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DEVELOPMENT AND CHARACTERIZATION OF NANOEMULSION OF IPRIFLAVONE FOR THE TREATMENT OF OSTEOPOROSIS

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Abstract

This study investigates the physicochemical properties, formulation optimization, and stability of Ipriflavone nanoemulsions aimed at enhancing its therapeutic efficacy in osteoporosis treatment. Ipriflavone, characterized as a white to off-white crystalline powder with a melting point of 172–175°C, exhibited favorable flow properties with a bulk density of 0.58 g/cm³, tapped density of 0.46 g/cm³, compressibility index of 13.79%, and a Hausner ratio of 1.16, indicating moderate flowability. Five nanoemulsion formulations (NS-1 to NS-5) were evaluated for drug loading efficiency, yielding values ranging from 43.47% to 48.88%, with NS-3 achieving the highest yield at 75.32%. Particle size analysis revealed that NS-1 had a particle size of 436 nm, while NS-5 demonstrated the smallest size at 251 nm, correlating with improved stability. Spreadability and viscosity assessments indicated that NS-5 provided optimal ease of application (5.8 g•cm²/sec) and acceptable viscosity (8125 cps). A three-month stability study at 30 ± 2°C and 75 ± 5% relative humidity confirmed that the optimized ocular insert formulation maintained its appearance and drug content, slightly decreasing from 93.15% to 93.05%. These results underscore the potential of Ipriflavone nanoemulsions for effective delivery systems, providing a foundation for future clinical applications.

Keywords: Ipriflavone, nanoemulsion, physicochemical properties, stability study,

INTRODUCTION

Osteoporosis is a common skeletal disorder characterized by reduced bone mass and structural deterioration of bone tissue, resulting in increased susceptibility to fractures. It primarily affects postmenopausal women and older adults, leading to significant morbidity and healthcare costs globally. The World Health Organization (WHO) defines osteoporosis as a bone mineral density (BMD) that is 2.5 standard deviations or more below the mean value for young healthy women. With increasing life expectancy, osteoporosis poses a growing challenge, particularly in developed and developing countries, where aging populations are prevalent.[1]

The pathophysiology of osteoporosis involves an imbalance between bone resorption and bone formation. As individuals age, the rate of bone resorption by osteoclasts exceeds the rate of bone formation by osteoblasts. This imbalance leads to thinning of the bone trabeculae, reduced bone strength, and increased risk of fractures, particularly in the spine, hip, and wrist. Traditional therapies include bisphosphonates, selective estrogen receptor modulators (SERMs), and hormone replacement therapy (HRT), but these treatments often come with limitations such as poor patient compliance, side effects, and suboptimal efficacy.[2]

Ipriflavone, a synthetic isoflavone, has been extensively studied for its potential to prevent bone resorption and enhance bone formation. It acts by inhibiting osteoclast activity and stimulating osteoblast function, offering a dual mechanism of action in the treatment of osteoporosis. Ipriflavone has shown promise in clinical trials, where it was found to reduce bone loss and increase bone mineral density. However, its clinical application has been limited due to poor bioavailability and rapid first-pass metabolism in the liver, which significantly reduces its therapeutic efficacy. Oral administration of ipriflavone results in low plasma concentrations, necessitating higher doses that may lead to side effects such as gastrointestinal discomfort and liver enzyme elevation.[3]

Osteoporosis, a condition marked by reduced bone mass and structural degradation, remains a major global health concern. Traditional treatments, such as bisphosphonates, have shown effectiveness but often present limitations in terms of side effects, patient adherence, and insufficient bone mass improvement.[4]

Ipriflavone, a synthetic isoflavone derivative, has been investigated for its ability to inhibit bone resorption and stimulate bone formation by acting on osteoclasts and osteoblasts. Several clinical studies have demonstrated its potential in improving bone mineral density (BMD). However, the major drawback of ipriflavone is its poor bioavailability and rapid metabolism, reducing its overall therapeutic efficacy.[5-6]

Nanotechnology offers an innovative solution through nanoemulsions, which improve drug delivery by enhancing solubility, stability, and absorption. Nanoemulsions, characterized by their small droplet size (20-200 nm), have shown promise in improving the bioavailability of poorly soluble drugs. By incorporating ipriflavone into a nanoemulsion system, researchers have aimed to enhance its pharmacokinetic profile, potentially making it a more viable treatment for osteoporosis.[7-8]

Studies on nanoemulsions for drug delivery have highlighted improved absorption, sustained drug release, and increased therapeutic efficacy. This formulation strategy could address the challenges associated with traditional ipriflavone delivery and may offer a better therapeutic option for osteoporosis patients.[9-10]

Material used

The materials used for the study included Ipriflavone, which was obtained from Sigma Aldrich. Tween 80 and Insulin were also sourced from Sigma Aldrich. Distilled water used in the experiments was prepared in the college laboratory. Polyethylene Glycol 200 was acquired from Sigma Aldrich. Phosphate buffer saline (PBS 7.4), distilled water, and ferric chloride solution were prepared in the laboratory. Ethanol and methanol were procured from Sigma Aldrich, India, along with sodium bicarbonate. All other chemicals utilized in the study were of analytical grade.

The drug, Ipriflavone, used in this study was collected as a gift from a nearby Pharmacy shop.

Methodology

Physical Appearance

Physical appearance of was determined by visual observation.

pH

The pH of 1% aqueous solution of Ipriflavone was checked by using pH meter (EQ-610, Equiptronics, Mumbai).

Determination of melting point

The melting point of the drug was determined using a capillary tube, closed at one end, dipped in a liquid paraffin bath within a melting point apparatus (VMP-D, Veego, Mumbai). The temperature at which the drug melted was recorded.

Determination of flow properties of pure drug

Bulk density

Bulk density is calculated by placing a known weight of sieved powder in a graduated cylinder without compaction and measuring the volume. It is determined by dividing the mass by the total volume, including particle, inter-particle void, and pore volumes. Bulk density varies with vibration, making it dependent on the material's treatment.

Bulk density= Mass of powder/volume of powder

Tapped density

Tapped density is measured by mechanically tapping a drug sample in a graduated cylinder until the volume stabilizes. The cylinder is tapped at a fixed rate of 300 drops per minute, with the volume recorded after 500 taps and again after 100 more taps. The final volume is used to calculate the tapped density.

Tapped density= Mass of powder/Tapped volume

Compressibility index and Hausner ratio

Powder compressibility is typically assessed using the Carr index in pharmaceutics. A low Carr index indicates good flowability, where bulk and tapped densities are similar. In contrast, a higher Carr index, above 25, suggests poor flowability due to greater interparticle interactions. The Hausner ratio, calculated by dividing tapped density by bulk density, also measures flowability. A ratio around 1.2 indicates good flow, while values above 1.25 to 1.4 suggest poor flowability. Both the Carr index and Hausner ratio are key indicators of powder flow properties.

Carr's index= TD-BD/TD×100

Where,

TD=Tapped density

BD=Bulk density

Hausner ratio= TD/BD

Hausner ratio, <=1.25(good flow)

Angle of repose

The angle of repose is crucial for assessing a powder's flowability. It is measured as the angle between the horizontal plane and a sloping line on a heap formed by dumping the powder. This technique allows for quick and accurate measurement of flowability. A higher angle of repose indicates greater cohesiveness and poor flow, while a lower angle signifies better flow characteristics.

$$\text{Tan } \theta = h/r$$

$$\theta = \text{tan-1}h/r$$

Where,

h= height of pile (cm)

r= radius of pile (cm)

Method of preparation of Nanoemulsion

To prepare a nanoemulsion suspension, first dissolve the active drug (e.g., Ipriflavone) in an oil phase, such as soybean oil, applying gentle heat if necessary. Meanwhile, create the aqueous phase by dissolving a surfactant like Tween 80 in purified water. Slowly add the oil phase to the aqueous phase while stirring to form a coarse emulsion. This emulsion is then homogenized using high-pressure or ultrasonic methods to reduce droplet size to below 200 nm. Finally,

analyze the nanoemulsion for droplet size, zeta potential, and stability to ensure it meets the required specifications.

Table : Optimization of Components of Ipriflavone Loaded Formulation

S.NO	Formulation Code (Nanoemulsion Suspension-NS)	Tween 80	Propylene glycol:Distilled Water	Ipriflavone (mg)
1.	NS-1	4%	20:80	20
2.	NS-2	6%	30:70	20
3.	NS-3	8%	25:75	20
4.	NS-4	4%	30:70	20
5.	NS-5	6%	25:75	20

Physicochemical characterization of Nanoemulsion

Drug loading and loading efficiency

Drug loading efficiency is the ratio of the drug in nanoparticles to the total drug used. To evaluate this, 2 mg of nanoparticles was suspended in 2 ml of a 40:60 (v/v) water-acetonitrile solution, vortexed for five minutes, and incubated at 37°C for three to four hours. After centrifugation at 10,000 rpm for five minutes, the supernatant's absorbance was measured at 218 nm. A blank formulation was used for comparison. The difference in absorbance determined the actual drug content, allowing for the calculation of drug loading percentage and efficiency:

$$\textbf{Percentage of loading} = \frac{Amount\ of\ in\ present\ drug\ nanoparticles}{Weight\ of\ nanopartic\ le\ analyzed} \textbf{ x 100}$$

$$\textbf{Percentage of efficiency} = \frac{Actual\ loading\ drug}{Theoretical\ \ drug\ loading} \textbf{ x 100}$$

Yield Percentage

The quantity of nanoemulsion produced was calculated based on the total amount of raw materials used. The lyophilized nanoemulsion was weighed, and the percentage yield of the formulations was determined using the following formula:

$$\textbf{Percentage of loading} = \frac{\textit{Amount of nanopartic obtained}}{\textit{Total of polymer and drug used}} \textbf{ x 100}$$

Particle size, size distribution and zeta potential

The stability and efficacy of nanoparticle-based therapeutics depend on particle size distribution and zeta potential. Particle size is measured using techniques like dynamic light scattering (DLS) and electron microscopy. Zeta potential indicates electrostatic repulsion; high values suggest stability, while low values indicate potential aggregation. Controlling these parameters is essential for optimal drug delivery and effectiveness Overall, understanding particle characteristics is crucial for developing successful nanoparticle formulations.

Preparation of nanoparticle Gel

Once the nanoemulsion suspension is ready, incorporate it into a gel matrix. Start by dispersing a gelling agent like Carbomer 940 in purified water using a high-shear mixer to prevent clumping. Allow the gelling agent to hydrate, forming a gel base. Neutralize the gel with triethanolamine and adjust the pH to 6-7 for the desired consistency. Gradually add the nanoemulsion while stirring to ensure even distribution, adjusting the volume with additional purified water if necessary. Mix thoroughly and use a homogenizer for a smooth texture. Add preservatives, such as propyl paraben, to prevent microbial contamination. Conduct quality control checks on viscosity and pH, and evaluate gel stability. Finally, package the nanoemulsion gel into sterile, sealed containers for use.

Table : Preparation of Nanoemulsion Gel

S.NO	Formulation Code (Nanoparticle Gel -	Carbomer 940	Purified Water	Propyl paraben	Triethanolamine	Nanoemulsion Suspension

	NG)					
1.	**NG-1**	**10**	**20**	**As much as suffices**	**2**	**Nanoemulsion Suspension 1**
2.	**NG-2**	**15**	**20**	**As much as suffices**	**4**	**Nanoemulsion Suspension 2**
3.	**NG-3**	**18**	**20**	**As much as suffices**	**2**	**Nanoemulsion Suspension 3**
4.	**NG-4**	**15**	**20**	**As much as suffices**	**4**	**Nanoemulsion Suspension 4**
5.	**NG-5**	**11**	**20**	**As much as suffices**	**2**	**Nanoemulsion Suspension 5**

Characterization & Evaluation of Formulation

Evaluation of Gel Formulation

All prepared formulations of gel were characterized for:

Physical Evaluation

Physical parameters such as color and appearance of the herbal gel were observed manually.

Measurement of pH

Skin pH is crucial for the stability of topical preparations, particularly gels, as human skin typically has a pH ranging from 5.5 to 6. Therefore, the pH of gels should align with this range. The pH of various gel formulations was measured using a digital pH meter. One gram of gel was dissolved in 100 ml of distilled water and allowed to stand for two hours. Each formulation's pH was measured in triplicate, and the average value was calculated.

Spreadibility

Spreadability was determined using a wooden block apparatus with a pulley. About 2 g of gel was placed on a ground slide and sandwiched between it and another glass slide with a hook. A 1 kg weight was applied for 5 minutes to create a uniform gel film. Excess gel was scraped off, and then the top slide was pulled with an 80 g weight. The time (in seconds) taken to cover 7.5 cm was recorded, with shorter times indicating better spreadability. Spreadability was calculated using a specific formula:

$$S = M \times L / T$$

Where,

S = Spreadibility,

M = Weight in the pan (tied to the upper slide),

L = Length moved by the glass slide

T = Time (in sec.) taken to separate the slide completely each other.

Consistency

The consistency of the gels was measured by dropping a cone attached to a rod from 10 cm onto the center of a glass cup filled with gel. After 10 seconds, the penetration depth from the gel surface to the cone tip was recorded.

Homogeneity

All the developed gels were tested for homogeneity by visual inspection after setting the gels in the container. They were observed for their appearance and presence of any aggregates.

Viscosity

Viscosity was measured using a Brookfield viscometer with spindle No. 7 at 50 rpm and room temperature. The gels were rotated at 0.3, 0.6, and 1.5 rpm, recording the corresponding dial readings at each speed. Viscosity was calculated by multiplying the dial reading by the factor provided in the Brookfield viscometer manual.

Stability Studies

In stability studies, the optimized formulations were subjected to storage conditions simulating different temperature and humidity levels to assess their stability over time. The formulations were stored at two distinct conditions:

1. **30°C ± 2°C / 65% ± 5% RH**
2. **40°C ± 2°C / 75% ± 5% RH**

Results

Pre-formulation studies

Physical Appearance

Ipriflavone is found in the form of a white to off-white crystalline powder...

Determination of melting point

Melting point ranging from 172°C to 175°C.

Determination of flow properties of pure drug

Bulk density and tapped density

The flow properties of the pure drug were determined by assessing key parameters. These values are essential for understanding the material's handling and processing characteristics in pharmaceutical formulations.

Table : Determination of flow properties of pure drug

Parameters	Values
Bulk density	0.50
Tapped density	0.58

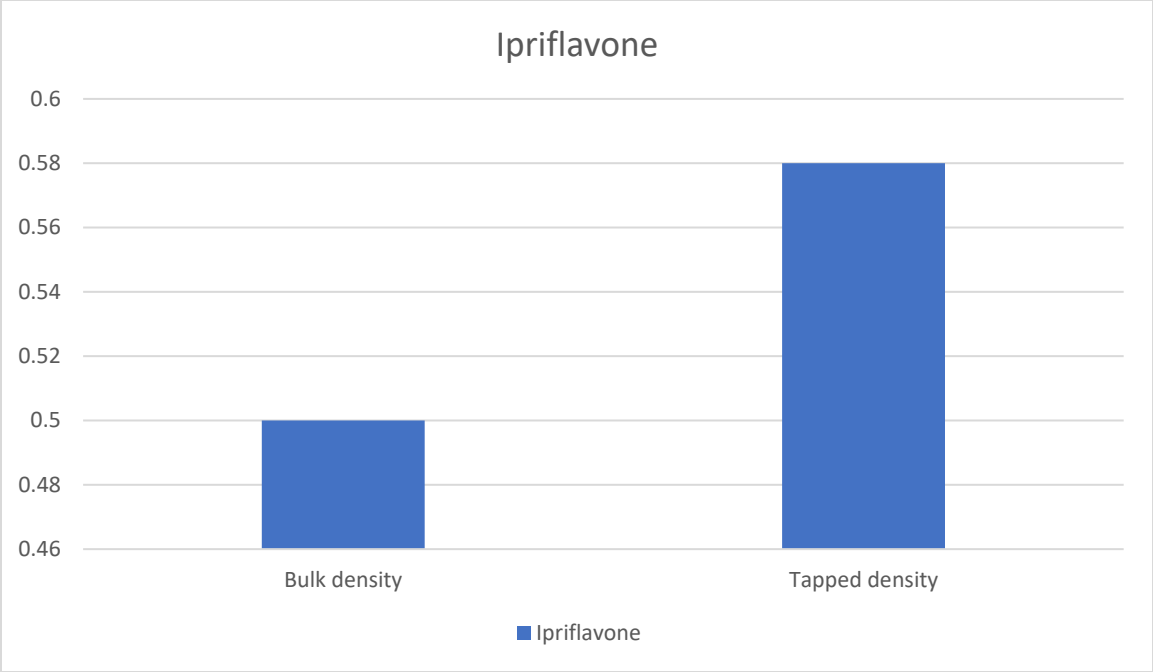

Figure : Graph of flow properties of Ipriflavone

5.4.2 Compressibility index, hausner ration and angle of repose

The compressibility index (CI) is a measure of the powder's ability to be compressed. An angle of repose of 25.68° indicates that the powder has good flow properties, as lower angles suggest better flowability.

Table : Determination of flow properties of pure drug

Parameters	Values
Compressibility index	13.79%
Hausner ratio	1.16
Angle of repose	25.68^0

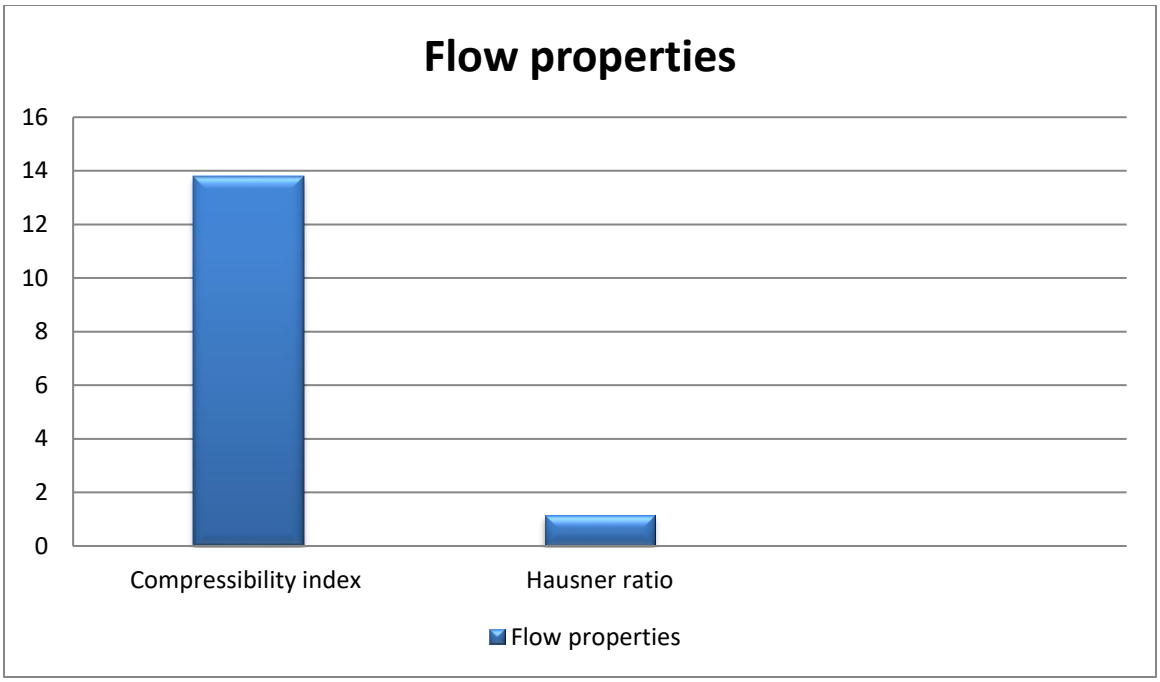

Figure : Graph of flow properties of Ipriflavone

Physicochemical characterization of nanoparticles

The physicochemical characterization of various nanoemulsion suspensions (NS) was conducted to assess their performance.

Table Physicochemical characterization of Nanoemulsion

Formulation (Nanoemulsion Suspension-NS)	Drug Loading (%)	Loading Efficiency (%)	Yield Percentage (%)
NS-1	47.34	62.57	66.27
NS-2	45.46	60.28	64.48
NS-3	44.54	59.44	75.32
NS-4	43.47	57.37	54.36
NS-5	48.88	61.34	59.58

Table Physicochemical characterization of nanoparticles

Formulation	Particle Size (nm)	Zeta Potential (mV)
NS-1	436	22.34
NS-2	214	24.67
NS-3	381	25.22
NS-4	356	26.88
NS-5	251	25.67

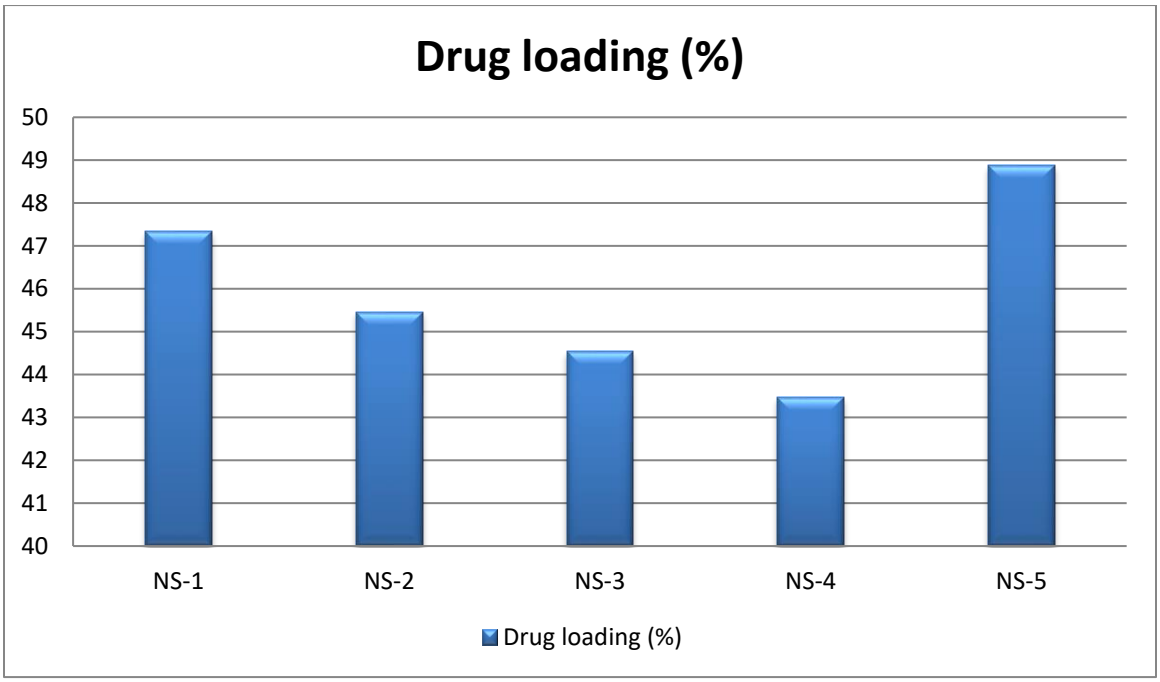

Figure : Graph of Drug loading

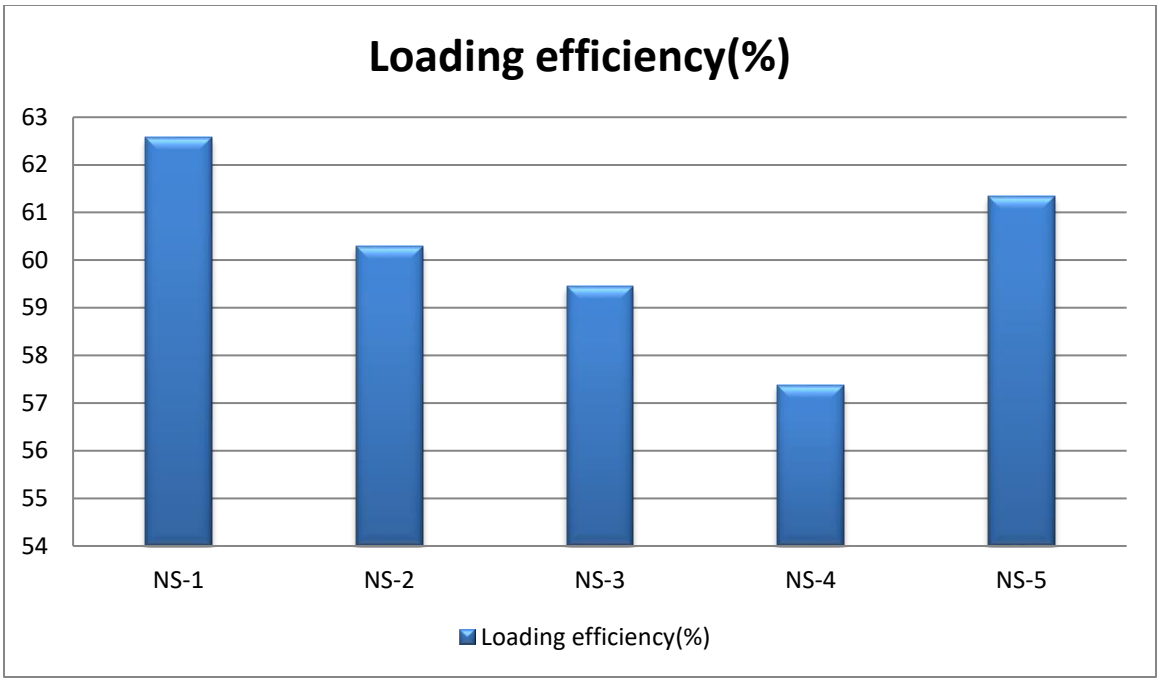

Figure 5.23 Graph of Loading efficiency

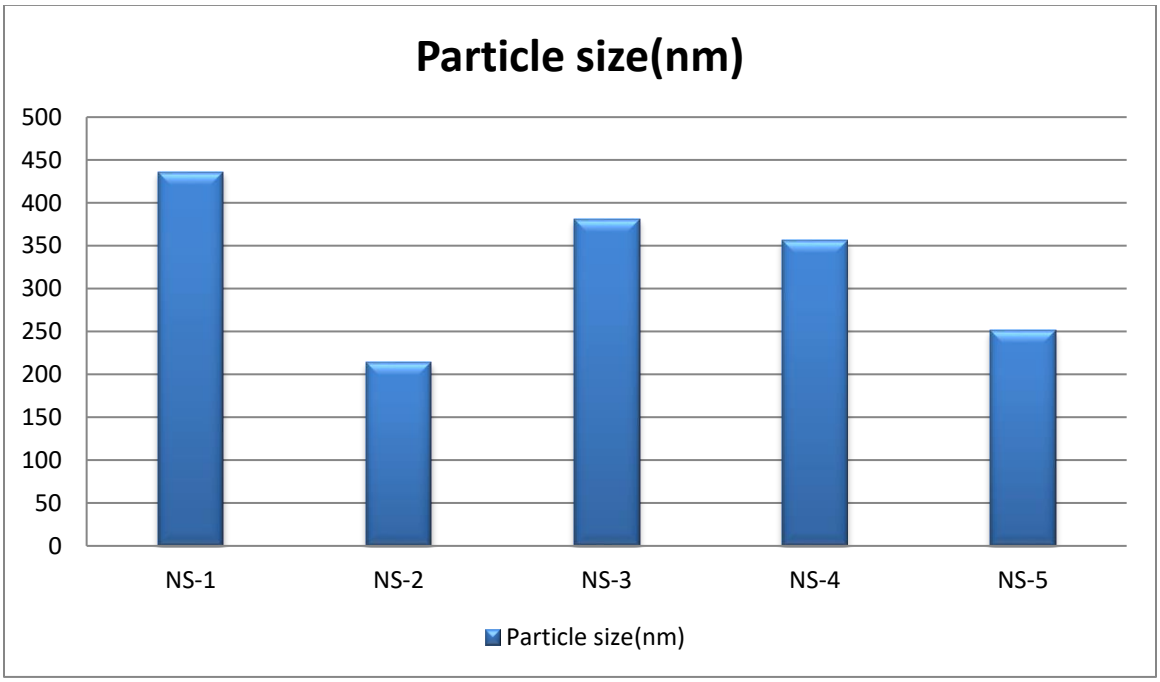

Figure : Graph of Particle size

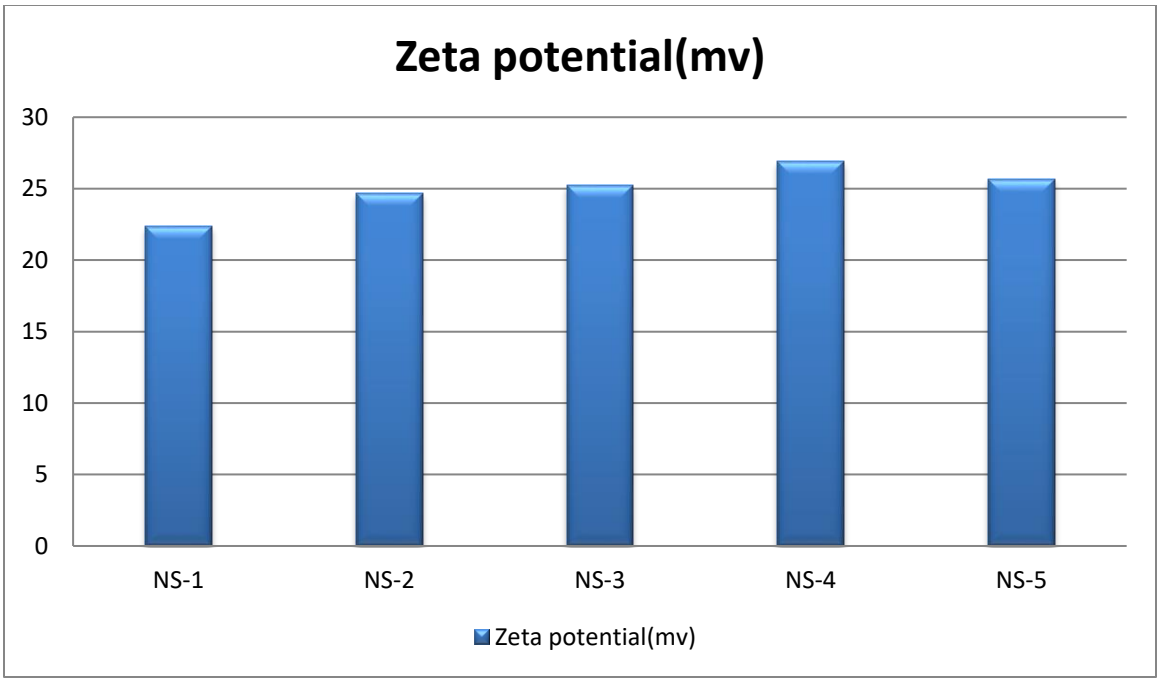

Figure : Graph of Zeta potential

Evaluation of Gel Formulation

The physicochemical properties of the formulations were further evaluated for spreadability and viscosity. These findings provide valuable insights into the performance and usability of each gel formulation.

Table : Evaluation of Gel Formulation

Formulation	**pH**	**Consistency(mm)**	**Homogeneity**
NS-1	**6.6**	**5.4**	Homogenous
NS-2	**5.5**	**5.4**	Homogenous
NS-3	**5.5**	**5.6**	Homogenous
NS-4	**6.5**	**6.3**	Homogenous
NS-5	**5.5**	**4.5**	Homogenous

Table : Evaluation of Gel Formulation

Formulation	**Spreadability (g cm²/sec)**	**Viscosity**
NS-1	5.5	8470
NS-2	5.7	8440
NS-3	5.2	7582
NS-4	5.1	7404
NS-5	5.8	8115

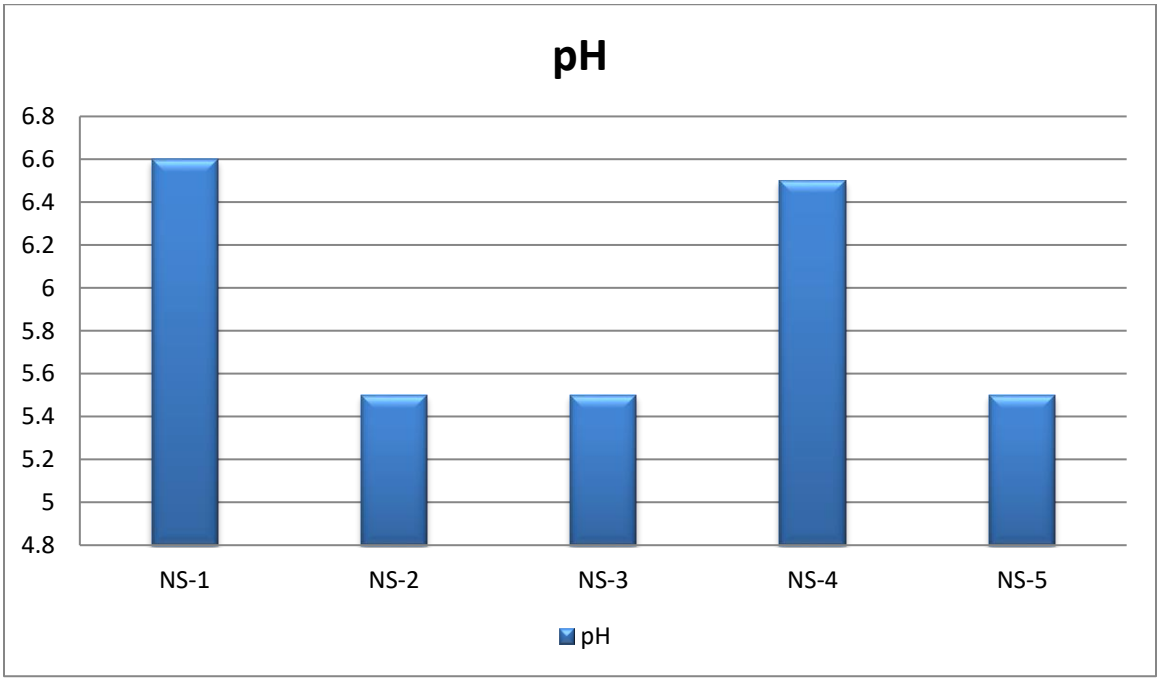

Figure : Graph of pH

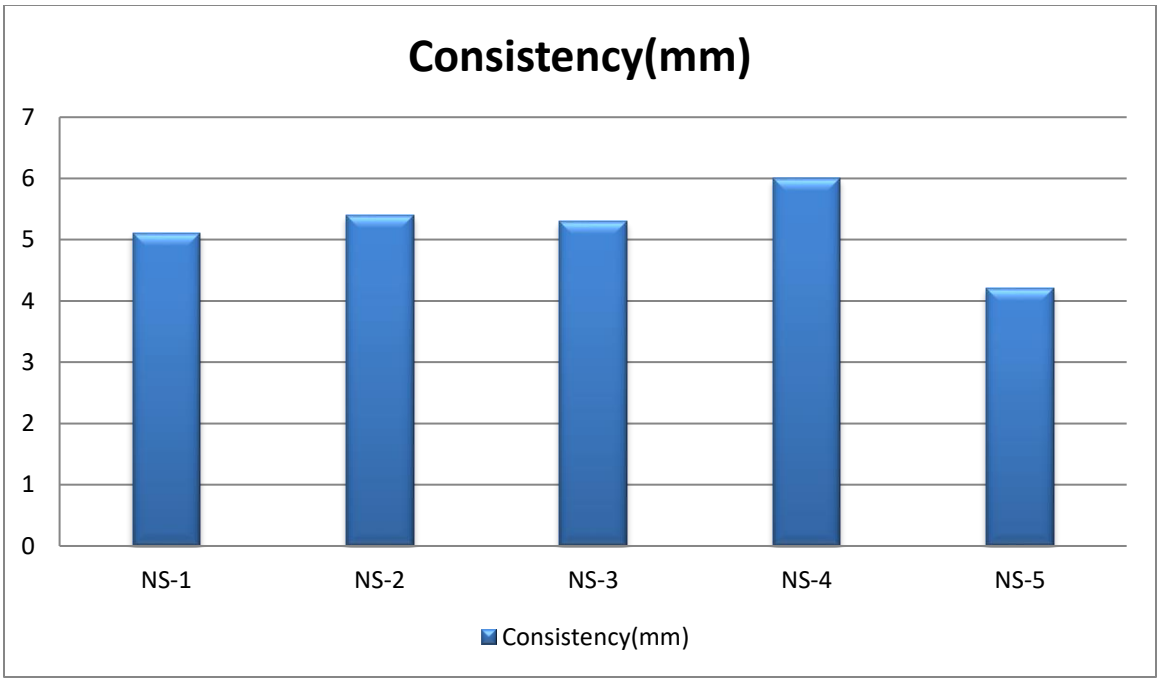

Figure : Graph of Consistency

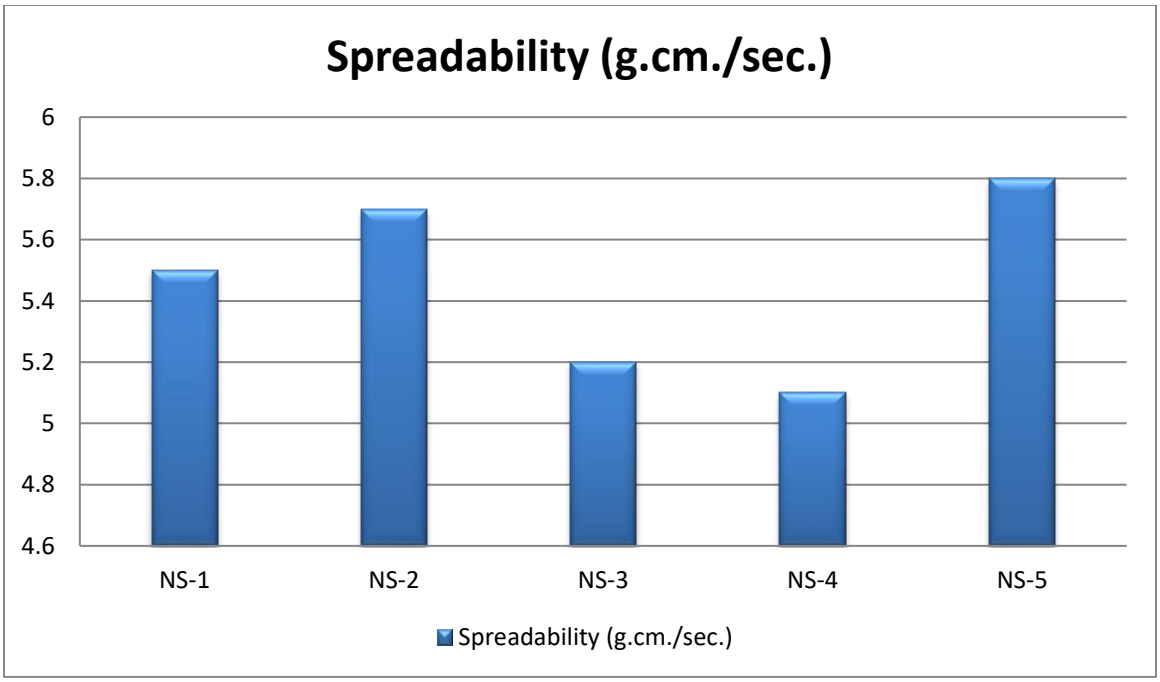

Figure : Graph of Spreadability

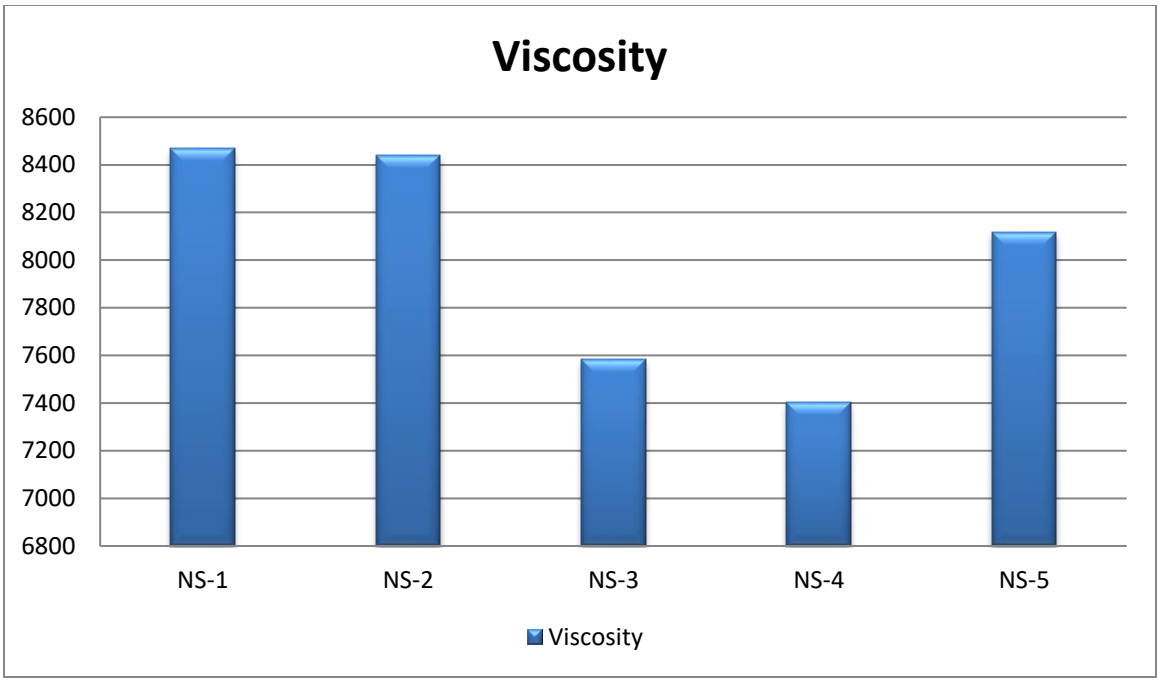

Figure : Graph of Viscosity

Accelerated Stability Studies

Stability study was carried out on optimized ocular inserts formulation for three months.

Table : Stability Study Data

Time in Month	Temperature / %RH	Appearance	% Drug content
1	40 ± 2ºC/ 75 ± 5	Smooth, transparent Surface	93.15
2	40 ± 2ºC/ 75 ± 5	Smooth, transparent Surface	93.15
3	40 ± 2ºC/ 75 ± 5	Smooth, transparent surface	93.05

CONCLUSION

In conclusion, the study of Ipriflavone and its nanoemulsion formulations demonstrates promising potential for therapeutic applications, particularly in the treatment of osteoporosis. The physicochemical properties of Ipriflavone indicate good flowability and compressibility, which are favorable for formulation development. The varying drug loading efficiencies across different formulations highlight the importance of optimization in achieving desired therapeutic outcomes. Notably, the particle size and zeta potential analyses suggest that smaller particle sizes correlate with enhanced stability, which is crucial for maintaining the efficacy of the formulations. Additionally, the evaluations of spreadability and viscosity reveal that formulations like NS-5 offer excellent ease of application while maintaining appropriate thickness, enhancing their usability. Finally, the stability study confirms that the optimized ocular insert formulation remains stable over three months under controlled conditions, with minimal loss in drug content, ensuring its suitability for clinical application. Overall, these findings contribute valuable insights into the development of effective nanoemulsion-based delivery systems for Ipriflavone, paving the way for further research and potential clinical implementation.

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