Curcumin-loaded chitosan nanoemulsion: Evaluation of stability and release kinetics

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ABSTRACT

Curcumin, a potent bioactive compound derived from *Curcuma longa*, exhibits significant antibacterial, antioxidant, and anti-inflammatory properties, indicating broad applications in the food and agricultural industries. However, its practical utility is constrained by inherently low water solubility, poor bioavailability, and chemical instability when exposed to environmental factors. This study addresses these limitations by nanoencapsulating curcumin within a chitosan-based nanoemulsion, curcumin-loaded chitosan nanoemulsions (CurChiNEM), formulate through an emulsification process followed by ionotropic gelation using sodium tripolyphosphate (TPP). Initial extraction from turmeric yielded 8.29% curcumin, confirmed by a maximum absorption wavelength at 425 nm. Formulation 1 (2.5 mg/mL curcumin concentration) achieved the highest encapsulation efficiency (77.82 ± 1.2%) and resulted in the smallest particle size (664 ± 0.467 nm), determined using ImageJ. Scanning electron microscopy further revealed that the formulate nanoemulsions resulted in smooth, quasi-spherical particles. Fourier transform infrared spectroscopy further confirmed the effective crosslinking of chitosan to TPP and loading of curcumin. Moreover, the formulated nanoemulsion significantly enhanced curcumin's stability, retaining 87.56% of its content after 28 days of ambient storage, 77.02% under prolonged ultraviolet light exposure, and approximately 67.71% when subjected to 100°C treatment. In contrast, free curcumin degraded rapidly under identical conditions, exhibiting near-total loss. *In vitro* release studies conducted at pH 7.4 elucidated a diffusion-controlled release mechanism, optimally described by the Higuchi release kinetics model ($R^2 = 0.9736$). These compelling findings affirm the chitosan/TPP nanoemulsion as a highly effective and promising delivery system for substantially enhancing the stability and facilitating the controlled release of curcumin, thereby broadening its potential for diverse applications.

Keywords:
Curcumin; Chitosan; Bioactive compound; Release kinetics; Stability assessment

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How to cite this article:
Lazaro LJC, Eugenio PJG, Garvida FJA, Sarong MM. Curcumin-loaded chitosan nanoemulsion: Evaluation of stability and release kinetics. *Biomater Transl.* **2025**

doi: 10.12336/bmt.25.00056

1. Introduction

Curcumin is the principal bioactive compound found in the rhizomes of turmeric (*Curcuma longa*). Extensive research has demonstrated that this substance has antibacterial, anti-inflammatory, and antioxidant properties, providing excellent applications in various fields.[1] Specifically, curcumin is highly valued in the agricultural area for its defense mechanisms against plant diseases and pests. Curcumin has been established to effectively protect crops from a range of microbiological pathogens and invasive insects that cause damage to vegetation.[2] However, the practical uses of curcumin have been limited due to its low water solubility, poor bioavailability, and chemical instability. Curcumin is also highly susceptible to metal ions, physiological pH, heat, light, and other external factors during processing and storage.[3] Among the strategies developed to overcome these challenges, encapsulation is considered one of the most promising technologies, as it can extend the shelf life of bioactive compounds by protecting them from degradation.[4-6] Furthermore, maximizing

the therapeutic and functional potential of curcumin requires its efficient delivery to specific target sites, which necessitates the use of an appropriate and reliable delivery system.

Several curcumin delivery strategies have been established, but they are not without limitations. For instance, solid dispersions and inclusion complexes (e.g., cyclodextrin and polyethylene glycol-based carriers) enhance solubility and dissolution rate but often require high polymer loads, risk recrystallization, and entail manufacturing complexity, limiting scalability and long-term stability.[7,8] High-temperature exposure in spray drying risks degradation, and spray-chilling may result in core leakage or poor encapsulation efficiency (EE).[9] There are still emerging techniques, such as deep eutectic solvents and magnetically triggered release systems, that show promise for enhanced solubility and controlled release, but still require extensive validation, especially for *in vivo* applicability and safety profiles. In this study, a chitosan/sodium tripolyphosphate (TPP) modified nanoemulsion, curcumin-loaded chitosan nanoemulsions (CurChiNEM), prepared through ionic gelation, addresses the limitations of existing systems: (i) enables high EE under gentle, aqueous processing conditions, avoiding high-temperature or organic solvents, (ii) provides enhanced thermal and storage stability, overcoming lipid phase-separation or crystallization issues common to lipid-based systems, and (iii) offers diffusion-controlled release (as evidenced by Higuchi kinetics) in a vehicle that balances sustained release performance with improved solubility and stability. These attributes differentiate CurChiNEM from conventional self-nanoemulsifying drug delivery systems, polymeric nanoparticles, and microencapsulation systems, supporting its scientific novelty and practical relevance.

Over the years, the agricultural sectors have faced various challenges, including production losses, the implications of global climate change, and the rapid depletion of natural resources.[10] Green nanotechnology can contribute to the sustainability of agricultural productivity.[11] Recently, agrochemicals based on nanoemulsions have demonstrated their capacity and efficacy to control the transport of active substances during application.[12] Nanoencapsulation through nanoemulsion has been regarded as a feasible method for the controlled delivery of bioactive substances, as it offers better loading capacity for the component and shows great potential for harnessing the therapeutic activities present against external stimuli.[13] Nanoemulsions are colloidal dispersions of two immiscible liquids stabilized using an interfacial film of surfactant and cosurfactant molecules, having a droplet size of 10–1,000 nm.[14] Water, oil, and emulsifier are the three primary components of a nanoemulsion droplet. These phases can be water-in-oil or oil-in-water droplets stabilized with an amphiphilic surfactant.[15] Nanoemulsions exhibit important roles in encapsulating sensitive substances. They enhance the stability of the component against environmental stressors, its solubility in water during application, and the bioavailability of the enclosed compound.[16]

Incorporating chitosan in nanoemulsification yields multifaceted benefits and also offers room to integrate bioactive components from botanicals for a synergistic effect. Chitosan acts as an emulsifier or emulsion stabilizer by adsorbing the protective layer at oil-water interfaces, enhancing viscosity, and interacting with surface-active agents.[17] In addition, the release kinetics of encapsulated active substances from nanoemulsions can be adjusted by varying the molecular weight, degree of deacetylation, or crosslinking of chitosan.[18] Therefore, chitosan nanoemulsion provides a feasible approach to protect curcumin from external influences that affect its stability and to improve the release kinetics for controlled distribution. In this study, curcumin extracted from turmeric was encapsulated with a chitosan-based nanoemulsion system. We aimed to investigate the effectiveness of this approach in maintaining curcumin's stability when exposed to light, time, and temperature.

2. Methods

2.1. Materials and reagents

In this study, turmeric powder (Soils and Plant Analytical Laboratory, Central Luzon State University [CLSU], Philippines) was used for curcumin extraction. Ethanol (95% purity) (Physical and Material Science Research Laboratory [PMS], CLSU, Philippines), polysorbate 20 (Tween-20) (PMS, CLSU, Philippines), low-molecular-weight chitosan (PMS, CLSU, Philippines), TPP (PMS, CLSU, Philippines), and glacial acetic acid (PMS, CLSU, Philippines) were used to prepare the nanoemulsions. Moreover, the phosphate-buffered saline (PBS) was also acquired for the release kinetics study. Solutions were prepared using distilled water. All chemicals and reagents were used without any further modifications.

2.2. Extraction of curcumin

Curcumin was extracted from turmeric powder using the method outlined by Kumar *et al.*,[19] with minor adjustments. Briefly, 100 g of powdered turmeric was dissolved in 700 mL of 95% ethanol. The solution was then transferred to an amber bottle and stored for 2 days with occasional shaking. Using filter paper (Whatman No. 1, Cytiva, USA), the mixture was filtered. It was then transferred to an amber bottle and kept in the refrigerator for 2 days. To remove the ethanol, the solution was concentrated using a rotary evaporator (DLAB RE100-Pro, DLAB Scientific, China) operating at reduced pressure in a 40°C water bath at 80 rpm. The solution was stored in a conical container covered with aluminium foil. The concentrated curcumin oleoresin (11 μL) was diluted with 5 mL of ethanol. Afterward, the resulting solution was diluted 40 times using 95% ethanol before absorbance reading. All measurements were performed in triplicate to ensure accuracy. The curcumin

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content was measured using a spectrophotometer (NanoDrop 2000C, Thermo Fisher Scientific Inc., USA) at 425 nm with 95% ethanol as a blank. The curcumin content was calculated using Equation I, considering that 0.42 absorbance at 425 nm = 0.0025 g of curcumin.

$$Curcumin\ content\ (\%) = \frac{0.0025 \times Abs \times V \times DF \times 100}{0.42 \times W(g) \times 1{,}000} \quad (I)$$

where V is the volume made up, DF is the dilution factor, W is the sample weight, and Abs is the absorbance measured at 425 nm.

2.3. Preparation of reagents

A 10% (w/w) Tween 20 solution was prepared by weighing 10 g of Tween 20 and adding distilled water until the total weight reached 100 g. Then, varying concentrations of curcumin extract were prepared before mixing with the surfactant. Separately, a chitosan solution was prepared by dissolving 0.45 g of chitosan powder in 50 mL of 1% v/v aqueous acetic acid and stirring with a magnetic stirrer (MR-HEI standard, Heidolph Instruments, Germany) for 30 min. In addition, a TPP solution was prepared by dissolving 0.45 g of TPP in 250 mL of distilled water.

2.4. Formulation of CurChiNEM

The curcumin-loaded nanoemulsion was formulate according to the methodology outlined by Chin *et al.*[20] with minor modifications. The solvent phase consisted of absolute ethanol as the organic solvent and Tween 20 surfactant as the emulsifying agent. The curcumin extracts at different concentrations were dissolved in absolute ethanol and mixed with 10% Tween 20, followed by continuous stirring for 1 h. After that, a chitosan solution was added, and the mixture was stirred for 30 min using a magnetic stirrer. Under continuous stirring, 250 mL of TPP was added dropwise to the mixture using a syringe (19G, Cosmomedical, Inc., Philippines). The final solution was then filtered using filter paper. The filtrate was then retained for additional testing, while the residue was carefully scraped off using a spatula and stored in centrifuge tubes. Following that, the formulate CurChiNEMs were freeze-dried using a freeze-drier (VaCo 2, Zirbus Technology, Germany) under −53.8°C and a pressure of 0.001 mbar. After that, a vortex mixer was used to grind the freeze-dried nanoemulsions into a fine powder. The powdered nanoemulsions were then kept in a vial for further analysis. The formulations are listed in **Table 1**.

2.5. EE of the CurChiNEM

Initial solutions for each formulation were made to calculate the EE of the nanoemulsion. The curcumin extract (1 mL) was dissolved in 39 mL of distilled water. A dilution factor of five was applied to the initial solutions and the filtrate obtained from each formulation for ultraviolet (UV)-visible light (Vis) analysis. The absorbances of the diluted solutions (initial and filtrate) were measured using a UV-Vis Spectrophotometer (UH-5100, Hitachi, Japan) and were measured in triplicate. After comparing the absorbances, the %EE was calculated using Equation II.

$$\%EE = \frac{total\ amount\ of\ curcumin - amount\ of\ free\ curcumin}{total\ amount\ of\ curcumin} \times 100 \quad (II)$$

Table 1. Nanoemulsion formulations with varying curcumin concentrations

Formulation	Curcumin concentration
Formulation 0	No curcumin
Formulation 1	2.5 mg/mL curcumin extract
Formulation 2	5.0 mg/mL curcumin extract
Formulation 3	10.0 mg/mL curcumin extract

2.6. Characterization of CurChiNEM

2.6.1. Surface morphology

The morphological structure of CurChiNEM was analyzed using a scanning electron microscope (SEM; FlexSEM 1000-S2000, Hitachi, Japan). Four samples were prepared for the characterization: three for the encapsulation formulation and a blank sample. The powder was secured onto a SEM mount using double-sided carbon adhesive tape in a vacuum setting for sample preparation. The prepared sample was positioned on the SEM stage, and images were obtained using an accelerating voltage of 5.00 kV at various working distances and magnifications. Secondary electrons were utilized to generate grayscale micrographs of the samples.

Particle size analysis was performed using ImageJ software (National Institutes of Health, USA). SEM images of the samples were captured at a magnification of 2,500 × and a working distance of 20 µm. ImageJ measurements were calibrated using the 20-µm scale bar embedded in the SEM images, and 10 manual measurements were subsequently taken on quasi-spherical particles.

2.6.2. Functional group

Fourier-transform infrared spectroscopy (FTIR) was employed to detect distinct functional groups in the CurChiNEMs. The FTIR analysis of the samples was conducted using a spectrometer equipped with a quantum-attenuated total reflectance (ATR) attachment (IRSpirit, Shimadzu Corporation, Japan). Samples were uniformly distributed on the dry ATR surface, and their infrared spectra were collected over a range of 700–4,000 cm^{-1} with a resolution of 4 cm^{-1} and 20 scans. Before examining each sample, a background spectrum was obtained utilizing identical experimental conditions. All measurements were performed at ambient temperature.

2.7. Stability assessment of CurChiNEM

2.7.1. Light stability

The light stability of curcumin extract and CurChiNEMs was determined using the method described by Li and Lu[21] with slight modifications. Curcumin extract (6.40 mL) and nanoemulsion (5 g)

were stored in a glass bottle and exposed to near-sunlight for 8 h daily over 28 days. Samples were taken from the glass bottle at designated intervals (0, 7, 14, 21, and 28 days). Solutions were

stirred for 5 min and subsequently centrifuged for another 5 min before analysis. The absorbance values obtained from the spectrophotometer were used to ascertain the percentage retention of curcumin, defined as the ratio of curcumin content retained in the sample to the initial amount. The retention % of curcumin was determined using Equation III.

$$Curcumin\ retention\ (\%) = \frac{Curcumin_{retained}}{Curcumin_{initial}} \times 100 \quad \text{(III)}$$

2.7.2. Storage stability

The storage stability of the CurChiNEMs was evaluated over 28 days, and curcumin retention was compared to that of the curcumin extract. The nanoemulsions (5 g) were placed in glass vials wrapped with aluminium foil to shield the sample from light and were maintained at room temperature (28 ± 2°C).[22] At specified intervals (0, 7, 14, 21, and 28 days), the quantity of curcumin remaining in each sample was assessed according to the methodology outlined in Equation III.

2.7.3. Temperature stability

The temperature stability of the curcumin extract and nanoemulsion at various temperatures was tested using the methodology described by Wang *et al.*,[23] with some modifications. The curcumin extract and nanoemulsion were subjected to a water bath at specified temperatures (0, 25, 50, 75, and 100°C) for 15 min. The nanoemulsion was mixed with ethanol, stirred for 5 min, and subsequently centrifuged for 5 min. The absorbance values of the curcumin extract and nanoemulsion were measured using a spectrophotometer at 425 nm, and these values were employed to calculate the percentage retention of curcumin in both samples. The percentage of curcumin retention was assessed as outlined in the initial stability study (Equation III).

2.8. *In vitro* release study of CurChiNEM

The *in vitro* release of curcumin from chitosan nanoemulsion was assessed following the approach outlined by Das *et al.*,[24] with modifications. Approximately 600 mg of the CurChiNEMs was suspended in a 12 mL PBS solution (release medium) at pH 7.4. Triplicate was prepared for a time-dependent release study at intervals of 0.0, 0.5, 1.0, 2.0, 4.0, 6.0, 12.0, 24.0, and 48.0 h. The solutions were kept at room temperature with occasional agitation using a vortex shaker. At designated time intervals, curcumin-loaded nanoparticles underwent centrifugation for 15 min, followed by spectrophotometric quantification of curcumin concentration, with absorbance measured at 425 nm. The cumulative percentage of release is calculated using Equation IV.

$$Cumulative\ release\ of\ curcumin\left(\%\right) = \frac{E}{E_o} \times 100 \quad \text{(IV)}$$

where E is the amount of curcumin extract released and E_0 is the amount of curcumin extract initially loaded.

2.9. Release kinetics

The possible release mechanism of the CurChiNEMs was identified by fitting the release data from *in vitro* experiments into various kinetic models. The models were evaluated based on the regression coefficients produced to determine the most suitable model for curcumin release. The model that most accurately aligns with the experimental data will yield the R^2 value nearest to one.[25]

2.9.1. Zero-order

In the zero-order model, the cumulative percentage of curcumin release was plotted against time. The equation is shown in Equation V.

$$Q_{(t)} = k_0 \times t_n \quad \text{(V)}$$

where $Q_{(t)}$ is the cumulative fraction of the curcumin release at time t, k_0 is the constant rate, and n is geometrically dependent.

2.9.2. First-order

The acquired data were displayed in the first-order model as a cumulative log percentage of curcumin remaining versus time. The compound is released from the formulation exponentially, as presented in Equation VI.

$$Log\ C = Log\ C_0 - \frac{K_t}{2.303} \quad \text{(VI)}$$

where C_0 is the initial curcumin concentration, t is time, and K_t is the first-order rate constant.

2.9.3. Higuchi

In the Higuchi model, the cumulative percentage of curcumin release was plotted against the square root of time and is expressed in Equation VII.

$$Q_t = \frac{K_H}{\sqrt{t}} \quad \text{(VII)}$$

where Q_t is the cumulative amount of curcumin released at time t and K_H is the Higuchi rate constant.

2.9.4. Korsmeyer-Peppas

Finally, the log of cumulative curcumin release against the log of time was plotted in this model (Equation VIII).

$$\frac{M_t}{M_\infty} = K_{KP} t^n \quad \text{(VIII)}$$

where M_t/M_∞ is the cumulative amount of the curcumin released at time t and K_{KP} is the rate constant. By determining the value of the exponent n based on the best match with experimental data (for $t < 60\%$) and the system dimension, the major physical mechanism of curcumin release will be identified.

3. Results and discussion

3.1. Curcumin extraction and yield

Curcumin was extracted from *C. longa* (turmeric) using the solvent extraction method. This procedure resulted in a concentrated viscous extract characterized by a deep reddish-brown color and an aromatic scent, as shown in **Figure 1**. The color of the concentrated extract is mainly due to curcumin and

other associated curcuminoids, including desmethoxycurcumin and bisdemethoxycurcumin, which are naturally occurring substances in turmeric. Curcumin, the primary bioactive constituent of turmeric, displays a yellow polyphenolic color in its pure state.[26] However, the color intensity is affected by the extract concentration, with higher concentrations typically displaying a darker color. The aromatic fragrance of the extract further confirms the presence of curcumin, as the aroma generally originates from essential oils and other volatile chemicals in turmeric released during extraction.[27]

The extraction process in this study yielded a curcumin of 8.29%, comparable with the literature findings. Turmeric powder usually comprises curcumin and its derivatives in concentrations of 3–15%, with curcumin constituting 71.5%, desmethoxycurcumin 19.4%, and bisdemethoxycurcumin 9.1%. The results of this study correspond with those of Kusumaningrum *et al.*,[28] who documented a curcumin concentration of 8.28% in turmeric powder. These findings also align with a study on *Curcuma xanthorrhiza*, which reported an 8.08% curcumin concentration in the starting powdered material.[29] In contrast, Kwon and Chung[30] obtained a significantly higher yield, indicating a curcumin concentration of 13.71% from turmeric. Various parameters, including the extraction method, duration, temperature, solvent type, and solvent-to-sample ratio, may affect the yield of curcumin extraction.[31]

Furthermore, a UV-Vis spectrophotometer revealed that the curcumin extract obtained exhibited a pronounced absorption peak at 425 nm, as depicted in **Figure 2**. This peak aligns with the established absorption profile of curcumin, proving the extraction of the chemical from turmeric powder. The absorption spectra of curcumin generally display a broad band, with peak absorbance at 425 nm, indicating low-energy π–π^* transitions inside the curcumin molecule. This aligns with the absorption maximum of the extract, as documented in the research of Kim *et al.*[32] The 425 nm peak in the UV-Vis spectrum is often linked to the conjugated aromatic system of curcumin, which accounts for its significant light absorption characteristics (**Figure 2**). This peak is characteristic of curcumin dissolved in organic solvents, such as ethanol.[33]

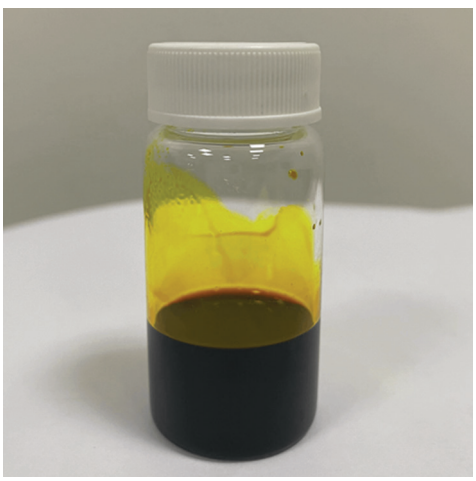

Figure 1. Curcumin extract

3.2. Surface morphology of CurChiNEM

The surface morphology of both blank and curcumin-loaded chitosan-based nanoemulsions was analyzed using SEM (**Figures 3-6**). SEM micrographs demonstrated that all formulations produced quasi-spherical nanoemulsions with smooth surfaces. This observation aligned with the SEM image in the study of Nair *et al.*[34] The SEM micrographs of the optimized curcumin nanoparticles displayed a spherical morphology with a smooth surface texture. A study by Mofazzal Jahromi *et al.*[35] reported that curcumin-loaded chitosan nanoparticles displayed a spherical shape and smooth surface with enhanced drug release, showing high encapsulation properties and improved release efficiency.

The observed surface morphology in **Figures 3-6** is highly advantageous, especially for its release patterns. Spherical, smooth nanoparticles typically provide a slower release of compounds than their non-spherical or rough-surfaced equivalents. The spherical form and smooth surfaces decrease

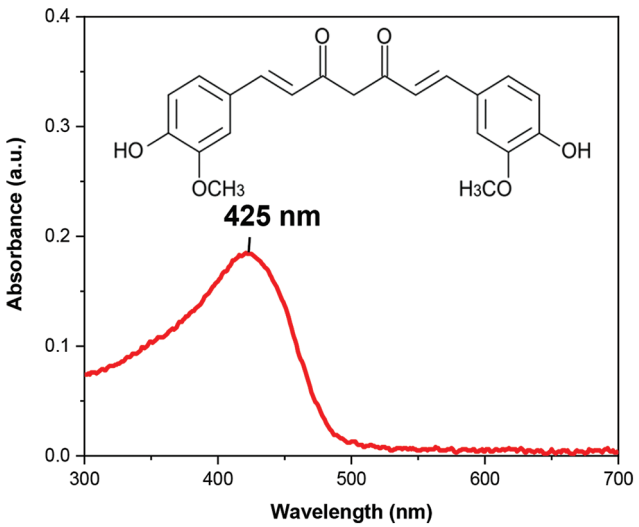

Figure 2. Ultraviolet-visible spectra of curcumin extract

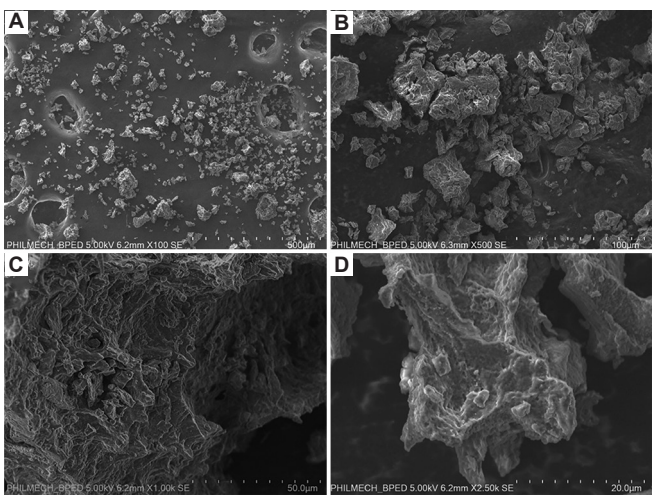

Figure 3. Scanning electron micrographs of blank chitosan nanoemulsion at different magnification levels. (A) 100× (scale bar: 500 µm, (B) 500× (scale bar: 100 µm), (C) 1,000× (scale bar: 50 µm), and (D) 2,500× (scale bar: 20 µm).

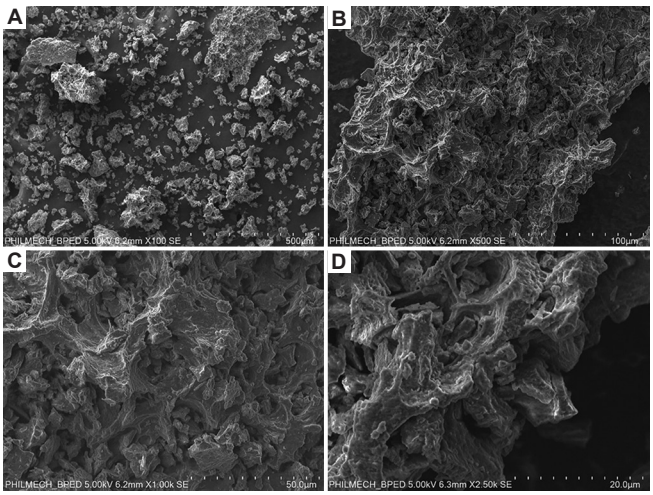

Figure 4. Scanning electron micrographs of Formulation 1 at different magnification levels. (A) 100× (scale bar: 500 μm), (B) 500× (scale bar: 100 μm), (C) 1,000× (scale bar: 50 μm), and (D) 2,500× (scale bar: 20 μm).

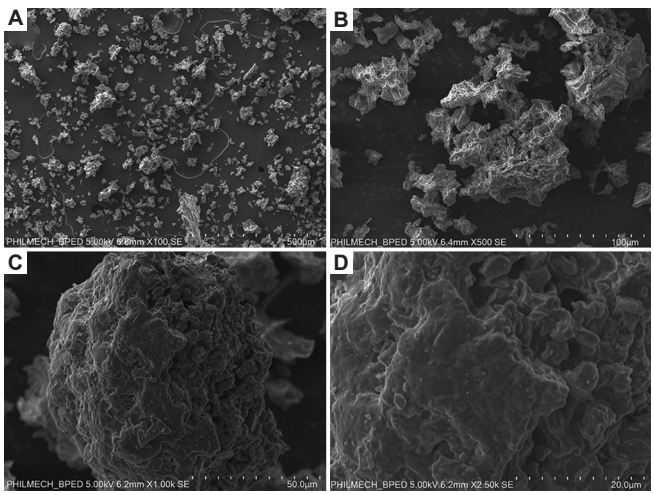

Figure 5. Scanning electron micrographs of formulation 2 at different magnification levels. (A) 100× (scale bar: 500 μm), (B) 500× (scale bar: 100 μm), (C) 1,000× (scale bar: 50 μm), and (D) 2,500× (scale bar: 20 μm).

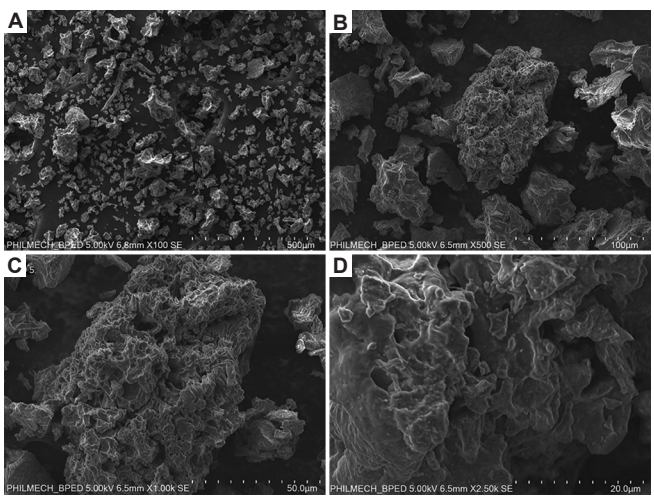

Figure 6. Scanning electron micrographs of formulation 3 at different magnification levels. (A) 100× (scale bar: 500 μm), (B) 500× (scale bar: 100 μm), (C) 1,000× (scale bar: 50 μm), and (D) 2,500× (scale bar: 20 μm).

the overall surface area, minimizing interactions between the nanoparticle and its environment, which consequently reduces the release rate of the encapsulated substance.[36]

Table 2 lists the estimated mean particle sizes, based on SEM images, of chitosan nanoemulsions containing varying quantities of curcumin. The particle size of the blank (no curcumin) was 478 nanometers (nm), which progressively increased with the loading of curcumin, attaining sizes of 664 nm, 750 nm, and 977 nm for Formulations 1, 2, and 3, respectively. When compounds are incorporated into a system, they occupy space within the particle, pushing apart the polymer chains and leading to an expansion of the overall structure. The extent of this size increase depends on several factors, such as the type of compound, its concentration, and the preparation method.[37]

The generated nanoemulsion size was clearly within the region of 400–1,000 nm. Polymeric nanoparticles formulate from biocompatible polymers, such as chitosan, typically measure between 1 and 1,000 nm. These can be achieved using many techniques, including solvent evaporation, nanoprecipitation, emulsification, and salting out.[38] Calvo *et al.*[39] pioneered the production of chitosan-polyethylene oxide (PEO) nanoparticles using ionotropic gelation, revealing particle sizes ranging from around 300 to 1,000 nm, dependent on varying concentrations of PEO and PEO-polyphenylene oxide.

Among the curcumin-loaded treatments, formulation 1 demonstrated the smallest mean particle size at 664 nm, making it the most effective formulation. Particle size is a critical determinant in the efficacy of nanoemulsions, as reduced droplet dimensions provide several benefits, especially in improving stability, delivery efficacy, and overall quality.

The primary benefit of a smaller droplet size in nanoemulsions is the enhancement of stability and bioavailability of the encapsulated compounds. Nanoemulsions with small droplets exhibit less susceptibility to gravitational separation, such as creaming or sedimentation, due to the predominance of Brownian motion over gravitational forces. The small size maintains uniform droplet dispersion during storage. This enhances kinetic stability, indicating that the emulsion shows greater resistance to alterations in droplet size, phase separation, or degradation over time.[40]

Moreover, smaller droplets enhance the delivery performance of the active compound—specifically curcumin—by increasing the surface area available for interaction with the surrounding medium. This promotes accelerated diffusion, improved absorption, and enhanced distribution, making nanoemulsions ideal for applications requiring controlled or sustained release.[41]

Table 2. Particle size analysis of curcumin-loaded chitosan nanoemulsion formulations

Formulation	Particle size (nm)
Formulation 0	478±0.285
Formulation 1	664±0.467
Formulation 2	750±0.361
Formulation 3	977±0.554

Furthermore, nanoemulsions with reduced particle sizes have enhanced solubilization capacity, especially for lipophilic or oil-soluble substances, such as curcumin. This enables them to be useful in systems requiring the dispersion of these compounds in water environments.[42] The results indicate that Formulation 1 offers the most efficient formulation for administering curcumin in an aqueous medium, which poses difficulties due to the limited water solubility of curcumin.

3.3. Functional group through FTIR analysis of CurChiNEM

The FTIR graph, shown in **Figure** 7, reveals several characteristic peaks common across the blank and treated samples, indicating the presence of specific functional groups. The distinct peaks observed confirm the effective crosslinking of chitosan to TPP and loading of curcumin. In both the blank and treated samples, a peak around 883–888 cm^{-1} was observed corresponding to N–H deformations. Specifically, this peak range pertains to the bending or twisting motion of the N–H bond within the chitosan structure, particularly the wagging vibration of the saccharide structure.[43] The C–C–C stretching vibrations constantly showed approximately 1,066–1,068 cm^{-1} across all formulations, signifying that the polysaccharide backbone of chitosan remained predominantly intact after the production of the nanoemulsion and the incorporation of curcumin. The peaks at 1632–1637 cm^{-1} are attributed to C=O stretching (amide I) in the chitosan structure, whereas the peaks at 2,862–2,919 cm^{-1} correspond to the aliphatic C–H stretching vibrations of the chitosan backbone. Minor to no shifts are observed in between formulations, but the functional groups remain largely present. Comparable findings were also reported in several studies.[44,45]

The notable peak at 1,533–1,541 cm^{-1}, linked to N–O–P stretching vibrations, confirms ionic interactions between the protonated amine groups of chitosan and the negatively charged phosphate groups of TPP in the nanoemulsion system.

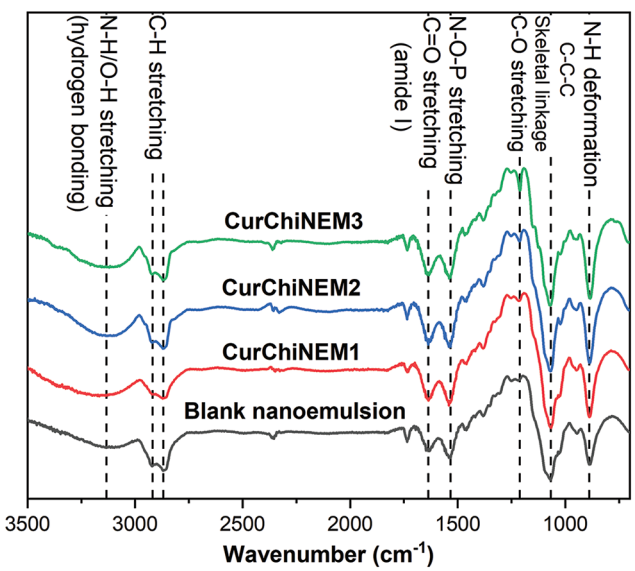

Figure 7. Fourier-transform infrared spectroscopy spectra of curcumin-loaded chitosan nanoemulsion

The peak correlates with the findings of Loutfy *et al.*,[46] wherein the FTIR spectra of the produced chitosan-TPP nanoparticles had a peak at 1,539 cm^{-1}, indicating an N–O–P stretching vibration associated with the crosslinking of chitosan and TPP.

Finally, the broad O–H/N–H stretching peak showed a slight shift from 3,133 cm^{-1} in the blank to 3,135 cm^{-1} in all formulations loaded with curcumin. A transition to higher wavenumbers indicates that either new, potentially stronger hydrogen bonds are forming or that the geometry of those that already exist changed.[47]

3.4. EE of the nanoemulsion

EE is a critical parameter in evaluating the performance of nanoemulsion systems, as it determines the proportion of the active compound incorporated within the carrier matrix. In this study, chitosan-based nanoemulsions were formulated with varying concentrations of curcumin to assess the effect of different drug loads on EE.[48] The results obtained from these formulations are summarized in **Table 3**. At a curcumin concentration of 2.5 mg/mL, the EE reached a peak value of 77.82 ± 1.2%, indicating an optimal loading capacity of the system. However, an increase in curcumin concentration resulted in a gradual decrease in EE, yielding values of 72.04 ± 0.86% at 5 mg/mL and 65.41 ± 0.98% at 10 mg/mL.

The observed inverse relationship implies that the nanoemulsion encapsulation capacity for curcumin is limited, likely due to the chitosan matrix achieving saturation at higher concentrations. When the concentration of curcumin surpasses a specific threshold, excess curcumin molecules may struggle to penetrate the interior of the encapsulation matrix. Excess curcumin molecules might begin to aggregate or clump together rather than remaining individually encapsulated, which diminishes the overall efficiency.[49] This observation is consistent with the findings that increasing curcumin concentration beyond optimal levels may lead to a decrease in EE due to droplet aggregation and inadequate emulsifier coverage.[50]

3.5. Stability assessment of CurChiNEM

3.5.1. Light stability

The light stability of CurChiNEM was evaluated in comparison to free curcumin extract under prolonged light exposure. The results showed that the free curcumin extract underwent rapid photodegradation, with its curcumin content decreasing to 6.6% within 7 days. In contrast, the CurChiNEM nanoemulsion demonstrated remarkable stability, retaining 77.02% of its initial curcumin content even after 28 days under the same conditions, as shown in **Figure 8**.

Table 3. Encapsulation efficiency of curcumin-loaded chitosan nanoemulsion

Formulation	Curcumin concentration (mg/mL)	Encapsulation efficiency (%)
1	2.5	77.82±1.20[a]
2	5.0	72.04±0.86[b]
3	10.0	65.41±0.98[c]

Note: [a,b,c]Mean values with different letters denote significant difference (p>0.05, analysis of variance followed by Tukey's honestly significant difference test).

Chitosan has been reported to offer UV-protective characteristics, due to its capacity to absorb UV radiation. The absorption is attributed to the presence of particular chemical structures within the chitosan molecule, including conjugated double bonds and aromatic rings. These structures function as chromophores, efficiently absorbing UV radiation and obstructing its penetration into the interior substance.[51] Numerous investigations have demonstrated the UV-protective characteristics of chitosan, including the research by Lin *et al.*,[52] which showed that utilizing gelatine and chitosan as composite wall materials effectively shielded curcumin from degradation under UV-Vis light and enhanced its storage stability. In the study conducted by Rachtanapun *et al.*,[53] the integration of curcumin extract into chitosan films markedly reduced the transmission of UV and visible light compared to the control films. This indicates that the encapsulation of curcumin within the chitosan/TPP matrix successfully addresses the photosensitivity of the bioactive component.

3.5.2. Storage stability

Figure 9 illustrates the storage stability of CurChiNEM compared to free curcumin extract over 28 days. The nanoemulsified formulation retained 87.56% of its curcumin content, while the free extract exhibited significant degradation, decreasing to approximately 14% at day 28. The findings suggest that the chitosan nanoemulsion provides effective protection for curcumin against degradation when stored under standard conditions. Chitosan possesses the ability to create protective films that serve as a barrier. The barrier effect has the potential to impede oxidation, microbial proliferation, and various degradation processes, thereby prolonging the shelf life of products.[54]

The results are consistent with the study by Lee *et al.*,[55] which reported that curcumin-loaded nanoparticles demonstrated strongly enhanced photo and storage stability compared to free curcumin, attributed to the protective effects of the carrier system. Furthermore, the research conducted by Chen *et al.*[56] indicated that the particle size of cur-chitosomes remained consistent over a 28-day incubation period, with a notable stability value of 85.3% under degradation conditions at 25°C. This indicates that the formation of liposomes and the incorporation of chitosan in curcumin can prolong the storage duration.

3.5.3. Temperature stability

As shown in **Figure 10**, the free extract retained only 14.67% of its curcumin concentration at 100°C, whereas the nanoemulsion preserved approximately 67.71%, highlighting the effective thermal protection of chitosan encapsulation.

Figure 10 demonstrates that both variables exhibit a simultaneous declining trend; nevertheless, the curcumin retention of CurChiNEMs remains consistently higher than that of curcumin extract alone. Chitosan coatings appear to be a successful strategy for boosting the resistance of curcumin to heat-induced conditions. This observation aligns with the findings of Chen *et al.*,[53] which demonstrated that chitosan incorporation lessens curcumin's vulnerability to heat, and that cur-chitosomes performed well at elevated temperatures.

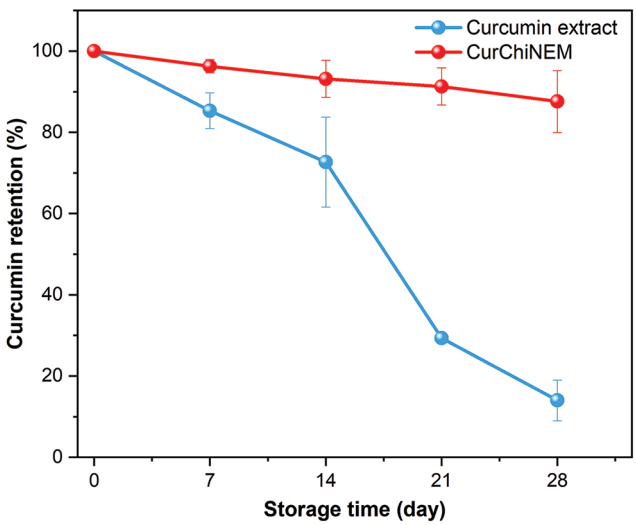

Figure 9. Storage stability of curcumin-loaded chitosan nanoemulsion

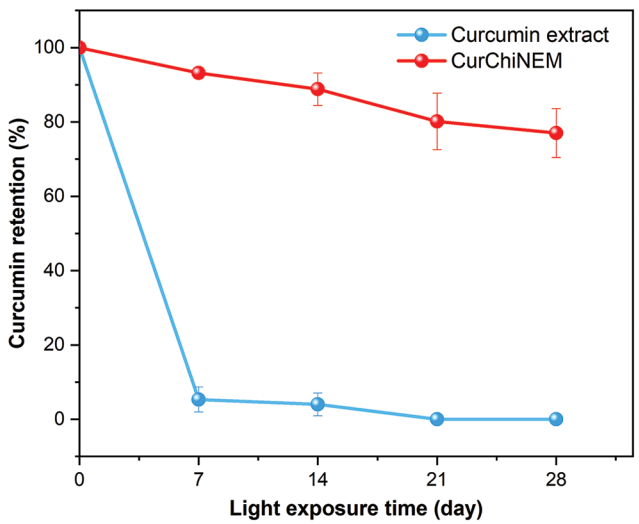

Figure 8. Light stability of curcumin-loaded chitosan nanoemulsion

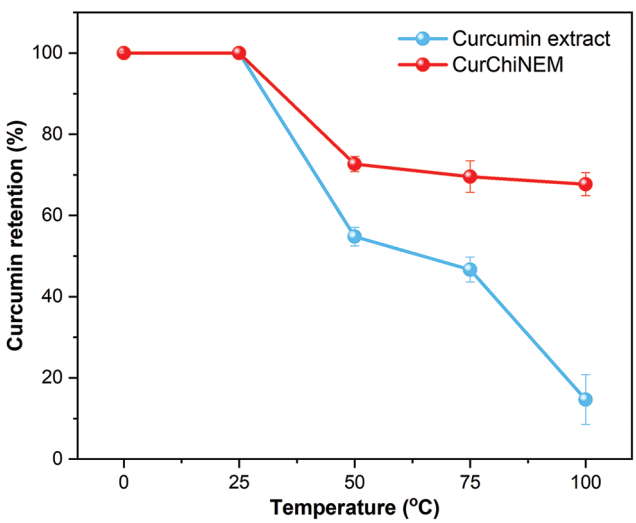

Figure 10. Temperature stability of curcumin-loaded chitosan nanoemulsion

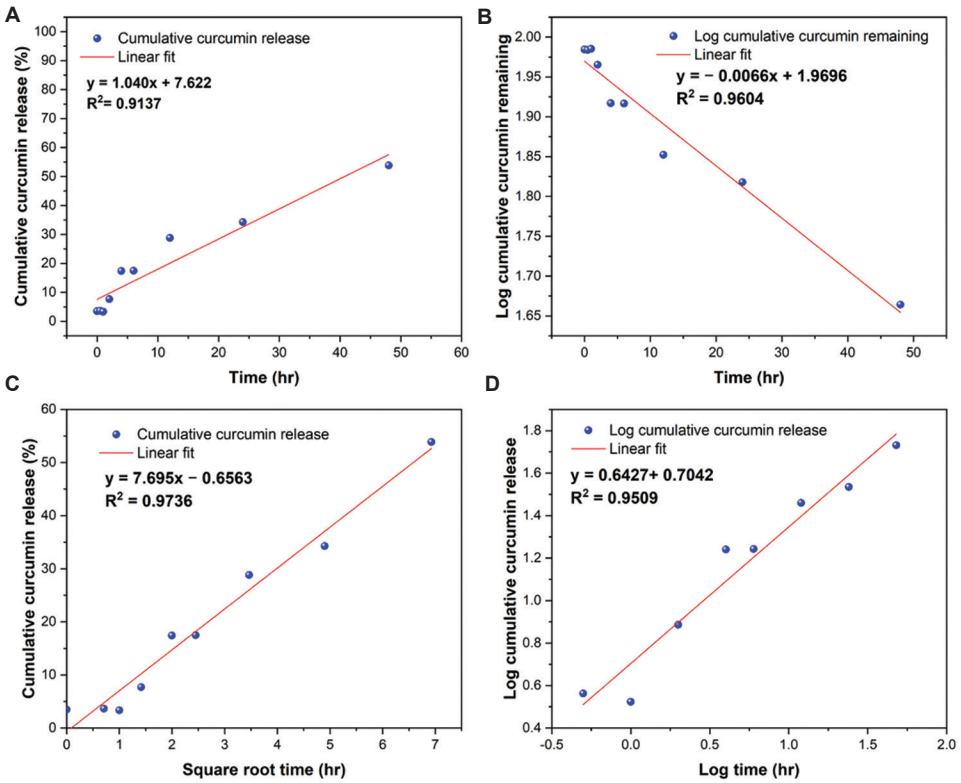

Figure 11. Curcumin-loaded chitosan nanoemulsions release data fitted to various kinetic models. (A) Zero-order, (B) first order, (C) Higuchi model, and (D) Korsmeyer-Peppas.

Similarly, Zhu *et al.*[54] reported that a quaternized chitosan-coated nanoemulsion offered enhanced thermal stability to encapsulated curcumin compared to uncoated versions, effectively shielding it from degradation during heating. These suggest that biopolymer coatings significantly improve the temperature stability of heat-sensitive compounds, such as curcumin.

Overall, the results demonstrate that the encapsulation of curcumin in the chitosan/TPP matrix significantly enhances its stability under various conditions, including exposure to light, elevated temperatures, and prolonged storage. Compared to the free curcumin extract, which rapidly degraded under exposure to light, heat, and prolonged storage, the nanoemulsion formulation consistently preserved a significantly higher percentage of curcumin content across all tested conditions. Therefore, encapsulating curcumin in a nanoemulsion network offers a practical and effective solution to these limitations. Based on these findings, the use of nanoemulsions produced through the ionic gelation of chitosan and TPP presents a highly promising strategy for enhancing the stability of sensitive bioactive compounds, such as curcumin, particularly when products are subjected to fluctuating environmental conditions throughout the entire shelf life.

3.6. *In vitro* release study of CurChiNEM

The *in vitro* release profile of the CurChiNEMs was assessed by fitting the data to various established kinetic models, as shown in **Figure 11**. The Higuchi model exhibited the best fit with an R^2 = 0.9736, indicating that the release of curcumin predominantly adhered to a diffusion-controlled mechanism. Chitosan usually follows the Higuchi model for drug release, as it involves the diffusion of the drug through the polymer matrix. The Higuchi model describes the release of drugs from an insoluble matrix where diffusion is the primary mechanism. In chitosan-based systems, the drug is typically distributed within the polymer matrix, and drug release occurs through diffusion as it moves from the matrix into the surrounding solution. These results correspond with the study conducted by Nair *et al.*,[34] which generated curcumin-encapsulated chitosan nanoparticles using a chitosan/TPP ratio of 5:1. The release data at pH 7.4 exhibited optimal alignment with Higuchi's model, suggesting a diffusion process with an R^2 of 0.9510. The 5:1 ratio exhibited a marginally improved drug release, likely attributable to the better swelling capacity of the nanoparticles and the reduced TPP concentration. The crosslinking agent significantly influences drug release. The expansion of chitosan facilitates the penetration of the release medium into the polymer matrix, functioning as a plasticizer that transforms the glassy polymer into an elastic form, hence enhancing drug release from the nanoparticle structure. Furthermore, the release of curcumin from chitosan-curcumin liposomes also followed the Higuchi model, with an R^2 of 0.99 at 23°C, in accordance with the study by Lee *et al.*[55]

Moreover, the first-order kinetic model showed the next strongest correlation with an R^2 = 0.9604, indicating that the release rate of curcumin decreased as the drug concentration in the system decreased. The Korsmeyer-Peppas model was closely followed, with an R^2 of 0.9509, indicating a mixed mechanism involving diffusion and possible structural changes in the

chitosan nanoemulsion over time, such as matrix softening or swelling. In contrast, the zero-order model displayed the least fit, with an R^2 of 0.9137. This model, which assumes a constant release rate independent of drug concentration, did not accurately represent the observed release behavior, suggesting that a steady, time-based mechanism did not govern the release of curcumin from the nanoemulsion. Instead, the release of curcumin was predominantly controlled by the diffusion through a polymer matrix.[56,57]

3.7. Limitations of the experimental design

While this study successfully demonstrates enhanced curcumin stability and controlled release, several limitations in experimental design warrant consideration for future research.

First, particle size analysis relying on manual ImageJ measurements of SEM images may not fully represent hydrodynamic size or polydispersity in hydrated states. The reported sizes (664–977 nm) are also at the larger end of the nanoemulsion range, potentially affecting bioavailability.

Second, the 28-day stability assessments, while informative, are insufficient for assessing long-term shelf life. Future studies should extend these evaluations over longer periods and under broader environmental conditions.

Third, this study is entirely *in vitro*. Rigorous *in vivo* validation is crucial to assess the CurChiNEM system's ultimate efficacy, safety, and bioavailability in complex biological environments.

Finally, EE was evaluated across only three curcumin concentrations, using a curcumin extract instead of pure curcumin. An extensive optimization study would provide a comprehensive understanding and improve generalizability.

4. Conclusions

In this study, encapsulating curcumin was achieved using a chitosan-nanoemulsion complex. Stability assessments indicated that CurChiNEMs significantly enhanced the storage, photostability, and thermal stability of curcumin. The nanoemulsion significantly improved curcumin's stability, retaining 87.56% of its content after 28 days of storage, 77.02% under UV light exposure, and approximately 67.71% at 100°C. In contrast, free curcumin degraded rapidly under identical conditions. *In vitro* release studies at pH 7.4 revealed a Higuchi release kinetics model (R^2 = 0.9736), suggesting a diffusion-controlled release mechanism. The findings of this research and inferences suggest several recommendations to optimize the CurChiNEM system and broaden its potential applications in biomedical, pharmaceutical, and agricultural domains: (i) to employ the nanoemulsion system in seed nanopriming and enhance the delivery of bioactive compounds, such as curcumin, and improve seed vigor; (ii) to broaden the therapeutic potential of the nanoemulsion by co-loading additional bioactive compounds or incorporating them as active ingredients; (iii) to evaluate various polymers for curcumin encapsulation to assess their biocompatibility, release profiles, and protective capabilities; and (iv) to assess the shelf life and practical usability of the nanoformulation by storing it under varying humidity levels for periods exceeding 25 days.

Acknowledgement
The authors would like to extend their sincere gratitude to the following institutions for their invaluable support and contributions to this research: Bio-Processing Engineering Division of the Department of Agriculture-Philippine Center for Postharvest Development and Mechanization, Department of Agriculture-Livestock Biotechnology Center, Philippine Science High School-Ilocos Region Campus, College of Fisheries, Central Luzon State University, and to Asst. Prof. Darwin F. Reyes of Nueva Ecija University of Science and Technology.

Financial support
This research was supported by Central Luzon State University under the project titled "Development, Testing and Field Evaluation of Nano-enabled Seed Technologies (NeST) to Increase Productivity of Major Agricultural Crops" with grant number (164-648-458-A-012).

Conflicts of interest statement
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions
Conceptualization: PJGE and MMS; *Data curation*: MMS; *Formal analysis*: LJCL; *Investigation*: LJCL; *Supervision*: PJGE; *Writing–original draft:* LJCL; *Writing–review & editing:* FJAG. All authors read and approved the final manuscript

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Availability of data
Data will be made available on request from the corresponding author.

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Received: June 3, 2025
Revised: August 11, 2025
Accepted: August 12, 2025
Available online: September 11, 2025