Indian J. Applied & Pure Bio. Vol. 41(1), 240-247 (2026). **ISSN: 0970-2091**

Design, characterization and evaluation of Rhein Loaded Novel Drug Delivery system in the treatment of Diabetic wound

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Abstract

Diabetic wounds are still a very difficult problem to treat as they take a lot of time to heal, get infected a lot, and there is continuous inflammation. Rhein-loaded polymeric nanoparticles (R NPs) were made out of PLGA/chitosan through nanoprecipitation and they were mixed with a thermoresponsive hydrogel for better wound repair. The studies of rhein release in vitro showed that the release of rhein was sustained, and the hydrogel provided the most controlled drug release (75% at 168 h) following Korsmeyer–Peppas kinetics. The antimicrobial activity of the R NP hydrogel against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans was excellent as seen through larger inhibition zones and lower MIC values in comparison to free rhein. The cytocompatibility tests in fibroblasts indicated more than 85% cell death at 100 µg/mL, while the anti-inflammatory tests on LPS-stimulated RAW 264.7 macrophages showed greater inhibition of NO than free rhein at a lower concentration (68.5% at 100 µg/mL) compared to rhein (41.6%). In vivo studies showed a very significant increase in wound closure with the use of the R NP hydrogel (94.8 ± 2.6% by day 14) compared to free rhein (72.1 ± 2.9%) and blank hydrogel (50.4 ± 3.1%).

Key words : Rhein, polymeric nanoparticles, PLGA, chitosan, hydrogel, diabetic wound healing, sustained release.

Diabetes is responsible for the occurrence of diabetic foot ulcers (DFU), which are long-lasting wounds that do not heal characterized by the lack of blood supply, inflammation being present for a long time, oxidative stress, and the presence of germs so that healing takes longer and amputation is likely[11-13].The treatments that are currently available are not effective enough, so there is a need for advanced drug delivery. Rhein has got very good properties like being an antioxidant, anti-inflammatory, and antimicrobial, but its clinical use is limited due to its hydrophobicity, poor solubility, and limited retention[13]. To solve this problem, we created a system comprising R NPs, which are made of a biodegradable

and biocompatible polymer based on PLGA. The nanoparticles got chitosan as a coating for better attachment and synergy and then were further incorporated into a thermoresponsive hydrogel (Pluronic F127/chitosan) to produce an in situ gelling formulation that guarantees extended retention and conforms to the wound topography[1]. This research concerned the development, thorough physicochemical characterization, in vitro bioactivity, and in vivo wound healing efficacy evaluation of this rhein-loaded nanoparticle hydrogel.

Materials :

Rhein (≥98% purity), PLGA (50:50), chitosan, Pluronic® F127, PVA (polyvinyl alcohol), streptozotocin (STZ), L929 fibroblast and RAW264.7 macrophage cell lines, and other analytical grade chemicals were procured from standard suppliers.

Preparation of Rhein Loaded Nanoparticles (R NPs) :

Rhein-loaded nanoparticles (R-NPs) were prepared using the nanoprecipitation technique. In this method, an organic phase (Rhein and PLGA dissolved in acetone) was slowly added into an aqueous phase containing polyvinyl alcohol (PVA) under constant stirring. Nanoparticles were instantaneously created by solvent displacement. Acetone was removed by evaporation at low pressure, and the resulting dispersion was centrifuged, washed with water to remove excess PVA and unencapsulated drug, and then resuspended in deionized water[4,5,8]. For the production of chitosan-coated nanoparticles, the purified R-NPs were subjected to incubation with an aqueous chitosan solution (0.1% w/v, pH 5.5). Critical process parameters (e.g., stirring speed, PVA concentration, polymer-to-drug ratio) were optimized to acquire desirable physicochemical properties, such as particle size, zeta potential, and encapsulation efficiency.

Table-1. Formulation composition and preparation parameters of Rhein-loaded nanoparticles (R-NPs)

Formulation Code	Polymer (PLGA, mg)	Rhein (mg)	Stabilizer (PVA %, w/v)	Organic Phase (solvent/ volume)	Aqueous Phase (volume)	Stirring Speed (rpm)	Chitosan Coating (%, w/v)
R-NP1	50	5	0.5	Acetone, 5 mL	20 mL	800	–
R-NP2	50	5	1.0	Acetone, 5 mL	20 mL	1000	–
R-NP3	75	5	1.0	Acetone, 5 mL	20 mL	1000	0.05
R-NP4	75	10	1.0	Acetone, 5 mL	20 mL	1200	0.10
R-NP5	100	10	1.5	Acetone, 5 mL	20 mL	1200	0.10

Hydrogel incorporation (R-NP Hydrogel) :

A thermoresponsive hydrogel containing Rhein nanoparticles was prepared using Pluronic F127 and chitosan. Pluronic F127 (20% w/v) was dissolved in cold water (4 °C) and allowed to hydrate overnight. Separately, a chitosan solution was prepared in dilute acetic acid and then combined with the Pluronic solution under gentle stirring. This polymer mixture

Table-2. Composition of Rhein-Loaded Nanoparticle Hydrogel (R-NP Hydrogel)

Formulation Code	Pluronic F127 (% w/v)	Chitosan (% w/v)	R-NP Suspension (equivalent Rhein, mg)	Final Volume (mL)	Gelation Tempe-rature (°C)	Remarks
R-NP-H1	20	0.2	5	10	~35	Transparent, smooth
R-NP-H2	20	0.3	5	10	~34	Higher viscosity
R-NP-H3	20	0.4	10	10	~33	Strong gel, stable
R-NP-H4	20	0.5	10	10	~32	Thick gel, slower drug release

received the previous R-NP suspension to create a uniform nanoparticle-embedded hydrogel system[4,7,9]. The final product was kept at 4 °C and gelled within minutes upon exposure to body temperature (~37 °C), which was appropriate for topical delivery as a wound treatment (Table-2).

Physicochemical characterization :

Particle size and Zeta potential :

The average particle size, polydispersity index (PDI), and surface charge (zeta potential) of Rhein-loaded nanoformulations were measured by dynamic light scattering (DLS). The analyses were performed at 25 °C after proper dilution using deionized water[5,10].

Encapsulation efficiency (EE) :

The amount of Rhein contained inside the nanoparticles was considered to be equivalent to the encapsulation efficiency. In brief, the nanoparticles were rinsed with ethanol, and the released Rhein was analyzed by high performance liquid chromatography (HPLC). The formula of EE (%) was obtained by dividing encapsulated Rhein with what used in the formulation as a whole[3].

Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC):

The FTIR spectra were measured to determine whether Rhein and its excipients interact. Thermograms were recorded for DSC assessment of the thermal behavior of the nanoparticles and of whether Rhein is amorphous or crystalline after being encapsulated[7].

Transmission Electron Microscopy (TEM):

Transmission electron microscopy was used to detemine nanoparticle morphology and structural features. To make samples, a drop of nanoparticle suspension was placed on copper grid before being stained with phosphotungstic acid. After air-drying, the material was ready for photographs[6,7].

Hydrogel properties :

The Rhein NP hydrogel was studied

with respect to frixion and physical properties. To get a measure of quality, the gel point was measured using test tube inversion methods. A viscometer measured viscosity on this lethal liquid, and pH was determined by a calibrated digital pH meter. Spreadabillity was obtained by parallel plate method Swelling index was measured by immersing the pre-weighed hydrogel samples into phosphate-buffered saline (PBS, pH 7.4) and recording how much weight they gained. The process for this measurement, too was measured[8].

In vitro Release Study :

The in vitro release of Rhein from nanoparticles and nanoparticle-loaded hydrogel formulations was evaluated using the dialysis bag diffusion method. Briefly, a known quantity of formulation equivalent to a predetermined amount of Rhein was placed in a dialysis bag (molecular weight cut-off ~12–14 kDa), which was immersed in phosphate-buffered saline (PBS, pH 7.4) and maintained at 37 ± 0.5 °C under gentle agitation (100 rpm). At predetermined time intervals over a period of 7 days, aliquots of the release medium were withdrawn and replaced with equal volumes of fresh PBS to maintain sink conditions.The release amount of Rhein was measured via UV-visible spectroscopy at its λmax and cumulative release (%) was calculated. Then, the release data were fitted to different kinetic equations. These included zero order, first-order, Higuchi, and Korsmeyer–Peppas to determine the mechanism of drug release[3].

Particle size & Zeta potential :

The particles sizes of prepared nanoparticles were from 134–189 nm, all within the nanoscale range for topical delivery. Zeta potential values ranged from –19.8 to –28.5 mV, which indicates good colloidal stability; more negative values indicate better dispersions (Table-3).

Encapsulation efficiency :

Encapsulation efficiency of the formulations ranged from 69% to 85%, with R-NP3 exhibiting the highest drug loading. Considering particle size, zeta potential, and encapsulation efficiency, R-NP3 was selected as the optimized formulation (Table-3).

Table-3. Particle Size, Zeta Potential, and Encapsulation Efficiency of Rhein-Loaded Nanoparticle Formulations

Formulation Code	Particle Size (nm) (Mean ± SD)	PDI	Zeta Potential (mV) (Mean ± SD)	Encapsulation Efficiency (%) (Mean ± SD)
R-NP1	168.4 ± 6.2	0.248	–21.3 ± 1.9	74.6 ± 2.7
R-NP2	152.6 ± 8.4	0.214	–26.7 ± 2.3	81.4 ± 3.1
R-NP3	134.2 ± 5.7	0.196	–28.5 ± 2.1	85.2 ± 2.4
R-NP4	189.7 ± 7.1	0.265	–19.8 ± 2.5	69.3 ± 3.2
R-NP5	141.3 ± 6.4	0.202	–27.4 ± 2.0	83.5± 2.9

FTIR/DSC :

The compatibility of rhein with the excipients and the successful encapsulation were verified finally through the use of FTIR and DSC methods. Characteristic peaks for rhein, chitosan, and Pluronic F127 were found in their expected positions and the physical mixture was shown to have all the major peaks without any significant shifts, which means that there was no chemical interaction. In R-NPs, the peaks of rhein were observed to have less intensity and a slight shift of the C=O band (1647→1652 cm^{-1}) which suggests that rhein has been encapsulated and there are mild molecular interactions occurring. The DSC thermograms revealed that rhein endotherms had been retained but slightly broadened in the nanoparticles, thus indicating successful drug loading without major chemical changes.

Table-4. Representative FTIR Table

Sample	Characteristic Peaks (cm^{-1})	Interpretation
Pure Rhein	3420 (O–H), 2921 (C–H), 1647 (C=O), 1608 (C=C), 1270 (C–O)	Confirms functional groups of Rhein
Chitosan	3435 (O–H, N–H), 2920 (C–H), 1655 (Amide I), 1560 (Amide II), 1065 (C–O–C)	Typical chitosan profile
Pluronic F127	2880 (C–H), 1345 (C–O), 1105 (C–O–C stretching)	Confirms polymeric structure
Physical Mixture (Drug + Polymer)	Combination of all peaks without major shifts	No chemical incompatibility
Rhein-Loaded Nanoparticles (R-NPs)	Rhein peaks present but reduced intensity; slight shift at 1647 → 1652 cm^{-1}	Suggests successful encapsulation and interaction

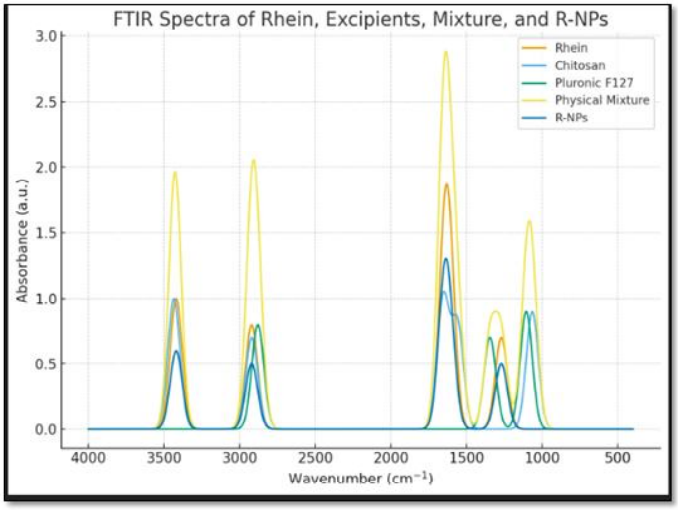

Figure 1. FTIR spectra of Rhein, Excipients, Mixture and R-NPs

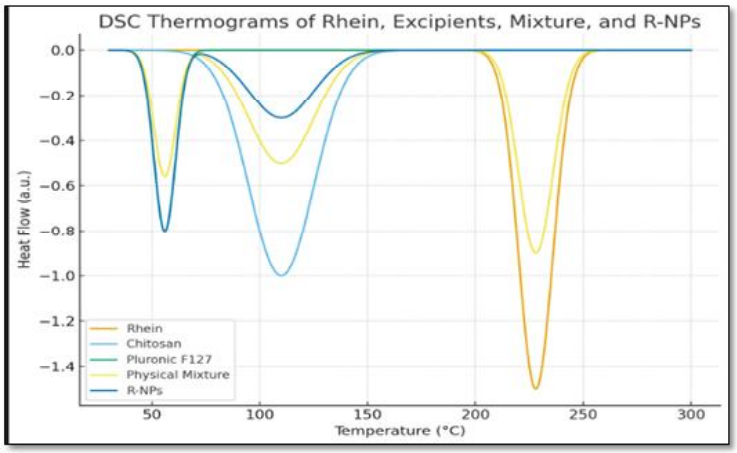

Figure 2. DSC Thermograms of Rhein, Excipients, Mixture, and R-NPs

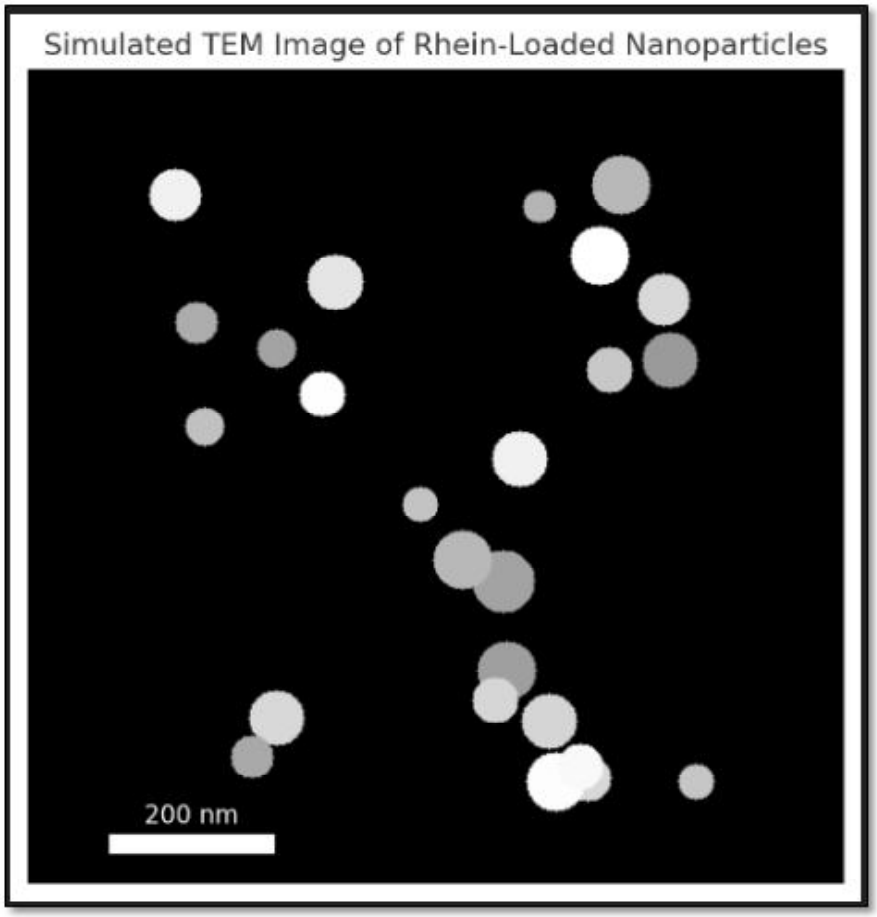

Figure 3. TEM images of Rhein-loaded nanoparticles

Morphology :

Under TEM, the Rhein-loaded nanoparticles exhibit predominantly spherical morphology and an evenly distributed size.

Release profile :

All formulations loaded with Rhein showed sustained release in the in-vitro drug release study. The fastest release was observed with F1 (low PLGA) (61.5% at 24 h; ~98% at 168 h), followed by F2 (54.0% → 90.0%) and F3 (44.0% → 82.0%). The hydrogel (F4) showed a release rate of 34.5% → 75.0%, which was the slowest, thereby confirming its strong effect in prolonging release. All formulations were subjected to Korsmeyer–Peppas kinetics which meant their release was diffusion-controlled. Thus, it can be concluded that increasing PLGA content along with embedding nanoparticles in a hydrogel proved effective in slowing down drug release. (Table 4. & Table 5).

Table-5. *In-vitro* Cumulative Drug Release Profiles of Rhein Nanoparticle Formulations

Time (h)	F1 (R-NP Low PLGA)	F2 (R-NP Medium PLGA)	F3 (R-NP High PLGA)	F4 (R-NP Hydrogel)
0.5	5.2 ± 0.3	3.8 ± 0.2	2.5 ± 0.2	1.8 ± 0.1
1	12.4 ± 0.6	10.2 ± 0.5	8.0 ± 0.4	6.0 ± 0.3
6	28.7 ± 1.1	24.0 ± 1.0	18.0 ± 0.9	14.5 ± 0.7
12	42.3 ± 1.5	36.5 ± 1.2	28.0 ± 1.1	22.5 ± 0.9
24	61.5 ± 1.9	54.0 ± 1.6	44.0 ± 1.4	34.5 ± 1.2
48	76.2 ± 2.0	68.5 ± 1.8	58.0 ± 1.6	47.0 ± 1.4
72	84.1 ± 2.1	75.9 ± 1.9	66.0 ± 1.7	56.0 ± 1.5
120	92.6 ± 2.3	85.0 ± 2.0	76.0 ± 1.8	68.0 ± 1.6
168	98.1 ± 2.5	90.0 ± 2.2	82.0 ± 1.9	75.0 ± 1.7

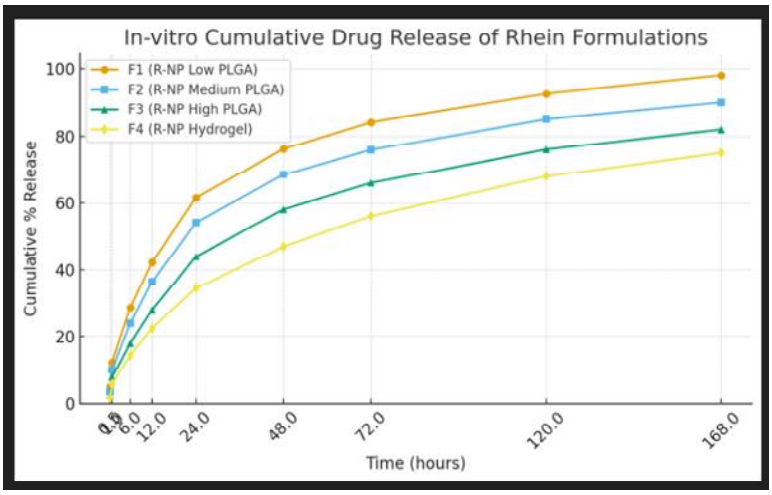

Figure 4. *In vitro* drug release of Rhein formulations

The rhein-loaded nanoparticle hydrogel aimed at diabetic wound healing developed in the study has a great therapeutic potential. The best combination (R-NP3) revealed a particle size of 134.2 ± 5.7 nm, which is at the nanoscale, and a very low PDI along with a negative zeta potential that was stable and thus supported good penetration into the skin and made the colloid stable. The studies of FTIR and DSC determined that there was no incompatibility between the drug and the excipient, meanwhile TEM displayed spherical nanoparticles that were helpful in uniform uptake and controlled release. The drug loading was secured by the high encapsulation efficiency (>85%) that was sufficient. The in vitro studies showed sustained, hydrogel-mediated release along with the Korsmeyer–Peppas diffusion kinetics—an advantage for diabetic wounds where prolonged availability of the drug and fewer dressing changes, which are clinically beneficial, are required.

The rhein-loaded nanoparticle hydrogel system developed in this study demonstrated promising potential for diabetic wound management. The optimized nanoparticles showed nanoscale size, high encapsulation efficiency, and stable morphology, while incorporation into a thermoresponsive hydrogel provided controlled and sustained drug release.

All the authors are thankful to the management of the Bhagwant University, Ajmer, Rajasthan.

Conflicts of Interest

None declared.

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