface charge of the materials CNPs and G-CNPs to determine the validity of their use for movement within the body. The test was done by a zeta potential analyzer(Zeta PLUS analyzer BROOKHAVEN USA) with a charge range of (+150 - -150mV) (Al Saadi *et al.,* 2024).

FTIR Analysis:

Identifies functional groups and chemical bonds post-adsorption using IR spectroscopy (400–4000 cm^{-1}) after centrifugation, washing, and drying (TENSOR 27, Germany). Samples were mixed with KBr, pressed into discs, and analyzed to detect spectral peaks representing molecular features (Tugarova *et al*., 2018; Al Saadi *et al*., 2024).

Experimental animals

Forty male albino rats were housed in the laboratory animal facility of the College of Veterinary Medicine. The animals were randomly divided into four groups, with ten rats per group. Ten animals comprised the control group, while the remaining thirty animals were allocated into three experimental groups. The treatments were administered orally via gavage directly into the stomach for a period of 60 days, according to the following experimental groups:

CNG: Rats in this group received no treatment and served as the negative control group.

PHZ-G: Rats in this group were injected intraperitoneally with phenylhydrazine at 20 mg/kg body weight for two consecutive days to induce anemia, serving as the positive control group (Al-Yasari *et al.,* 2024).

SPG: Rats were injected with phenylhydrazine at 20 mg/kg body weight for two consecutive days to induce anemia and subsequently treated daily with spirulina extract at 100 mg/kg body weight.

SP-NPS: Rats were injected with phenylhydrazine at 20 mg/kg body weight for two consecutive days to induce anemia and subsequently received daily treatment with spirulina-loaded chitosan nanoparticles at 100 mg/kg body weight.

Statistical analysis: The Microsoft Program (SPS) was used to statistically evaluate the data and showed how the T-test was used to compare the mean of variance at $P<0.05$ (Mosa *et al*., 2022; Al-Yasari *et al*., 2024).

RESULTS

Characterization of Nanoparticles

The particle size was measured by laser rays that penetrated the liquid containing the molecules of spirulina encapsulated within chitosan. Our data show that the range of particle sizes of spirulina after delivery by polymers was an average of 93.2 nm, as shown in Table 1 and Figure 1. The polymer dispersion index (PDI), also known as the polydispersity index, is a measure of the distribution of molecular mass of a polymer. Data appear to show uniformity of nanoparticles, with a perfect polydisperssion index at 0.005 that refers to good stability.

Table 1: Effect of percent of chitosan to extract and type of emulsifier on the polydispersity index (PDI), particle size distribution.

Polymer Chitosan	extract	TPGS	Particle size (nm)	PDI*
2	1	0.01	93.2 ±3.2	0.05
1	1	0.01	255± 6.5	0.264
2	1	Without TPGS	476.6±13.5	0.278

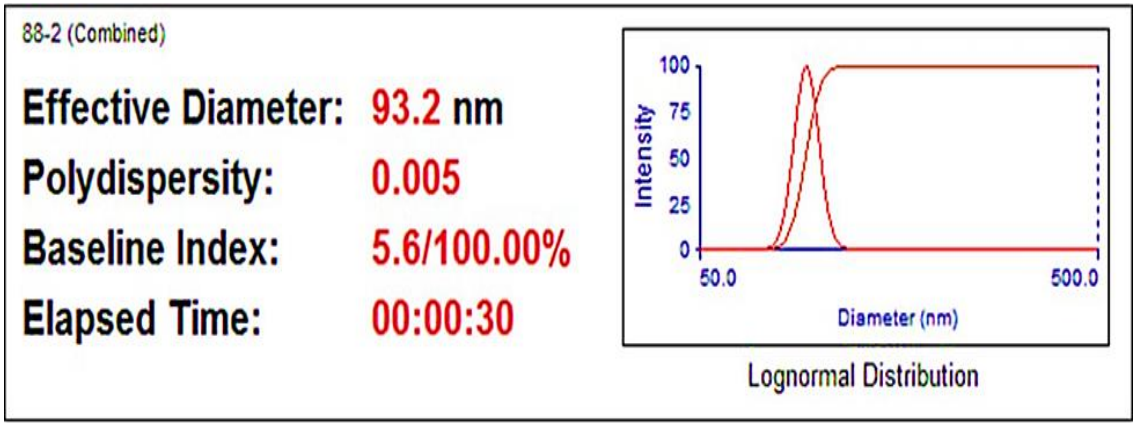


Fig. 1: Effective diameters of spirulina loading chitosan nanoparticles showed 93.2nm with polydispersion index 0.005.

Zeta potential analysis

Measuring the zeta potential includes knowing the surface charge of particles and the substances adsorbed on their surface. The zeta potential of chitosan in aqueous solution was +30.04 mV, indicating a good surface charge, while the zeta potential of Tripolyphosphate (TPP) was -19.62 mV, suggesting strong bonding with chitosan due to the charge difference between them (Figure 2 a, b). As for the zeta potential curve of the pure spirulina particles in aqueous solution, the highest potential of the surface was +30 mV, while loading of spirulina molecules in the colloidal solution of chitosan (CNPG) showed zeta potential increased in the direction of positivity to reach +153.97 mV (Figure 3), confirming stable positive charge after attachment of the extract molecules to the surface of the chitosan particles, which gives more stability to the solution.

X-ray diffraction

According to the XRD pattern in the file (visual estimation), the major diffraction peaks appear approximately at $2\theta \approx 31.8°$ $2\theta \approx 34.4°$ $2\theta \approx 36.2°$ $2\theta \approx 47.5°$ $2\theta \approx 56.6°$ $2\theta \approx 62.8°$ $2\theta \approx 67.9°$ $2\theta \approx 69.1°$ $2\theta \approx 72.6°$ We'll use the most intense peak (around $2\theta \approx 36.2°$) as an example to calculate the crystallite size.
Calculating Crystallite Size (Using Scherrer Equation)
X-ray wavelength (λ) = 1.5406 Å (Cu Kα) $\theta = 36.2° \div 2 = 18.1° = 0.316$ radians.
Estimated Full Width at Half Maximum (FWHM), $\beta \approx 0.2° = 0.0035$ radians

Scherrer Equation: D = \frac (K Kafshgari *et al.*){\beta \cos \theta}
Crystallite size (in nm) Shape factor (typically 0.9), X-ray wavelength, FWHM in radians, Bragg angle in radians

Using the Scherrer equation, the estimated crystallite size was found to be approximately 42 nanometers, confirming that the sample is in the nanometer scale (<100 nm).

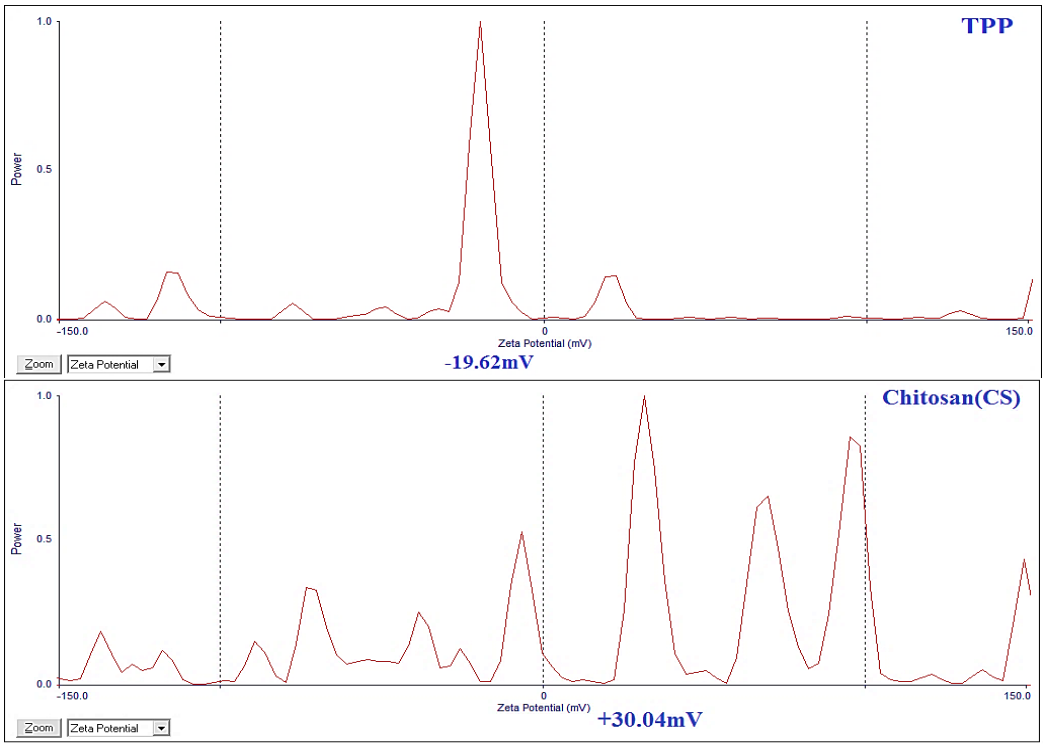


Fig. 2: Zeta potential of TPP chitosan nanoparticles (CNP), and

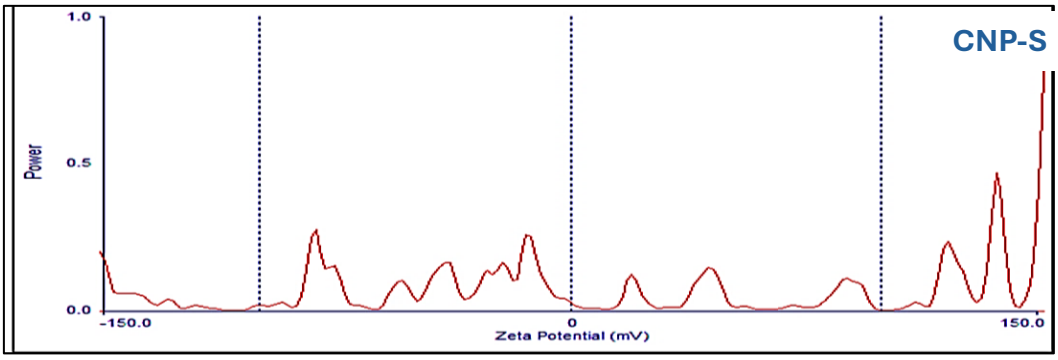


Fig. 3: Zeta potential of chitosan nanoparticles loaded with Spirulina.

FTIR analysis

The infrared spectrum of the chitosan-TPP solution (Figure 4) indicates the presence of several peaks located at many wavenumbers. The absorption peak at 896.9 cm^{-1} can be associated with the bending vibrations of aliphatic C–H bonds. The peak at 1653 cm^{-1} is due to the stretching vibrations of C=C bonds. The band at 1035.77 cm^{-1} is indicative of C=O stretching vibrations. Peaks at 1082.07, 1156.36, and 1259.51 cm^{-1} are attributed to C–O stretching vibrations, likely arising from alcohol groups present in the chitosan-TPP polymer structure. Additionally, the peaks at 1379.10, 2873.94, 3851.85, 3917.43, and 3417.86 cm^{-1} are associated with O–H stretching vibrations from alcohol functional groups. The bands at 3417.86, 3429.43, and 2873.94 cm^{-1} also correspond to N–H stretching vibrations of amine groups. In addition to that, the structure of the CNP in Figure 5 was confirmed by

FTIR, where the CNP's spectrum shows more broad absorption bands at 3358.07 cm^{-1}. This broad band could potentially represent the stretching vibrations of the hydroxyl group (OH) in water molecules, as well as the stretching vibrations of the OH and NH groups in free amino groups. The two bands observed at 2927.94 and 2879.72 cm^{-1} correspond to the asymmetric stretching of CH_3 and CH_2 in both CNPs and CNP-G. The observed band at 2221 cm^{-1} is attributed to the C–N group of C–NH_2. In addition, the stretching band of C–O in the chitosan spectrum was observed at 1038.48 cm^{-1} in all types of NPs.

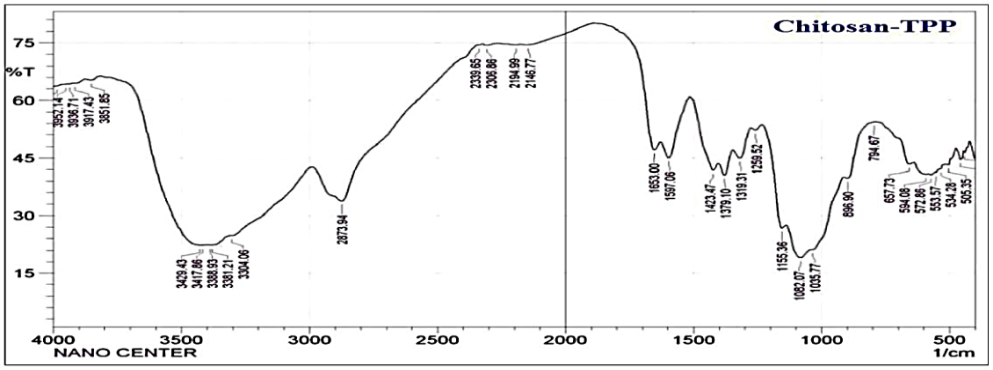


Fig. 4: FTIR spectroscopy of Chitosan-TPP solution.

The FTIR (Fourier Transform Infrared) spectrum of Spirulina provides insight into the various functional groups that reflect its chemical makeup. A broad peak in the range of 3300–3400 cm^{-1} is usually linked to O–H and N–H stretching vibrations. This means that there are hydroxyl groups from water or polysaccharides and amine groups from proteins. Peaks around 2920 and 2850 cm^{-1} are associated with C–H stretching in aliphatic chains, indicating lipid content. The presence of proteins is confirmed by a strong band near 1650 cm^{-1}, known as the amide I band, caused by C=O stretching of peptide bonds, along with the amide II band around 1540 cm^{-1}, which results from N–H bending and C–N stretching. Bands observed in the 1400–1450 cm^{-1} region often represent C–H bending or symmetric stretching of carboxylate groups, which may come from fatty acids or amino acids. Phosphate groups, likely from nucleic acids or phospholipids, are identified by a peak between 1240 and 1280 cm^{-1} due to P=O stretching. Carbohydrates are commonly detected by strong absorption between 1040 and 1070 cm^{-1}, attributed to C–O stretching vibrations. Additionally, the region around 875–900 cm^{-1} may show signals from aromatic C–H bending, which can be linked to pigments like chlorophyll or phycocyanin. Overall, the FTIR spectrum of Spirulina indicates a complex mix of biomolecules, including proteins, lipids, sugars, phosphate compounds, and pigments.

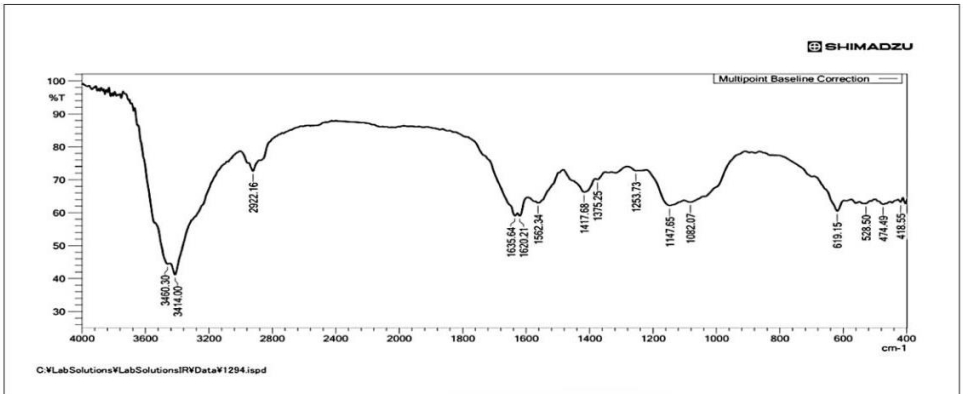
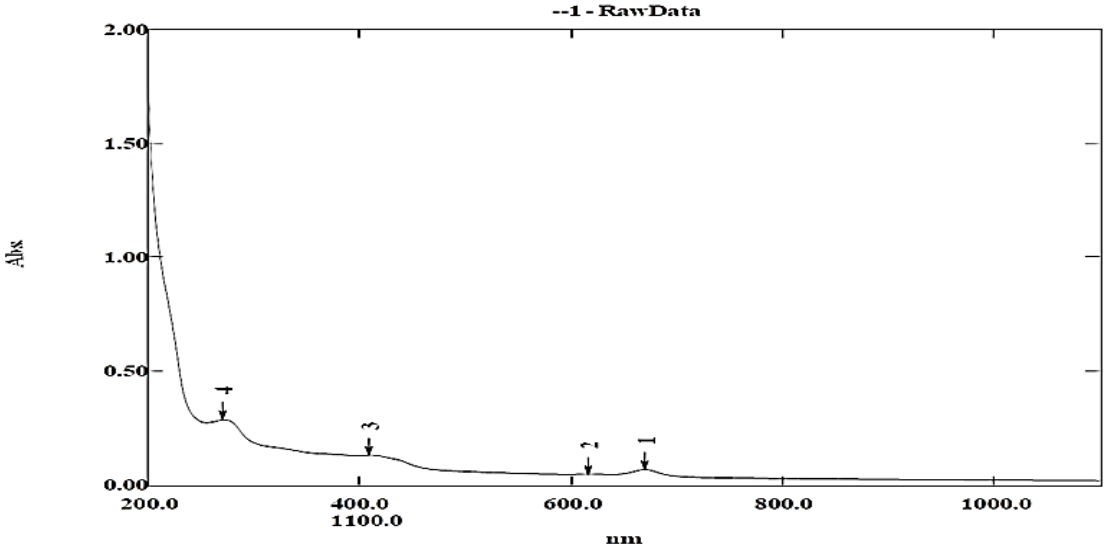


Fig. 5: FTIR spectrum of Spirulina loaded chitosan NPS appear different functional groups.

UV- spectrum

The UV-Vis spectrum of the chitosan-loaded pure spirulina shows key absorbance peaks, indicating the presence of important bioactive compounds. A strong peak at 271 nm indicates that there are aromatic compounds in the sample, such as phenolics or amino acids. Peaks at 409 nm, 617 nm, and 670 nm represent the presence of spirulina pigments. The 409 nm peak may correspond to the Soret band linked to porphyrin structures, while the 617 nm and 670 nm peaks indicate phycocyanin and chlorophyll-a, respectively. These pigments are known for their antioxidant and therapeutic properties. The retention of these peaks confirms that spirulina's active components remain intact after being incorporated into chitosan. This suggests a successful formulation, potentially suitable for pharmaceutical or nutraceutical applications, offering both stability and enhanced bioactivity. The analysis supports the use of chitosan as an effective carrier for spirulina-derived compounds, as shown in Figure 6.



Threshold: 0.001
Number of Points: 4

No.	P/V	Wavelength nm.	Abs.	Description
1	↑	670.0	0.069	
2	↑	617.0	0.048	
3	↑	409.0	0.130	
4	↑	271.0	0.287	

Fig. 6: UV spectrum of the chitosan-loaded spirulina

Field Emission Scanning Electron Microscopy (FESEM)

The image (Figure 7) included in the document shows chitosan-loaded Spirulina at a magnification of 60,000X. Size: Based on the scale bar (1 µm), the individual particle sizes range from approximately 40 to 300 nm, which falls within the nanoscale to low microscale range; however, some aggregation may affect the accuracy of this size estimation. Based on the image and scale bar: Morphology: The particles appear to be irregular and somewhat aggregated, with a rough surface texture. This kind of surface may suggest efficient encapsulation or interaction between chitosan and Spirulina biomass.

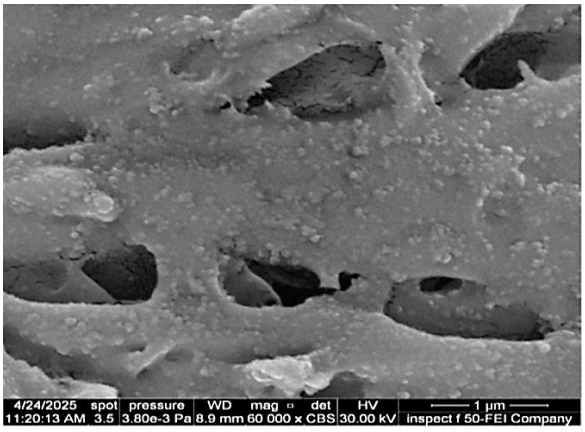


Fig. 7: FSEM nanoparticles of spirulina loaded chitosan appear irregular rounded to oval shape less than 100 nm.

Hemolytic study of spirulina -NPs

The haemato-compatibility of chitosan-NPs in diverse concentration extents (5, 2.5, and 1 mg/ml) was determined in Table 2. The outcome exhibited slightly hemolytic action in the formulations containing spirulina in all concentrations. It was observed at the higher concentration but did not reach a significant difference $P<0.05$ when compared with the control. On the other hand, full hemolysis was seen in samples treated with Triton (100%). Our data indicated that different concentrations of chitosan-loaded spirulina are candidates for oral administration without affecting blood circulation due to high safety.

Table 2: Effect of different chitosan loading spirulina concentration on rats' blood hemolysis *in vitro.*

Chitosan loading spirulina concentration	Percent of hemolysis
C	2.88±0. 04 a
C1	2.9±0.01 a
C2	2.7±0.069 a
C3	3.1±0.7 a
C4	3.3±0.6 a
C5	3.1±0.2 a

The data represented mean ±SE. *N=3 for each group. *Different capital letters appear significant ($P<0.05$) among groups. *C; represent normal saline 0.9%. *
C1: Spirulina at concentration 5 mg/ml. *C2: Spirulina loading chitosan at concentration 1mg/ml. *C3: Spirulina loading chitosan at concentration 2.5 mg/ml *C4: Spirulina loading chitosan at concentration 5mg/ml. *empty Chitosan at concentration 5 mg/ml

Biomarkers evaluation

The present data in Figure (8) demonstrate significantly elevated serum vitamin B12 levels in the treatment group that received spirulina-loaded chitosan nanoparticles compared to the hemolytic anemic group. In contrast, the SPG group showed non-significant ($P>0.05$) differences when compared to the negative control group.

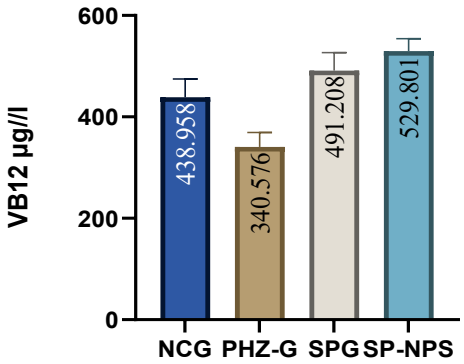


Figure 8: Effect of spirulina pure and spirulina loaded chitosan nanoparticles on vitamin B12 of anemic male rats. CNG: Control Negative Group, PHZ-G: Anemic group injected with phz, SPG: An anemic group treated with spirulina pure group, SP-NPS: An anemic group treated with spirulina loading chitosan nanoparticles.

Figure (9) confirms a significant increase in serum ferritin levels in the SP-NPS group compared to the anemic group, while serum ferritin in the SPG group showed only a slight increase compared to the negative control group. Furthermore, animals that received phenylhydrazine exhibited a significant reduction in haptoglobin levels, whereas treatment with spirulina extract and spirulina-loaded chitosan nanoparticles resulted in a significant restoration of haptoglobin levels to normal values (Figure 10).

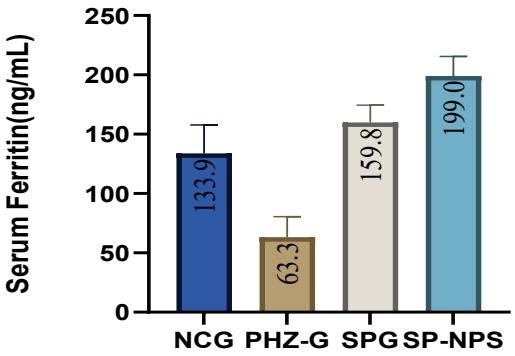


Figure 9: Effect of spirulina pure and spirulina loaded chitosan nanoparticles on total serum ferritin. CNG: Control Negative Group, PHZ-G: Anemic group injected with phz, SPG: An anemic group treated with spirulina pure group, SP-NPS: An anemic group treated with spirulina loading chitosan nanoparticles.

DISCUSSION

This study successfully developed and characterized Spirulina-encapsulated chitosan nanoparticles (SCNPs) through an ionic gelation approach. Detailed analyses, including particle size measurement, zeta potential, FTIR, UV-Vis, XRD, and FESEM, confirmed the efficient loading and physical stability of the nanoparticles, highlighting their promise as drug delivery carriers. The SCNPs had an average particle diameter of approximately 93.2 ± 3.2 nm, with a low polydispersity index (PDI = 0.05), reflecting a uniform size distribution. This aligns with the work of Vaezifar *et al*. (2013), who showed that the ionic gelation technique allows control over nanoparticle size through modulation of the chitosan-to-TPP ratio (Vaezifar et al., 2013).

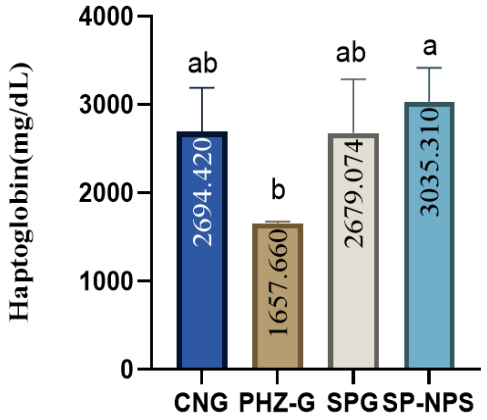


Figure 10: Effect of spirulina pure and spirulina loaded chitosan nanoparticles on Haptoglobin capacity (HP) level. CNG: Control Negative Group, PHZ-G: Anemic group injected with phz, SPG: An anemic group treated with spirulina pure group, SP-NPS: An anemic group treated with spirulina loading chitosan nanoparticles.

Nanoparticles smaller than 100 nm typically exhibit improved cellular uptake and bioavailability, key attributes for therapeutic applications (Al-Yasari *et al*., 2024). The increased size following Spirulina loading, compared to bare chitosan nanoparticles, supports successful encapsulation of Spirulina's active constituents. Zeta potential analysis showed a significant surface charge increase from +30.04 mV in plain chitosan nanoparticles to +153.97 mV in SCNPs. This enhancement suggests greater colloidal stability, which reduces aggregation and facilitates consistent biological interaction. Similar results reported by Imam *et al*. (2021) emphasize the correlation between a high positive charge and improved particle dispersion and interaction (Adnan and Alqaraghuli, 2019). This stability stems from strong ionic interactions between the negatively charged TPP and the positively charged chitosan matrix. Fourier transform infrared spectroscopy confirmed the encapsulation of spirulina compounds within the chitosan matrix. The shifts and broadening of chitosan's characteristic absorption bands (OH, NH_2, C=O, C–O) in SCNPs suggest the formation of new chemical interactions. Amide I and II bands, visible around 1650 cm^{-1} and 1540 cm^{-1}, respectively, further indicate the presence of Spirulina-derived proteins and peptides (Tugarova *et al*., 2018). Retention of these biochemical features is essential for maintaining Spirulina's antioxidant and therapeutic activities (Sachdeva *et al*., 2023). XRD patterns revealed a semi-crystalline nature in the SCNPs, with an estimated crystallite size of 42 nm. The presence of sharp diffraction peaks suggests that Spirulina integration does not disrupt the crystalline regions of chitosan. This level of crystallinity contributes to mechanical strength and enables a sustained release profile, as also demonstrated by Kairytė *et al*. (2022) in similar composite systems. UV-Vis spectra displayed peaks at 271, 409, 617, and 670 nm, attributed to phenolic compounds, porphyrins, phycocyanin, and chlorophyll-a, respectively. These peaks verify the retention of Spirulina's key bioactive molecules within the nanoparticles. The findings are consistent with the study by Jasim *et al.* (2021), which emphasized the therapeutic benefits of these compounds, particularly in antioxidative and anti-

inflammatory contexts. Their stability suggests that the chitosan matrix effectively shields them from degradation. Field Emission Scanning Electron Microscopy showed that the SCNPs possessed a roughly spherical morphology with slight aggregation. The observed particle sizes, ranging from 40 to 300 nm, correspond well with the DLS results, supporting the formation of a stable nanoparticulate system (Kafshgari *et al*., 2011). These structural features are consistent with other biopolymer-based nanoparticle systems designed for biomedical applications (Abbas *et al*., 2022). The study highlights SCNPs as promising candidates for drug delivery systems. Spirulina contributes valuable pharmacological attributes, including antioxidant, anti-inflammatory, and immune-modulatory effects (Ansari et *al.*, 2023). Encapsulation in chitosan not only improves the stability and bioavailability of these compounds but also facilitates targeted and controlled delivery, as supported by prior nanoparticle-based therapeutic research (Jafernik *et al*., 2023). The favorable surface charge and biocompatibility further enhance mucosal adhesion and cellular uptake, making SCNPs suitable for oral and parenteral administration. This study explored the impact of spirulina extract and chitosan-based formulations on hematological recovery in rats induced with anemia. The treatment groups showed significant improvements in important blood parameters, which suggests that both spirulina and chitosan are good for you, especially when used together. Spirulina is recognized for its rich nutritional profile, including high levels of iron, phycocyanin, and essential vitamins (Gogna *et al*., 2023). These components are thought to enhance red blood cell synthesis and improve hemoglobin levels. In this study, anemic rats receiving spirulina showed measurable increases in red blood cell count, hemoglobin concentration, and hematocrit, supporting its role as a hematinic agent (mentioned in other study). The antioxidant properties of spirulina may also contribute to protecting bone marrow cells from oxidative stress, which often impairs erythropoiesis in anemic conditions (Eltantawy *et al*., 2022). When spirulina was loaded by chitosan as a drug delivery, the results were more pronounced than with spirulina alone. The chitosan matrix likely facilitated a controlled and enhanced release of spirulina's active components, improving their absorption and efficacy. Additionally, chitosan may influence intestinal iron uptake and improve nutrient bioavailability, indirectly supporting hematopoietic recovery. The combined formulation not only improved iron status but also appeared to minimize some limitations associated with direct iron supplementation, such as gastrointestinal distress or poor absorption. The bio-adhesive properties of chitosan might enhance retention time in the gastrointestinal tract, allowing more sustained uptake of the therapeutic agents (Ahmad *et al*., 2024). Despite these promising results, the precise mechanisms by which chitosan enhances spirulina’s activity are not yet fully understood. Further studies involving molecular analyses would be valuable in elucidating how these substances interact at the cellular level to promote hematological health. In fact, the combination of spirulina extract with chitosan presents a promising natural strategy for managing anemia. This approach supports hematological recovery by enhancing iron bioavailability, stimulating red cell production, and providing antioxidant protection, offering a potential alternative to conventional therapies, particularly in cases of iron intolerance or deficiency-related anemia.

CONCLUSION

This study shows that chitosan nanoparticles loaded with spirulina are a very effective treatment for phenylhydrazine-induced anemia. They work better than regular spirulina extract because they are more bioavailable and can

be delivered to the right place. The nanoformulation significantly ameliorated hematological parameters, restored critical nutritional biomarkers including vitamin B12 and ferritin levels, and provided robust antioxidant protection against oxidative cellular damage. The comprehensive therapeutic benefits observed encompass multiple synergistic pathways, including enhanced erythropoiesis, improved iron metabolism, immunomodulatory effects, and systematic reduction of oxidative stress markers. These compelling findings establish spirulina-loaded chitosan nanoparticles as a promising alternative therapeutic strategy that warrants rigorous clinical evaluation for the management of anemia and related hematological disorders.

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REFERENCES

Abbas, F.G., Jasim, A.M.; Jawad, M.J.; Hassan, S.M.; Jawad, M.J. and Hadi, N.R. (2022): TPGS In Drug Delivery Systems And Pharmaceuticals Of Veterinary And Human Applications. *Bull Natl Inst Heal Sci,* 140**,** 1421-32.

Adnan, M.J. and Alqaraghuli, H.F.H. (2019): Effect of oral PLGA/TPGS nanoparticles on stability of vorapaxar and atherosclerosis markers in male albino rats. *Online Journal of Veterinary Research,* 23**,** 337-344.

Ahmad, A.M.R.; Intikhab, A.; ZAFAR, S.; Farooq, U.; Shah, H.B.U.; Akram, S.; Abid, J.; Parveen, Z. and Iqbal, S. (2023): Spirulina, an FDA-approved functional food: Worth the hype? *Cellular and Molecular Biology,* 69**,** 137-144.

Ahmad, K.; Zhang, Y.; Chen, P.; Yang, X. and Hou, H. (2024): Chitosan interaction with stomach mucin layer to enhances gastric retention and mucoadhesive properties. *Carbohydrate Polymers,* 333**,** 121926.

AL-Yasari, A.M.R.; Aboktifa, M.A.; Ahmed, Z.A.; Almaliki, H.S.; Abbas, R.K. and Jasim, A.M. (2024): The physiological role of phytosomal curcumin to mitigate hemolytic anemia and cytogenic effect induced by phenylhydrazine in rats. AIP Conference Proceedings,. AIP Publishing.

Al Saadi, A.; Raut, N.; Mousa, H.; Vakili-Nezhaad, G.R. and Vaidya, R. (2024): Advanced Modeling and Comparative Analysis of Nanofiltration Membrane Parameters: NF90 vs. NP010. *Nanoscience and Nanotechnology-Asia*.

Ali, M.E.A.; Aboelfadl, M.M.S.; Selim, A.M.; Khalil, H.F. and Elkady, G.M. (2018): Chitosan nanoparticles extracted from shrimp shells, application for removal of Fe (II) and Mn (II) from aqueous phases. *Separation Science and Technology,* 53**,** 2870-2881.

Almukainzi, M.; El-Masry, T.A.; Ibrahim, H.A.; Saad, H.M.; El Zahaby, E.I.; Saleh, A. and El-Nagar, M.M. (2024): Ameliorative Effect of Chitosan/Spirulina platensis Ethanolic Extract Nanoformulation against Cyclophosphamide-Induced Ovarian Toxicity: Role of PPAR-γ/Nrf-2/HO-1 and NF-kB/TNF-α Signaling Pathways. *Marine Drugs,* 22**,** 395.

Almukhtar, J.G.J. and Karam, F.F. (2020): Preparation characterization and application of Chitosan nanoparticles as drug carrier. Journal of Physics: Conference Series. IOP Publishing, 012071.

Ansari, R.; Foroughinia, F.; Dadbakhsh, A. H.; Afsari, F. and Zarshenas, M.M. (2023): An overview of pharmacological and clinical aspects of Spirulina. *Current Drug Discovery Technologies,* 20**,** 74-88.

Balkrishna, A.; Dev, S.N.C.; Joshi, B. and Mishra, R.K. (2023): Spirulina: A Miraculous alga with Pharmaco-nutraceutical Potential as Future Food. *International Journal of Food,* 11(3), 128-136.

Eltantawy, F.; Mohameed, S.; Verginia, F. and Mohamed, S. (2022): Protective Effects of Spirulina against Cyclophosphamide Induced Bone Marrow Toxicity in Mice. *Biointerface Res. Appl. Chem,* 12**,** 1085-1095.

Gogna, S.; Kaur, J.; Sharma, K.; Prasad, R.; Singh, J.; Bhadariya, V.; Kumar, P. and Jarial, S. (2023): Spirulina-an edible cyanobacterium with potential therapeutic health benefits and toxicological consequences. *Journal of the American Nutrition Association,* 42**,** 559-572.

Ibrahim, A.E.; Ibrahim, S.A.A.; Fadhel, D.H. and Hussein, A.A. (2014): Sedimentation levels of red blood cells (ESR) and its effect on viscosity of blood cells (PVC) and glucose in elderly people. *Al-Nahrain Journal of Science,* 17**,** 9-12.

Imam, S.S.; Alshehri, S.; Ghoneim, M.M.; Zafar, A.; Alsaidan, O.A.; Alruwaili, N.K.; Gilani, S.J. and Rizwanullah, M. (2021): Recent advancement in chitosan-based nanoparticles for improved oral bioavailability and bioactivity of phytochemicals: Challenges and perspectives. *Polymers,* 13**,** 4036.

Jafernik, K., Ładniak, A., Blicharska, E., Czarnek, K., Ekiert, H., Wiącek, A. E. and Szopa, A. 2023. Chitosan-based nanoparticles as effective drug delivery systems—a review. *Molecules,* 28**,** 1963.

Jalloob, E.D.; Heider, R.; AL Zahid, A.A.; Jaber, I.J.; Jasim, A.M.; Dirwal, A.R. and Neamah, D.A. (2022): Characterization and synthesis of milk thistle nanoparticles to overcome oxidative stress induce testicular damage in male rats. *Research Journal of Pharmacy and Technology,* 15**,** 1664-1670.

Jasim, A.; Dirwal, A. and Al-Yassri, A. (2019): Role of zontivity loaded by PLGA coated by TPGS in ameliorating induced cardiovascular and pulmonary disorder in adult male. *East African Medical Journal,* 96.

Jasim, A.M.; Alhtheal, E.D.; Raheem, S.S.; Rawaa, K.J. and Hamad, A. (2021): Characterization and Synthesis of Selenium-TPGS Nanoparticles for Target Delivery Clove to Minimize Cytogenic and Liver Damage Induced in Adult Male Rats. *Nano Biomedicine and Engineering,* 13.

Kafshgari, M.H.; Khorram, M.; Khodadoost, M. and Khavari, S. (2011): Reinforcement of chitosan nanoparticles obtained by an ionic cross-linking process. *Iranian Polymer Journal,* 20**,** 445-456.

Kairytė, A.; Vaitkus, S.; Vėjelis, S.; Balčiūnas, G. and Kremensas, A. (2022): Recycling liquid glass modified waste-lignin into bio-based polyurethane foam composites for building materials industry.

Kou, S.G.; Peters, L.M. and Mucalo, M.R. (2021): Chitosan: A review of sources and preparation methods. *International Journal of Biological Macromolecules,* 169**,** 85-94.

Kumar, R.; Sharma, V.; Das, S.; Patial, V. and Srivatsan, V. (2023): Arthrospira platensis (Spirulina) fortified functional foods ameliorate iron and protein malnutrition by improving growth and modulating oxidative

stress and gut microbiota in rats. *Food and Function,* 14**,** 1160-1178.

Malik, S.; Niazi, M.; Khan, M.; Rauff, B.; Anwar, S.; Amin, F. and Hanif, R. (2023): Cytotoxicity study of gold nanoparticle synthesis using Aloe vera, honey, and Gymnema sylvestre leaf extract. *ACS omega,* 8**,** 6325-6336.

Mosa, A.H.; Hamzah, K.J. and Aljabory, H.A. (2022): First study on the molecular prevalence of caprine arthritis encephalitis virus in goats in Babylon, Iraq. *Veterinary World,* 15**,** 1129.

Neamah, G.A.K.; Hamza Alalwany, E.A.; Rabee, F.B.; Jasim, A.M. and Abukhomra, A.S. (2024): Histopathological Detection of the Protective Role of Hydroxytyrosol Against the adverse Effect of Azithromycin in Rats. *Advancements in Life Sciences,* 11**,** 386-391.

Pires, C. T., Vilela, J. A. and Airoldi, C. (2014): The effect of chitin alkaline deacetylation at different condition on particle properties. *Procedia Chemistry,* 9**,** 220-225.

Refai, H.; El-Gazar, A.A.; Ragab, G.M.; Hassan, D.H.; Ahmed, O.S.; Hussein, R.A.; Shabana, S.; Waffo-Téguo, P.; Valls, J. and Al-Mokaddem, A.K. (2023): Enhanced wound healing potential of Spirulina platensis nanophytosomes: Metabolomic profiling, molecular networking, and modulation of HMGB-1 in an excisional wound rat model. *Marine Drugs,* 21**,** 149.

Sachdeva, B.; Sachdeva, P.; Negi, A.; Ghosh, S.; Han, S.; Dewanjee, S.; Jha, S.K.; Bhaskar, R., Sinha, J.K. and Paiva-Santos, A.C. (2023): Chitosan nanoparticles-based cancer drug delivery: Application and challenges. *Marine drugs,* 21**,** 211.

Salman, S.A.K.; Taki, M.M.; Hadi, S.J. and Jasim, A.M. (2022): Green synthesis and Characterization of Zinc Nanoparticles using Herbal plant Extracts with their Influence on some Bacterial Infection. *Research Journal of Pharmacy and Technology,* 15**,** 3147-3152.

Selvakumar, P.; Seenivasan, S.; Vijayakumar, G.; Panneerselvam, A. and Revathi, A. (2025): Application of Nanotechnology on Medicine and Biomedical Engineering. *Nanomaterials and the Nervous System.* IGI Global.

Suhail, M., Khan, A., Rahim, M.A., Naeem, A., Fahad, M.; Badshah, S.F., Jabar, A. and Janakiraman, A.K. 2022. Micro and nanorobot-based drug delivery: an overview. *Journal of Drug Targeting,* 30**,** 349-358.

Tugarova, A.V.; Mamchenkova, P.V.; Dyatlova, Y.A. and Kamnev, A.A. (2018): FTIR and Raman spectroscopic studies of selenium nanoparticles synthesised by the bacterium Azospirillum thiophilum. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 192**,** 458-463.

Vaezifar, S.; Razavi, S.; Golozar, M.A.; Karbasi, S.; Morshed, M. and Kamali, M. (2013): Effects of some parameters on particle size distribution of chitosan nanoparticles prepared by ionic gelation method. *Journal of Cluster Science,* 24**,** 891-903.

توصيف وتخليق بوليمرات توصيل الأدوية لتحميل سبيرولينا باستخدام جزيئات الكيتوزان النانوية

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يُعد الكيتوسان من البوليمرات الحيويه مشتق من الكيتين، من المواد التي تكتسب اهتمامًا بحثيا متزايدًا نظرًا لخصائصه الفريدة وتطبيقاته المتنوعة. إذ إن تحميل الكيتوسان بمستخلص الإسبيرولينا يُعزز من خصائصه المضادة للأكسدة والمضادة للميكروبات والمحفزة على التئام الجروح، مما يجعله نظامًا طبيعيًا ومتوافقًا حيويًا مع الجسم يمكن استخدامه في توصيل الأدوية، والهندسة النسيجية، وتطبيقات العناية بالبشرة، مع تحسين الفعالية البيولوجية والاستدامة (الافراز لفتره أطول). حضرت الجسيمات النانوية لتحميل المواد بطريقه تسمى (التجلط الأيوني): حيث يتم تحميل الإسبيرولينا على جسيمات الكيتوسان النانوية :(SCNPs) لتحضير جسيمات SCNPs، ثم يتم إذابة ٢٠٠ ملغم من مسحوق الإسبيرولينا في ٥ مل من الماء المقطر، ومن ثم تضاف بشكل تدريجي إلى محلول الكيتوسان مع التحريك بسرعة ٩٠٠ دورة في الدقيقة لمدة ٣٠ دقيقة. بعدها يوضع الخليط في جهاز الموجات فوق الصوتية لمدة دقيقة واحدة، تلتها إضافة الصوديوم متعدد الفوسفات TPP (٠,٢٥٪) بنسبة ١:٥. بعد مزيد من التحريك والتصويت، تم ترشيح المحلول ثم وضع في جهاز الطرد المركزي بسرعة ١٠,٠٠٠ دورة في الدقيقة لمدة ١٥ دقيقة. تم جمع الرواسب الناتجة (SCN) وحُفظت في درجة حرارة الثلاجة وبعدها تمت عملية توصيف الجسيمات النانوية باستخدام تقنيات متعددة، شملت قياس حجم الجسيمات، تحليل الأشعة فوق البنفسجية (UV)، تحليل الجهد السطحي (zeta potential)، حيود الأشعة السينية (XRD)، التحليل الطيفي بالأشعة تحت الحمراء (FTIR)، المجهر الإلكتروني الماسح الباعث للإلكترونات (FESEM)، واختبار النشاط الانحلالي الدموي. أظهرت نتائج الدراسة الحالية أن استخدام الكيتوسان بمفرده أو بعد استخدام فيتامين أي المضاف مع استات متعدد الاثيلين كمستحلب ساهم في تقليل حجم الجسيمات النانوية إلى ٩٣,٢ نانومتر مقارنة باستخدام الكيتوسان فقط. كما أدى إلى تعزيز استقرار الجسيمات من خلال زيادة قابلية التشتت، وتحسين الجهد السطحي، وإنتاج جسيمات نانوية ذات شكل بيضوي ومتجانس. ومن ناحية أخرى، لم تُظهر الجسيمات النانوية المحمّلة بالإسبيرولينا أي تأثيرات سلبية على مكونات ووضائف الدم، ولم تتسبب في أي مضاعفات أو تأثيرات غير مرغوب فيها. وفي الدراسة الحيوية داخل الجسم، لوحظ تحسن واضح بعد المعالجة بجسيمات الكيتوسان المحمّلة بالإسبيرولينا في العديد من المؤشرات، حيث سجلت زيادة معنوية في مستويات فيتامين B12 في المصل، وكذلك في الفيريتين لدى المجموعة المعالجة مقارنة بالمجموعة المصابة بفقر الدم. إضافة إلى ذلك، أظهرت الحيوانات التي تم استحداث تحلل الدم فيها بدون معالجة بالفينيلهايدرازين انخفاضًا كبيرًا في الهبتوغلوبين، في حين أدت المجاميع العلاجية أدت الى رجوع الدم الى مستوياته الطبيعية بعد المعالجة بخلاصة بالإسبيرولينا أوالمحملة على الكيتوسان.