RESEARCH PAPER

Preparation And Characterization Of Naringenin-Loaded Zinc Oxide Nanoparticles For Enhanced Therapeutic Activity

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Abstract

Naringenin is a naturally occurring flavanone with antioxidant, anti-inflammatory and anticancer potential; however, its practical pharmaceutical application is constrained by poor aqueous solubility and limited oral bioavailability. The present work investigated the preparation and characterization of naringenin-loaded zinc oxide (ZnO) nanoparticles as a nano-enabled approach for improving the physicochemical and dissolution characteristics of naringenin. ZnO nanoparticles were prepared by a precipitation method using zinc acetate dihydrate as the zinc precursor and sodium hydroxide as the precipitating agent. The zinc precursor solution was treated with NaOH under continuous stirring, followed by heating, centrifugation, washing and drying. Naringenin was subsequently loaded onto the prepared ZnO nanoparticles at drug-to-nanoparticle ratios of 1:20, 1:10 and 1:5. Tween 80 was used as a stabilizer during loading. Naringenin was authenticated by appearance, melting point and spectroscopic methods. UV-visible analysis showed a maximum absorption at 289 nm in methanol, with linearity over 2–16 µg/mL and a regression equation of $y = 0.0825x - 0.1279$ ($R^2 = 0.9982$). The aqueous solubility of naringenin-loaded nanoparticles was 186.55 ± 0.21 µg/mL compared with 34.65 ± 0.29 µg/mL for pure naringenin and 47.24 ± 0.24 µg/mL for the physical mixture. The nanoparticle formulation produced 93.16 ± 2.06% cumulative drug release at 12 h, whereas pure naringenin and the physical mixture showed 32.71 ± 0.51% and 42.74 ± 1.14%, respectively. The reported optimized nanoparticle exhibited a particle size of 143.2 ± 5.6 nm, PDI of 0.270 ± 0.07 and zeta potential of −40.0 ± 0.4 mV. FTIR findings indicated retention of characteristic naringenin functional groups with slight shifts and reduced intensity, while XRD confirmed the crystalline ZnO structure and successful loading without evident impurity peaks. Overall, the findings demonstrate that ZnO nanoparticle loading markedly improved naringenin solubility and in-vitro dissolution performance, supporting further investigation of this system as a potential nano-drug delivery platform.

Keywords: Naringenin; Zinc oxide nanoparticles; Precipitation method; Nano-drug delivery; Solubility enhancement; In-vitro drug release; FTIR; XRD; Zeta potential.

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Introduction

Nanotechnology has become an important platform in pharmaceutical and biomedical research because nanoscale materials can provide high surface area, altered interfacial behavior and opportunities for improved drug delivery. Zinc oxide nanoparticles (ZnO NPs) are metal-oxide nanomaterials investigated for pharmaceutical, biomedical, cosmetic and antimicrobial applications. Their nanoscale surface characteristics can support interaction with active pharmaceutical ingredients and may influence dissolution and delivery behavior. The thesis describes ZnO as a material of interest for drug delivery and notes that ZnO nanoparticles can be prepared by chemical, physical or biological approaches.

Naringenin (4,5,7-trihydroxyflavanone) is a flavanone mainly associated with citrus fruits and occurs in glycosidic and aglycone forms. The aglycone is pharmacologically active and has been investigated for antioxidant, anti-inflammatory, anticancer, antidiabetic, cardioprotective, hepatoprotective and neuroprotective activities. Its pharmaceutical development is limited by physicochemical and pharmacokinetic barriers, particularly poor aqueous solubility, extensive metabolism and low oral bioavailability.

The present study therefore focused on preparing naringenin-loaded ZnO nanoparticles by precipitation and evaluating the resulting system using solubility, particle size, PDI, zeta potential, FTIR, XRD and in-vitro drug-release studies. The work is positioned primarily as a formulation and physicochemical characterization study; biological efficacy was not experimentally established in the thesis data supplied.

1. Rationale and Need of the Study

Naringenin's limited aqueous solubility can restrict the amount of drug available in dissolved form for absorption. The thesis reports that poor solubility, gastrointestinal degradation, first-pass metabolism and limited membrane transport contribute to reduced oral bioavailability. Nanoparticle formation can increase effective surface area and modify the physical state and interfacial properties of a drug, potentially improving dissolution. The reported literature also supports nano-delivery approaches for naringenin because of its low solubility and bioavailability.

The selected ZnO nanoparticle platform was investigated as a carrier capable of incorporating naringenin and improving its aqueous solubility and in-vitro release. The thesis results provide direct evidence for these

physicochemical improvements, while further in-vivo, cellular and safety studies would be required before therapeutic claims can be established.

Objectives

1. To synthesize ZnO nanoparticles using zinc acetate dihydrate and sodium hydroxide by the precipitation method.
2. To load naringenin onto ZnO nanoparticles and investigate drug-to-nanoparticle ratios of 1:20, 1:10 and 1:5.
3. To characterize the prepared nanoparticles using particle size, PDI, zeta potential, FTIR, DSC and XRD.
4. To evaluate the solubility and in-vitro drug-release behavior of naringenin-loaded ZnO nanoparticles in comparison with pure naringenin and physical mixture.
5. To assess whether the nanoparticle system improves the physicochemical and dissolution characteristics of naringenin.

4. Materials and Methods

4.1 Materials

Material	Role / description	Source reported in thesis
Naringenin	Active pharmaceutical ingredient	Sisco Research Laboratories Pvt. Ltd., Mumbai
Zinc acetate dihydrate	Zinc precursor	Thesis material list
Sodium hydroxide	Precipitating agent	Thesis material list
Ethanol (96%)	Organic solvent	Thesis material list
Distilled/deionized water	Aqueous medium	Thesis material list
Tween 80	Stabilizer during loading	Thesis material list

4.2 Instruments

Instrument	Application
UV-visible spectrophotometer (UV mini-1800, Shimadzu, Japan)	UV method development and drug quantification
pH meter	pH adjustment/monitoring
Magnetic stirrer / hot plate	Reaction and loading under controlled stirring/heating
Sonicator	Nanoparticle dispersion
Analytical balance	Accurate weighing
Centrifuge	Nanoparticle separation
Particle size analyzer	Particle size and PDI
ATR-FTIR	Functional-group and compatibility assessment
DSC	Thermal characterization
Powder XRD	Crystalline structure assessment
SEM	Surface morphology assessment

4.3 Authentication of Naringenin

Naringenin was visually examined for appearance, colour and odour. Melting point was determined by the capillary method, with triplicate determinations. The reported melting-point range was 251–258 °C.

4.4 UV-Visible Analytical Method

Naringenin stock solution (100 µg/mL) was prepared by accurately weighing 100 mg naringenin into 100 mL methanol followed by sonication for 15 min. The absorption maximum was determined by scanning suitable concentrations in the UV-visible range. The maximum absorption in methanol was observed at 289 nm. A calibration range of 2–16 µg/mL was reported, with the regression equation $y = 0.0825x - 0.1279$ and $R^2 = 0.9982$.

Concentration (µg/mL)	Absorbance (mean ± SD)
Concentration (µg/mL)	Absorbance (mean ± SD)
2	0.065 ± 0.010
4	0.173 ± 0.009
6	0.350 ± 0.010
8	0.520 ± 0.005
10	0.704 ± 0.006

12	0.849 ± 0.012
14	1.019 ± 0.007
16	1.214 ± 0.012

4.5 Preparation of ZnO Nanoparticles

Zinc acetate dihydrate (2.1 g) was used to prepare 100 mL of 0.1 M zinc acetate solution in ethanol (96%). A 0.2 M NaOH solution was prepared by dissolving 0.4 g NaOH in 50 mL distilled water. NaOH was added dropwise to the zinc acetate solution under continuous stirring until pH 7.0. Stirring was continued for 1–2 h at room temperature. The mixture was then heated at approximately 60–80 °C with continued stirring for 3–4 h. After cooling, the suspension was centrifuged at 10,000 rpm for 10 min. The separated nanoparticles were washed with ethanol and dried at 60–80 °C for 4–6 h or until completely dry.

4.6 Loading of Naringenin onto ZnO Nanoparticles

For each formulation, 500 mg of previously prepared ZnO nanoparticles was dispersed in 100 mL ethanol and sonicated for 10–15 min. Naringenin was added at three drug-to-ZnO ratios. Tween 80 (1% w/v as reported in the thesis) was incorporated as stabilizer. The mixtures were stirred for 1–2 h at room temperature and subsequently heated gently at 40–50 °C. The loaded nanoparticles were separated by centrifugation at 10,000 rpm for 10 min, washed with ethanol to remove unloaded naringenin and dried in a vacuum oven at 60 °C for 4–6 h or in a desiccator.

Formulation	Naringenin:ZnO ratio	Naringenin	ZnO NPs
F1	1:20	25 mg	500 mg
F2	1:10	50 mg	500 mg
F3	1:5	100 mg	500 mg

4.7 Characterization

Solubility was determined after equilibration in distilled water followed by centrifugation and filtration; filtrates were analyzed spectrophotometrically. Percentage yield was calculated from the recovered dried nanoparticle mass relative to the drug/material quantity as described in the thesis. Particle size, PDI and zeta potential were determined after dispersing nanoparticles in distilled water and sonication. FTIR spectra were recorded over 4000–400 cm^{-1} using KBr pellets. XRD was used to evaluate crystalline characteristics. The thesis also describes DSC as part of the characterization plan, although no numerical DSC result is reported in the extracted results section.

4.8 In-vitro Drug Release

In-vitro release was studied using USP dissolution apparatus II. Pure naringenin, physical mixture and naringenin-loaded nanoparticles were tested at 5 mg/mL in phosphate buffer pH 6.8 using a dialysis bag (MWCO 3500 Da). The dissolution medium volume was 900 mL, temperature 37 ± 0.5 °C and stirring speed 100 rpm. Samples were withdrawn at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 h and replaced with equal volumes of fresh medium. Samples were filtered and analyzed spectrophotometrically at the appropriate wavelength.

5. Results and Discussion

5.1 Authentication of Naringenin

The drug was described as a crystalline, solid, white to light-yellow powder with a distinct odour. The observed melting-point range was 251–258 °C, supporting the identity and crystalline nature of the supplied naringenin sample as reported in the thesis.

5.2 UV Method and Linearity

Naringenin showed maximum absorbance at 289 nm in methanol. The calibration data demonstrated a strong linear relationship over 2–16 µg/mL. The regression coefficient (R^2 = 0.9982) indicates excellent linear association within the studied range. The calibration equation can be used for spectrophotometric quantification of naringenin under the stated analytical conditions.

Parameter	Reported result
λmax in methanol	289 nm
Linear range	2–16 µg/mL
Regression equation	y = 0.0825x − 0.1279
R^2	0.9982

Figure 1 : UV-visible spectra of naringenin displaying λmax at 289 nm

5.3 Calibration in Water and PBS

The thesis additionally reports solvent-specific calibration studies. In water, the reported regression equation was y = 0.0949x + 0.082 with R^2 = 0.999. In PBS pH 6.8, the reported equation was y = 0.0694x − 0.0533 with R^2 = 0.999. These data indicate linear analytical response under the respective studied conditions.

Medium	Reported wavelength	Regression equation	R^2
Methanol	289 nm	y = 0.0825x − 0.1279	0.9982
Water	322 nm	y = 0.0949x + 0.082	0.999
PBS pH 6.8	322 nm	y = 0.0694x − 0.0533	0.999

5.4 Solubility Enhancement

A marked improvement in aqueous solubility was observed after nanoparticle formation. Pure naringenin showed a solubility of 34.65 ± 0.29 µg/mL, while the physical mixture showed 47.24 ± 0.24 µg/mL. Naringenin-loaded

nanoparticles showed 186.55 ± 0.21 µg/mL. Relative to pure naringenin, the reported nanoparticle system therefore increased measured solubility by approximately 5.38-fold. The thesis attributes this improvement to nanoparticle formation and a transition from a predominantly crystalline state toward altered/amorphous characteristics; FTIR, XRD and DSC were cited as supporting characterization techniques. The current numerical results directly establish increased measured solubility, while the precise amorphous fraction is not quantified in the supplied data.

Sample	Water solubility (µg/mL)	Approx. fold vs pure NRG
Naringenin	34.65 ± 0.29	1.00
Physical mixture	47.24 ± 0.24	1.36
Naringenin-loaded nanoparticles	186.55 ± 0.21	5.38

5.5 In-vitro Drug Release

Time (h)	Pure Naringenin (%)	Physical mixture (%)	Naringenin-loaded NPs (%)
0	0.00 ± 0	0.00 ± 0	0.00 ± 0
0.5	5.59 ± 0.61	7.24 ± 1.07	9.11 ± 1.06
1	8.47 ± 0.36	11.37 ± 1.50	23.12 ± 2.22
2	12.45 ± 0.40	16.69 ± 0.40	37.63 ± 1.21
3	16.25 ± 0.31	19.29 ± 1.67	49.36 ± 2.63
4	20.21 ± 0.51	24.21 ± 1.50	61.55 ± 2.69
5	25.23 ± 0.42	31.20 ± 2.21	69.87 ± 2.46
6	28.23 ± 0.57	34.56 ± 0.78	78.56 ± 1.76
7	29.14 ± 0.31	36.22 ± 1.22	87.19 ± 1.50
8	31.24 ± 0.62	38.39 ± 1.18	91.42 ± 2.68
10	33.45 ± 0.40	39.45 ± 1.35	94.54 ± 2.31
12	32.71 ± 0.51	42.74 ± 1.14	93.16 ± 2.06

The nanoparticle formulation demonstrated substantially higher cumulative release throughout the dissolution study. At 12 h, naringenin-loaded nanoparticles released 93.16 ± 2.06%, compared with 42.74 ± 1.14% for the physical mixture and 32.71 ± 0.51% for pure naringenin. At 8 h, the nanoparticle formulation had already reached 91.42 ± 2.68%. The improved release is consistent with the thesis interpretation that increased effective surface area and nanoscale dispersion enhanced dissolution of naringenin. The release profile also indicates that the nanoparticle system maintained a high release level between 8 and 12 h rather than showing the limited release observed for pure drug.

Figure 2. In-vitro cumulative drug-release profile of pure naringenin, physical mixture and naringenin-loaded ZnO nanoparticles in PBS pH 6.8 (37 ± 0.5 °C; 100 rpm).

5.6 Particle Size, PDI and Zeta Potential

The reported optimized naringenin-loaded nanoparticle showed a mean particle size of 143.2 ± 5.6 nm, PDI of 0.270 ± 0.07 and zeta potential of −40.0 ± 0.4 mV. The low PDI indicates a relatively narrow particle-size distribution under the reported measurement conditions. The magnitude of zeta potential is consistent with electrostatic stabilization and supports the reported interpretation of good physical stability. These characteristics are also within the nanoscale range relevant to modern drug-delivery research.

Quality attribute	Reported result
Particle size	143.2 ± 5.6 nm
PDI	0.270 ± 0.07
Zeta potential	−40.0 ± 0.4 mV

5.7 FTIR Compatibility Study

Pure naringenin showed characteristic FTIR bands attributed to O–H stretching (~3400 cm^{-1}), C–H stretching (~2900 cm^{-1}), C=O stretching (~1650 cm^{-1}), aromatic C=C stretching (~1600–1500 cm^{-1}) and C–O stretching (~1200–1000 cm^{-1}). The naringenin-loaded nanoparticle spectrum retained similar characteristic bands with slight shifts and reduced intensity. The thesis interprets these changes as evidence of successful incorporation without major chemical incompatibility. Because exact peak positions for the nanoparticle spectrum are not numerically tabulated in the thesis, the manuscript does not invent additional peak assignments.

Figure 3 : FTIR spectra of naringenin

Figure 4 : FTIR spectra of naringenin loaded nanoparticle

5.8 X-Ray Diffraction

The XRD pattern of the naringenin-loaded ZnO nanoparticles showed characteristic diffraction peaks associated with crystalline ZnO and its hexagonal wurtzite structure. Reduced intensity and slight broadening of peaks were reported after loading, while no additional impurity peaks were observed. These findings support successful formation of the ZnO nanoparticle system and loading of naringenin without evidence of major structural contamination. Exact 2θ values were not

numerically tabulated in the thesis and are therefore not reproduced here.

The literature similarly describes ZnO nanoparticles as commonly exhibiting the wurtzite crystal structure, and contemporary ZnO nanoparticle research frequently combines XRD, FTIR, particle-size analysis and microscopy for physicochemical characterization.

Figure 5 : XRD of naringenin

Figure 6 : XRD of naringenin loaded nanoparticles

6. Comparative Summary of Major Findings

Parameter	Pure NRG	Physical mixture	Naringenin-loaded ZnO NPs
Water solubility (μg/mL)	34.65 ± 0.29	47.24 ± 0.24	186.55 ± 0.21
Cumulative release at 12 h (%)	32.71 ± 0.51	42.74 ± 1.14	93.16 ± 2.06
Particle size	Not reported	Not reported	143.2 ± 5.6 nm
PDI	Not reported	Not reported	0.270 ± 0.07
Zeta potential	Not reported	Not reported	−40.0 ± 0.4 mV

7. Discussion

The central outcome of the study is the improvement in naringenin's measured aqueous solubility and dissolution following incorporation into ZnO nanoparticles. The approximately 5.38-fold increase in solubility compared with pure naringenin and the increase in 12-h release from 32.71% to 93.16% indicate a substantial formulation effect. At the nanoscale, increased surface area and improved dispersion can facilitate contact between drug particles and aqueous medium, which provides a plausible explanation for the enhanced dissolution observed in this study.

The reported particle size of approximately 143 nm and PDI of 0.270 indicate a nanosized and comparatively uniform dispersion. The zeta potential of −40 mV suggests strong surface charge and supports colloidal stability. FTIR findings indicate retention of the principal naringenin functional groups without major spectral disruption, whereas XRD confirms crystalline ZnO and the absence of evident impurity peaks. Together, the physicochemical results support successful development of a naringenin-loaded ZnO nanoparticle system.

The findings are consistent with broader research showing that nanoformulation can address naringenin's low solubility and bioavailability. Reviews of naringenin nano-delivery systems describe polymeric, lipid-based, micellar, nanosuspension, nanoemulsion and metal-oxide approaches as strategies for improving delivery characteristics. cite turn0search0 turn0search10 However, the present thesis does not contain cellular cytotoxicity, animal pharmacokinetics, in-vivo efficacy, long-term toxicity or clinical data. Therefore, the present results support physicochemical and in-vitro dissolution enhancement rather than a definitive therapeutic efficacy claim.

8. Research Novelty and Significance

The study combines naringenin, a poorly water-soluble bioactive flavanone, with ZnO nanoparticles prepared through a relatively straightforward precipitation process. The novelty of the work lies in the reported formulation-specific combination and its demonstrated improvement in solubility and dissolution. Recent literature confirms substantial interest in naringenin nanoformulation, but the available evidence includes many different carrier systems; therefore, the present ZnO-based approach adds a distinct metal-oxide nanocarrier perspective. cite turn0search0 turn0search6

For a stronger journal submission, future work should include complete formulation optimization data, drug-loading capacity, entrapment efficiency, morphology by SEM/TEM, thermal analysis, release-kinetic modelling, statistical comparison, accelerated stability and appropriate biological/safety evaluation. These are identified as recommended next steps and are not presented as completed experiments in the supplied thesis.

9. Limitations

1. The supplied thesis does not provide complete numerical data for entrapment efficiency and loading capacity
2. Although DSC is included in the methodology, a numerical DSC result/thermogram interpretation is not present in the extracted results section.
3. SEM is listed as an instrument and characterization method, but a numerical or detailed SEM morphology result is not reported in the available results text.
4. The exact 2θ peak values from XRD are not tabulated in the thesis
5. Only physicochemical and in-vitro dissolution outcomes are available; no in-vivo pharmacokinetic, pharmacodynamic, cytotoxicity or long-term toxicity results are reported
6. The formulation designated as 'optimized' is reported with particle-size/PDI/zeta-potential results, but the thesis text does not explicitly provide a statistical optimization design or clearly map that characterization result to F1, F2 or F3.

10. Conclusion

Naringenin-loaded ZnO nanoparticles were successfully prepared by the precipitation approach described in the thesis. The developed system exhibited nanoscale particle size (143.2 ± 5.6 nm), relatively low PDI (0.270 ± 0.07) and a zeta potential of −40.0 ± 0.4 mV. Most importantly, the formulation increased measured water solubility from 34.65 ± 0.29 μg/mL for pure naringenin to 186.55

± 0.21 µg/mL and increased cumulative drug release at 12 h from 32.71 ± 0.51% to 93.16 ± 2.06%. FTIR and XRD findings supported incorporation of naringenin into the ZnO nanoparticle system without major chemical incompatibility or evident impurity formation. These results establish a promising physicochemical basis for further development of naringenin-loaded ZnO nanoparticles; however, biological efficacy, safety, pharmacokinetics and long-term stability require additional experimental confirmation before therapeutic application can be claimed.

11. References

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