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Formulation And Evaluation of Curcumin Loaded with Piperine Nanogel Against Skin Cancer

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ABSTRACT

This study presents the successful development and characterisation of a nanogel formulation encapsulating curcumin and piperine for potential application in skin cancer therapy. The nanogel was prepared using the solvent evaporation method with carbopol polymer, and its physicochemical attributes were systematically investigated. Fourier-transform infrared spectroscopy (FT-IR) confirmed the encapsulation of curcumin and piperine within the carpool matrix, while scanning electron microscopy (SEM) and differential scanning calorimetry (DSC) elucidated the nano gel's morphology and thermal behaviour. Zeta potential and particle size analyses revealed a stable colloidal system with well-defined nanoscale characteristics. Drug release kinetics were also assessed, revealing a controlled and sustained release of curcumin and piperine from the nanogel over time. The anticancer activity of the nanogel was assessed using MTT assays on A231 skin cancer cells, demonstrating significant inhibition. The findings highlight the formulation's potential as an effective and targeted therapy for skin cancer.

KEYWORD: curcumin, piperine, nanogel, skin cancer, drug release, MTT assay, cell viability.

INTRODUCTION

Skin cancer, a malignancy arising from the uncontrolled proliferation of skin cells, poses a significant global health concern.[1] Among the various forms of skin cancer, melanoma, basal cell carcinoma, and squamous cell carcinoma are predominant.[2-4] Despite notable progress in cancer research and treatment modalities, the quest for innovative and productive therapeutic approaches for skin cancer remains paramount.[5,6]

In recent years, natural compounds have garnered attention for their potential in cancer therapy due to their multifaceted pharmacological properties.[7-10] Curcumin, the principal polyphenolic component of turmeric (Curcuma longa), has long been recognised for its anti-inflammatory, antioxidant, and anti-cancer activities.[11] Its anti-inflammatory properties are attributed to its ability to modulate various molecular targets involved in inflammation, including transcription factors, enzymes, and cytokines. Moreover, curcumin has demonstrated the ability to trigger apoptosis, or programmed cell death, in tumour cells and impede their growth, thereby positioning it as a highly potential contender for cancer treatment.[12] Curcumin encounters challenges in clinical utilisation due to its limited aqueous solubility, diminished bioavailability, and fast metabolism.[13] Incorporating curcumin into a nanogel formulation could address these limitations, enhancing its stability and ensuring sustained release for prolonged therapeutic effects.[14,15]

Piperine, an alkaloid found in black pepper (Piper nigrum), is known for its bioenhancing properties. It has increased the bioavailability of various drugs and nutrients, including curcumin. Piperine achieves this by inhibiting enzymes responsible for metabolising these compounds in the liver and intestine, enhancing their absorption.[16,17] Moreover, piperine possesses inherent anti-cancer properties. Studies have indicated its potential to induce apoptosis, inhibit angiogenesis (the formation of blood vessels supporting tumour growth), and modulate signalling pathways involved in cancer progression. The synergy between curcumin and piperine, often called the "bioenhancement effect," highlights their complementary roles in enhancing therapeutic outcomes.[18]

Nanotechnology, particularly the development of nanogels, offers a promising avenue for overcoming these challenges. Nanogels, three-dimensional networks of hydrogel nanoparticles, present an ideal platform for encapsulating and delivering therapeutic agents.[19] Carbopol, a widely used biocompatible polymer in pharmaceutical formulations, exhibits excellent mucoadhesive properties and has proven effective in topical drug delivery systems.[20]

This research endeavours to bridge the gap between the therapeutic potential of curcumin and piperine and their clinical application in skin cancer treatment. Incorporating these natural compounds into a nanogel matrix, utilising Carbopol polymer, aims to address issues related to their solubility, bioavailability, and controlled release. This innovative approach promises to enhance the therapeutic efficacy of curcumin and piperine against skin cancer while minimising systemic side effects.

MATERIALS

The curcumin has been bought from Loba Chemie Pvt. Ltd, located in Mumbai, India. Piperine was sourced from Sigma-Aldrich in St. Louis, USA. Ethanol was purchased from Changsh Hongsheng Fine. Carbopol 934 was received from Sisco Research Laboratories Pvt. Ltd in Mumbai, India. Triethanolamine and propylene glycol are sourced from Nice Chemical Pvt. Ltd, located in Mumbai, India. All remaining compounds were of analytical quality and utilized without additional purification.

METHODS

Fabrication of Curcumin and Piperine loaded nano gel.

A modified emulsion solvent diffusion process is employed in preparing the nanogel. In the organic phase, a precisely measured amount of the medicine curcumin and the bioenhancer piperine are dissolved while stirring in ethanol and propylene glycol. Using a magnetic stirring apparatus (Rem Elektrotechnik Ltd.), dissolve Carbopol -934 in water and stir continuously while heating it for 20 minutes to prepare the aqueous phase. The medicinal component is subjected to sonication in an ultrasonic bath using a Sonicator for ten minutes. For 30 minutes at 6000 rpm, the drug phase is slowly added to the water phase using high-speed homogenisation to create an emulsion. Figure 1 shows the process of creating nanogel by using a homogeniser (Rem Elektrotechnik Ltd) to transform the emulsion into a nanodroplet. Then, the o/w emulsion is homogenised for 1 hour at 8000 rpm to make nanogel.

Figure 1: formulation of Nanogel

Characterisation of Nanogel

Fourier transform infrared spectroscopy (FTIR)

Piperine and plain curcumin were tested using Fourier transform-infrared spectroscopy (FT-IR) by an FT-IR spectrometer manufactured by Shimadzu Corporation and distributed by

Binion Scientific Inc. in the United States. The resultant spectra were collected within the range of scanning of 4000-400 cm^1 after a laser beam came into direct touch with weighed quantities of drug-loaded and plain nanogel.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (Pyris 4 DSC, Perkin Elmer, Waltham, MA, USA) was used to explain the variations in the enthalpies of curcumin, piperine, and carbopol 934, in addition to the formulation combination. Once both pans were prepared, the samples (5 mg each) were transferred to them, and the reference standard was placed on top. Dry nitrogen gas was used to heat both pans simultaneously at a scanning rate of 20˚C/min (20-250˚C).

Drug release study

The medication is released as water seeps through the nanogel network's porous particle surface. When an aqueous medium is supplied to the nanogel network, it swells, causing the medication to be released. The swelling behaviour of nanogels in a specific media determines the drug release rate. The drug-loaded Nanogel was submerged in a predetermined volume of buffer solutions with pH values of 4.5, 6.8, and 7.4 in a shaker incubator operating at 100 rpm at 37˚C. Piperine and curcumin, which were liberated from nanogel, were separated from the solution by centrifuging them at 4000 rpm for 10 minutes at predetermined intervals. To find out how much curcumin and piperine were in each solution, a UV-vis spectrophotometer (UV-vis spectrophotometer, Varian 4000, USA) was used to collect 5 millilitres of each. So that the volume remained consistent, the same medium was added again in an equal volume. A calibration curve using curcumin and piperine was used to ascertain the concentration of the released medicines. Here is how the release was calculated using the equation:

Percentage drug release = Released amount of drugs in nanogel / total amount of drug in nanogel ×100

Scanning electron microscopy

A morphological study using an SEM (scanning electron microscope) validated the dimension of the nanoparticles.

Zeta potential measurement

The surface charges of the NGs in water at room temperature were measured using a zeta potential analyser (Malvern Instruments Zetasizer ver. 7.11 MAL 500999). We sonicated and diluted the NG dispersion with ultrapure water before testing.

Zeta particle size measurement

A dynamic light scattering approach called Zetasizer Nano ZSP was employed to measure the size of the drug-loaded nanogel. Malvern Instruments of Worcestershire, UK, developed this instrument. After the sonication process, 10 µL of the sample volume was spread out over the copper grid that had a carbon surface. The grid was then negatively stained with 1% phosphotungstic acid. The nanogel containing the drug (~2 mg) was mixed with 50 mL of deionised water.

ANTICANCER ACTIVITY

Cell line and culture:

This study's A431 cell line was acquired from the NCCS in Pune, India. At 37 °C, the cells were cultured in a humidified environment with 50 µg/ml CO2, in DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml).

In Vitro assay for anti-cancer activity: (MTT assay) [21]

Inoculation was carried out at 37^0C with 5% CO_2 using 24-well plates that contained 1 × 105 cells per well. The cells were cultured for 24 hours after they attained confluence before different doses of substances were introduced. Following incubation, the sample was removed from the well and rinsed with either DMEM devoid of serum or phosphate-buffered saline (pH 7.4). 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added to 100µl/well (5mg/ml) of the solution and left to incubate for 4 hours. Each well was then treated with 1 ml of Solvent following incubation. Using DMSO as a blank, the absorbance at 570 nm was measured with a UV- -spectrophotometer. A graphic representation of the data showing the concentration necessary to achieve a 50% inhibition (IC50) was generated from the measurements taken. This is the formula that was used to determine the cell viability percentage:

$$\% \text{ Cell viability} = A570 \text{ of treated cells} / A570 \text{ of control cells} \times 100$$

Graphs are created by plotting the sample concentration on the X-axis and the percentage of cell viability on the Y-axis. Each experiment includes a cell control and a sample control to compare the complete cell viability assessments.

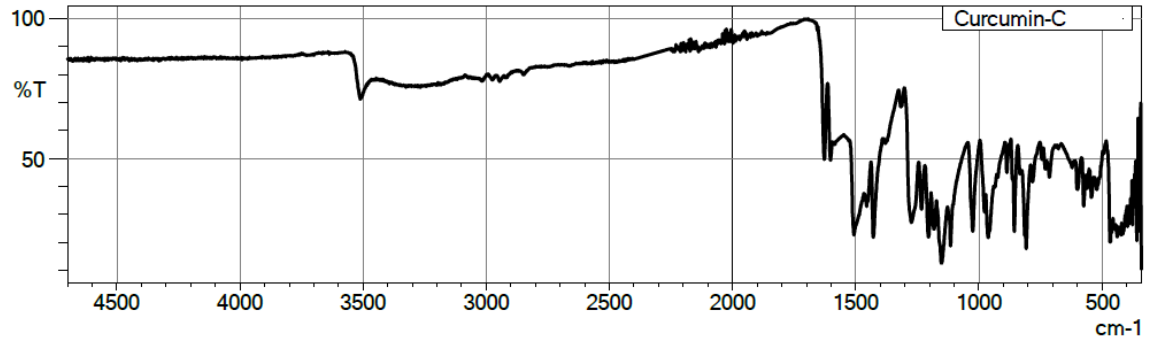
RESULTS AND DISCUSSION

In this work, we created curcumin and piperine-loaded nano gel using a relatively simple and repeatable preparative technique that did not require crosslinkers. The assembly method for curcumin and piperine is carried out in an aqueous solution, doesn't require harsh conditions, and is often biomaterial-friendly.

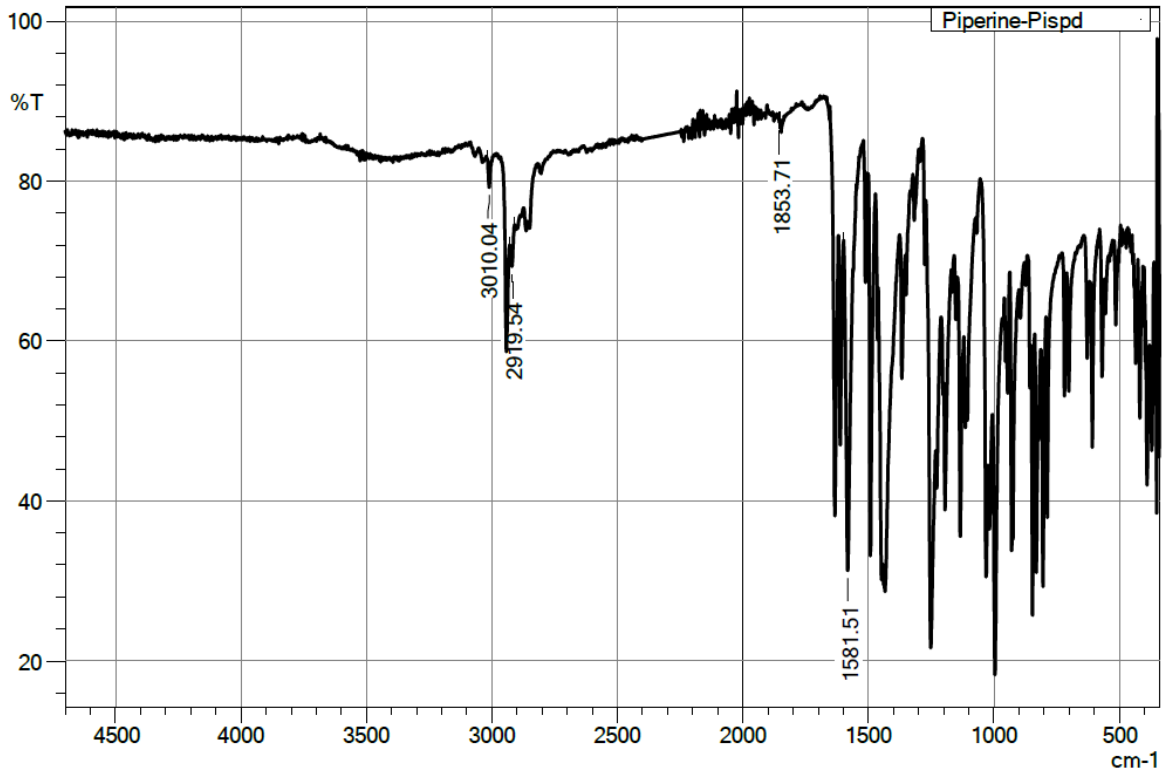
Characterisation of nanogel

FTIR spectra

Infrared spectroscopy shows that the entrapment in the nanogel is chemically stable. Figure 2a,b displays the FT-IR spectra of curcumin and piperine, while Figure 3 depicts the nanogel loaded with a combination of the two. In addition to curcumin and piperine, the combination nanogel exhibited these absorption bands. Curcumin and piperine seem to flatten out, though, which could mean that they aren't interacting with any of the other excipients in the formulation and are instead entirely stuck in the nanogel. This proves that curcumin and piperine are chemically stable in the nanogel.



(a)



(b)

Figure 2: (a) FT-IR of plain curcumin, (b) FT-IR of plain piperine

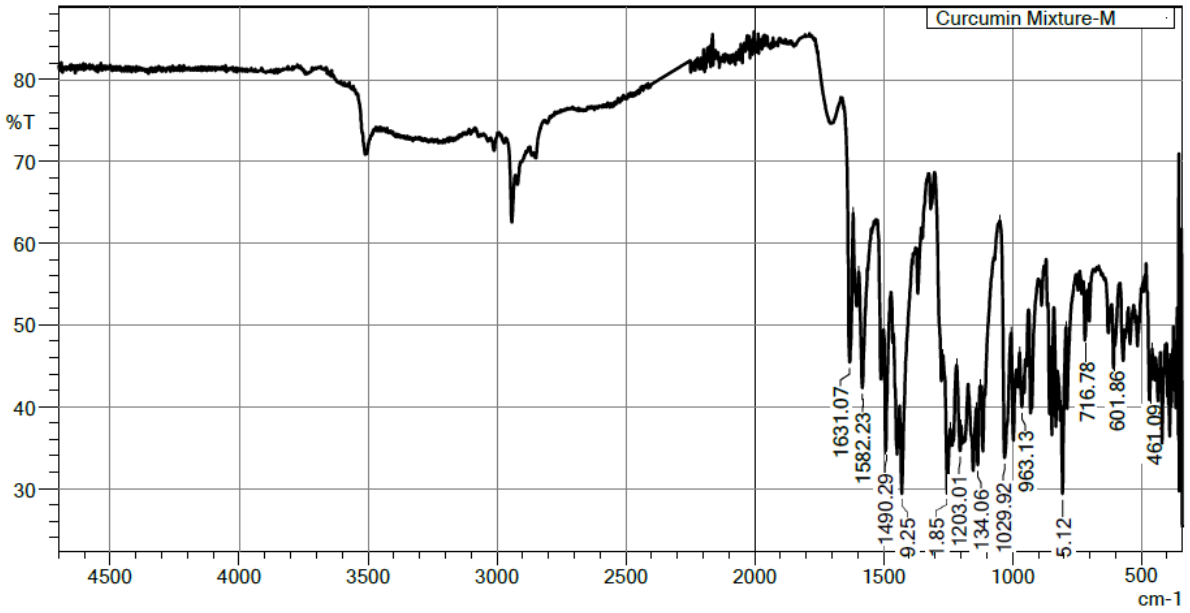
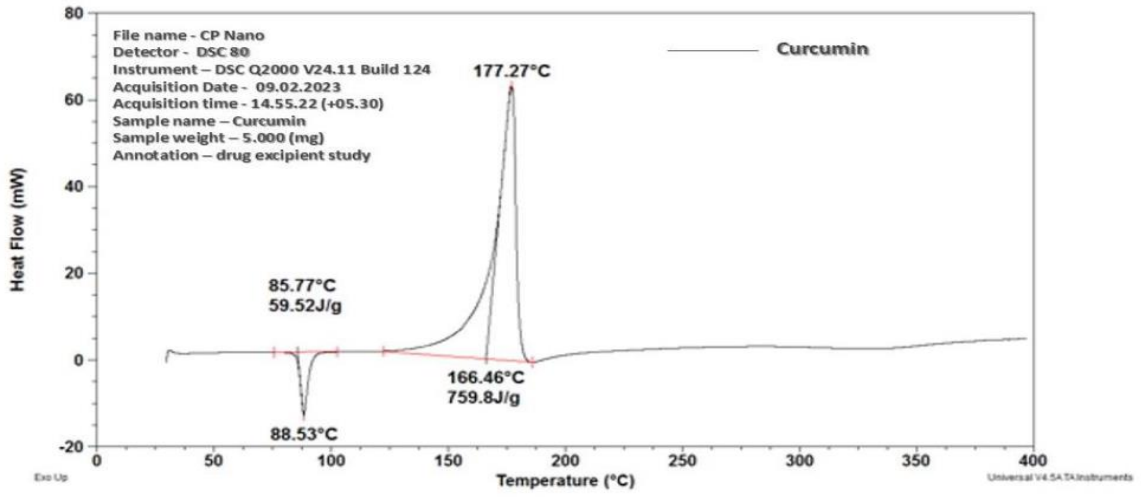


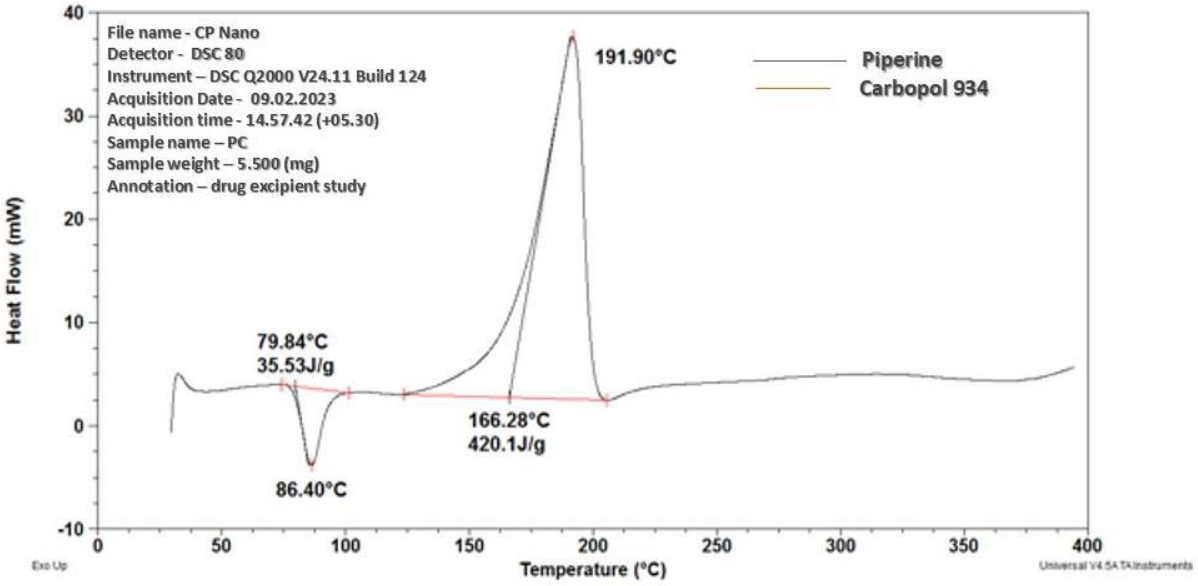
Figure 3: FT-IR spectra of curcumin and piperine-loaded nanogel

DSC of drug-loaded nanogel

A differential scanning calorimeter (DSC) thermogram is essential for determining the composition and changes to the melting point range of the physical mixture's excipients, such as Carbopol, simple curcumin and piperine. Figures 4 a and b display the thermal analysis results for carpooling with piperine and simple curcumin. The DSC thermogram showed that curcumin alone has a crystalline structure, with a melting point of 177.27 C being the strong endothermic peak. Figure 5 shows a mixture of curcumin and piperine-loaded nanogel. We can see that the medicine and excipients do not interact or cause incompatibility by looking at the DSC chart, which shows no noticeable variations between the two peaks.



(a)



(b)

Figure 4: (a) DSC of plain curcumin (b) DSC of piperine in carbopol

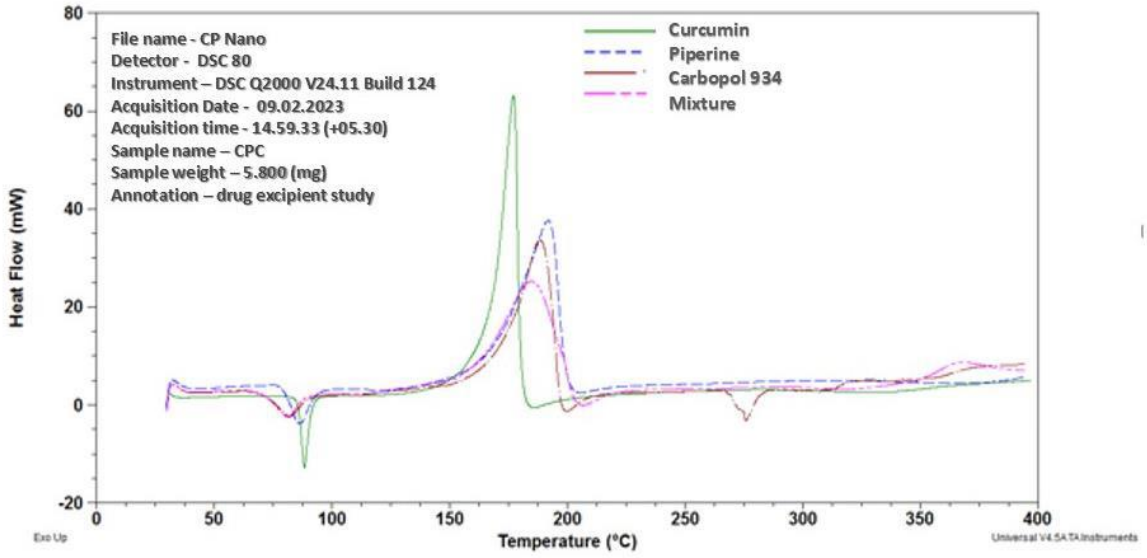


Figure 5: Mixture of curcumin and piperine-loaded nanogel

DRUG RELEASE STUDY

The investigation into the drug release kinetics of five distinct formulations (QP1 to QP5) incorporating a nanogel loaded with curcumin and piperine has yielded compelling results. Graphical representation of the cumulative drug release profiles unveiled a distinct superiority in the QP 5 formulation, which exhibited an impressive release rate of 94.95% (figure 6). This finding highlights the efficiency of the chosen nanogel delivery system and underscores the potential of this particular formulation for optimised drug release. Comparative analysis among the formulations revealed a statistically significant difference, further substantiating the robustness of the QP 5 formulation in achieving maximal drug release. The observed performance is particularly noteworthy as it aligns to enhance therapeutic efficacy through controlled and sustained drug delivery. In the ensuing discussion, we will dissect the key attributes contributing to the remarkable drug release in the QP 5 formulation and consider its translational significance in drug delivery applications.

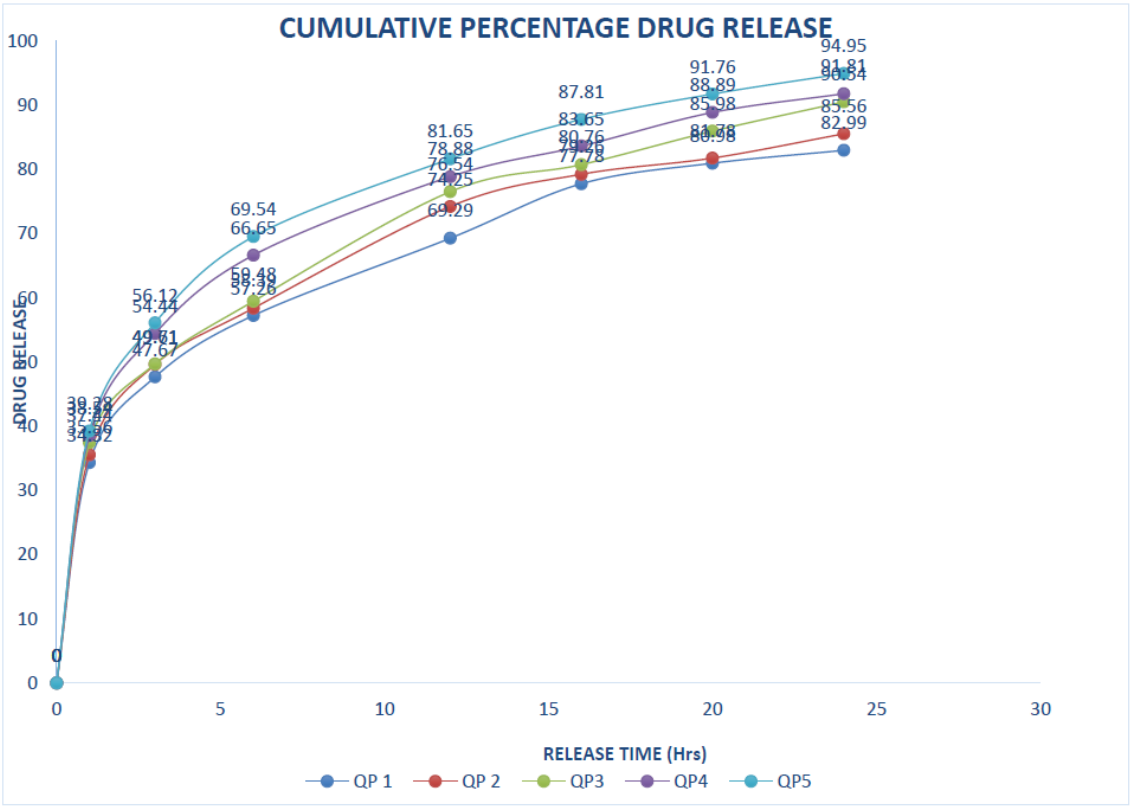


Figure 6: Cumulative percentage of drug release study of curcumin and piperine-loaded nanogel

Scanning electron microscope of nanogel

Figure 7 displays a scanning electron micrograph demonstrating the nanogel optimal formulation. The image reveals that the nanoparticles are consistent, spherical, well-dispersed, uniform in size, and disaggregated. The nanogel that has been produced contains

curcumin and piperine, which have excellent efficacy in encapsulating drugs. Additionally, it has a consistent and reproducible size. The particle size in the polymeric system was effectively regulated using sonication, a method employed during the preparation process.

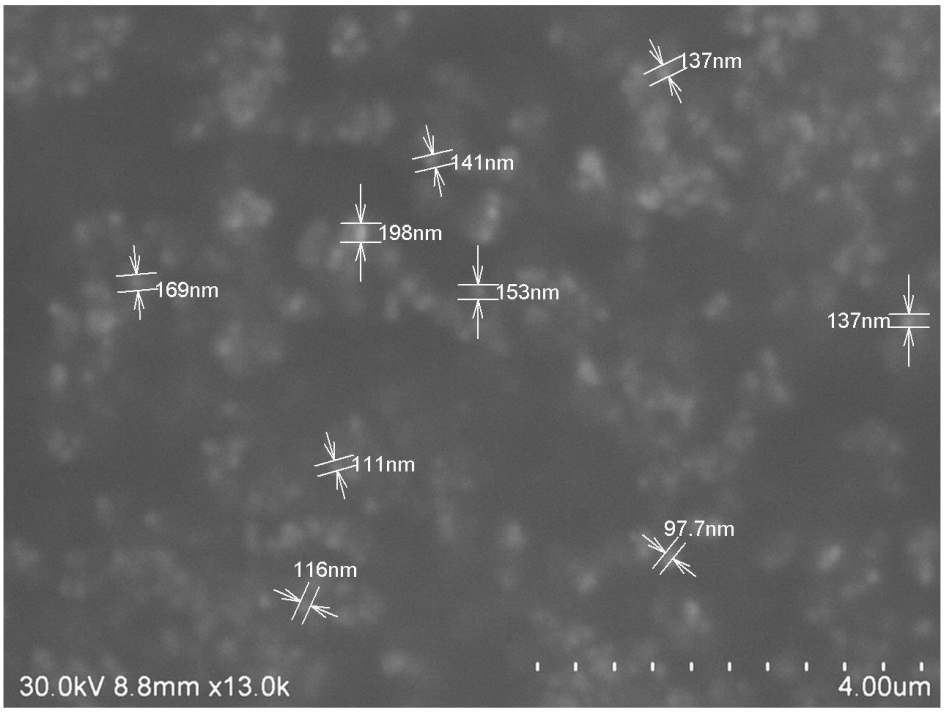


Figure 7: SEM image of drug-loaded nanogel

Zeta potential and Zeta particle size measurement of drug-loaded nanogel

The physicochemical attributes of Formulation incorporating curcumin and piperine within a nanogel matrix were systematically evaluated through zeta potential and particle size analyses. Zeta potential measurements, conducted using dynamic light scattering (DLS), disclosed a robust negative zeta potential of -34.6 mV (figure 8), indicating a high degree of electrostatic repulsion among particles and emphasising the formulation's colloidal stability. Parallelly, particle size analysis yielded a mean particle size of 344.8 nm and a low polydispersity index (PDI) of 0.466 (figure 9), underlining the uniformity and homogeneity of the nanogel population within this formulation. The interplay between these characteristics positions Formulation as a promising candidate, offering stability and a well-defined and controlled nanoscale architecture. These results contribute valuable insights into the physical attributes of Formulation, laying a solid foundation for its potential applications in various nanomedicine contexts.

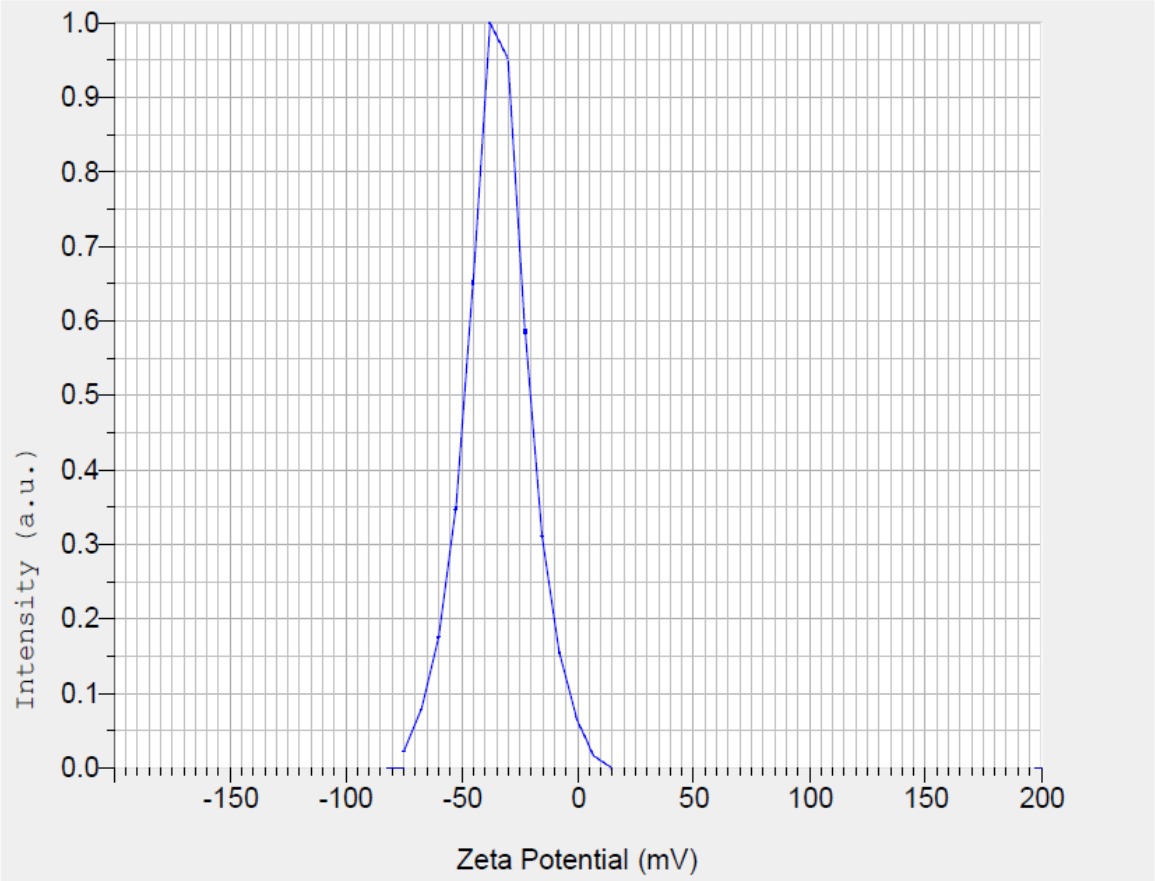


Figure 8: zeta potential of drug-loaded nanogel

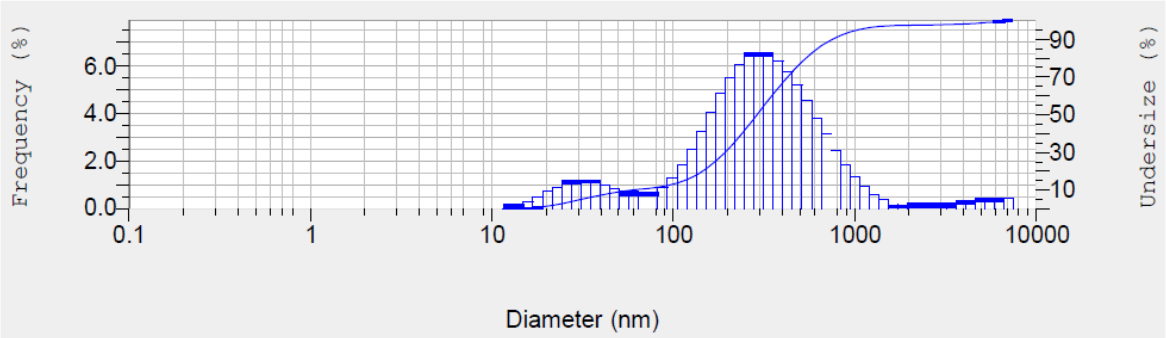


Figure 9: zeta particle size of drug-loaded nanogel

INVITRO CYTOTOXICITY ASSAY

Cell viability was assessed using the MTT test. The cell viability of the prepared nanogel was evaluated at various concentrations in an A431 cell line. The developed nanogel exhibited a reduction in cell viability dependent on the dosage, with concentrations ranging from 1000 to 7.8 µg/ml. The decrease in viability was statistically significant when comparing the control group to the test group. The IC50 value of the test samples loaded with curcumin and piperine nanoparticles was determined to be 51.48 at a 125 µg/ml concentration, as presented

in Table 1 and Figure 10. Figure 11 displays the optical microscopic image of the cell line at various concentrations.

Table 1: Anticancer activity of samples on the A431 cell line

S.No	Concentration (µg/ml)	Absorbance (O.D)	Cell viability (%)
1	1000	0.262	35.40
2	500	0.303	40.94
3	250	0.342	46.21
4	125	0.381	51.48
5	62.5	0.422	57.02
6	31.2	0.463	62.56
7	15.6	0.504	68.10
8	7.8	0.545	73.64
9	Cell control	0.740	100

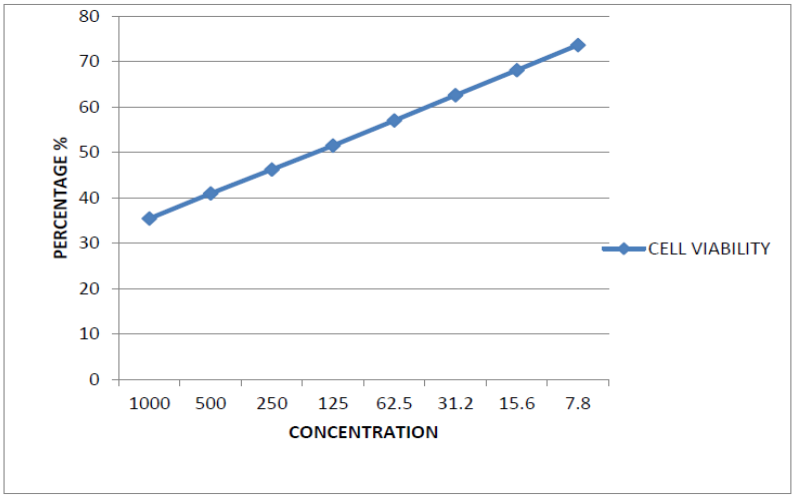
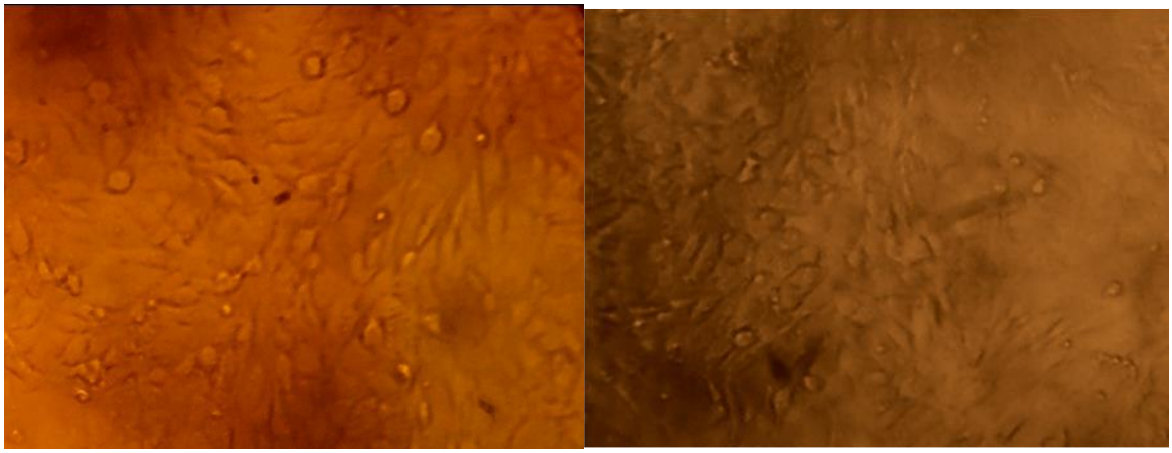
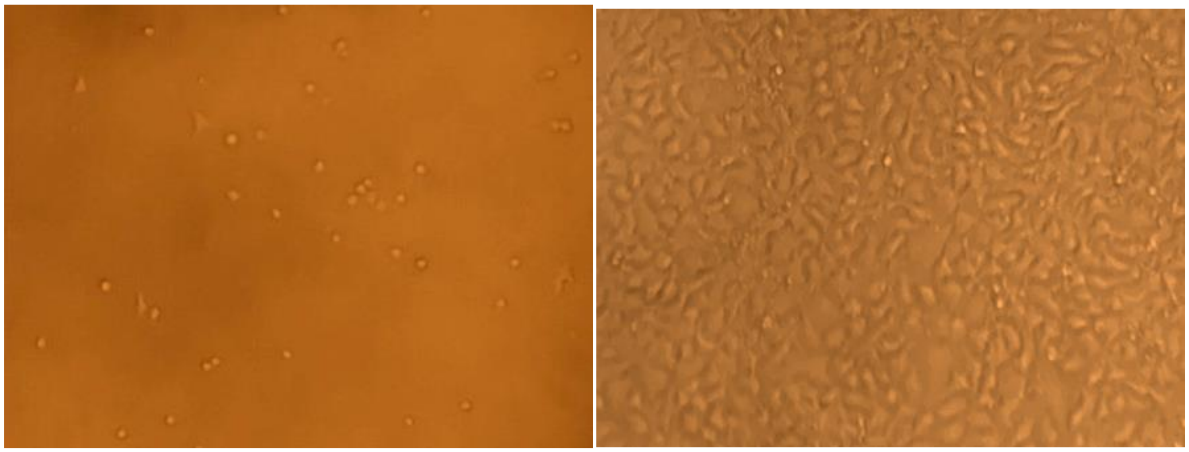


Figure 10: Graphical representations of IC50 values of curcumin and piperine loaded nanogel on the A 431 cell line in MTT assay.



(a) (b)



(c) **(d)**

Figure 11: In vitro cytotoxicity represented by optical microscopic images of A 431 cells (a) control, (b) 125 µg/ml concentration, (c) 1000 µg/ml concentration, (d) 7.8 µg/ml

CONCLUSION

In conclusion, the successful formulation of a nanogel containing curcumin and piperine using the solvent evaporation method with carbopol polymer has been demonstrated. The thorough characterization through FT-IR, SEM, DSC, zeta potential, and zeta particle size analyses has provided a comprehensive understanding of the nanogel's physicochemical attributes. FT-IR confirmed the successful encapsulation of curcumin and piperine within the carpool matrix, while SEM and DSC shed light on the nano gel's morphology and thermal behaviour. Zeta potential and particle size measurements indicated a stable colloidal system with a well-defined nanoscale architecture. The nanogel exhibited significant anticancer activity against A431 skin cancer cells, as demonstrated through MTT assays. These findings underscore not only the robustness of the nanogel formulation but also its potential application in skin cancer therapy. The presented results contribute to advancing our knowledge of nanogel-based drug delivery systems and position this formulation as a promising candidate for further development in skin cancer treatment. The integration of curcumin and piperine, known for their synergistic anticancer effects, makes this nanogel a noteworthy asset in pursuing effective and targeted skin cancer therapies.

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