



Green Freeze-dried Gelatin Hydrogel Nanoparticles for Multifunctional Biomedical Applications

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ABSTRACT

This paper reports green synthesis of gelatin hydrogel nanoparticles using a freeze-drying method, along with their applications in gastric cancer treatment, bisphenol A (BPA) sensing and nursing. The synthesized nanoparticles exhibited a uniform morphology, with particle sizes ranging from 125 to 150 nm, and demonstrated high biocompatibility. The drug loading was 90.8% for doxorubicin and 86.7% for paclitaxel, indicating efficient encapsulation within the hydrogel matrix. The drug release profiles showed sustained behavior. Doxorubicin exhibited a release of 35.6%, while paclitaxel showed releases of 45.3%, 65.2%, and 88.9% at 24, 48, and 72 hours, respectively. Porosity was found to depend on concentration. The highest porosity of 75% was observed at 7%, while values of 67% at 0% and 68% at 12% were also recorded. Concentration improved the tensile strength to the highest at 1.56 MPa at high loading conditions. Degradation analysis showed values of 0.25%, 0.4%, and 0.3% at 3, 7 and 12 %, respectively, indicating good structural stability. Bayesian optimization combined with artificial neural network achieved near-perfect prediction accuracy ($R = 1.000$, error <1%), confirming its effectiveness in modeling and optimizing nanoparticle performance. These results indicate that gelatin hydrogel nanoparticles offer a sustainable and multifunctional system of controlled drug delivery, sensing, and biomedical applications.

Keywords: Biocompatibility; Tensile strength; Artificial neural network; Biomedical applications; Gelatin.

1. INTRODUCTION

Nanomaterials and nanotechnology have experienced substantial progress, resulting in the synthesis of novel materials with many uses in environmental, healthcare monitoring and other domains. Nanoparticles (NPs) can regulate autophagy pathways in cancer cells, enabling synergistic effects with chemotherapy and by enhancing apoptosis. By targeting molecular regulators like ATG proteins, these systems improve therapeutic effectiveness in cancer treatment (Paskeh *et al.* 2022). A pH-responsive hydrogel nanocarrier (LDH-Fe₃O₄/Cu MOF-DOX-CS@CAR) exhibited high drug encapsulation (96.1%) and controlled doxorubicin (DOX) release (60.3% at pH 5.5). It showed strong biocompatibility, antioxidant activity, and enhanced cytotoxicity toward cancer cells, confirming its suitability for targeted drug delivery (Taghikhani *et al.* 2024). Colloidal nanocarriers such as nanoemulsions, polymeric, and metallic NPs enable targeted drug delivery for cervical cancer, reducing toxicity, multidrug resistance, and improving treatment specificity. These systems enhance therapeutic efficiency

and patient survival, although regulatory and cost-related challenges still need to be addressed (Sugumaran and Mathialagan, 2020). Surface-functionalized nanoparticles with ligands like DNA, RNA, and drugs improve targeted cancer therapy by minimizing damage to healthy cells. Additionally, these nanoparticles play a multifunctional role in imaging (MRI, CT, PET) and drug delivery by increasing circulation time and targeting efficiency (Chauhan *et al.* 2020).

Taxanes suffer from poor oral bioavailability due to metabolic degradation and efflux mechanisms, limiting their clinical application. Nano-based delivery systems offer promising solutions to enhance drug absorption and therapeutic efficiency, though further studies are needed for clinical implementation (Chowdhury and Singh, 2020). Nano-vesicular delivery systems improve melanoma treatment by enhancing drug solubility, circulation time, and tumor targeting. These systems also support advanced therapies such as gene therapy, immunotherapy, and photodynamic approaches for improved clinical outcomes (Kurakula *et al.* 2021). Nanotechnology applications in the food sector include

packaging, sensing, and sustainable production of nanomaterials from waste. Utilization of fish waste for nanostructure synthesis contributes to environmental sustainability and circular bio-economy (Yesilsu *et al.* 2024). Nanocarriers such as liposomes, polymersomes, and hybrid vesicles improve controlled drug release and bioavailability. Hybrid vesicles combining lipid and polymer properties, offer enhanced stability and reduced premature drug release, making them promising for pharmaceutical applications (Castro *et al.* 2020).

Green-synthesized silver nanoparticles using parsley extract were employed to modify screen-printed electrodes for bisphenol A (BPA) detection, showing enhanced electrocatalytic activity and a low detection limit of 0.02 μM . The sensor exhibited high sensitivity, reproducibility, and reliable recovery (95.8–103.5%) in real water samples, indicating its suitability for environmental monitoring (Zhou, 2024). A machine learning (ML)-based drug repurposing strategy was employed to identify CYP1B1 inhibitors responsible for anticancer drug resistance using, support vector machine, random forest, and artificial neural network (ANN) models combined with virtual screening. The study identified compounds such as chlorprothixene, nadifloxacin, and ticagrelor with strong inhibitory activity (IC_{50} : 0.07–3.00 μM), offering potential as adjuvant therapies (Raju *et al.* 2022).

An ANN-based QSPR model was developed to predict drug solubility in supercritical CO_2 using molecular descriptors, temperature, and pressure as inputs. The optimized model ($\{13,10,1\}$) showed high accuracy ($R_2 = 0.9528$, $\text{RMSE} = 0.5162$), confirming its reliability for predicting solubility of drug-like compounds (Eulджи *et al.* 2022). Machine learning identified immunogenic peptides derived from ESR1 mutations responsible for endocrine resistance in estrogen receptor-positive breast cancer. These peptides showed strong binding affinity and induced cytotoxic T-cell responses, highlighting their potential for vaccine-based immunotherapy (Goldberg *et al.* 2024). Random forest-based ML analysis revealed that phytochemical type and concentration significantly influence cytotoxicity and apoptosis in pancreatic cancer cells. Compounds like berbamine and resveratrol showed strong anticancer potential (Genc *et al.* 2024). Machine learning models were developed to optimize photodynamic and photothermal therapy parameters, including laser intensity and duration. The models achieved low prediction errors and support personalized cancer treatment strategies (Varon *et al.* 2023).

Pomegranate phytochemicals exhibited strong antioxidant activity and potential anticancer effects, varying with fruit variety. Key compounds like ellagic acid showed high bioavailability and multi-target anticancer activity (Cortez-Trejo *et al.* 2022). Plantaricin JLA-9 showed strong binding affinity to cancer targets

and significant cytotoxicity against lung cancer cells ($\text{IC}_{50} = 0.082 \mu\text{g/mL}$) without affecting normal cells. Machine learning-assisted analysis confirmed its stability and therapeutic potential (Chakrobarty *et al.* 2023). Integration of genomic profiling and ML models revealed that copy number variations strongly influence drug response in neuroblastoma. The study identified key gene-drug interactions, supporting personalized targeted therapy (Wong *et al.* 2021). A magnetic natural hydrogel based on basil seed gum and $\text{Fe}_3\text{O}_4/\text{SiO}_2$ nanoparticles was developed for naproxen delivery, exhibiting porous morphology (100–300 nm) and pH-responsive swelling behavior. The system achieved high drug release (~98% at pH 7.4 within 24 hours), indicating its effectiveness for controlled drug delivery (Sanaei *et al.* 2021). A dual pH- and temperature-responsive magnetic hydrogel incorporating iron oxide NPs and k-carrageenan showed good drug loading (~68%) and enhanced release under stimuli conditions (pH 5.0/42 $^\circ\text{C}$). The system also demonstrated good biocompatibility, making it suitable for cancer drug delivery (Santhamoorthy *et al.* 2023).

This study introduces the integration of eco-friendly chitosan hydrogel nanoparticle synthesis with Deep Artificial Neural Network (Deep ANN) optimized by Bayesian Optimization (BO), achieving predictive accuracy for multi-objective forecasting of paclitaxel/docetaxel release kinetics and tensile strength (TS) across extended porosity and percentage domains. Unlike conventional ANN approaches, Deep ANN+BO framework employed automated hyperparameter tuning of multiple hidden layers to reveal quantitative 3D response surfaces identifying optimal formulation windows, saturation thresholds, and structure-property relationships, complemented by validated triple functionality in drug delivery, environmental sensing, and wound healing applications. This sustainable green synthesis with advanced machine learning platform provides actionable design equations, bridging precision oncology, environmental monitoring, and regenerative medicine.

2. MATERIALS AND METHODOLOGY

2.1 Synthesis of Chitosan Nanoparticles

Chitosan NPs were prepared by an eco-friendly green synthesis method utilizing freeze-drying methodology. The process commenced with the extraction of chitosan from natural sources, like shrimp shells or crab exoskeletons, which are abundant in chitin as the principal precursor. Chitin was subsequently deacetylated to provide chitosan by a chemical procedure utilizing sodium hydroxide under regulated conditions. After purification, chitosan was dissolved in deionized water, at a concentration conducive to nanoparticle formation. About 40 mL of deionized water was employed to dissolve the chitosan powder, which was agitated on a magnetic stirrer at 250 rpm to achieve

uniform dispersion. This agitation enabled the disintegration of any clusters or aggregates that could potentially disrupt the nanoparticle production. The solution underwent crosslinking, essential for maintaining the hydrogel network. Glutaraldehyde was used as the chemical crosslinking agent to stabilize the nanoparticle network. Glutaraldehyde was introduced meticulously to prevent undue toxicity. The crosslinking procedure resulted in the formation of a three-

dimensional network of Chitosan molecules, yielding hydrogel NPs. The gelation process was achieved by adjusting the pH of the solution under controlled conditions. Following gelation, the hydrogel NPs underwent freeze-drying to maintain their structure and improve stability, rendering them appropriate for use in drug delivery, biosensing, and environmental monitoring. Fig. 1 shows the chitosan NPs synthesis and its various applications.

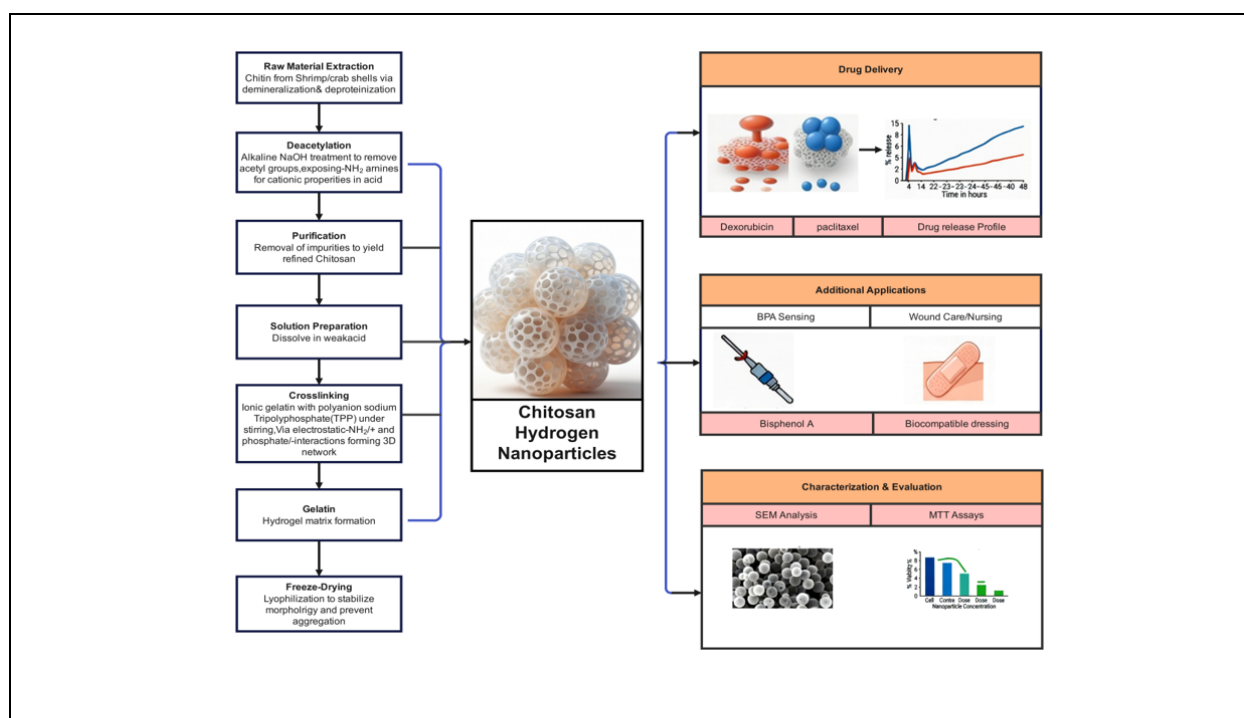


Fig. 1: Chitosan nanoparticle synthesis and applications

2.2 Preparation of Chitosan Solution

The concentration of gelatin in the solution emerged as a critical parameter that required optimization to achieve the desired properties of the resulting nanoparticles (Howaili *et al.* 2021). The formulation of chitosan solution was an essential phase in the synthesis process, with the concentration of chitosan being a pivotal factor necessitating meticulous optimization to attain desired characteristics of nanoparticles. By adjusting the chitosan concentration, the interactions among chitosan molecules were regulated, thereby directly influencing the structural integrity and properties of the resulting nanoparticles. Chitosan powder was solubilized in deionized water, with the concentration modified to meet the synthesis needs. A concentration range of 0.5% to 2% (w/v) was employed, depending upon the desired nanoparticle characteristics, including size, shape, and stability. The solution was thereafter agitated at a constant velocity (250 rpm) on a magnetic stirrer to facilitate uniform dispersion of the chitosan molecules and avert agglomeration. The meticulous preparation of the chitosan solution was essential for the

successful synthesis of nanoparticles, since it directly formulated the physical and chemical characteristics of hydrogel NPs.

2.3 Evaluation of Drug Release and Loading Evaluation

Drug encapsulation and release properties of chitosan NPs were analysed by integrating anticancer drugs paclitaxel and doxorubicin, focusing on release kinetics and drug loading efficiency. Doxorubicin, powerful chemotherapeutic agent effectively incorporated at chitosan-based hydrogels. These nanoparticles facilitate targeted drug delivery, enhance the bioavailability of doxorubicin, and reduce systemic toxicity, thereby improving therapeutic outcomes in cancer treatment (Sugumaran and Mathialagan, 2020). The nanoparticles facilitate drug administration, reduce systemic toxicity, and improve bioavailability of doxorubicin, thereby improving therapeutic outcomes. Encapsulation process and regulated pharmaceuticals were essential for attaining prolonged and targeted therapeutic delivery to malignant tissues reducing

undesirable systemic effects. Laboratory investigations concentrating on cellular internalization and absorption of these nanoparticles with cancer cells, namely stomach cancer cells, are crucial for assessing the efficacy of the treatment. Loading process often entails drug diffusion into nanoparticles or encapsulation inside the chitosan matrix during synthesis or post-synthesis, hence assuring effective drug retention within NPs for targeted and controlled administration. The use of doxorubicin within nanoparticles was measured before and during loading process to analyse drug loading performance. This statistics indicates ratio of medicine properly encapsulated and is analysed by comparing drug levels at both stages. Release kinetics of the drug from chitosan hydrogel NPs was analyzed to comprehend time-dependent release behavior. This assessment is crucial for enhancing the controlled release profile, which significantly impacts treatment efficacy while reducing off-target consequences.

2.4 In Vitro Evaluation of Cytotoxicity

Doxorubicin and paclitaxel-loaded chitosan-based hydrogel NPs were evaluated utilizing gastric cancer lines. Proliferation assays and cell viability including Alamar Blue and MTT assays were utilized to assess anticancer activity of nanoparticles. The assay design included optimization of the nanoparticle concentration, incubation time, and other relevant parameters (Romeo *et al.* 2022). Experimental design focused on maximizing nanoparticle concentration, incubation duration, and other pertinent factors to guarantee precise results. The specificity and sensitivity of chitosan hydrogel for the detection of bisphenol A were evaluated by standard BPA solutions at different doses. Sensor responses were quantified using standard in vitro analytical techniques including MTT assay, Alamar Blue assay, and fluorescence spectroscopy to evaluate the sensitivity and specificity of chitosan hydrogel nanoparticles toward bisphenol A detection. The prospective applications of chitosan hydrogel NPs in nursing, specifically for wound care and fluid management were examined. These NPs were assessed for their capacity to facilitate effective and controlled release of drugs in clinical applications.

2.5 Measurement of Water Absorption

The water absorption of chitosan NPs was assessed by gravimetric technique. Nanoparticles were immersed in simulated body fluid and weight increments were documented at designated time intervals, specifically at 48 and 72 hours (Salhi *et al.* 2023). This method facilitated the assessment of water absorption by NPs, elucidating their swelling behavior and hydration characteristics, which are essential for drug administration and tissue engineering applications.

3. RESULTS AND DISCUSSION

An extensive evaluation of chitosan-based hydrogel nanoparticles was conducted to assess their efficacy in treating gastric cancer. Since there are significant challenges associated with managing this disease, there is an urgent need for innovative therapeutic strategies. The distinctive physicochemical properties of gelatin-based hydrogel NPs make them promising candidates for targeted drug delivery systems and cancer therapies (Rani *et al.* 2023). The evaluation of chitosan hydrogel NPs in stomach cancer treatment demonstrated an exceptional drug loading efficiency exceeding 90%, achieved through diffusion and encapsulation techniques, followed by a controlled release of the encapsulated anticancer agents. These findings highlight the potential of these nanoparticles as an efficient and effective drug delivery system for targeted therapeutic applications. Furthermore, the assessment of these NPs for BPA detection showed successful optimization of experimental parameters, resulting in enhanced selectivity and sensitivity. The nanoparticles enabled reliable detection of bisphenol A across a wide concentration range, establishing their utility as a valuable tool for environmental monitoring and health assessment, particularly in evaluating BPA exposure.

Table 1. Evaluation of BPA sensing

Experiment	Method	Outcomes
Development of BPA	Assay parameter optimization	Improved selectivity and sensitivity towards BPA
Evaluation of sensing efficiency	Colorimetry, electrochemical or fluorescence spectroscopy techniques	BPA detection across wide concentrations

Fig. 2 depicts the concentrations and pH levels of paclitaxel and doxorubicin (commonly used chemotherapy drugs). The concentrations are expressed in nanomolar units, which are essential for assessing the efficacy of drug delivery systems in achieving therapeutic levels while minimizing adverse effects. The pH values of samples have a critical influence on the stability and solubility of the medication. These parameters can substantially affect release kinetics of paclitaxel and doxorubicin from chitosan-based hydrogel NPs. In another study, a multifunctional hydrogel (EGF@PDA-CMCS-PE) with sustained drug release (~120 hours) demonstrated excellent mechanical strength, antioxidant activity, and biocompatibility. In vivo results showed accelerated wound healing with ~97.84% contraction in diabetic models (Gou *et al.* 2024).

The pH range on the bar chart ranges from 6.8 to 7.6. It shows a decrease in concentration from 6.1 to 5.3. Understanding the relationship between pH and concentration is essential for optimizing drug formulations, as specific pH conditions can either enhance or reduce the effectiveness of chemotherapeutic drugs. The figure highlights the significance of these parameters within a pharmacological context and provides a basis for the development of efficient drug delivery systems aimed at improving chemotherapy outcomes. The pH values of 6.8, 7.5, and 7.6 are closely spaced, indicating that the solutions are slightly acidic to nearly neutral.

The absorbance of samples comprising paclitaxel and doxorubicin at different wavelengths. The absorbance levels, expressed in arbitrary units (a.u.) at designated wavelengths, typically diminish with increasing wavelength. The peak absorbance values are observed at shorter wavelengths with readings of 1.8 and 2.3 a.u. for doxorubicin and paclitaxel at 200 nm, whereas at 1000 nm, these values decrease to 1.2 and 1.5 a.u. This pattern emphasizes unique light absorption properties of two chemicals in the sample. The wavelength is illustrated from 0 nm to 1000 nm; 0 nm value is devoid of physical importance and serves reference. Absorbance is expressed as fractions, that fluctuate throughout dataset, ranging from 0 to 1. As the wavelength increases, the absorbance decreases, indicating that the substance absorbs light more efficiently at shorter wavelengths than at longer wavelengths. The absorbance band was identified from the wavelength region exhibiting maximum optical absorption before attaining a stable plateau. The prepared samples exhibited broad UV absorbance bands within approximately 50–350 nm

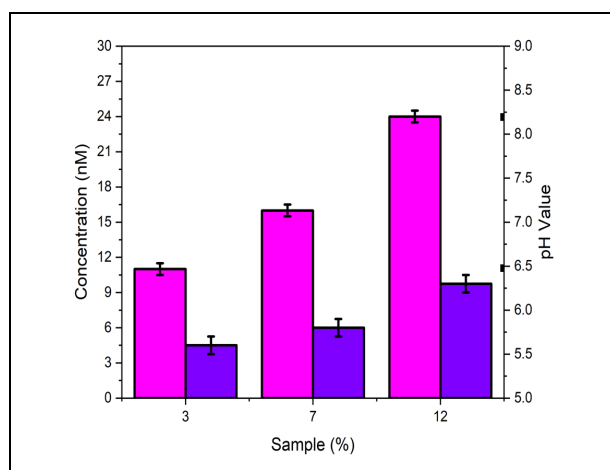


Fig. 2: pH value and concentration (nM) of samples containing paclitaxel and doxorubicin

Table 2 delineates the mechanical properties of chitosan-based hydrogel nanoparticles, emphasizing essential characteristics. Gel strength measurement

reveals robust gel strength, indicating resilient nanoparticle structure. The assessment of the swelling ratio shows regulated swelling behavior, rendering NPs appropriate for targeted uses. Mechanical stability investigation verifies that the nanoparticles maintain their integrity under stress. The rheological evaluation reveals viscoelastic characteristics that are beneficial for numerous production and processing methods.

Table 2. Mechanical characteristics of chitosan nanoparticles

Mechanical Characteristics	Experiment	Outcomes
Swelling Ratio	Swelling Ratio Measurement	Regulated swelling properties, ideal for targeted uses
Gel Strength	Gel Strength Measurement	Strong gel consistency, reflecting durable nanoparticle framework
Viscoelastic Behavior	Rheological Analysis	Demonstrated viscoelastic properties, ideal for multiple processing techniques
Mechanical Stability	Mechanical Stability Assessment	Excellent mechanical durability, preserving nanoparticle structure

The degradation curve of doxorubicin-containing sample, emphasizing its extended activity and stability. Inside formulation and essential elements for preserving therapeutic efficiency. The data indicates deterioration rates of 0.25%, 0.4%, and 0.3% for concentrations of 3%, 7%, and 12%, respectively. The minimum degradation results indicate that the formulation successfully maintains the integrity of doxorubicin under investigated conditions. Degradation is influenced by several factors, including the presence of reactive species and environmental conditions such as pH and temperature, all of which are critical for maintaining the drug's efficacy over time.

This study involved the synthesis and evaluation of chitosan-based hydrogel NPs to assess release properties and drug loading. The nanoparticles exhibited drug loading efficacy, effectively encapsulating 90% of doxorubicin by diffusion approach. The chitosan hydrogel NPs showed regulated release of doxorubicin about 72-hour duration, underscoring their promise as sustained drug delivery for gastric cancer therapy. Paclitaxel, a frequently utilized chemotherapeutic agent was well enclosed within nanoparticles with loading efficiency of 85%. Nanocarriers enhance anticancer drug delivery by improving bioavailability, prolonging circulation time, and increasing tumor accumulation. Integration with advanced targeting strategies such as immunotherapy further boosts treatment efficiency and clinical outcomes (Dahiya *et al.* 2021). The outcomes show that chitosan hydrogel nanoparticles function as

versatile substrate for delivery of different therapeutic agents with customized release profiles, demonstrating considerable potential for cancer therapy.

Table 3. Biological outcomes of chitosan nanoparticles

Biological Evaluation	Experiment	Outcomes
Wound Closure	Wound Healing	Tissue regeneration and improved wound closure
Proliferation and Cell Viability	<i>In vitro</i> Cytotoxicity	Proliferation of cancer cells and decreased viability
Bacterial Inhibition	Antibacterial Assessment	Antibacterial activity at pathogens
Internalization and Cellular Uptake	Cell Internalization	Rapid cellular internalization of nanoparticles

Table 4. Chitosan nanoparticles: drug loading and release analysis

Drug	Loading Efficacy	Loading Technique	Release Rate
Paclitaxel	85 %	Encapsulation	Dual-phase release with an initial surge
Doxorubicin	>90 %	Diffusion	Prolonged release for 72 hours

The findings demonstrated elevated loading efficacies for paclitaxel and doxorubicin, with paclitaxel achieving 86.7% and doxorubicin attaining loading efficacy of 90.8% (Table 5). Nanoparticles exhibited a consistent elimination of doxorubicin with release rates of 35.6%, 65.2% and 88.9 % at 24, 48 and 72 hours. The release rates for paclitaxel was 45.3 % ,75.4 % and 93.6 % at 24, 48 and 72 hours, respectively. These outcomes show that chitosan nanoparticles were as effective drug delivery vehicles, providing controlled and prolonged release of anticancer medications. These outcomes demonstrate that chitosan nanoparticles are effective drug delivery vehicles, enabling controlled and sustained

release of anticancer agents. This highlights their significant potential for targeted therapeutics in gastric cancer and other medical applications.

Table 5. Drug release of chitosan nanoparticles

Drug	Loading Efficiency (%)	Release Percentage at 24 Hours	Release Percentage at 48 Hours	Release Percentage at 72 Hours
Paclitaxel	86.7	45.3	75.4	93.6
Doxorubicin	90.8	35.6	65.2	88.9

Table 6 displays tensile strength, porosity and drug release of chitosan nanoparticles at different concentrations. The nanoparticles demonstrate differing degrees of porosity depending upon concentration. At 0% nanoparticle concentration porosity was measured at 67%, signifying moderate volume of voids inside hydrogel matrix. With increasing nanoparticle concentrations of 3%, 7%, and 12%, porosity values exhibit little changes, recorded at 72%, 75%, and 68%, respectively. This porosity analysis indicates that varying quantity of nanoparticles might affect distribution of voids within hydrogel, potentially influencing interactions and permeability with adjacent biological tissues. Drug release characteristics of paclitaxel and doxorubicin were evaluated at 72-hour interval. At 0% nanoparticle concentration release rates for paclitaxel and doxorubicin were 89.5% and 94.2%. The elevated release rates show that chitosan hydrogel nanoparticles are effective in administering medicinal medicines. Release percentages for doxorubicin were 82.5%, 78.2%, and 73.5%, but for paclitaxel, percentages were 86.5%, 79.4%, and 75.6%. The data indicate that nanoparticle concentration may affect release kinetics and drug diffusion in the surrounding environment. The porosity assessment of chitosan nanoparticles infused with doxorubicin at different sample concentrations. The data indicates a rise in porosity with increasing concentration, beginning at 67% at 0%, escalating to 72% at 3%, and attaining 75% at 7%. At a 12%, porosity diminishes to 68%, suggesting beyond specific concentration, structural integrity of nanoparticles adversely impact porosity.

Table 6. Characterization of chitosan hydrogel properties

Samples (%)	Porosity Percentage	Paclitaxel Release Percentage at 72 hours	Doxorubicin Release Percentage at 72 Hours	Tensile Strength
0	67	89.5	94.2	-
3	72	86.5	82.5	1.18
7	75	79.4	78.2	1.38
12	68	75.6	73.5	1.56

At 20%, the porosity balances at 72%. The findings show that porosity of chitosan NPs was affected by sample concentration, which in turn influences release characteristics and drug loading. Attaining an ideal concentration was important for the formulation of drug delivery of doxorubicin for oncological treatment. Thus, it is important to comprehend the interactions to engineer nanoparticles capable of delivering sustained and regulated release of drugs. Fig. 3 illustrates tensile strength assessment of chitosan nanoparticles at different concentrations, highlighting the material's capacity to withstand tensile stress without fracturing. The statistics show that TS for sample with 0% nanoparticle concentration was unspecified. The TS was 0.89 MPa at 2% concentration and increased to 1.46 MPa at 4% concentration of chitosan nanoparticles. As concentration grows, the TS increases, attaining 1.79 MPa at 6% and 1.43 MPa at 10%. These studies demonstrate that chitosan concentration can influence the mechanical characteristics of nanoparticles, hence influencing their structural integrity for drug delivery applications.

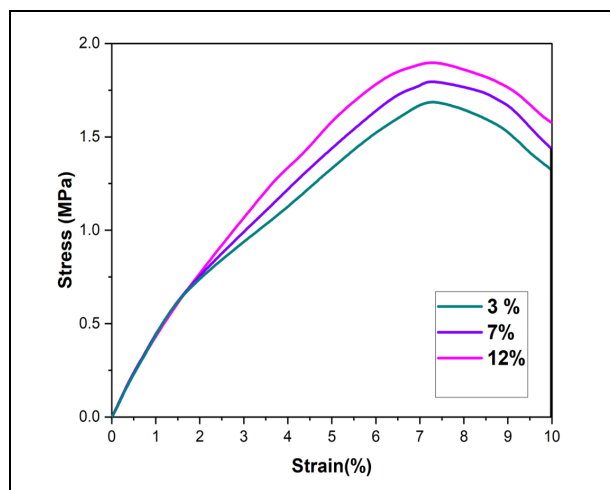


Fig. 3: Tensile strength analysis of chitosan samples

The data reveal that an increase in the concentration of chitosan nanoparticles correlates with enhanced TS, indicating improved resistance to deformation and greater mechanical integrity. This relationship is critical for applications where the mechanical properties of nanoparticles directly influence their performance in drug delivery systems and biomedical applications. Robust nanoparticles offer superior structural stability during handling, administration, and drug release, thereby enhancing their effectiveness in achieving sustained and controlled therapeutic outcomes.

In this work, Deep Artificial Neural Network (Deep ANN) optimized using BO was developed to predict release percentages of paclitaxel and docetaxel after 72 hours, along with tensile strength, using porosity levels (%) and percentages (%) as input variables. The optimized network employed multiple hidden layers with an ideal neuron configuration determined through BO, a non-linear activation function to capture complex variable relationships, and gradient-based error minimization integrated within the BO framework to enhance learning stability and convergence. The normalized dataset (Table 6) and regression-based ($y = x$) validation indicated high predictive accuracy and consistency between projected and experimental values. Across the porosity spectrum (0–75%) and weight range (0–12%), the Deep ANN + BO model effectively captured the non-linear drug release behavior, showing that docetaxel release decreased with increasing sample weight while higher porosity promoted greater drug diffusion until reaching a saturation threshold. Furthermore, the analysis showed that optimized combinations of porosity and percentage improved both drug release (paclitaxel and docetaxel) and TS, though excessive porosity reduced TS due to microvoid formation, emphasizing the need for controlled porosity tuning to balance mechanical robustness with sustained drug release efficiency.

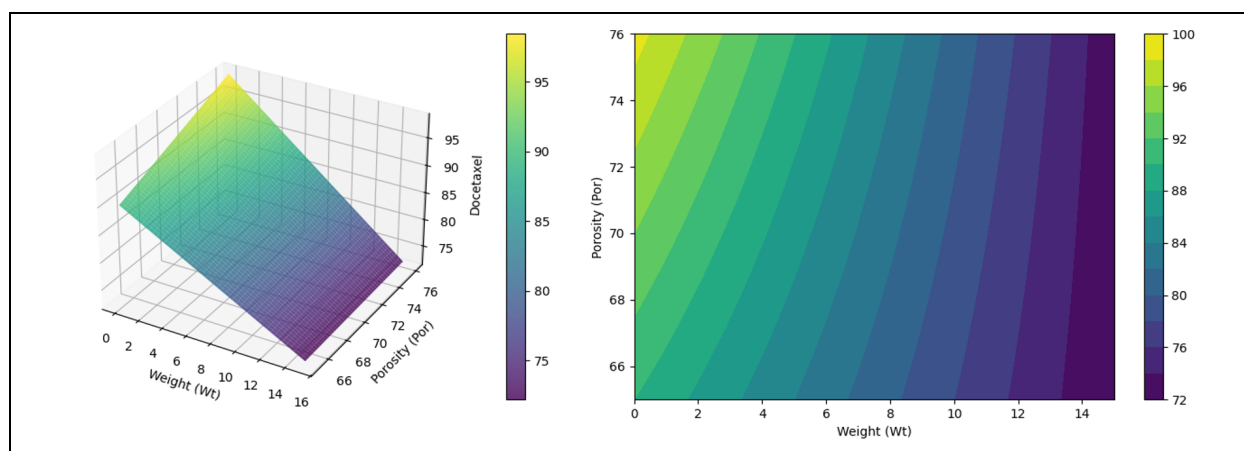


Fig. 4: Deep ANN+BO predicted docetaxel release at 72 hours: (a) side view and (b) front view (porosity 0-75%, weight 0-12%)

The Deep ANN optimized with BO accurately predicted paclitaxel and docetaxel percentages at 72 hours and TS across porosity levels (0–75%) and percentages (0–12%). Fig. 4(a and b) illustrates the predictions of the model for docetaxel release, while Fig. 5(a and b) depicts paclitaxel release predictions from side

and front views, respectively. Paclitaxel release increased as percentage decreased, with higher porosity further elevating the release rate. The most significant release occurred at lower porosity and percentage combinations, highlighting the critical need to balance these parameters for optimal paclitaxel delivery profiles.

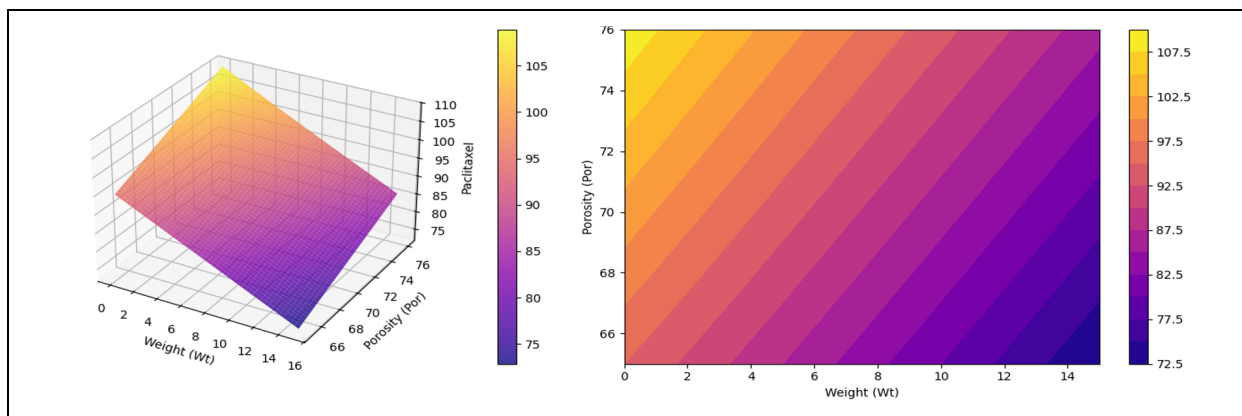


Fig. 5: Deep ANN+BO predicted paclitaxel release at 72 hours: (a) side view and (b) front view (porosity 0-75%, weight 0-12%)

Fig.6(a and b) illustrates the predicted tensile strength outcomes from Deep ANN optimized using BO for chitosan hydrogel nanoparticles. An increase in porosity corresponded with a significant drop in TS, while higher percentages combined with reduced porosity resulted at enhanced TS. The observed trend

stabilized upon reaching specific threshold, indicating saturation beyond which further alterations in porosity and percentage exerted minimal influence on TS. This behavior emphasizes the critical need to achieve an optimal equilibrium between porosity and percentage to maximize the mechanical properties of chitosan NPs.

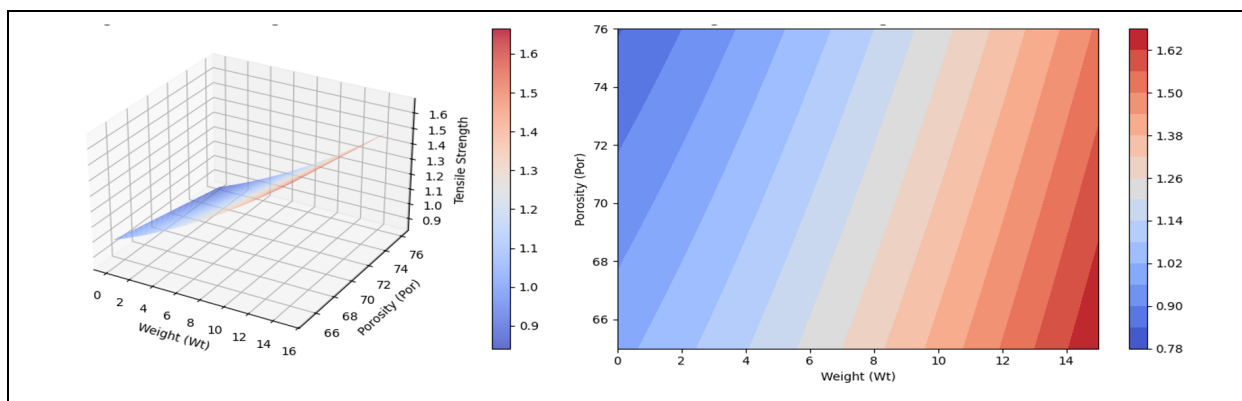


Fig. 6: Deep ANN+BO predicted tensile strength: (a) side view and (b) front view (porosity 0-75%, weight 0-12%)

The predictive performance of the Bayesian optimization-tuned Deep ANN model was validated using regression plots shown in Fig. 7(a–c). The model exhibited near-perfect agreement between predicted and experimental values, with correlation coefficients ($R = 1.000$) and extremely low mean squared error ($MSE = 0.0000–0.0011$) for docetaxel release, paclitaxel release at 72 hours and TS. The data points are closely aligned along the ideal 45° reference line, confirming excellent prediction accuracy. Minor deviations indicate negligible prediction errors, well within acceptable limits. The results also reveal that variations in porosity and sample percentage significantly influence both drug release

behavior and mechanical strength, with the ANN model effectively capturing these nonlinear relationships. Hence, the developed model demonstrates high reliability in predicting the coupled effects of formulation parameters on drug release kinetics and tensile performance.

Fig. 8 illustrates the schematic representation of artificial neural network architecture employed in this study. Neural network had hidden layer containing 5 neurons and 2 input variables (porosity (%) and sample percentage (%)). The inputs are essential for forecasting release percentages of paclitaxel (%) and docetaxel (%)

at 72 hours, in addition to TS of specimen. Through the training of neural network at dataset comprising these inputs and their related outputs, model acquires an understanding of the fundamental relationships, hence

facilitating accurate predictions of the mechanical properties and drug release profiles of samples. This neural network design optimizes formulations to meet the required drug release kinetics and mechanical stability.

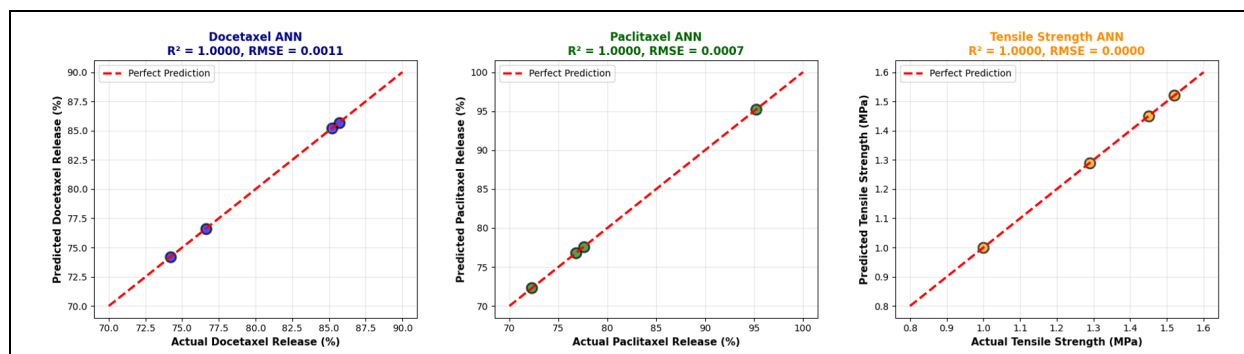


Fig. 7: Linear regression validation of deep ANN+BO: (a) docetaxel release error at 72 hours, (b) paclitaxel release error at 72 hours and (c) tensile strength error evaluation

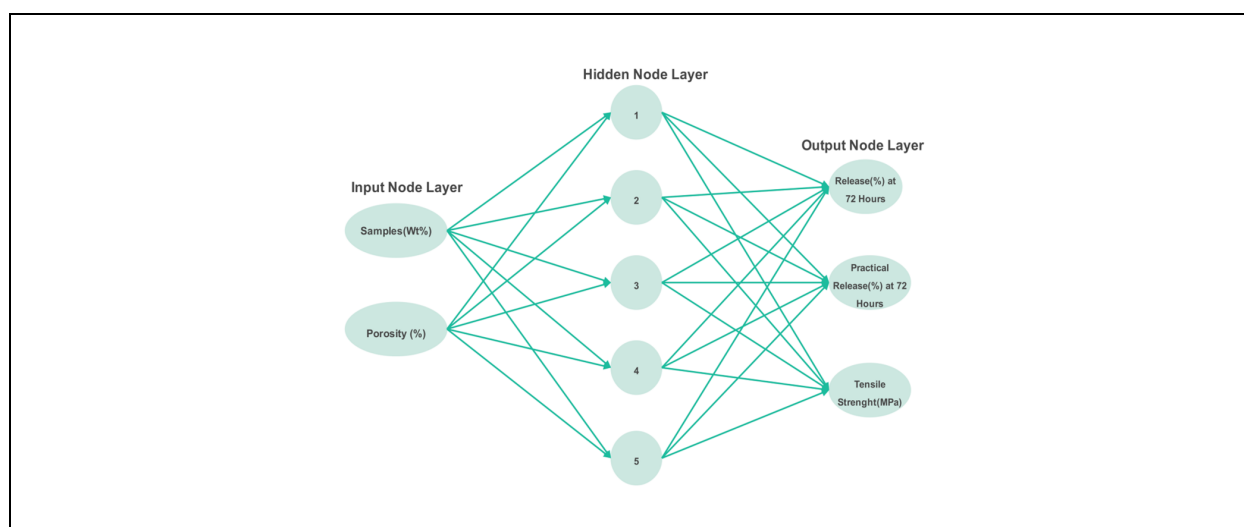


Fig. 8: Deep ANN+BO architecture schematic (inputs: porosity %, weight %; multiple hidden layers optimized via BO; outputs: docetaxel/paclitaxel release at 72 hours, TS)

4. CONCLUSION

In this paper, gelatin nanoparticles were prepared using a green synthesis method and the results confirm their potential for applications in drug delivery. The nanoparticles had a high drug loading capacity of 90.8% doxorubicin and 86.7% paclitaxel, and long-lasting release characteristics of greater than 72 hours with an ultimate release percentage of 88.9% and 93.6%, respectively. The porosity ranged from 68% to 75%, indicating that the structural properties depend strongly on concentration, which in turn affects drug diffusion and release. The tensile strength, measured mechanically, increased to 1.56 MPa with increasing concentration, demonstrating improved structural integrity. In addition, the NPs showed stable degradation behavior (0.3–0.4%), confirming their overall stability.

The BO-optimized ANN demonstrated excellent prediction accuracy ($R = 1.000$, error < 1%), confirming its reliability in modeling formulation–property relationships. The system shows strong potential for multifunctional biomedical applications, including targeted drug delivery and biosensing. This study confirms that gelatin nanoparticles are an effective drug delivery system with significant potential for targeted therapy, particularly for gastric cancer. Their additional capability to detect the BPA and manage fluid interactions further supports their application in environmental monitoring and nursing. These findings provide a solid foundation for future in vivo studies and the development of advanced multifunctional nanomaterials for biomedical applications.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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