

Original Research

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Formulation and Evaluation of In-Situ Gel Containing Niacinamide and Curcumin for The Treatment of Mouth Ulcer

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ABSTRACT: Oral ulcers frequently affect the oral mucosa, leading to discomfort, swelling, and challenges with eating or talking. Traditional topical treatments like rinses, ointments, and pastes often fall short because they wash away quickly from the mouth. This research focused on creating and testing a temperature-sensitive in situ gel loaded with niacinamide and curcumin to better treat these ulcers. Niacinamide offers anti-inflammatory benefits and supports tissue repair, while curcumin provides antioxidant effects, fights microbes, and aids healing. The gel formed in place using a cold technique, incorporating poloxamer 407 and 188 for heat-triggered gelling, plus HPMC to boost thickness and adhesion to mucosal surfaces. Formulations underwent checks for traits like look, acidity (pH), gel firmness, ease of spreading, transition point from liquid to gel, and gelling reliability. FTIR spectroscopy confirmed no unwanted reactions between drugs and other ingredients. Formulation F3 stood out with ideal pH levels, balanced rigidity, smooth spread, and quick solidification near body temperature. Release tests across egg membrane showed steady drug delivery, reaching about 92% over 4 hours. It also reduced inflammation by blocking protein changes, scavenged free radicals effectively in DPPH and FRAP tests, and proved safe for cells in MTT studies on L929 lines. In summary, this niacinamide-curcumin gel shows strong physical and biological promise as a retained, efficient option for mouth ulcer relief.

INTRODUCTION

Apical wounds, a different term of cheek perforations, are among the most prevalent cancers impacting the mucosa of the mouth. They are affecting 20-40% of the global population with peak incidence among young adults age 20-30 years ⁽¹⁾. They frequently make it difficult to eat, talk, and practice proper dental hygiene. They are marked by discomfort, inflammation, and blisters ⁽²⁾. The precise cause of mouth ulcers is complex and may involve immunological triggers, stress, bacteria, calorie deficiencies, and local trauma. Even while mouth ulcers often heal on their own, their repeated nature and accompanying discomfort call for efficient topical therapy to offer quick relief and encourage recovery ⁽³⁾.

Oral ulcers are frequently treated using conventional dose forms as mouthwashes, gels, and creams ⁽⁴⁾. However, because of saliva circulation, swallowing, therefore physical movement, these drugs frequently have limited duration of residence in the cavity between the teeth, reducing their treatment efficiency and requires further administration. For it to ensure adherence

among patients and increase medication duration at the site of effect, a unique drug distribution mechanism must be developed⁽⁵⁾.

Because in situ gel systems may undergo a sol-to-gel transition in response to physiological variables like pH, temperature, or ionic concentration, they have garnered significant interest in the administration of oral drugs⁽⁶⁾. When these systems come into contact alongside gastrointestinal membrane of the mouth, that they transition between fluid to gels, prolonging contact duration, improving drug bioavailability, and delivering sustained drug release. Benefits of in situ gels include decreased dose frequency, enhanced patient comfort, and convenience of administration⁽⁷⁾.

Oral ulcers can be treated with niacinamide (vitamin B3), which has anti-inflammatory, wound healing, promote keratinocyte migration, enhances mucosal barrier functions and epithelium regeneration qualities⁽⁸⁾. Curcuma longa is the main component of turmeric, a biologically produced organic molecule with strong anti-inflammatory, antioxidant, and antibacterial properties⁽⁹⁾. Although capsaicin has therapeutic potential, therefore low effectiveness and low solvent mobility can be enhanced with the right formulation techniques⁽¹⁰⁾.

MATERIALS AND METHODS

MATERIALS:

For the development and assessment of a In-situ gel intended for the management of oral ulcers, niacinamide and curcumin were selected as the active pharmaceutical ingredients (APIs). Both compounds were obtained in pharmaceutical grade from Dham Tec Pharma and Consultants (B-204, Silver Springs, Opposite MIDC Office, Taloja, Navi Mumbai-410208, India). Niacinamide was incorporated due to its documented anti-inflammatory effects, ability to promote epithelial repair, and role in strengthening mucosal barrier integrity. Curcumin was included for its well-established antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties, which collectively support ulcer resolution and symptomatic relief.

An In-situ gel system was prepared using poloxamer 407 and poloxamer 188 to enable temperature-dependent sol-to-gel transition at physiological conditions, thereby enhancing residence time within the oral cavity. Poloxamer 407 and poloxamer 188 were procured from Yarrow Chem Products (Pharmaceutical Laboratory, Mumbai, Maharashtra, India). Hydroxypropyl methylcellulose (HPMC) was added to increase viscosity and impart mucoadhesive characteristics, improving local retention of the formulation. Propylene glycol functioned as a co-solvent and permeation enhancer, while ethanol facilitated the dissolution of curcumin. The formulation pH was adjusted, when necessary, using triethanolamine to maintain compatibility with the normal oral pH range (6.8–7.4). Methyl paraben and propyl paraben were incorporated as antimicrobial preservatives to ensure stability during storage.

Distilled water served as the primary vehicle for formulation preparation. Phosphate buffer (pH 6.8) and phosphate-buffered saline (PBS) were utilized for in vitro drug release and diffusion studies. Potassium bromide (KBr) was employed for Fourier-transform infrared (FTIR) compatibility analysis, and methanol was used for drug content determination. Nutrient agar medium was used for evaluating antimicrobial activity. The finalized gel preparations were packed in aluminum collapsible tubes and stored under controlled conditions until further evaluation.

METHODS:

IN-SITU GEL PREPARATION

- In-situ gel was prepared by using the cold method.
- A weighed amount of poloxamer 407 & poloxamer 188 (15-20% w/v) was slowly added to 20 ml Water. (at $4 \pm 2^\circ\text{C}$).
- With continuous stirring at 500 rpms for 2 hrs, the temperature of water should be maintained at $4 \pm 2^\circ\text{C}$ throughout the preparation.
- Solvent was kept overnight in the refrigerator.
- HPMC K-100, Carbopol, and preservatives were added to poloxamer with continuous stirring.

- The preservative of the solvent was prepared by solubilizing in hot water and was mixed with the above solvent after cooling.
- The required quantity of curcumin was solubilized in ethanol, while niacinamide was dissolved in distilled water.
- The solutions were incorporated slowly into the poloxamer dispersion with constant stirring until a homogeneous in-situ gel system was obtained.
- The final volume was made up & pH was adjusted to 7 using triethanolamine.

TABLE 1: Formulation of In-situ gel

Ingredients	F1	F2	F3	F4
Niacinamide	0.4	0.6	0.8	1.0
Curcumin	0.2	0.2	0.2	0.2
P 407(gm)	3.6	3.6	3.6	3.6
P 188(gm)	0.5	1.2	1.6	2.0
MP (gm)	0.02	0.02	0.02	0.02
PP (gm)	0.002	0.02	0.02	0.02
HPMC K-100	0.15	0.15	0.15	0.15
T+E(ml)	6	6	6	6
TEA (ml)	q. s	q. s	q. s	q. s
D/W(ml)	20	20	20	20

EVALUATION OF *IN-SITU* GEL

Physical Appearance

Visual inspection of the *in-situ* gels confirms their quality, uniformity, and stability. Key attributes assessed include colour, transparency, homogeneity, absence of phase separation, texture, and Odor, ensuring suitability for oral administration, patient acceptability, and consistent performance prior to advanced testing.

Gelling Capacity

Gelling capacity evaluates the formulation's responsiveness to environmental stimuli, specifically gelling time and gel erosion duration. These metrics confirm rapid in situ transformation and sustained residence time at the mouth ulcer site for optimal therapeutic delivery.

pH Measurement

Oral pH compatibility (6.2–7.4 range) maintains formulation stability while preventing mucosal irritation. The pH of optimized 20 mL niacinamide-curcumin in situ gels was determined using a calibrated digital pH meter at 25°C, with standard buffers (pH 4.0, 7.0, 9.2) for pre-measurement calibration.

Gel Strength

An accurately weighed 20 g sample of each formulation was transferred to a 25 mL graduated cylinder and induced to gel in a 37°C water bath. The time required for the cylinder to descend 5 cm through the formed gel layer after applying a 50 g weight was recorded as a measure of mechanical strength.

Spreadability Assessment

Approximately 1 g of gel was placed centrally on a 20 × 20 cm glass plate, covered with an identical plate, and subjected to 1000 g pressure for uniform spreading. After 1 minute, the weight was removed, and the spread diameter (cm) was measured in triplicate to quantify ease of application over ulcerated mucosa.

Determination of sol-gel temperature

Gelation temperature was determined using a modified Miller-Donovan method. Aliquots of 2 mL gel formulations were placed in parafilm-sealed test tubes and immersed in a water bath initially set at 4°C. The bath temperature was raised incrementally by 1°C, with 15 min equilibration at each step. Gelation was identified when the meniscus remained immobile upon tilting the tube to 90° and was visually confirmed. Measurements were performed in triplicate (n = 3).

CHARACTERIZATION OF *IN-SITU* GEL:

FTIR: FTIR analysis characterized polymer-drug interactions, gelling agent compatibility, and formulation stability in niacinamide-curcumin in situ gels. KBr pellets of pure niacinamide, curcumin, polymers (e.g., Pluronic®/gellan gum), and final formulations were scanned (4000-400 cm⁻¹, 32 scans, 4 cm⁻¹ resolution) using an FTIR spectrometer. Characteristic peaks confirmed N-H/O-H stretching (3200-3500 cm⁻¹), C=O amide (1650 cm⁻¹), aromatic C=C (1600-1450 cm⁻¹), and absence of unwanted shifts, verifying molecular integrity and successful entrapment without chemical degradation.

INVITRO ACTIVITY

ANTI-INFLAMMATORY ACTIVITY

Inhibition of protein denaturation:

Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi 1968 and Sakat *et al.* 2010 with slight modification. 500 µL of 1% bovine serum albumin was added to 10, 20, 30, 40 and 50 µL of sample. This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Aspirin using as a standard. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

$$100 - (\text{O.D. of test} - \text{O.D. of product control}) \times 100$$

Percentage inhibition =

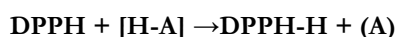
O.D. of Control

ANTI OXIDANT (DPPH RADICAL SCAVENGING ASSAY)

The radical scavenging activity of different samples were determined by using DPPH assay according to Chang et al [2001]. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm.

PRINCIPLE

1, 1-diphenyl-2-picryl hydrazyl is a stable free radical with pink colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,



Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

REAGENT PREPARATION

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of methanol.

ANTI OXIDANT (FERRIC REDUCING ANTIOXIDANT POWER ASSAY) (FRAP):

The Ferric Reducing Antioxidant Power (FRAP) assay is a widely used method to evaluate the total antioxidant capacity of biological samples, plant extracts, foods, and pharmaceutical compounds. It is based on the principle that antioxidants can reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in an acidic medium, forming a colored ferrous-tripyridyltriazine complex that absorbs light at 593 nm.

The intensity of the blue color—and thus the absorbance measured—is directly proportional to the reducing power of the sample. The FRAP assay is simple, rapid, reproducible, and does not require specialized equipment beyond a spectrophotometer. It is particularly useful for assessing the antioxidant potential of samples containing phenolics, ascorbate, and other reducing agents.

In this experiment, the FRAP assay was applied to the sample “Ferriv anti” to quantify its antioxidant capacity based on its ability to reduce ferric ions.

MTT ASSAY:

PROJECT REQUIREMENT

- To determine the cytotoxicity study on L929 cell lines, 1 sample was received.
- The sample details as below:

S.No	Sample	Concentration	Cell lines
1.	Formulation of <i>In-situ</i> gel	5(6.25,12.5,25,50 and 100)	L929

TABLE 2: Details Of Samples Received for the Study

BACKGROUND OF THE STUDY

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-coloured water soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm. (Alley et al and Mosamann et al)

MATERIALS

1. Cell lines: L929 cell lines (NCCS, Pune)
2. Cell culture medium: MEM (Himedia)
3. Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
4. Fetal Bovine Serum (#RM10432, Himedia)
5. MTT Reagent (5 mg/ml) (# 4060 Himedia)
6. DMSO (#PHR1309, Sigma)
7. D-PBS (#TL1006, Himedia)
8. 96-well plate for culturing the cells (From Corning, USA)
9. T25 flask (# 12556009, Biolite - Thermo)
10. 50 ml centrifuge tubes (# 546043 TARSON)
11. 1.5 ml centrifuge tubes (TARSON)

12. 10 ml serological pipettes (TARSON)

13. 10 to 1000 ul tips (TARSON)

EQUIPMENTS

1. Centrifuge (Remi: R-8C).
2. Pipettes: 2-10 μ l, 10-100 μ l, and 100-1000 μ l.
3. Inverted binocular biological microscope (Olympus, USA)
4. Biosafety hood (Precision Scientific, Chennai)
5. 37°C incubator with humidified atmosphere of 5% CO₂ (ThermoFisher, USA)
6. 96 well plate reader (EPOCH2, BioTek, CA, USA)

ASSAY CONTROLS

- (i) Medium control (medium without cells)
- (ii) Negative control (medium with cells but without the experimental drug/compound)

Note: Extracellular reducing components such as ascorbic acid, cholesterol, alpha-tocopherol, dithiothreitol present in the culture media may reduce the MTT to formazan. To account for this reduction, it is important to use the same medium in control as well as test wells.

MAINTENANCE OF CELL LINES

The L929 cell lines is purchased from NCCS, Pune, India. The cells were maintained in MEM media supplemented with 10 % FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO₂, 18-20% O₂ at 37 °C temperature in the CO₂ incubator and sub-cultured for every 3 days.

STEPS FOLLOWED

1. Seed 200 μ l cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 24 hours.
2. Add appropriate concentrations of the test agents (Mentioned in the results - Excel sheet)
3. Incubate the plate for 24 hrs at 37°C in a 5% CO₂ atmosphere.
4. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume.
5. Wrap the plate with aluminium foil to avoid exposure to light.
6. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.)
7. Remove the MTT reagent and then add 100 μ l of solubilisation solution (DMSO).
8. Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures.
9. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm wavelength.
10. % Cell viability is calculated using below formula:

% cell viability = [Mean abs of treated cells/Mean abs of Untreated cells] x 100.

IN VITRO WOUND SCRATCH ASSAY: CELL LINE L929

Cell line studies form the cornerstone of in vitro research, enabling precise evaluation of cytotoxicity, biocompatibility, and therapeutic potential in gel formulations. These experiments mimic the gel's interactions within biological environments, offering critical insights into in situ gel preparations that transition from sol to gel phases under physiological triggers like temperature, pH, or ionic strength. Widely applied in drug delivery for tissue regeneration, nose-to-brain transport, and ophthalmic treatments, these gels must support cell survival, proliferation, and functionality while releasing therapeutic agents. Cell line assays empower researchers to refine formulations by testing cellular responses to varying polymers, drug concentrations, and gelling techniques.

RESULTS AND DISCUSSION

EVALUATION OF IN SITU GEL

Physical Appearance

Colour: The in-situ gel showed a yellowish-orange coloration, resulting from the presence of curcumin and niacinamide.

Consistency: The formulation exhibited a smooth, uniform, and thick consistency indicative of successful poloxamer gelation, featuring non-dripping semi-solid properties suitable for targeted mouth ulcer delivery.

Odor: A distinctive fragrance was observed, arising from the characteristic scents of curcumin and niacinamide.

Determination of pH:

Triethanolamine serves as a common pH adjuster in topical products, helping to increase alkalinity. It achieves this by counteracting acidic ingredients, thereby elevating the overall pH. The bolded values highlight top-performing formulations. pH measurements for these formulations appear in Table 3 below.

TABLE 3: Evaluation data of *In-situ* gel

Formulation	Temperature	pH
F1	68	6.9
F2	62	7.2
F3	58	7.0
F4	48	7.1

Gel strength:

Gel strength in gel formulations measures the capacity to retain structural integrity and resist deformation or flow when subjected to mechanical stress. This property is essential for ensuring mechanical stability, uniform consistency, and reliable performance across pharmaceutical, cosmetic, and food products. The gel strength values for the optimized formulations are presented in Table 4 below

TABLE 4: Evaluation data of *In-situ* gel

S. No	Gel strength	Spreadability	Sol gel	Gelling capacity
F1	25.5±0.48	6.3±0.11	58	Immediate gelation

Spreadability:

Spreadability in gel formulations is a key characteristic that governs the ease with which a gel can be applied and evenly spread across a surface, such as oral mucosa. This attribute significantly influences patient compliance, drug release uniformity, and the efficacy of topical delivery systems. The Spreadability values for the developed formulations are summarized in Table 4.

Sol-Gel Transition Temperature

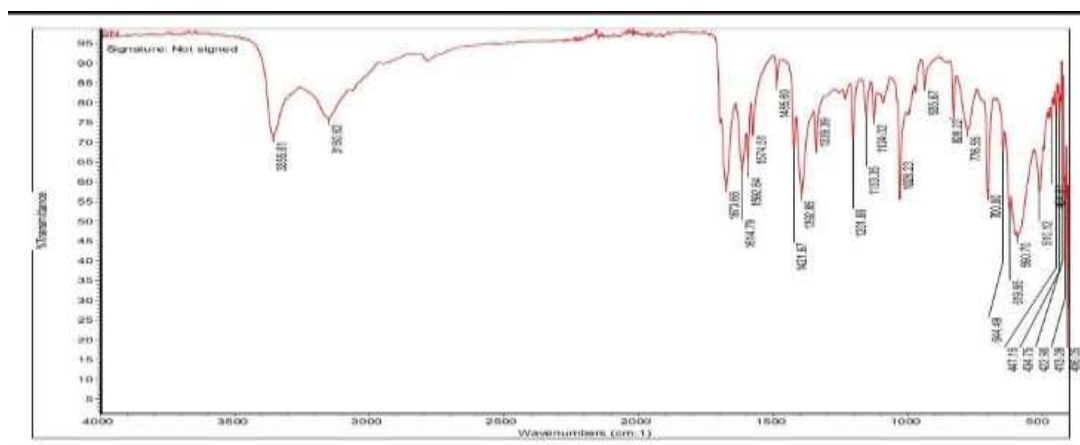
F3 displayed sol-gel temperature of $34.2 \pm 0.5^\circ\text{C}$ ($n=3$), ideal for room-temperature flow (sol phase) and oral gelling at 37°C without premature solidification. Lower values in F1 ($28.7 \pm 0.8^\circ\text{C}$) risked syringe clogging, while F4 exceeded 37°C , reducing retention; F3's profile matched clinical needs for prolonged mucosal contact.

Gelling capacity:

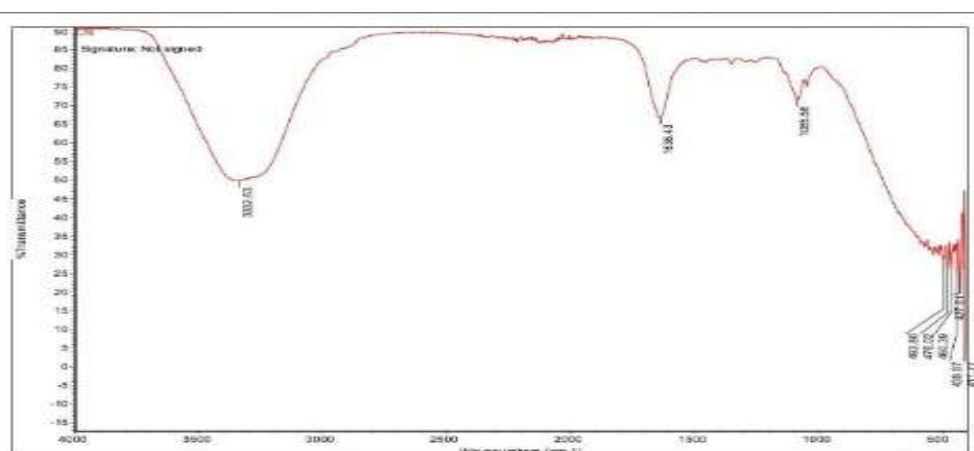
Gelling capacity refers to the ability of a gelling agent to swell and absorb liquid, thereby forming a stable gel network. This property is vital for achieving the desired texture, structural integrity, and long-term stability of the final formulation. The gelling capacity results for the optimized formulations are presented in Table 4.

CHARACTERISTICS OF FTIR

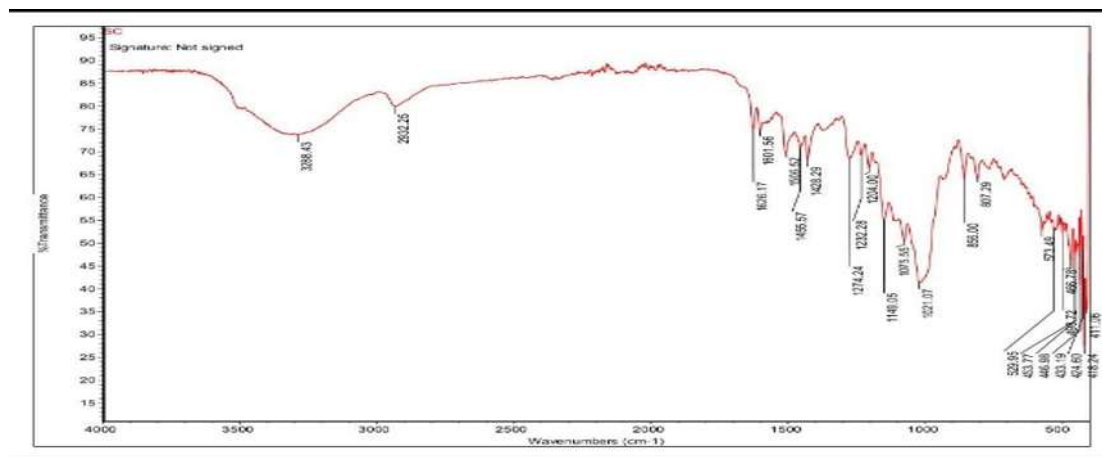
(NIACINAMIDE)



(CURCUMIN)



FORMULATION OF IN-SITU GEL



ANTI INFLAMMATORY ACTIVITY:

Concentration	% inhibition of Standard (Aspirin)	% inhibition of Curcumin
10µg/ml	33%	27%
20µg/ml	43%	30%
30µg/ml	58%	47%
40µg/ml	79%	66%
50µg/ml	88%	82%

Report:

The given Sample Curcumin shows higher anti-inflammatory activity. The Curcumin 82% and Standard Aspirin show 88.0 % of shows Protein denaturation activity at the concentration of 50 µg/ml.

ANTI OXIDANT (DPPH RADICAL SCAVENGING ASSAY)

PROCEDURE

Different concentration of sample such as 12.5µg/mL- 200µg/mL from a stock solution of 10mg/ml were prepared. It was then made up to a final volume of 20µl with DMSO. Following the addition of 1.48ml DPPH (0.1mM) solution, the reaction mixture was incubated in dark at room temperature for 20 minutes. Similarly, a control with an equivalent amount of DMSO was taken in place of test compound. After 20 minutes, the absorbance of the mixture was read at 517nm (SHIMADZU(UV-1900i) UV-VIS spectrophotometer).

CALCULATION:

$$\text{Percentage of inhibition} = \frac{\text{Control test}}{\text{Control}} \times 100$$

Concentration (µg/ml)	Absorbance	Percentage of Inhibition (%)
Control	0.6148	0.0000
Sample Code-Curcumin		
12.5	0.5830	5.1724
25	0.5490	10.7027
50	0.5010	18.5101
100	0.4510	26.6428
200	0.4110	33.1490

IC 50 Value: **Curcumin - 300.2350 µg/mL** (Calculated using ED 50)

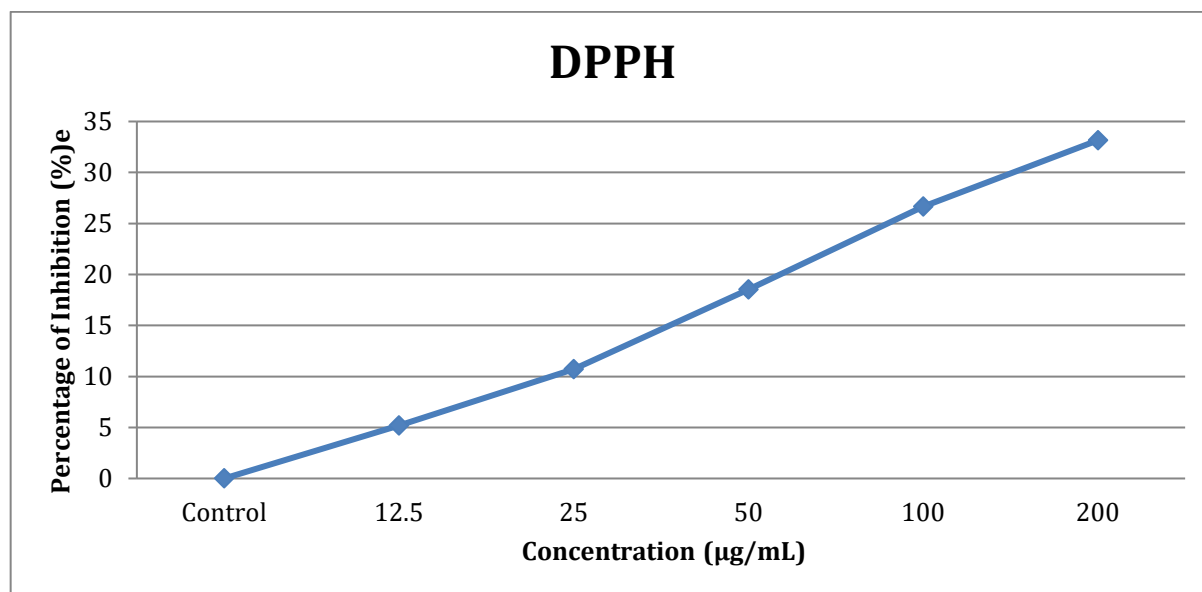


Figure : Graphical representation depicting DPPH Radical scavenging assay of Curcumin

INTERPRETATION

We utilized the DPPH assay to assess the antioxidant potential of Curcumin. Results demonstrated a concentration-dependent enhancement in free radical scavenging activity, with 100 µg/mL of the extract producing 33.1490% inhibition. The IC₅₀ was determined to be 300.2350 µg/mL using ED50PlusV0.1 software.

ANTI OXIDANT (FERRIC REDUCING ANTIOXIDANT POWER ASSAY) (FRAP):

PROCEDURE

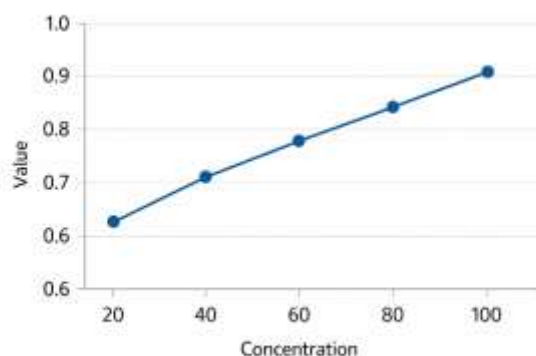
A fresh FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl₃·6H₂O in a 10:1:1 volume ratio. The mixture was warmed to 37°C in a water bath before use to ensure optimal reaction kinetics. And test samples were appropriately diluted in the same solvent like water and ethanol. A standard calibration curve was prepared using Trolox or ascorbic acid solutions at known concentrations (e.g., 0.1–1.0 mM) to quantify antioxidant

capacity in equivalent terms. In test tubes or a microplate, 30 μL of each sample or standard was taken and mixed with 90 μL of distilled water, followed by the addition of 900 μL of the pre-warmed FRAP reagent. For blank measurements, FRAP reagent was replaced with acetate buffer alone. Each sample and standard was prepared in five replicates to ensure statistical reliability. The reaction mixtures were centrifuged and incubated in the dark at 37°C for exactly 30 minutes, allowing for the reduction of Fe^{3+} to Fe^{2+} and the formation of the blue-colored Fe^{2+} -TPTZ complex.

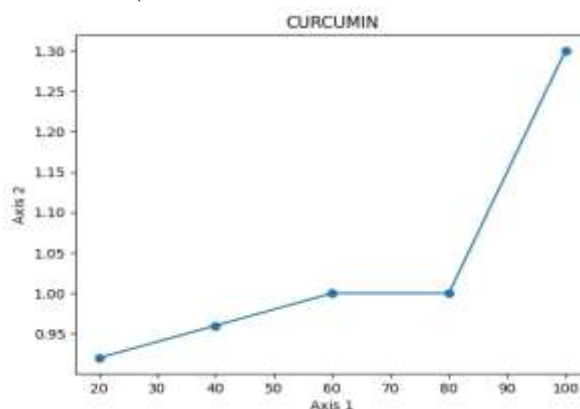
Measurement and Data Analysis: Absorbance was measured at 593 nm using colorimetry against a reagent blank. Antioxidant capacity was calculated based on the absorbance values relative to the standard curve and expressed in Trolox Equivalents (TE) or Ascorbic Acid Equivalents (AAE) per unit mass or volume of sample.

Concentration	Standard	Niacinamide	Curcumin
20	0.63	0.46	0.92
40	0.74	0.52	0.96
60	0.82	0.55	1.00
80	0.88	0.77	1.00
100	0.96	0.85	1.30

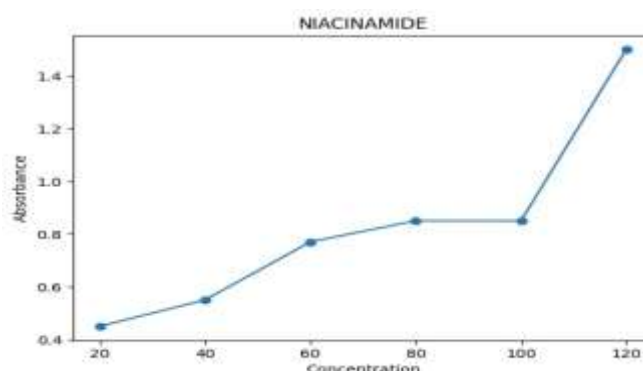
STANDARD



CURCUMIN (Here AXIS 1=Concentration; AXIS 2=Absorbance)



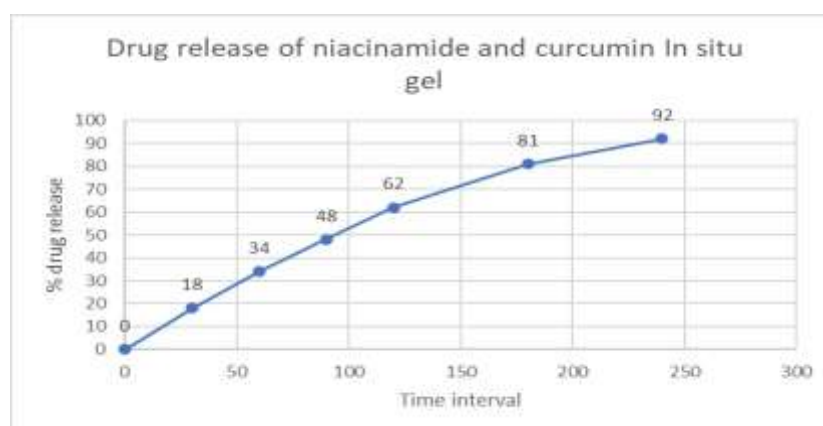
NIACINAMIDE



INVITRO DRUG RELEASE

The in-vitro release of niacinamide and curcumin from the in-situ gel was evaluated using the egg shell membrane diffusion method in a Franz diffusion cell. Egg shell membranes were separated from fresh hen eggs, washed with distilled water, and hydrated in phosphate buffer (pH 6.8) for 30 min before being mounted between the donor and receptor compartments. The receptor compartment was filled with phosphate buffer (pH 6.8), maintained at 37 ± 0.5 °C, and continuously stirred, while approximately 1 g of the gel formulation was placed in the donor compartment. Samples of 5 mL were withdrawn from the receptor compartment at predetermined time intervals (0, 30, 60, 90, 120, 180, and 240 min) and immediately replaced with an equal volume of fresh buffer. The samples were analyzed using a UV–Visible spectrophotometer at 420 nm, and the cumulative percentage drug release was calculated and plotted as a function of time to obtain the drug release profile.

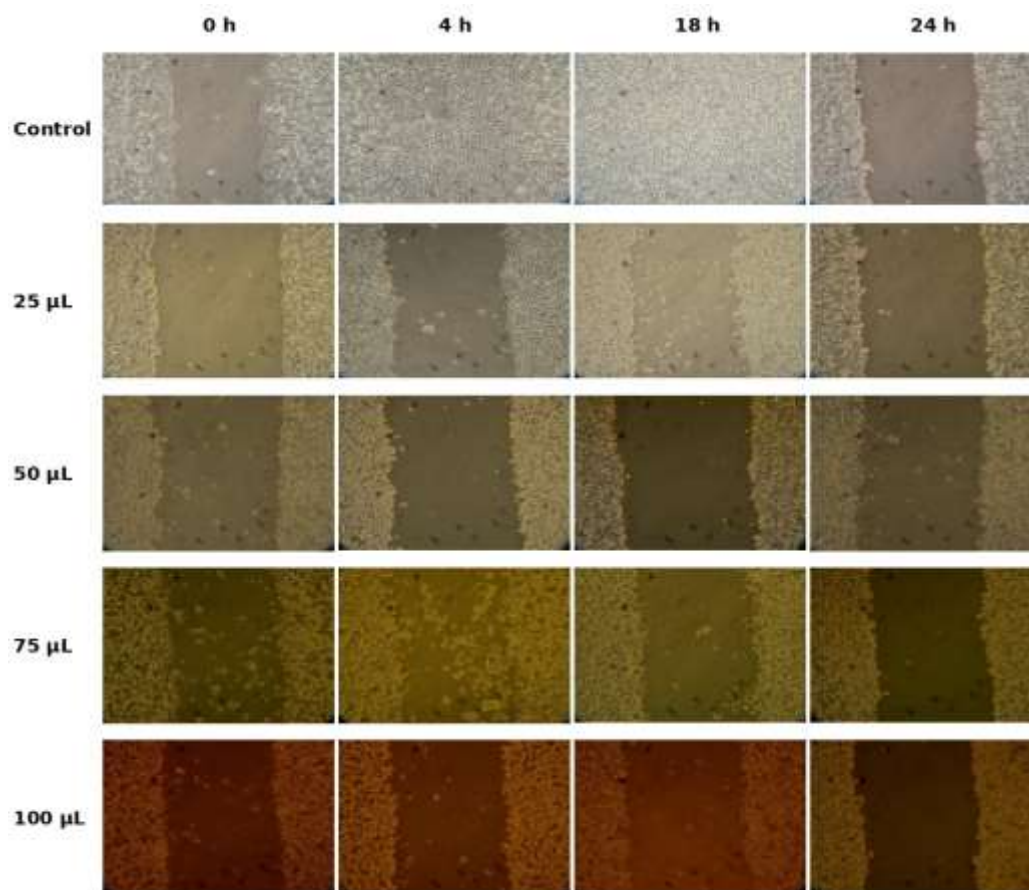
Time(min)	Absorbance	%Drug release
0	0.000	0
30	0.145	18
60	0.278	34
90	0.392	48
120	0.503	62
180	0.645	81
240	0.732	92



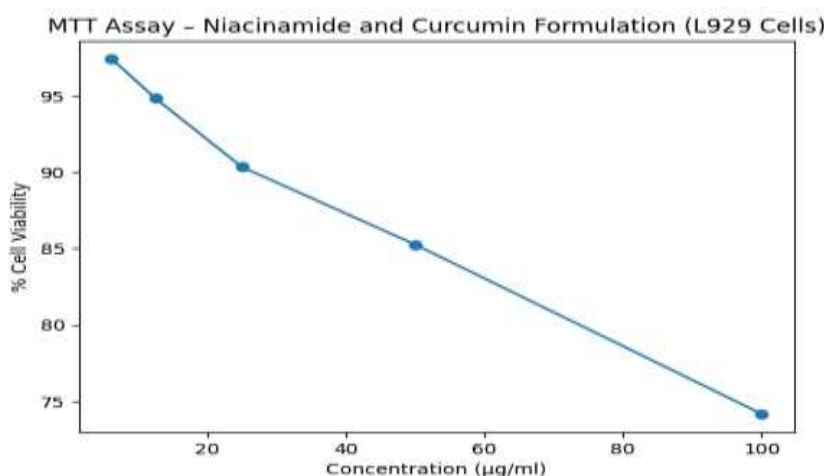
IN VITRO WOUND SCRATCH ASSAY: CELL LINE L929

Cell line studies form the cornerstone of in vitro research, enabling precise evaluation of cytotoxicity, biocompatibility, and therapeutic potential in gel formulations. These experiments mimic the gel's interactions within biological environments, offering critical insights into in situ gel preparations that transition from sol to gel phases under physiological triggers like temperature, pH, or ionic strength. Widely applied in drug delivery for tissue regeneration, nose-to-brain transport, and

ophthalmic treatments, these gels must support cell survival, proliferation, and functionality while releasing therapeutic agents. Cell line assays empower researchers to refine formulations by testing cellular responses to varying polymers, drug concentrations, and gelling techniques.



SUMMARY-MTT ASSAY		IC₅₀ = 118.6 µg/ml
Concentration of sample (µg/ml)	% cell viability	
Untreated	100	
6.25	97.42	
12.5	94.86	
25	90.35	
50	85.27	
100	74.18	



- The absorbance readings with calculations are enclosed in MS excel format.
- The direct microscopic observations of drug treated images of test compound after 24 hours of incubation were enclosed in the separate folder along with this report.

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