Glutaraldehyde-cross-linked gelatin nanoparticles incorporating *Yucca schidigera* extract as a green co-surfactant for sustained-release drug delivery

Akram Hoshyari[1], Reza Ahmadi[2], Mojgan Heydari[1]*, Mozhgan Bagheri[1], and Nader Nezafati[1]

ABSTRACT

Gelatin nanoparticles (GNPs) have been designed and characterized to enable the controlled release of tramadol, offering potential for improved drug delivery and sustained therapeutic effects. In this study, biocompatible GNPs for controlled release of water-soluble drug tramadol were prepared through the water-in-oil emulsion method using *Yucca schidigera* extract as an eco-friendly, natural green co-surfactant. The presence of an aldimine functional group in the structure of GNPs was confirmed using Fourier-transform infrared spectroscopy, indicating cross-linking of gelatin by glutaraldehyde. In addition, the NPs exhibited a uniform, spherical structure without cracks, and the average particle size increased from 70 to 350 nm as the percentage of the cross-linker agent decreased from 25% to 8% v/v. The ninhydrin test was used to study the degree of cross-linking, and the results showed that 8% and 25% v/v of glutaraldehyde were able to cross-link the gelatin structure. The swelling index of GNPs cross-linked with 25% v/v glutaraldehyde (798%) was lower than with 8% v/v glutaraldehyde (1,030%). The GNP-to-tramadol ratios and glutaraldehyde concentration were optimized for tramadol release, and the results showed that cross-linked gelatin with 25% v/v glutaraldehyde and a GNP-to-tramadol ratio of 1:5 exhibited the most optimal characteristics for controlled drug delivery. Drug release kinetics analysis revealed that the release mechanism is concentration-dependent and best described by a first-order model, indicating a non-Fickian, diffusion-controlled process. Moreover, tramadol released from GNPs showed controlled behavior compared to the commercial tablet. Furthermore, the use of *Yucca* extract with proven emulsifying and stabilizing properties enhanced NP formation, highlighting its potential as a sustainable alternative to synthetic surfactants. The results confirmed that the designed drug delivery system could be a potential candidate for the delivery and controlled release of drugs such as tramadol compared to available conventional tablets.

Keywords:
Gelatin nanoparticles; Water-in-oil emulsion; *Yucca schidigera* extract; Tramadol hydrochloride; Controlled release; Kinetic models

***Corresponding author:**
Mojgan Heydari,
m.heydari@merc.ac.ir

How to cite this article:
Hoshyari A, Ahmadi R, Heydari M, Bagheri M, Nezafati N. Glutaraldehyde-cross-linked gelatin nanoparticles incorporating *Yucca schidigera* extract as a green co-surfactant for sustained-release drug delivery. *Biomater Transl.* **2025**

doi: 10.12336/bmt.25.00020

1. Introduction

Tramadol, a highly water-soluble drug, is often used in the therapy of osteoarthritis when non-steroidal anti-inflammatory drugs, such as paracetamol, or cyclooxygenase-2 inhibitors alone do not provide adequate pain relief. The drug is available in the forms of drops, capsules, and sustained-release formulations for rectal administration, as well as in the form of a solution for intramuscular and intravenous injection. However, it is most commonly taken orally. In conventional drug delivery systems, after 4.9 h, the drug is completely released, necessitating repeated dosing to relieve pain. Rapid release of

tramadol in the body can cause many side effects, such as liver problems or addiction.[1] Therefore, a new oral drug delivery system is needed to enable controlled release while using a lower drug dose.

Oral drug delivery is among the most preferred routes of administration, as it allows drug dosages to be adjusted, is relatively safe, and provides ease of administration. The design of oral drug delivery should be based on the gradual release of a drug to avoid initial uncontrolled burst release.[2] Therefore, the development of oral tablets with continuous release for water-soluble drugs has attracted the attention of pharmacists.[3] Rapid drug release in conventional oral drug delivery methods leads to issues such as high-dose toxicity, adverse effects on organs like the liver, which is caused by high drug dose release, suboptimal therapeutic outcomes due to prolonged treatment durations, and increased costs.[4] Therefore, the development of polymer-based oral drug delivery systems is necessary, as they can be used as drug carriers and have the ability to gradually swell in aqueous environments.[5]

Tramadol is a widely used analgesic for the relief of moderate to severe pain. However, its clinical application faces limitations, including a short half-life, burst release, and adverse effects. The rapid release of tramadol reduces the duration of its effect, which in turn requires frequent administration of the drug to maintain pain relief. Therefore, the development of a biocompatible platform with controlled release capability, high efficacy, and minimal side effects is essential. Tramadol-containing drug delivery systems can reduce the frequency of administration by providing sustained and controlled release profiles. These advantages are particularly important in managing postoperative pain, controlling chronic pain, improving treatment efficacy, and reducing side effects.[6] Gelatin nanoparticles (GNPs) could serve as a reliable drug delivery system for the controlled release of tramadol. GNPs obtained from collagen are a popular hydrophilic polymer, known for their biocompatibility, biodegradability, non-toxicity, non-carcinogenic nature, low antigenicity, cost-effectiveness, high availability, and extensive use in parenteral formulations. In addition, GNPs have many biomedical and pharmaceutical applications, including in hydrogel scaffolds and tissue engineering.[7] However, a major limitation of GNPs in drug delivery is the rapid or burst release of the drug in aqueous solutions. To address this challenge and increase the stability of gelatin-based drug delivery, cross-linking agents such as carbodiimide, formaldehyde, and glutaraldehyde have been used.[8] Glutaraldehyde is a preferred cross-linker for gelatin due to its high reaction rate, affordability, excellent solubility in aqueous media, and ability to react with numerous amino acid groups in the gelatin structure.[9] Recently, emulsion-based drug delivery systems have gained attention for their ability to control drug release and prevent burst or rapid release.

Emulsions consist of two immiscible liquids, in which droplets of one phase are dispersed within a continuous phase. To disperse two immiscible liquids in each other, a suitable surfactant is needed to ensure the stability of the emulsion.[10] Surfactants play an important role in the emulsion polymerization process, particularly in the water-in-oil (W/O) emulsion method used for the preparation of GNPs. However, concerns about surfactant toxicity have triggered global regulatory scrutiny due to concerns about their ecological impact and biodegradability. Therefore, researchers are trying to develop safe surfactants using renewable resources. Many green surfactants with high biodegradability and low toxicity have been synthesized from renewable sources such as amino acids, sugars, and organic acids.[11] Studies have shown that the *Yucca schidigera* extract can be used as a natural surfactant in the food, pharmaceutical, and beverage industries. It is rich in steroidal saponins (mostly sarsasapogenin), and its extract is approved for use in the food industry (Food and Drug Administration registration no. 172.510).[12]

Khan *et al.*[13] prepared a gelatin-based drug delivery system using the W/O emulsion method, with cytarabine used as the model drug. Their results showed that approximately 88.4% of the drug was released after 180 min. Houshyari *et al.*[14] previously used GNPs as a carrier for tramadol hydrochloride (HCl) within a W/O nanoemulsion-based drug delivery system. They used sorbitan monooleate (Span 80) and Tween 80 as surfactants, glutaraldehyde as the cross-linker, and ethyl acetate as the continuous phase. However, these chemical and conventional surfactants can reduce the biocompatibility of the drug delivery system. *Yucca* extract acts as a natural biosurfactant with amphiphilic properties suitable for stabilizing drug delivery systems. *Yucca* contains high levels of steroidal saponins, which reduce surface tension and exhibit natural emulsifying properties. Compared to conventional surfactants such as Tween 80 or sodium dodecyl sulfate, *Yucca* offers superior biocompatibility and reduced toxicity, making it an attractive candidate for pharmaceutical applications. Its use in tramadol-loaded GNPs represents an innovative step toward the development of more sustainable and patient-friendly drug delivery platforms. Furthermore, the natural origin of *Yucca* aligns with current trends in green nanotechnology and the replacement of synthetic additives in biomedical formulations.

The primary aim of this study is to synthesize and characterize cross-linked GNPs for controlled drug delivery applications. For this purpose, tramadol-loaded GNPs were prepared using the W/O emulsion method, and *Yucca* was extracted and used as a green co-surfactant instead of conventional surfactants. Parameters such as cross-linker concentration, cross-linking process time, GNP-to-tramadol ratios on drug load, NP yield, entrapment efficiency, swelling index, cross-linking degree, and tramadol release profile were investigated. In addition, kinetic models for drug release were investigated to study the mechanism and kinetics of tramadol release. The novelty of the present research lies in achieving controlled release of tramadol (without any burst effect), regulated by the swelling index and

[1]Department of Nanotechnology and Advanced Materials, Materials and Energy Research Center, Karaj, Alborz, Iran; [2]Group Molecular and Industrial Biotechnology, Department of Chemical Engineering, School of Engineering, Polytechnic University of Catalonia, Terrassa, Barcelona, Spain

the cross-linking degree of GNPs. The results showed that GNPs cross-linked with 25% v/v glutaraldehyde and a GNP-to-tramadol ratio of 1:5 can provide a controlled drug delivery system superior to commercial samples currently available on the market.

2. Materials and methods

Gelatin type A (isoelectric point is 7.6) from porcine skin was purchased from Sigma-Aldrich (United States). Glutaraldehyde (Merck KGaA, Germany) was used as the cross-linker, ethyl acetate (Merck, Germany) as the oil phase, and sorbitan monooleate (Span 80, Merck, Germany) and Tween 80 (Merck, Germany) were used as co-surfactants. The plant of *Y. schidigera* was obtained from the glasshouse of the City Hall in Meshkindasht (Karaj, Iran). Tramadol HCl (99%) was purchased from Tehran Darou Pharmaceutical Company, Iran.

2.1. Preparation of the *Yucca* extract

First, the root of *Y. schidigera* was processed into chips and dried at an ambient temperature. Subsequently, the dried root was ground to obtain a powder. In the next step, 10 g of *Y. schidigera* was shaken in 30 mL of n-hexane solution at 50°C for 6 h. The obtained solution was filtered, and the residue was mixed with methanol (in a ratio of 1:2) and shaken at 50°C for 6 h. Then, the solution was placed in a rotary evaporator for 4 h to remove the solvent, resulting in a yellow powder.[15]

2.2. Preparation of the drug delivery system

For GNP preparation, 0.25 g of gelatin was dissolved in 25 mL of deionized (DI) water at 55°C for 10 min under magnetic stirring at 500 rpm. Then, 25 mL of acetone was added to the solution as a dissolving agent to precipitate gelatin with high molecular weight, and after centrifuging the solution for 10 min, the supernatant was removed. Subsequently, the gelatin precipitations were stirred in 25 mL of DI water, and varying amounts of tramadol HCl drug (GNP: tramadol HCl = 1:5, 1:10, 1:50, 1:100) were dissolved directly in the solution. In the next step, 1 mL of gelatin solution, 0.5 g of *Yucca* extract, and 0.25 g of Tween 80 were slowly added dropwise to 10 mL of ethyl acetate containing 0.25 g of Span 80, under magnetic stirring at 700 rpm at 45°C for 30 min. This was followed by an ultrasound for 10 min under ice. The setup was covered with aluminum foil to prevent ethyl acetate from evaporating during the emulsification diffusion step. Then, 8% and 25% v/v glutaraldehyde were added to the nanoemulsion and stirred for 24 h at room temperature to cross-link the gelatin. To remove impurities and remaining materials, the final solution was centrifuged five times with acetone at 4°C for 10 min at 4,000 rpm and then washed three times with DI water and kept in a desiccator. The synthesis process of the drug delivery system is presented in **Figure 1**.

2.3 Characterization of the drug delivery system

The chemical bonding structure of the drug delivery system was characterized using Fourier transform infrared spectroscopy (FTIR; Model RX1, Perkin Elmer, United States) in the range of 4,000–400 cm^{-1}. The morphology of GNPs was evaluated using field emission scanning electron microscopy (FESEM) images (VEGA/MIRA3, TESCAN, Czech Republic). The mean size of GNPs was measured using the dynamic light scattering method (Zetasizer Nano ZS, model 3000HAs, Malvern Instruments, United Kingdom). The ultraviolet-visible spectroscopy (UV-Vis; Perkin Elmer, United States) method was employed to investigate drug load and release profile in the drug delivery system

2.4. Evaluation of cross-linking degree and swelling index of GNPs

Ninhydrin assay was used to evaluate the degree of cross-linking by measuring the percentage of free amino groups. For this purpose, 10 mg of cross-linked GNPs were heated with 3 mL of 0.5% w/v ninhydrin solution for 20 min at 100°C. After cooling the solution to an ambient temperature, its optical absorption was measured using a UV-Vis spectrophotometer at 570 nm. The cross-linking index of GNPs was calculated using Equation I:

$$Crosslinking\ degree\left(\%\right)=\frac{C_b-C_a}{C_b}\times 100 \quad \text{(I)}$$

where C_b and C_a are concentrations of free amino groups in the gelatin before and after cross-linking, respectively.

In addition, the swelling index of GNPs was assessed using the weighing method. For this purpose, 10 mg of dried GNPs were weighed and immersed in the phosphate-buffered saline (PBS) solution for 24 h at 37°C and a pH of 7.4. Finally, the swollen NPs were weighed, and the swelling index was calculated using Equation II:[16]

$$Swelling\ index\left(\%\right)=\frac{W_s-W_d}{W_d}\times 100 \quad \text{(II)}$$

where W_d and W_s are the weights of GNPs before and after swelling, respectively.

2.5. Evaluation of drug load, nanoparticle yield, entrapment efficiency, and drug release of GNPs

Key parameters for oral drug delivery, including drug load, NP yield, and entrapment efficiency, must be evaluated for each drug delivery system. After the cross-linking stage, the solution was centrifuged at 11,000 rpm for 15 min to obtain a two-phase solution. Then, the supernatant was removed, and the concentration was measured using UV-vis spectroscopy at 271 nm. NP yield, drug load, and entrapment efficiency were calculated using Equations III, IV, and V,[17] respectively:

$$Nanoparticle\ yield\left(\%\right)=\frac{weight\ of\ nanoparticles}{weight\ of\ Gelatin\ and\ drug\ fed\ initially}\times 100 \quad \text{(III)}$$

$$Drug\ load\left(\%\right)=\frac{weight\ of\ drug\ in\ nanoparticles}{weight\ of\ nanoparticles}\times 100 \quad \text{(IV)}$$

$$Entrapment\ efficiency\left(\%\right)=\frac{weight\ of\ drug\ in\ nanoparticles}{weight\ drug\ fed\ initially}\times 100 \quad \text{(V)}$$

To evaluate the drug release profile, a certain amount of synthesized GNPs containing tramadol was placed in a dialysis

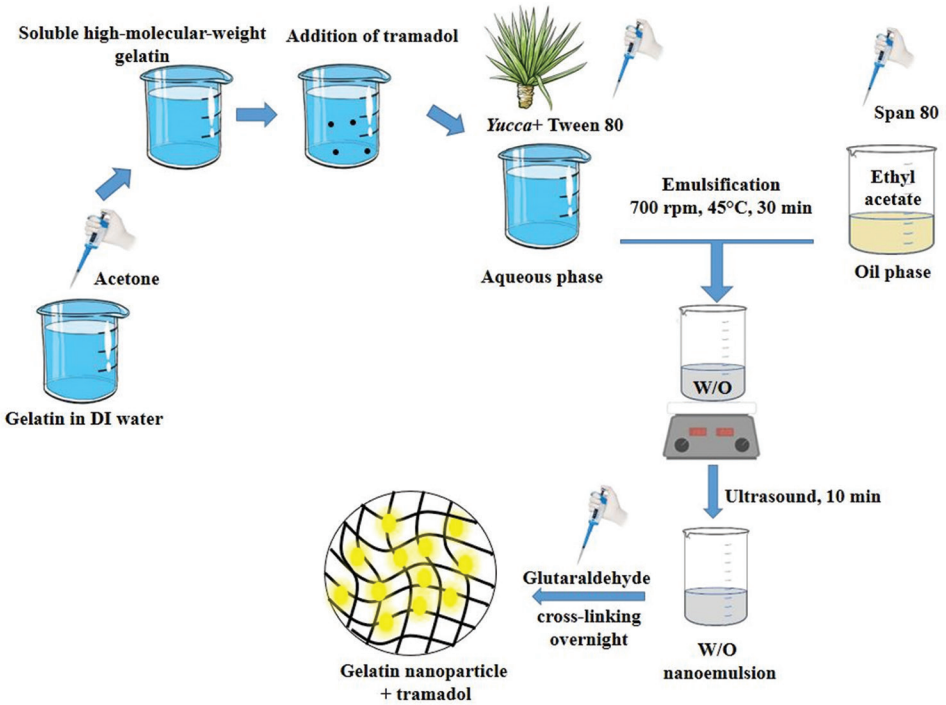

Figure 1. The synthesis process of the drug delivery system. Image created with Microsoft PowerPoint.
Abbreviations: DI: Deionized water; W/O: Water-in-oil.

bag (Dialysis tubing flat width 43 mm, Sigma-Aldrich D9527, molecular weight cutoff 14,000K). Then, the dialysis bag was transferred into 100 mL of PBS solution in a water bath at 37°C and a pH of 7.4, followed by stirring at 100 rpm. In the first 60 min, every 12 min, and thereafter every 60 min, 4 mL of the release medium was collected and replaced by an equal volume of fresh medium. The amount of tramadol was determined using UV-vis spectrophotometry at 271 nm.[18] In addition, the release of tramadol from the synthesized drug delivery system was compared with the commercial sample of tramadol (100 mg) purchased from Daroupakhsh Company (Iran). The volume of PBS solution used for the commercial sample was adjusted to match the conditions in terms of the weight of the synthesized GNPs and the commercial tablet. For comparison, the tramadol tablet was placed in 900 mL PBS solution in a 37°C water bath and stirred at 100 rpm. At certain time intervals, 4 mL of the sample was removed from the solution and 4 mL of fresh PBS was added. This process was repeated for 24 h. Finally, the concentration of the drug released from the tablet was recorded using a UV-vis spectrophotometer at 271 nm, and the percentage of cumulative release was calculated using Equation VI:

$$Cumulative\ drug\ release(\%) = \frac{\left(V_e \sum_1^{n-1} C_i + V_o C_n\right)}{m_{drug}} \times 100 \quad \text{(VI)}$$

where m_{drug} represents the mass of tramadol in GNPs, V_0 is the whole volume of the release media, V_e is the volume of the replaced media, and C_i is the concentration of tramadol in the release media. The experiments were performed in triplicate.

2.6. Assessment of drug release kinetics

Kinetics and mechanism of tramadol release from GNPs were investigated by fitting the drug release profile to various kinetic models consisting of the zero-order release model, the first-order release model, the Higuchi model, and the Korsmeyer–Peppas model. Usually, the initial 60% drug release is sufficient to determine the best release model. For each model, a graph was drawn, and the constant velocity and dependent values were obtained by applying a suitable line. The zero-degree kinetic model was obtained by plotting the release rate of cumulative drug versus time. The first-order kinetic model was obtained by plotting the logarithm of the percentage of drug remaining over time. The Higuchi model was obtained by plotting the percentage of cumulative drug release versus the second root of time, whereas the Korsmeyer–Peppas model was analyzed by plotting the logarithm of the cumulative drug release percentage versus the logarithm of time.[19] The first-order model describes drug release as a concentration-dependent process (Equation VII):[20]

$$LogC = LogC_0 - \frac{kt}{2.303} \quad \text{(VII)}$$

where C_0 represents the initial drug concentration, C is the drug concentration, t is time, and k is the release rate constant.

Hence, the release rate in a first-order process is directly proportional to the concentration of the drug (the higher the concentration, the faster the drug is released). First-order kinetics occur when a constant proportion of the drug is eliminated per unit time, resulting in a significantly slower and more variable release rate over time.

3. Results and discussion

3.1. FTIR spectroscopy spectral analysis

FTIR spectroscopy spectral analysis is a simple tool to ascertain chemical compounds, molecular structure, and functional groups of a material. FTIR spectra obtained from the uncross-linked gelatin, the cross-linked GNPs, the tramadol-loaded GNPs, and the *Yucca* extract are presented in **Figure 2**. According to the spectrum of gelatin (**Figure 2**), the broad peak at position 3,373 cm^{-1} and the low-intensity peak at position 2,950 cm^{-1} are related to N–H stretching of the amide group of gelatin and aliphatic C–H (asymmetric stretching of CH_2 groups of gelatin), respectively. In addition, the peak at wavelengths of 1,653 cm^{-1}, 1,549 cm^{-1}, and 1,311 cm^{-1} is related to amide I (C=O stretching bond), amide II (N–H bending bond), and C–N stretching, respectively.[21] In the spectrum of cross-linked GNPs (**Figure 2**), all the previously mentioned bands are present, confirming the presence of gelatin in this sample. However, in the GNPs' spectrum, a new peak appeared at wavenumber 1,445 cm^{-1} due to the formation of the aldimine group during the cross-linking process. The aldimine functional group (CH=N) is formed through the reaction of the aldehyde group (–CHO) in glutaraldehyde with the amino-functional group ($–NH_2$) in gelatin.[22] This reaction increases the stability of gelatin and reduces its dissolution rate in aqueous environments. In addition, the intensity of the band of amino groups of GNPs in the range of 3,200–3,400 cm^{-1} due to the cross-linking process decreased compared to the uncross-linked gelatin. Therefore, FTIR spectra confirmed the cross-linking of GNPs with glutaraldehyde.

The spectrum of tramadol-loaded GNPs not only showed the above-mentioned peaks of gelatin and GNPs, but also exhibited strong peaks at 547 cm^{-1}, 844 cm^{-1}, 985 cm^{-1}, 1,242 cm^{-1}, and 2,457cm^{-1}, which are related to O–H, CH_2, C–O, O–H, and C–H stretching and bending of the tramadol molecule, respectively.[23] FTIR spectrum analysis was also conducted to study the structure of the *Yucca* extract. According to the spectrum of the *Yucca* extract, the intense peak at 1,024 cm^{-1} is related to the C–O–C bending band, and the low-intensity peaks at positions 1,636 cm^{-1}, 1,726 cm^{-1}, and 2,931 cm^{-1} are related to C=C, C=O, and C–H bands, indicating the presence of saponin. Moreover, a broad peak is observed at position 3,390 cm^{-1}, which is associated with the hydroxyl group (OH) in the structure of the *Yucca* extract. The peaks in the spectrum of the *Yucca* extract confirmed its presence, consistent with previous studies.[24,25]

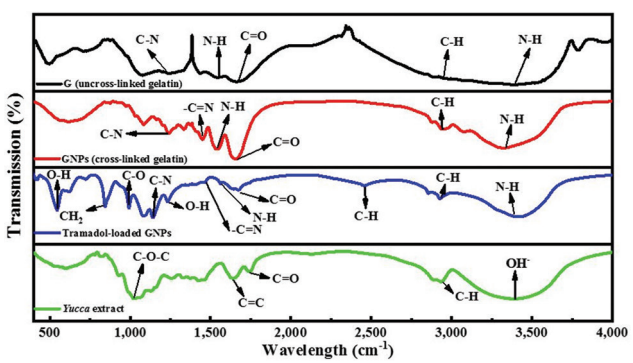

Figure 2. The Fourier transform infrared spectroscopy spectra of gelatin (G; uncross-linked gelatin), GNPs; cross-linked gelatin, tramadol-loaded GNPs, and *Yucca* extract.
Abbreviation: GNPs: Gelatin nanoparticles.

3.2. Morphology and particle size of tramadol-loaded GNPs

The morphology of tramadol-loaded GNPs was studied through FESEM images, and the obtained results are presented in **Figure 3**. Although the SEM image (**Figure 3**) displayed some GNPs adhesion, this is likely caused by aggregation during the sample drying process, which is known to occur during SEM preparation. To compare the effect of cross-linker amount on the morphology and particle size of GNPs, 8% and 25% v/v glutaraldehyde were used. **Figure 3A** and **B** show that tramadol-loaded GNPs cross-linked for 24 h (with a constant tramadol: GNPs ratio of 1:50) with 25% and 8% glutaraldehyde have a uniform spherical structure without cracks. However, the mean particle size of GNPs increased from 70 nm (**Figure 3A**) to 350 nm (**Figure 3B**) with a decrease in the quantity of glutaraldehyde from 25 to 8% v/v.[26] The decrease in particle size with an increasing glutaraldehyde cross-linker concentration is attributed to the formation of a more compact and denser structure resulting from the cross-linking reaction with aminated gelatin. This reaction forms a strong covalent bond between the polymer and the cross-linker. The degree of cross-linking directly influences the size of NPs; thus, a higher percentage of cross-linker leads to a reduction in the average size of the GNPs.[27]

In addition, to study the effect of cross-linking time on the size of GNPs, the cross-linking process was conducted for 6 (**Figure 3C**) and 24 h (**Figure 3A**) at room temperature with the amount of cross-linker agent (25% v/v and tramadol: GNPs ratio of 1:50) kept constant. The mean particle size of GNPs decreased from 400 nm to 70 nm when the cross-linking time increased from 6 h to 24 h. The results showed an increase in cross-linking degree with the formation of a more compact GNPs structure at a longer cross-linking time. Therefore, it can be deduced that the longer the cross-linking reaction time, the smaller the particle size of GNPs.

In addition, the effect of the amount of drug load on GNP size was evaluated through a dynamic light scattering assay (in aqueous medium). The percentage of glutaraldehyde, kept constant (25% v/v), and different ratios of GNP: tramadol (1:5, 1:10, 1:50, and 1:100) were investigated. The results are presented in **Figure 4**. According to the obtained results, the GNP's size increased dramatically with the increase in the amount of loaded drug. The average particle size of GNPs loaded with ratios of 1:5, 1:10, 1:50, and 1:100 was 118, 151, 410, and 575 nm, respectively. Increasing the amount of tramadol during the synthesis of the drug delivery system caused the formation of a more concentrated phase, which made it difficult to disperse and separate the aqueous from the organic phases. As a result, the size of the synthesized gelatin particles increased.[28] The polydispersity index of these nanosystems with different GNP-to-tramadol ratios was between 0.250 and

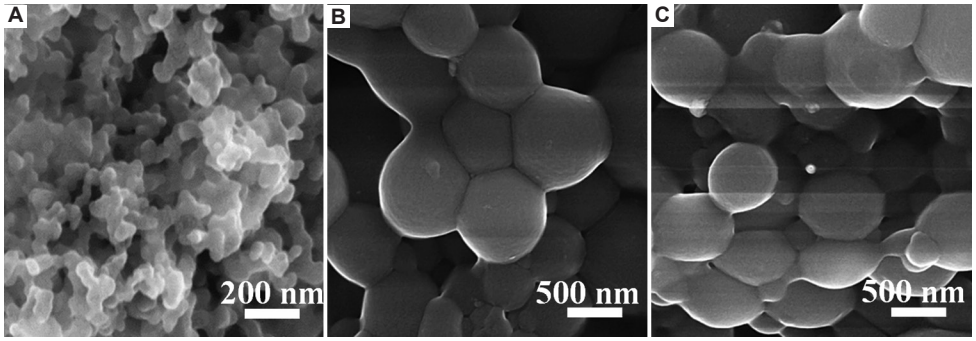

Figure 3. Field emission scanning electron microscopy image of tramadol-loaded GNPs. (A) GNPs prepared with 25% v/v glutaraldehyde with a 24 h cross-linking time. Scale bar: 200 nm, magnification 100,000×. (B) GNPs prepared with 8% v/v glutaraldehyde with a 24 h cross-linking time. Scale bar: 500 nm, magnification 50,000×. (C) GNPs prepared with 25% v/v glutaraldehyde with a 6 h cross-linking time. Scale bar: 500 nm, magnification 50,000×. Tramadol: GNP was 1:50 for all images.
Abbreviation: GNPs: Gelatin nanoparticles.

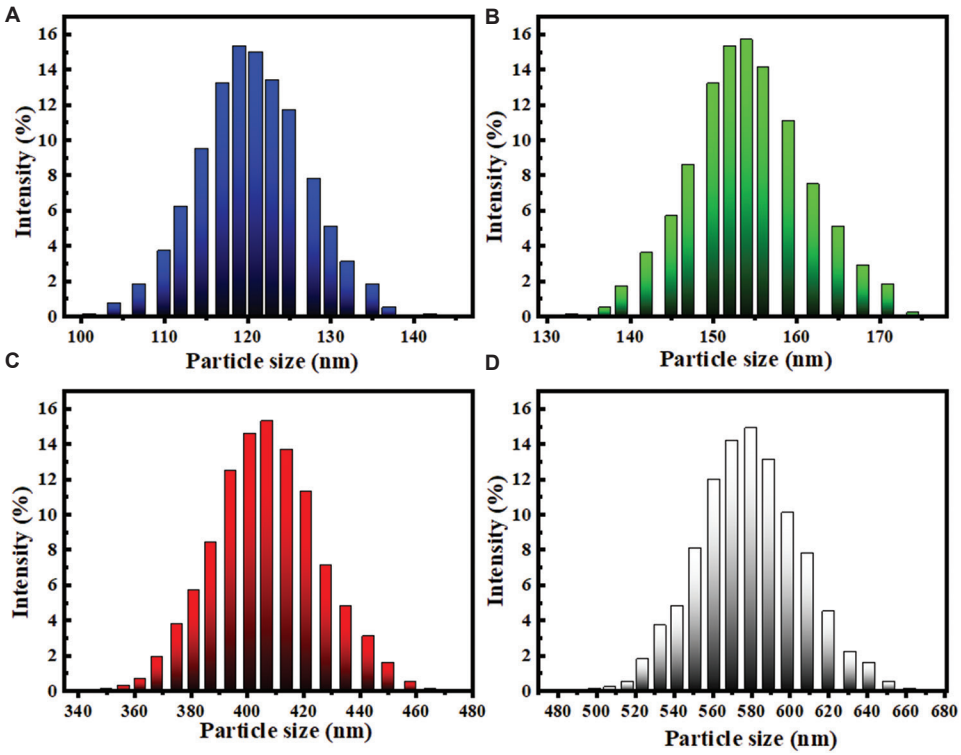

Figure 4. Particle size distribution of GNPs cross-linked (25% v/v glutaraldehyde) with varying GNP: tramadol. GNP-to-tramadol ratios of (A) 1:5, (B) 1:10, (C) 1:50, and (D) 1:100.
Abbreviation: GNPs: Gelatin nanoparticles.

0.330, suggesting a moderate level of polydispersity. This is typical for nanosystems incorporating natural biosurfactants, where some variability in size is expected. Despite this, the polydispersity index value remained within an acceptable range for stable colloidal dispersions, and the formulation demonstrated good potential for drug delivery applications.

3.3. Cross-linking degree and swelling index studies

The drug release rate depends on the swelling index and the degree of dissolution of the drug release system in the body. Gelatin, as a water-soluble substance, dissolves quickly inside the digestive system. Therefore, to reduce the dissolution of gelatin, it is necessary to use a cross-linking agent, which will lead to a continuous and prolonged drug release. In this study, glutaraldehyde was used as a cross-linking agent. Glutaraldehyde, one of the most well-known and strongest available cross-linkers, is widely used for medicinal applications. This cross-linker can completely bind to gelatin. Glutaraldehyde has an aldehyde functional group (–CHO), which reacts with the amino group ($–NH_2$) of gelatin and forms an aldimine bond (CH=N). This reaction increases the stability of gelatin and reduces its dissolution in aqueous environments.

The ninhydrin test was used to evaluate the cross-linking degree of GNPs with 8 and 25% v/v glutaraldehyde. Ninhydrin is used to detect free amine groups in amino acids, peptides, and proteins. Ninhydrin is a yellow powder that changes to

dark blue or purple due to a reaction with amine groups. Ninhydrin reacts with amino acids, releasing them in the form of ammonia and aldehyde compounds (RCHO), and turning itself into hydrindantin. Then, in the presence of ammonia, hydrindantin reacts with ninhydrin, producing a purple product.[29] As observed in **Figure 5A**, the uncross-linked gelatin has completely turned purple, indicating the presence of free amino acid groups, which are obtained through the reaction with ninhydrin. However, GNPs cross-linked with different percentages of glutaraldehyde did not change color, which indicates the absence of free amino groups in these samples. As a result, it is proven that the amino groups of GNPs completely reacted with the aldehyde group of glutaraldehyde, resulting in the attainment of the aldimine group, and confirming the cross-linking of GNPs with glutaraldehyde.

In addition, the UV-vis spectrum obtained from the ninhydrin test for uncross-linked gelatin and cross-linked GNPs is provided in **Figure 5B**. The UV-vis spectrum of uncross-linked gelatin has an absorption peak at 580 nm, indicating the presence of free amine groups. In contrast, this peak was absent in the cross-linked GNPs. Moreover, increasing the cross-linker percentage made the GNPs' structure denser, as proven through the evaluation of the swelling index. The higher the cross-linker percentage, the denser the structure and the lower the swelling index of the GNPs (**Figure 5C**).

Another parameter that influenced the drug release process is the swelling index of GNPs. The slow release of drugs from GNPs involves water penetration into the NPs, swelling of the matrix, and subsequent release of the water and drug from the swollen structure. To evaluate the swelling index, cross-linked GNPs were placed in PBS solution for 24 h at 37°C and a pH of 7.4. The swelling percentage was calculated using Equation II, with the results presented in **Figure 5C**. After 24 h, the swelling index decreased with an increase in cross-linker percentage. The index of GNPs cross-linked with 25% v/v glutaraldehyde (798%) was lower than that of 8% v/v glutaraldehyde (1,030%). GNPs swell as a result of absorbing water, termed hydration.[30] During the cross-linking stage of gelatin, bridges are formed between polymer chains, and the degree of cross-linking influences the swelling index. Therefore, GNPs that are more cross-linked have a denser structure. Such a structure can prevent the rapid penetration of the solvent into GNPs. As a result, the swelling index and release rate can be controlled by changing the degree of cross-linking (by changing the cross-linker concentration or cross-linking time).

3.4. Evaluation of drug load, nanoparticle yield, and entrapment efficiency of the drug delivery system

The effect of GNP-to-tramadol ratios and glutaraldehyde percentage on drug load, NP yield, and entrapment efficiency was investigated, and the obtained results are presented in **Figure 6**. For this purpose, the percentage of glutaraldehyde was kept constant at 25% v/v, and different GNP-to-tramadol ratios (1:5, 1:10, 1:50, and 1:100) were investigated (**Figure 6A**). By increasing the GNP-to-tramadol ratio from 1:5 to 1:100, the drug load parameter significantly decreased from 21.2% to 0.76%. When a large amount of drug is present in the loading system, it causes the formation of a drug agglomeration in the PBS solution, which is unable to penetrate the cross-linked network of GNPs. Therefore, increasing the amount of drug in the loading system leads to larger drug

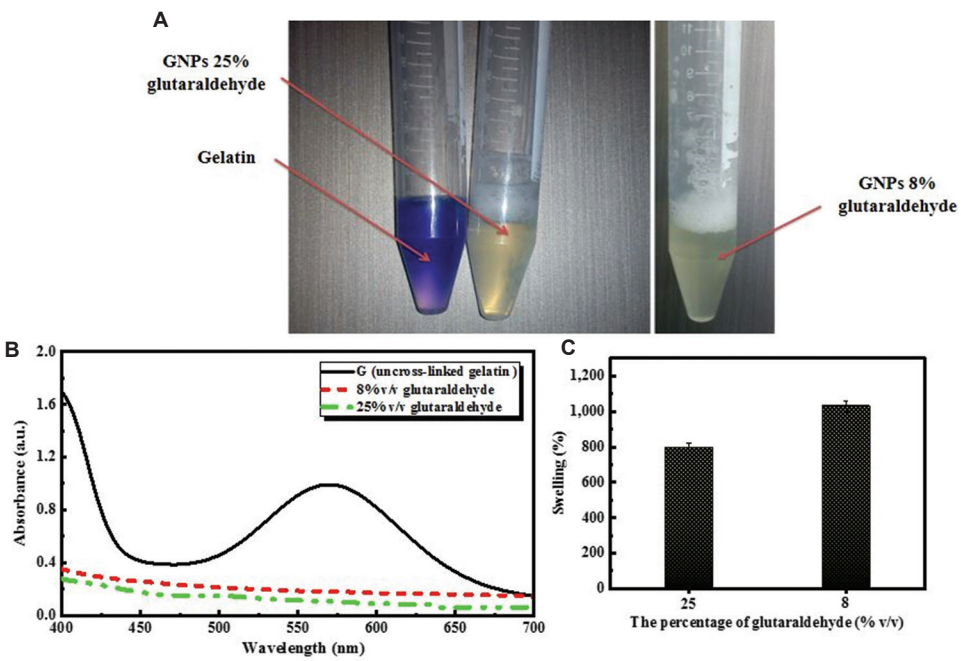

Figure 5. Characterization of uncross-linked and cross-linked GNPs using the ninhydrin test, UV-Vis spectroscopy, and swelling index. (A) Ninhydrin test of uncross-linked gelatin (G) and cross-linked GNPs with concentrations of 8% and 25% v/v glutaraldehyde. (B) UV-Vis spectroscopy spectrum of the samples after reaction with ninhydrin. (C) The swelling index of cross-linked GNPs with 8% and 25 % v/v glutaraldehyde for 24 h.
Abbreviations: GNPs: Gelatin nanoparticles; UV-Vis: Ultraviolet-visible.

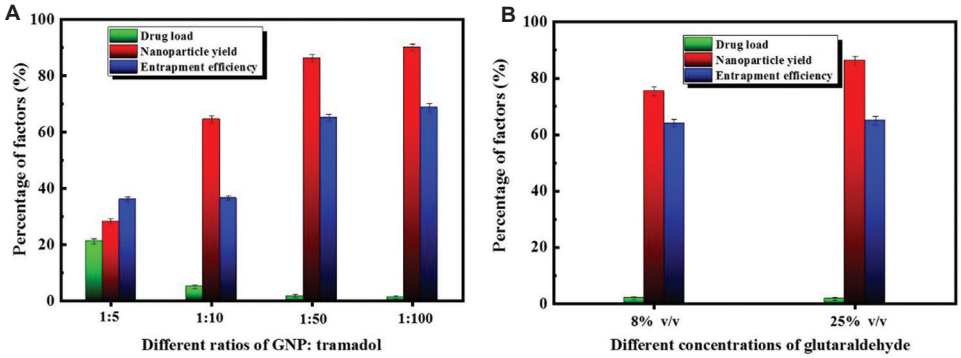

Figure 6. Drug load, nanoparticle yield, and entrapment efficiency for (A) different ratios of GNP: tramadol with 25% v/v glutaraldehyde, and (B) 1:50 ratio of GNP: tramadol with 8% and 25% v/v glutaraldehyde.
Abbreviation: GNP: Gelatin nanoparticle.

agglomerates and a reduced amount of drug loaded into the NPs. The NP yield parameter significantly increased with higher tramadol content. NP yields for ratios 1:5, 1:10, 1:50, and 1:100 were 28.3%, 64.5%, 86.3%, and 90.1%, respectively. In addition, entrapment efficiency also improved as the drug amount increased, due to a sufficient drug quantity available during synthesis. For ratios 1:5 and 1:10, entrapment efficiency remained at 36.5%, indicating considerable drug loss during synthesis. However, at ratios 1:50 and 1:100, entrapment efficiency rose to 65.1% and 68.8%. Therefore, increasing the GNP-to-tramadol ratio negatively affected drug load but improved NP yield and entrapment efficiency. Previous studies reported maximum NP yield (49%) and drug load (6.3%) at ratios of 1:100 and 1:5, which are lower compared to the current study. This improvement is likely due to the use of the emulsification method and *Yucca* extract as a surfactant during the synthesis of the drug delivery system.

In addition, the effect of cross-linking with 8% and 25% v/v glutaraldehyde was investigated while keeping the GNP-to-tramadol ratio constant (1:50). As shown in **Figure 6B**, the increase in glutaraldehyde did not significantly affect drug load and entrapment efficiency. Increasing the cross-linker agent concentration from 8% to 25% v/v reduced drug load insignificantly from 1.7% to 1.5% and increased the entrapment efficiency from 64% to 65%. Increasing the glutaraldehyde content leads to a higher cross-linking density. As the cross-linking density increases, the polymer network becomes less capable of accommodating the entire volume of liquid, resulting in the expulsion of solvent from the gel by contraction, a phenomenon known as syneresis. Consequently, drug load decreases, likely due to the drug being released from the GNPs during this process. However, increasing the cross-linker percentage can increase the NP yield from 75% to 86%, which can be attributed to the formation of a denser cross-linked network in the gelatin structure that effectively prevents drug loss during synthesis.[31]

3.5. Tramadol release profile

During the synthesis of the drug delivery system, the drug molecules penetrate the GNP matrix through the hydration of the polymer structure. This hydration allows the drug to either form a polyionic complex with GNPs or remain unbound within the GNP matrix. In the first case, where the drug forms a complex, release occurs through the destruction of the GNP matrix. In the second case, the uncomplexed drug molecules within the matrix are released more rapidly as the GNPs degrade, creating an explosive release effect in the initial release profile of the drug. This burst release can be undesirable for controlled drug delivery,[32] so strategies must focus on mitigating this phenomenon to achieve sustained release profiles. Therefore, evaluating drug release in such systems involves optimizing parameters such as cross-linker concentration and GNP-to-tramadol ratio. Hence, the release profiles of tramadol from GNPs were compared with those of commercial samples available in the market.

3.5.1. Effect of GNP: tramadol ratios and glutaraldehyde concentration on tramadol release

To investigate the effect of GNP-to-tramadol ratios on drug release in PBS solution (pH = 7.4 and 37°C), the cross-linker agent was fixed at 25% v/v, and different GNP-to-tramadol ratios (1:5, 1:10, 1:50, and 1:100) were evaluated. The results are presented in **Figure** 7. Drug concentration had a significant effect on drug release from the NPs. As shown in **Figure 7A**, in the first 30 min, all GNP-to-tramadol ratios showed a burst release, potentially due to the presence of tramadol molecules on the surface of GNPs. A higher amount of drug load corresponded to a higher drug release rate. The ratios of 1:100 and 1:50 released 96.6% and 86.6% of the drug within 480 min, respectively. The high drug release rate in these two ratios makes them unsuitable for slow or controlled drug delivery. In contrast, the GNP-to-tramadol ratios of 1:10 and 1:5 exhibited about 75.6% and 70.6% of drug release within 480 min. The decrease in drug release rate with increasing amounts of loaded drug can be attributed to the swelling of GNPs with high drug concentrations. Higher percentages of drug load cause more NPs to swell. Therefore, the release rate can be controlled by adjusting the drug concentration. The results also showed that all the samples exhibited similar release patterns independent of tramadol concentration.

Another parameter that can affect the release of tramadol from GNPs is the percentage of cross-linker, which determines the density of the cross-linked structure. To evaluate the effect of the concentration of cross-linker, the release of tramadol from

the cross-linked GNPs with 8% and 25% v/v glutaraldehyde (GNP: tramadol was 1:5) was measured. The results are presented in **Figure 7B**. In general, the release of tramadol decreased with an increase in the percentage of glutaraldehyde from 8% to 25% v/v. After 480 min, GNPs cross-linked with 8% and 25% v/v glutaraldehyde demonstrated 86% and 75% of tramadol release, respectively. This difference could be attributable to the denser structure of the cross-linked GNPs with 25% v/v glutaraldehyde compared to 8% v/v. Glutaraldehyde establishes cross-links between gelatin molecules; hence, it is expected that the drug release rate will decrease with increasing concentrations of glutaraldehyde.

A high drug release rate in a short time indicates a burst effect in the drug delivery system, implying uncontrolled release and undesirable drug delivery. However, the obtained results did not show a burst effect in the GNP drug delivery system-based drug release due to cross-linking and swelling control of GNPs. According to the results obtained in **Figure 7,** cross-linked GNPs with 25% v/v glutaraldehyde and a GNP: tramadol of 1:5 are the most optimal parameters for the drug delivery system, as they exhibited a more continuous and controlled release than the other samples.

3.5.2. Tramadol release from GNPs and commercial sample

To compare the performance of the drug delivery system, the release of tramadol from optimized GNPs (GNP: tramadol=1:5 and 25% v/v glutaraldehyde) was compared with a commercial tablet called tramadol DP sustained release 100 mg. Results are presented in **Figure 8**. For the commercial sample, nearly 30% of the drug was released within 60 min, and almost 100% of the drug was released within 420 min. Although the release in the commercial sample is similar to the optimized GNPs, this drug delivery system released a lower percentage of drugs within 420 min (about 65%). Comparing the release profiles of the GNPs with the commercial sample showed that the slow release of tramadol from the drug delivery system is suitable.

3.6. Controlled tramadol release mechanism and release kinetics

Gelatin swells upon exposure to water due to interactions between water molecules and the gelatin chains. In swelling-controlled drug delivery systems, the amount of water absorbed by the NPs determines the drug release rate. When tramadol-loaded GNPs are placed in an aqueous solution, water molecules penetrate the gelatin matrix, expanding its three-dimensional network. The permeated water subsequently disrupts the gelatin chains, releasing the encapsulated tramadol.[33] **Figure 9** shows the release mechanism of tramadol from GNPs.

Tramadol release from GNPs can be controlled using parameters such as the drug load and cross-linker percentage. Drug release depends on the swelling index of GNPs; the lower the swelling index, the more controlled and reduced the release of tramadol. In addition, the swelling index is dependent on the degree of cross-linking; the higher the degree of cross-linking, the denser the structure will be, and, as a result, the swelling index is reduced (enabling controlled drug release). Therefore, the cross-linker agent has a direct effect on the

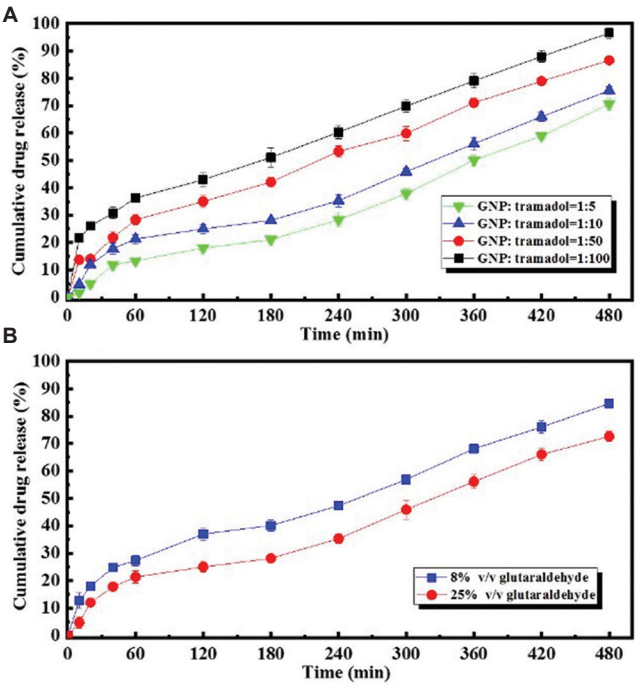

Figure 7. Tramadol release profiles. Release profile in (A) different ratios of GNP: tramadol, with glutaraldehyde concentration of 25% v/v, and (B) 8% and 25% v/v glutaraldehyde concentration with GNP: tramadol of 1:5 in phosphate-buffered saline at pH = 7.4 and 37°C.
Abbreviation: GNP: Gelatin nanoparticle.

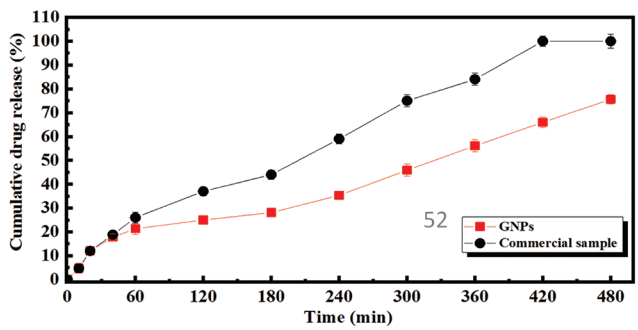

Figure 8. Tramadol release profile from optimized GNPs (GNP: tramadol=1:5 and 25% v/v glutaraldehyde) and commercial tablet (tramadol DP sustained release 100 mg), in phosphate-buffered saline at pH = 7.4 and 37°C.
Abbreviation: GNP: Gelatin nanoparticle.

internal molecular structure of the GNP network.[34] In addition, an increase in the amount of tramadol loaded raises the release rate of the drug from GNPs due to the expansion or opening of the gelatin structure. Therefore, the release of tramadol can be optimized or controlled through the degree of cross-linking and the amount of drug loaded.

In this research, four drug release kinetic models were investigated. To determine the best-fitting tramadol release model, the drug release data were plotted according to each model. A linear slope in these plots indicates compatibility with the release behavior. For this purpose, data obtained from the *in vitro* release study were fitted to four mathematical

kinetics models of zero-order, first-order, Higuchi, and Korsmeyer–Peppas, with results as presented in **Figure 10**. **Table 1** summarizes the correlation coefficients (R^2) and the release rate constants. The model with the highest R^2 value is considered suitable for the drug release process. The *in vitro* tramadol release profile best fitted the first-order model with the highest correlation coefficient R^2, indicating that the driving mechanism of drug release is non-Fickian.[35] The n value (0.5425) obtained from the Korsmeyer–Peppas model was more than 0.45. An $n = 0.45$ release corresponds to Fickian diffusion, while when $0.45<n<1$, the release mechanism is termed as non-Fickian.[35] Therefore, the mechanism of

Table 1. Correlation coefficients and release rate constants of different kinetic models

Kinetics models		Sample				
	GNP: tramadol	1:5	1:10	1:50	1:100	1:50
	Glutaraldehyde	25% v/v	25% v/v	25% v/v	25% v/v	8% v/v
Zero-order	K	36.031	27.361	18.915	13.501	26.258
	R^2	0.6176	0.6943	0.7469	0.7711	0.7557
First-order	K	1.9248	1.963	1.975	1.996	2.001
	R^2	0.9526[a]	0.9686[a]	0.9466[a]	0.9587[a]	0.999[a]
Higuchi	K	13.900	5.437	0.047	4.962	4.905
	R^2	0.8868	0.9288	0.9286	0.9270	0.9540
Korsmeyer–Peppas	n	0.4850	0.4790	0.5425	0.6840	0.4730
	R^2	0.8980	0.9196	0.8975	0.9147	0.9444

Note: [a]indicates the highest R^2 value across the parameters. K refers to the release rate constant, and R^2 refers to the correlation coefficient.

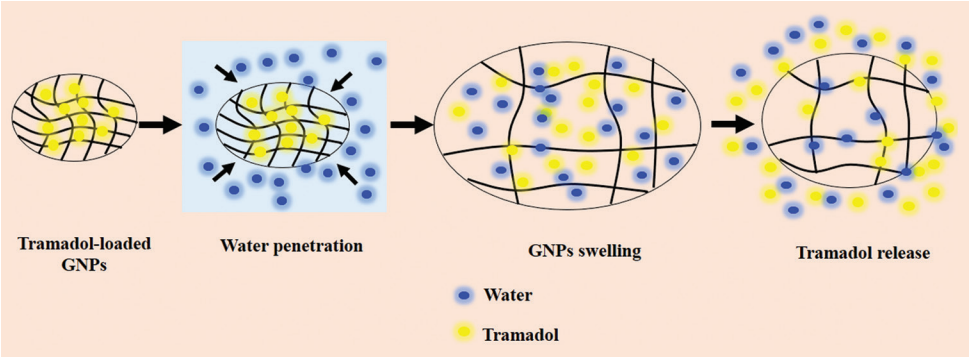

Figure 9. Release mechanism of tramadol from swollen GNPs. Image created with Microsoft PowerPoint.
Abbreviation: GNPs: Gelatin nanoparticles.

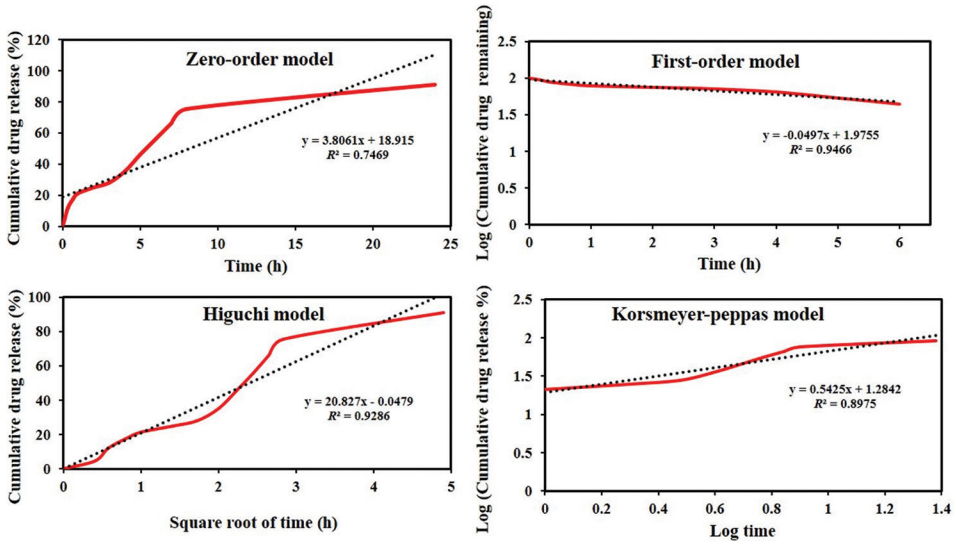

Figure 10. Plot of different kinetic release models of tramadol release from GNPs (25% glutaraldehyde, GNP: tramadol = 1:50, with 24 h of cross-linking time).
Abbreviation: GNPs: Gelatin nanoparticles.

tramadol release from the NPs was non-Fickian. In addition, different concentrations of glutaraldehyde and GNP-to-tramadol ratios were evaluated for all models, with the first-order model consistently providing the best fit. This suggested that the drug was adsorbed onto the surface of the GNPs and entrapped within these particles. Following the first-order model, the Higuchi and Korsmeyer–Peppas models yielded higher R^2 values than the zero-order model.

The controlled release of tramadol from GNPs occurs gradually, ensuring drug release over a prolonged period. This controlled release primarily depends on several factors. First, tramadol, as a polar drug with hydroxyl and amine groups, can interact with the gelatin matrix through hydrogen bonds and electrostatic interactions. These interactions help to retain the drug in the matrix, preventing rapid release and enabling a gradual release of the drug over time. In addition, *Yucca* extract, rich in amphiphilic saponins, can modify the matrix structure by affecting its hydration and porosity. This change in matrix structure leads to a controlled drug release. The release kinetics followed a first-order model, showing that drug release is concentration-dependent and gradually decreases with time. The combination of tramadol interactions with gelatin, the effect of *Yucca* extract, and the structural properties of the matrix plays a role in the controlled and sustained release of the drug.

4. Conclusions

Tramadol-loaded GNPs were prepared for the first time using *Yucca* extract as a surfactant, with a W/O emulsion process. FTIR spectral analysis and ninhydrin test results confirmed complete cross-linking of GNPs prepared with 8% and 25% v/v glutaraldehyde. FESEM images revealed that the GNPs had a uniform spherical structure without cracks. In addition, increasing the ratio of GNP-to-tramadol from 1:5 to 1:100 resulted in a decrease in drug load from 21.2% to 0.76% while increasing the NP yield and entrapment efficiency from 28.3% to 90.1% and 36.5% to 68.8%, respectively. Increasing the percentage of cross-linkers from 8% to 25% v/v increased the NP yield and entrapment efficiency from 75% to 86% and 64% to 65%, while drug load decreased slightly from 1.7% to 1.5%. The results of drug release experiments showed that tramadol release can be controlled and optimized using the swelling index. The swelling index is dependent on the cross-linking degree (dependent on glutaraldehyde concentration) and the GNP-to-tramadol ratio. Tramadol-loaded GNPs synthesized with 25% v/v glutaraldehyde and a GNP-to-tramadol ratio of 1:5 exhibited controlled behavior release, following a first-order kinetic model.

Although this study successfully demonstrated the formulation and short-term controlled release of tramadol-containing GNPs using *Yucca* extract, several limitations should be noted. First, the release behavior was only studied *in vitro*, and no pharmacokinetic or pharmacodynamic evaluations have been performed *in vivo*. Furthermore, the role of *Yucca* as a biosurfactant, although promising, would benefit from more detailed mechanistic studies and comparisons with other natural and synthetic surfactants. Future works are required to focus on conducting extensive stability tests, evaluating the biocompatibility and efficacy of the drug in animal models, and investigating the feasibility of a scale-up production. These efforts will help validate the clinical relevance and practical application of the developed drug delivery system.

Acknowledgement
None.
Financial support
This project was financially supported by the Materials and Energy Research Center (MERC, Iran) under the project number 771395064.
Conflicts of interest statement
The authors declare no competing interests.
Author contributions
Conceptualization: AH and MH; *Data curation*: AH, RA, and MB; *Methodology:* RA; *Supervision*: AA; *Writing – original draft:* RA, MB, and AB; *Writing – review & editing:* RA, MH, MB, AB, and NN. All authors read and approved the final manuscript.
Ethics approval and consent to participate
Not applicable.
Consent for publication
Not applicable.
Availability of data
All data analyzed have been presented in the paper.

References

1. Grond S, Sablotzki A. Clinical pharmacology of tramadol. *Clin Pharmacokinet.* 2004;43(13):879-923. doi: 10.2165/00003088-200443130-00004/METRICS
2. Bahoor A, Ahmadi R, Heydari M, Bagheri M, Behnamghader A. Synthesis and evaluation of cross-linked gelatin nanoparticles for controlled release of an anti-diabetic drug: Gliclazide. *Inorg Chem Commun.* 2023;154:110856. doi: 10.1016/J.INOCHE.2023.110856
3. Ghanbari M, Salavati-Niasari M, Mohandes F. Injectable hydrogels based on oxidized alginate-gelatin reinforced by carbon nitride quantum dots for tissue engineering. *Int J Pharm.* 2021;602:120660. doi: 10.1016/J.IJPHARM.2021.120660
4. Zhang M, Li Y, Wang L, Li S. Compatibility and mechanical properties of gelatin-filled polybutylene succinate composites. *J Appl Polym Sci.* 2020;137(29):48881. doi: 10.1002/APP.48881
5. Xie Z, Shen J, Sun H, Li J, Wang X. Polymer-based hydrogels with local drug release for cancer immunotherapy. *Biomed Pharmacother.* 2021;137:111333. doi: 10.1016/J.BIOPHA.2021.111333
6. Giuliano E, Paolino D, Fresta M, Cosco D. Drug-loaded biocompatible nanocarriers embedded in poloxamer 407 hydrogels as therapeutic formulations. *Medicines (Basel).* 2018;6(1):7. doi: 10.3390/MEDICINES6010007
7. Nezafati N, Farokhi M, Heydari M, Hesaraki S, Nasab NA. *In vitro* bioactivity and cytocompatablity of an injectable calcium phosphate cement/silanated gelatin microsphere composite bone cement. *Compos Part B Eng.* 2019;175:107146. doi: 10.1016/J.COMPOSITESB.2019.107146
8. Seera SDK, Kundu D, Gami P, Naik PK, Banerjee T. Synthesis and characterization of xylan-gelatin cross-linked reusable hydrogel for the adsorption of methylene blue. *Carbohydr Polym.* 2021;256:117520. doi: 10.1016/J.CARBPOL.2020.117520
9. Deng L, Li Y, Feng F, Wu D, Zhang H. Encapsulation of allopurinol by glucose cross-linked gelatin/zein nanofibers: Characterization and

release behavior. *Food Hydrocoll.* 2019;94:574-584.
doi: 10.1016/J.FOODHYD.2019.04.004
10. Jafari SM, Assadpoor E, He Y, Bhandari B. Re-coalescence of emulsion droplets during high-energy emulsification. *Food Hydrocoll.* 2008;22(7):1191-1202.
doi: 10.1016/J.FOODHYD.2007.09.006
11. Kandasamy R, Rajasekaran M, Venkatesan SK, Uddin M. New trends in the biomanufacturing of green surfactants: Biobased surfactants and biosurfactants. *ACS Symp Ser.* 2019;1329:243-260.
doi: 10.1021/BK-2019-1329.CH011
12. Ralla T, Salminen H, Tuosto J, Weiss J. Formation and stability of emulsions stabilised by *Yucca* saponin extract. *Int J Food Sci Technol.* 2018;53(6):1381-1388.
doi: 10.1111/IJFS.13715
13. Khan H, Shukla RN, Bajpai AK. Genipin-modified gelatin nanocarriers as swelling controlled drug delivery system for *in vitro* release of cytarabine. *Mater Sci Eng C.* 2016;61:457-465.
doi: 10.1016/J.MSEC.2015.12.085
14. Houshyari A, Heydari M, Bagheri M, Nezafati N. Preparation of gelatin nanoparticles by a water-in-oil emulsion method for water-soluble model drug encapsulation. *Mater Today Proc.* 2018;5(7):15800-15805.
doi: 10.1016/J.MATPR.2018.05.077
15. Uenobe F, Nakamura SI, Miyazawa M. Antimutagenic effect of resveratrol against Trp-P-1. *Mutat Res Mol Mech Mutagen.* 1997;373(2):197-200.
doi: 10.1016/S0027-5107(96)00191-1
16. Adu AA, Neolaka YAB, Riwu AAP, Iqbal M, Darmokoesoemo H, Kusuma HS. Synthesis, characterization and evaluation of swelling ratio on magnetic p53-poly(MAA-co-EGDMA)@GO-Fe3O4 (MIP@GO-Fe3O4)-based p53 protein and graphene oxide from Kusambi wood (*Schleichera oleosa*). *J Mater Res Technol.* 2020;9(5):11060-11068.
doi: 10.1016/J.JMRT.2020.08.003
17. Wei Y, Chang YH, Liu CJ, Chung RJ. Integrated oxidized-hyaluronic acid/collagen hydrogel with β-TCP Using proanthocyanidins as a crosslinker for drug delivery. *Pharmaceutics.* 2018;10(2):37.
doi: 10.3390/PHARMACEUTICS10020037
18. Okeyo PO, Rajendran ST, Zór K, Boisen A. Sensing technologies and experimental platforms for the characterization of advanced oral drug delivery systems. *Adv Drug Deliv Rev.* 2021;176:113850.
doi: 10.1016/J.ADDR.2021.113850
19. England CG, Miller MC, Kuttan A, Trent JO, Frieboes HB. Release kinetics of paclitaxel and cisplatin from two and three layered gold nanoparticles. *Eur J Pharm Biopharm.* 2015;92:120-129.
doi: 10.1016/J.EJPB.2015.02.017
20. Pal A, Bajpai J, Bajpai AK. Easy fabrication and characterization of gelatin nanocarriers and *in vitro* investigation of swelling controlled release dynamics of paclitaxel. *Polym Bull.* 2018;75(10):4691-4711.
doi: 10.1007/S00289-018-2291-4/METRICS
21. Hyun K, Lee J, Kang S, Kwon Y. Effect of the protection layer formed by cross-linked gelatin on the stability and performance of glucose and oxygen fuel cells. *J Energy Chem.* 2021;61:155-162.
doi: 10.1016/J.JECHEM.2021.01.033
22. Cebi N, Dogan CE, Mese AE, Ozdemir D, Arıcı M, Sagdic O. A rapid ATR-FTIR spectroscopic method for classification of gelatin gummy candies in relation to the gelatin source. *Food Chem.* 2019;277:373-381.
doi: 10.1016/J.FOODCHEM.2018.10.125
23. Ammar HO, Ghorab M, El-Nahhas SA, Kamel R. Polymeric matrix system for prolonged delivery of tramadol hydrochloride, part I: Physicochemical evaluation. *AAPS PharmSciTech.* 2009;10(1):7-20.
doi: 10.1208/S12249-008-9167-0
24. Kim SW, Park SK, Kang SI, *et al.* Hypocholesterolemic property of *Yucca schidigera* and *Quillaja saponaria* extracts in human body. *Arch Pharm Res.* 2003;26(12):1042-1046.
doi: 10.1007/BF02994756/METRICS
25. Cordeiro N, Faria M, Abraham E, Pothan LA. Assessment of the changes in the cellulosic surface of micro and nano banana fibres due to saponin treatment. *Carbohydr Polym.* 2013;98(1):1065-1071.
doi: 10.1016/J.CARBPOL.2013.07.002
26. Subara D, Jaswir I, Alkhatib MFR, Noorbatcha IA. Synthesis of fish gelatin nanoparticles and their application for the drug delivery based on response surface methodology. *Adv Nat Sci Nanosci Nanotechnol.* 2018;9(4):045014.
doi: 10.1088/2043-6254/AAE988
27. Pal A, Kumar Bajpai A, Bajpai J. Study on facile designing, swelling properties and structural relationship of gelatin nanoparticles. *J Macromol Sci Part A.* 2019;56(3):206-214.
doi: 10.1080/10601325.2019.1565542
28. Ray S, Mishra A, Mandal TK, Sa B, Chakraborty J. Optimization of the process parameters for the fabrication of a polymer coated layered double hydroxide-methotrexate nanohybrid for the possible treatment of osteosarcoma. *RSC Adv.* 2015;5(124):102574-102592.
doi: 10.1039/C5RA15859A
29. Lai JY. Interrelationship between cross-linking structure, molecular stability, and cytocompatibility of amniotic membranes cross-linked with glutaraldehyde of varying concentrations. *RSC Adv.* 2014;4(36):18871-18880.
doi: 10.1039/C4RA01930J
30. Liu J, Liu CK, Brown EM. Development and characterization of genipin cross-linked gelatin based composites incorporated with vegetable-tanned collagen fiber (VCF). *J Am Leather Chem Assoc.* 2017;112(12):410-419.
31. Papadimitriou S, Bikiaris D. Novel self-assembled core-shell nanoparticles based on crystalline amorphous moieties of aliphatic copolyesters for efficient controlled drug release. *J Control Release.* 2009;138(2):177-184.
doi: 10.1016/J.JCONREL.2009.05.013
32. Khan S, Anwar N. Gelatin/carboxymethyl cellulose based stimuli-responsive hydrogels for controlled delivery of 5-fluorouracil, development, *in vitro* characterization, *in vivo* safety and bioavailability evaluation. *Carbohydr Polym.* 2021;257:117617.
doi: 10.1016/J.CARBPOL.2021.117617
33. Carbinatto FM, de Castro AD, Evangelista RC, Cury BSF. Insights into the swelling process and drug release mechanisms from cross-linked pectin/high amylose starch matrices. *Asian J Pharm Sci.* 2014;9(1):27-34.
doi: 10.1016/J.AJPS.2013.12.002
34. Arumugam M, Murugesan B, Sivakumar PM, *et al.* Electrospun silk fibroin and gelatin blended nanofibers functionalized with noble metal nanoparticles for enhanced biomedical applications. *Process Biochem.* 2023;124:221-234.
doi: 10.1016/J.PROCBIO.2022.11.019
35. Naeem F, Khan S, Jalil A, *et al.* pH responsive cross-linked polymeric matrices based on natural polymers: Effect of process variables on swelling characterization and drug delivery properties. *Bioimpacts.* 2017;7(3):177.
doi: 10.15171/BI.2017.21

Received: April 28, 2025
Revised: August 5, 2025
Accepted: August 6, 2025
Available online: August 27, 2025