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Formulation & Evaluation of Nanosuspension of Luteolin

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KEYWORDS

Luteolin, Nanosuspension, Solvent–Antisolvent Method, Full Factorial Design, Characterization, Oral Bioavailability, Antibacterial Activity.

ABSTRACT:

Background: Luteolin (LUT), a flavonoid with antioxidant, anti-inflammatory, and anticancer properties, suffers from inadequate oral bioavailability and poor water solubility , limiting its clinical utility.

Aim: The goal of the study was to create and improve a luteolin nanosuspension in order to increase its oral bioavailability and solubility.

Method: A bottom-up solvent–antisolvent precipitation method was employed using Hydroxypropyl methylcellulose (HPMC) and sodium lauryl sulphate (SLS) as stabilizers. The formulation parameters were optimised using a 3^2 full factorial design. The prepared nanosuspensions were evaluated for particle size, zeta potential, polydispersity index (PDI), solubility, drug content, and in vitro dissolution. Characterization techniques included UV spectroscopy, FTIR, SEM, and X-ray powder diffraction (XRPD). The antibacterial activity was assessed using the agar well diffusion method.

Results: Optimized formulations showed particle sizes around 300–322 nm, with PDI values <0.5 and zeta potentials of up to -33.0 mV, indicating good stability. Solubility significantly increased in methanol and ethanol compared to water. FTIR and XRPD analyses confirmed the compatibility of excipients and a reduction in drug crystallinity, suggesting improved dissolution. Drug content was 97.2% ± 0.25, and in vitro studies showed enhanced dissolution. Antibacterial assays demonstrated activity against E.Coli .

Conclusion: The developed luteolin nanosuspension successfully enhanced solubility and oral bioavailability while showing promising antibacterial activity, making it a viable strategy for poorly water-soluble drugs.

1. Introduction

Any medication's solubility is considered regardless of how it is administered. Developing innovative pharmaceutical items is a huge problem for pharmaceutical masterminds. This is because the new paradigm high-output network found that over half of the active pharmaceutical ingredients (APIs) are either poorly soluble in water or insoluble in it. Many of the novel chemical realities are difficult to formulate due to their low oral bioavailability and weak solubility. Bioavailability is often impacted by the poor water response of about 60 of the recently developed and shovelled APIs. Nanosuspension is an emerging technology that overcomes these issues and significantly improves medicine delivery. Immersion of a systemic and clinical medicine is commensurable.{6}

Since the medicine is water insoluble, immersion may be increased with the addition of its dissolution rate. Clinical adverse goods with low answerable medicines experienced the non-steady immersion because it varies with the patient population and patient-by-patient dosing. The active pharmaceutical element must be separated from the capsule form former to immersion and have to dissolve within the fluid in the stomach. Substances supplied as of Low solubility and high permeability (BSC-II) are fragile to dissolve which causes coy immersion by the gastrointestinal tract. The dissolution

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rate raises while the particle size decreases to the nanoscale.This can also enhance the bioavailability.{6}

Flavonoids are polyphenolic phytochemicals widespread supplied across shops and vegetables. They have been said to hawk many natural products with similar effects such as antioxidant, free-radical scavenger, antiinflammatory, antiproliferative and antiestrogenic properties. These goods are good for mortal health, meaning they can prevent and cure diseases like cancer and heart disease. Celery, green pepper, camomile tea, and artichokes are generally found to contain the flavone luteolin in its glycosylated forms. It has been identified as a benefit of xanthine oxidase and protein kinase C and has been demonstrated to have antitumorigenic, anticancer, antiproliferative, and antioxidant properties. Using Caco-2 cells and microsomes isolated from the liver or gut of rats and humans, the luteolin absorption and metabolism have been measured in vitro.{5}

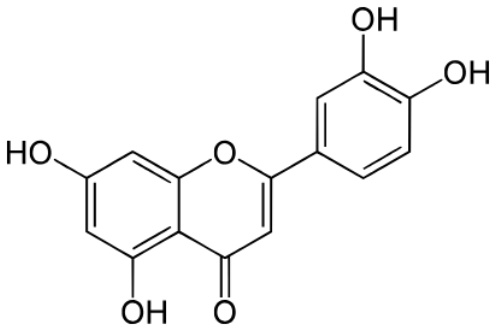

Figure no .1. Structure of Luteolin

Nanoparticle technologies have been utilized as major tools to deliver medicines such as peptides and proteins, vaccines and, more recently, nucleotides. In the field of pharmacy, nanosuspension, nanoemulsion, tone-nanoemulsifying medicine delivery system, solid lipid nanoparticle(SLN), etc., come under the nanotechnology domain. It can be used efficiently to counteract the problems associated with these conventional forms of solubility and bioavailability enhancement. Nanosuspensions are the biphasic systems which are equivalent of pure medicine patches suspended in a thirsty vehicle, stabilized by the assistance of surfactants. They exhibit several advantages, which range from an improvement in solubility, reduction of variability caused by ingestion of food to oral administration, improvement in cohesion to address cell membranes, improvement in bioavailability, ease of expression, ease of scale-up, limited size distribution of the drugs at the nanoscale, adjustable medicine quantum, and relationship in maximum medicines (most particularly medicines which are poorly accountable in both arid and non-arid media), and no interference with blood capillaries.{7}

Besides, the nanosuspension medicine delivery system can be utilized as a liquid capsule form or be transformed into solid capsule formulations such as cream, tablet, bullet, capsule, and film capsule forms. Therefore, nanosuspension may be delivered safely through multitudinous routes such as oral, intravenous, optical, dermal, pulmonary, etc. Nanocrystals are face stabiliser-stabilised liquid nanoparticles with a size of 200-500 nm. They improve the achromatism solubility, velocity of dissolution, and most likely mucoadhesion, leading to better oral medicine bioavailability by medicines whose bioavailability depends on dissolution rate. Additionally, nanocrystals preserve the advantages of high medicine lading, evasion from organic cleaners, improved stability and reduced bane compared to liposomes, polymer nanoparticles, lipid nanoparticles and cosolvent phrasings. According to FDA Nanoparticulate medicines aren't bioequivalent" to a product approved and thus can be patented and are intended as a new medicine because nanoparticulate medicines aren't bioequivalent to a microcrystalline or solubilised version of the same medicine taken at the same capsule. It also gives pharmaceutical companies a unique advantage of product line extension to the existing medicine phrasings. Similar product line extension may also benefit the consumer by providing new capsule formulations of medicines which can produce lower side goods, decreased boluses and rapid onset of action. The chic example is Rapamune ®.{4}

Foodborne infections are primarily caused by Escherichia coli, a facultative anaerobe that is Gram-negative (E. coli),which can also cause parenteral and gastrointestinal illnesses such diarrhoea, enteritis, bacteremia, urinary tract infections, etc. Even more concerning is the sharp rise in drug-resistant E. Coli that is generated from humans and animals as a result of the widespread use and misuse of antibiotics, which has already become a serious hazard to food safety and public health worldwide. E. Coli is currently multidrug-

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resistant (MDR E. Coli) due to its resistance to several antibiotics, including quinolones and polymyxins. as well as microorganisms that are extremely resistant to drugs. Additionally, statistics show that 80% of clinical infections are caused by drug-resistant bacterial biofilms . The creation of biofilms is closely linked to bacterial drug resistance, which is even more startling. The biofilm that forms is denser when bacteria are more resistant to drugs , making antibiotic treatment even more challenging. Numerous studies have focused on creating natural botanicals from plant extracts in an attempt to mitigate the effects of antibiotic overuse and drug-resistant E. coli. However, little research has been done on the antibacterial action of Gram-negative bacteria, especially foodborne E. coli, which has the highest level of treatment resistance.{3}

Materials and Methods:

Materials :

The supplier of Luteolin (LUT) was Dhamtech Pharma and Consultants in Mumbai, India. India's Research Lab Fine Chem Industries supplied the hydroxypropyl methylcellulose (HPMC).Methanol, Sodium Lauryl Sulphate and Mannitol were acquired from Loba Chemie LTD. in Mumbai, India. Every chemical and reagent employed was of analytical quality and didn't require any additional purification.

Formulation of Nanosuspension :

Preparation of nanosuspension :

There are two main approaches for creating nanosuspensions top- down and bottom up . The solvent-antisolvent approach is appertained to as bottom-up technology . In the solvent- antisolvent system, the drug is dissolved in a solvent and also added to a nonsolvent following the addition of polymer stabilisers and surfactant, the antisolvent is spun at 1000 rpm for 10 min . A hyperactivity is used to add the drug affect dropwise. After ten stabiliser beats, an ultraprobe sonicator tuned to 20 – 23 kHz is produced. Nine batches of nanosuspension were made after the trend was established.{6}

Optimization of Nanosuspension by using 3^2 full factorial design :

By using Minitab software 3^2 full factorial design was applied. attention of surfactant(X1) and attention of polymer(X2) are variable(Table no. 1) and 9 different expression are set. .{8,9}

Level	Minimal (-1)	Moderate (0)	High (+1)
X1 = SLS Amount (mg)	0.1	0.15	0.2
X2=amount of HPMC (mg)	0.3	0.4	0.5

Table No. 1: Independent variables

Pre-formulation study :

Determination of max :

1 mg Luteolin dissolves in ten millilitres of methanol. One millilitre of this solution is further diluted with ten millilitres of methanol.and absorbance is checked in 200-400 nm . .{11}

Calibration curve of Luteolin in Methanol :

The specific amount of the drug was dissolved into the methanol and the dilution of the solution was taken and various concentrations was prepared 2,4,6,8 & 10 $\mu l/ml$ and absorbance was taken at each concentration at 350 nm wavelength. {12}

Solubility studies :

Saturated solutions in a variety of media were prepared, In a water bath, maintained at 37◦C ± 0.5◦C, and constantly agitated in a rotary shaker for up to 24 hours in order to assess the solubility of LUT. At 350 nm, samples were extracted, filtered, and examined using a UV 1700 spectrophotometer. Three measurements were made for every sample. {6}

The Fourier transform infrared spectroscopy (FTIR)

The Fourier transform infrared spectroscopy (FTIR) spectrometer-430 (Shimadzu 8400) was used to produce the FTIR spectra of pure LUT as well as a physical mixture of LUT with all of the excipients, including sodium lauryl sulphate (SLS), hydroxyl propyl methyl cellulose (HPMC), mannitol, and methanol. Each sample

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was mixed with IR-grade potassium bromide in a 1:100 ratio, and then compressed using an IR pellet manufacturing equipment.{6}

Thermodynamic study :

Heating cooling cycle (25^0C-roomtemp) , ultracentrifugation (1000rpm) & freez-thaw cycle (-4^0C-room temp)was applied . .{13}

Evaluation of Nanosuspension :

Analysis of Particle Size ,PDI & Zetapotential :

Utilising the Malvern Zetasizer Nano ZS analyser, the particle sizes & zetapotential of batches of nanosuspension. To get an applicable attention for dimension, the samples must be adulterated with deionized water previous to dimension. The creation of nano- sized patches was vindicated using the particle size distribution data.{6}

Scanning Electron Microscopy :

Scanning electron micrography was used to examine the surface topography (JEOL, JSM-5800LV). Palladium was applied to the optimised batch's manufactured nanosuspension for five minutes after it was dusted onto double-sided tape on an aluminium stub. The surface was examined morphologically at 100000x magnification.{6}

Powder diffraction for X-rays:

Using an X-ray powder diffractometer, the crystalline form of the luteolin distributed in the nanosuspension was captured. Cu will be the target sludge for the X-ray diffraction pattern (XRD) investigations of luteolin nanosuspension utilising an X-ray diffractometer with a voltage/current of 40 KV/40 Ma. .{6}

Determination of drug content in nanosuspension :

A 10 mL volumetric flask was filled with methanol and a measured volume of nanosuspension (1 mL). After being sonicated for an hour, the material was filtered. Using a UV-visible spectrophotometer, the acquired samples were analysed at the wavelength (λ max) of 350 nm, where the drug present in methanol had the highest absorbance.{10}

The drug content was then determined using the following formula.:

D$\boldsymbol{rug\ content}$ %= ($\boldsymbol{Practical\ c}$)\ $\boldsymbol{Theaortical\ conc\ x\ 100}$

Anti-bacterial assay :

The diffusion method of agar wells:

A common technique for assessing the antibacterial activity of plants or extracts is the agar well diffusion method. [32,33]. Similar to the disc-diffusion approach, the bacterial inoculum (Escherichia coli) is spread out throughout the whole surface of the agar plate in order to inoculate it. A 6 to 8 mm diameter hole is then aseptically punched using a sterile cork borer or tip. The well is then filled with 20–100 mL of the extract solution or antibacterial agent at the proper concentration. After that, agar plates are incubated in the appropriate setting. The tested bacterial strain is prevented from developing by the antibiotic material, which disperses throughout the agar medium.{1,2}

In vitro dissolution :

The dissolving test outfit-paddle system (Electrolab TDT 08 L Plus, Mumbai, India) was used to evaluate the rate of dissolution of pure luteolin (100 mg) and optimised nanosuspension. The paddles were set to turn at 100 revolutions per minute. The medium of dissolution consisted of approximately 900 millilitres of 0.1 N HCl buffer and 0.1 N PBS at 37 ± 0.5 °C. One millilitre of samples was taken out and filtered at predetermined intervals, and one millilitre of blank dissolving media was added separately to replace the medium. Using a UV 1700 spectrophotometer, the amount of medication dissolved at 350 nm was determined. Both the standard deviation and the mean values were shown.{6,}

Stability study :

Visual examination was used to conduct stability trials. It was discovered that stable systems did not exhibit any physical alterations, including precipitation, flocculation, or phase separation. For a month, stability was noted between 4°C and 25°C. Additionally assessed were the formulations' drug concentration, zeta potential, particle size, and polydispersity index.{14}

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Results & discussion :

Preformulation study :

UV Spectroscopy :

10 cc of methanol is used to dissolve 1 milligramme of luteolin. Using UV-Vis spectroscopy, which has standard wavelength ranges of 200–400 nm, the absorbance of this result is tested after 1 ml is further attenuated in 10 ml of methanol. The wavelength outside of the medication luteolin was set to 350 nm, which is within an acceptable range, when the sample was analysed under UV-Vis Spectroscopy.

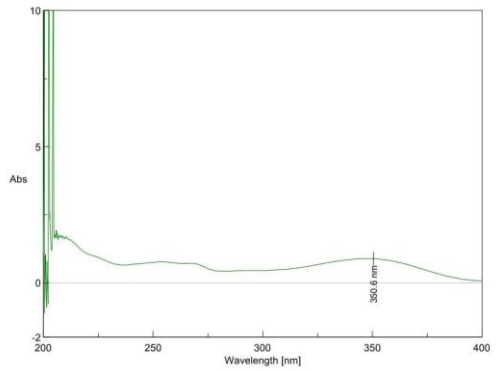

Figure No. 2: Luteolin UV-visible spectroscopy

Calibration curve of luteolin in methanol :

The specific amount of the drug was dissolved into the methanol and the dilution of the solution was taken and various concentrations was prepared 2,4,6,8 & 10 $\mu l/ml$ and absorbance was taken at each concentration at 350 nm wavelength and graph was plotted. By this the equation was taken for the further calculations.

Sr. NO	Concentration($\mu l/ml$)	Absorbance
1	0	0
2	2	0.0151±0.012
3	4	0.0236±0.008
4	6	0.0319±0.006
5	8	0.0448±0.020
6	10	0.0590±0.013

Table No. 2: Drug calibration curve in methanol

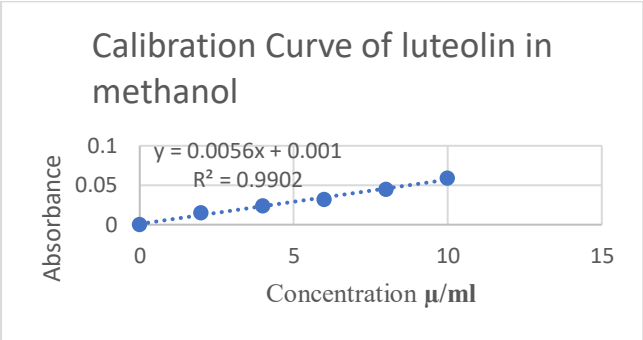

Figure 3: Drug calibration curve in methanol

Solubility Study of the luteoiln :

The saturation solubility study of the drug was performed in which the drug was added into the various solvent upto its saturation level and kept it into rotary shaker for 24 hrs then the supernatant was taken and absorbance was taken under UV Vis Spectroscopy and the wavelength was adjusted at 350 nm.

Solvent	**Observed Values (mg/ml)**
Methanol	0.28±0.01
Water	0.0064±0.01
DMSO	0.31 ±0.1
Ethanol	0.18±0.02

Table No. 3: Drug solubility in various solvents

FTIR Study :

FTIR study was conducted to determine whether the drug and excipients were compatible. All the excipients which was used in conformation of the nanosuspension are checked for the compatability. The drugs FTIR is done first and also physical combination of the drug and excipients IR is done. The drug contains C- H stretching at 686 cm-1, C = O stretching at 1612 cm-1, C-O stretching at 1257 cm-1, C=C stretching at 1512 cm-1, O-H stretching at 3425 cm-1 . Physical mixter of all the

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exicipents each done the all the mixture contains the all functional groups. Presence of All the Peak frequency of the drug Used States that Excipients Doesn't Interact with drug and Shows harmony. Which indicates the anti-bacterial is given by effective way.

Thermodynamic study :

- During the formulation of 9 batches 4 batches forms floccules so that they are cancelled out .
- Remaining 5 batches are undergo Freeze-thaw Cycling among them 3 batches particles were setteled down so that they were also cancelled out .
- Remaining 2 batches were passed for further evaluation parameters

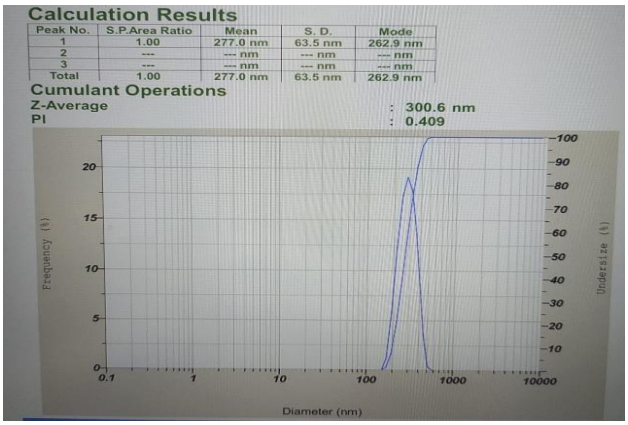
Evaluation of nanosuspension :

Analysis of Particle Size ,PDI & Zetapotential :

The molecule size, PDI & Zetapotential of 2 stable batches were performed. the results are set up as per below . We can infer from the results that the F2 batch is more stable than the F3 batch. such that only the F3 batch's XRD, SEM, drug content, and antibacterial activity were examined.

Formulation	molecule size (nm)	PDI	Zetapotential(mV)
F3	300.6	0.409	-4.1
F2	322.3	0.271	-33.0

Table 4. Analyses of particle size and zetapotential



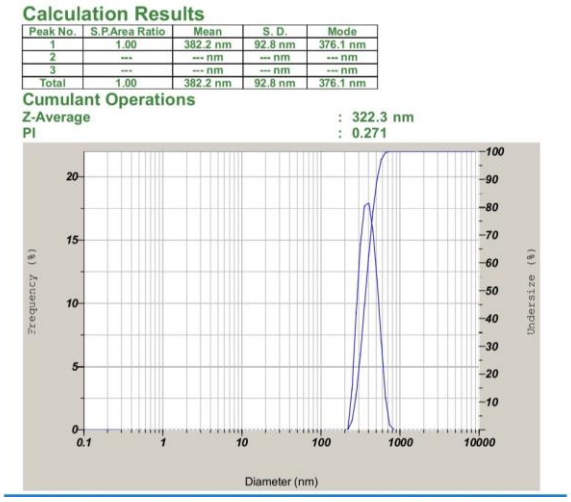


Figure No. 4: Particle size of formulation

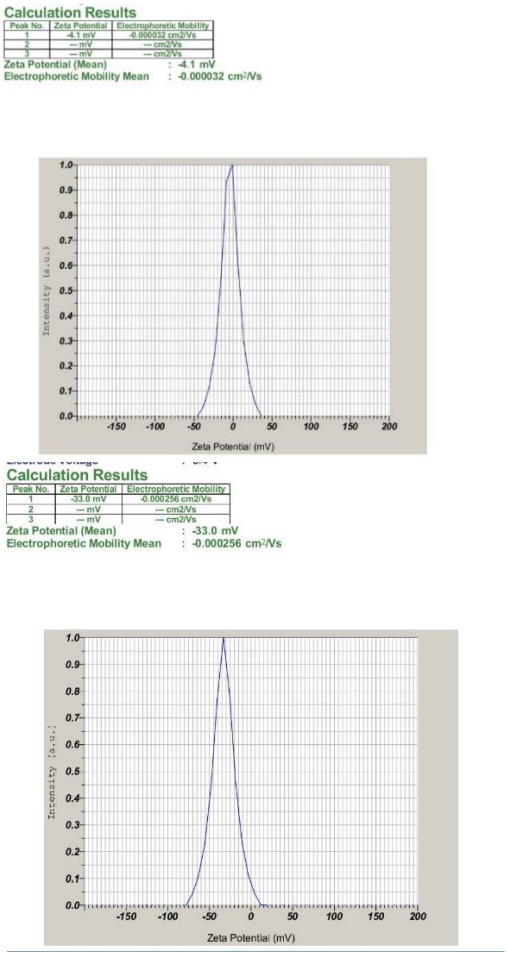


Figure No. 5: Zetapotential of formulation

SEM :

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The surface morphology of optimized formulation examined by SEM was illustrated in . The formulation appeared as amorphous particles. When at 100 000× magnification, it could be seen that presence of almost spherical structures of formulation .

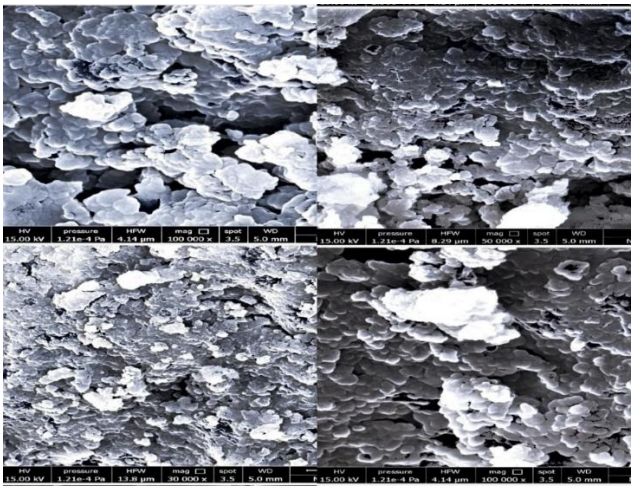
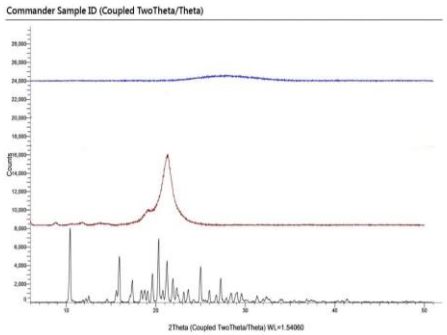


Figure No. 6: SEM of the formulation

X-ray powder diffraction :

Powder XRD image of pure luteolin , physical mixture of drug & excipients and optimized formulation . Numerous distinctive diffraction peaks in the diffraction pattern of the pure luteolin drug reflect its extremely crystalline structure. However, the formulation's X-ray diffraction pattern reveals a decrease in refraction peaks, indicating a drop in the degree of crystallinity. This shows that the drug's crystalline form has changed to an amorphous one.



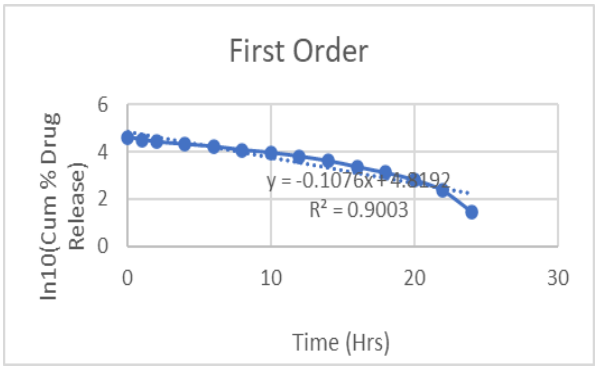


Figure No. 7 shows the formulation's XRD result.

Determination of drug content in nanosuspension :

A review of the chosen nanosuspension formulas revealed that their drug content percentage was 97.2% ± 0.2516. There was neither precipitation nor medication loss because they met British Pharmacopoeia (BP) criteria and fell within 95% and 110% of the permitted range.

Anti-bacterial assay :

Escherichia coli was used to test the anti-bacterial property of nanosuspension . the outcome of nanosuspension activity zone of inhibition is displayed in fig. it was discovered the prepared nanosuspension show zone of inhibition diameter 15 mm

Figure 8: Antibacterial action

In vitro dissolution :

Two different mediums were used to dissolve the optimum batch of nanosuspension and pure medication: 0.1 N HCl and PBS with a pH of 7.4. The rates at which nanocrystals dissolved had dramatically risen, as could be seen from a comparison of the dissolution profiles

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(Fig. 5). On the other hand, the optimised batch and pure drug showed a solubility rate of about 95.65%. The rate of dissolution has improved because to the rise in surface area and fall inparticle size. Additionally, compared to the drug in its pure form, the surfactants' enhanced surface wetting probably led to even greater rates of dissolution in the nanosuspension formulations.

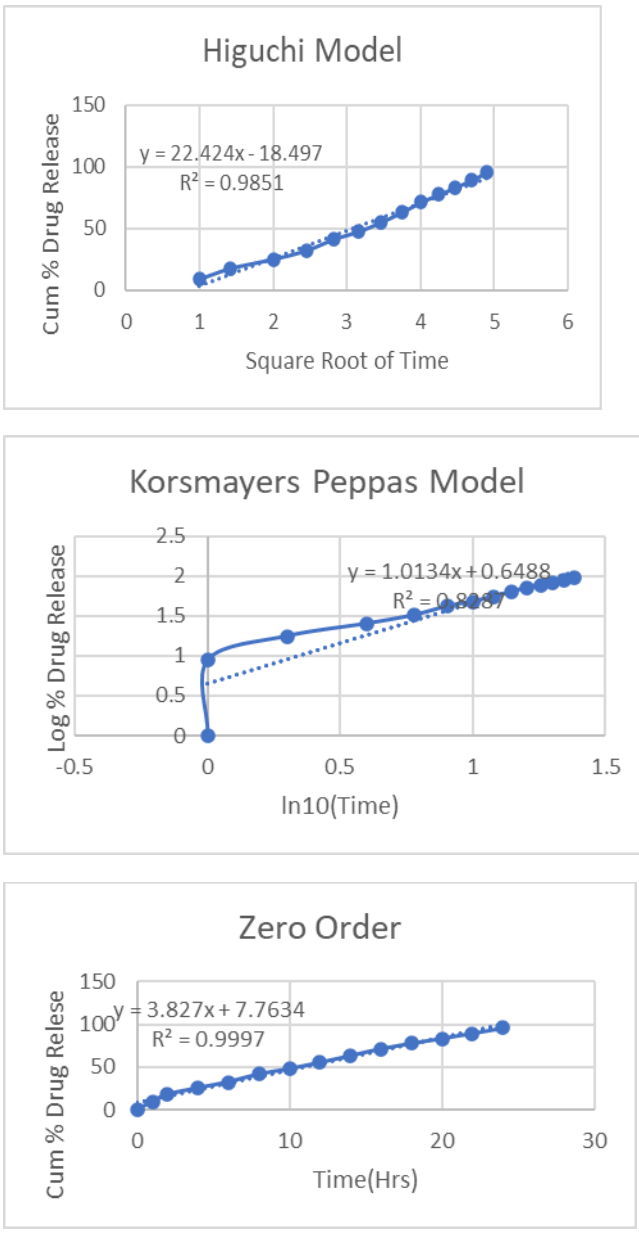

Figure No. 9: Drug release profile of formulation

According to the above drug release study the prepared formulation follows zero order

Stability study :

The physical appearance did not significantly change. Additionally, none of the samples experienced particle precipitation throughout a one-month period.

Temperature	Appearance	Particle size (nm)	PDI	Zeta potential (mV)	Drug content
4°C	No change	325.16	0.26	-34	92.01 %
25°C	No change	340.23	0.29	-36	90%

Table No. 5: Stability Analysis

Conclusion

- **Solubility & Calibration**
  - Luteolin shows good solubility in methanol (~0.28 mg/mL), and higher in DMSO (0.31 mg/mL), but poor in water (~0.006 mg/mL).
  - The UV-Vis calibration curve at 350 nm is linear and reliable across 2–10 µg/mL.
- **Compatibility & Physical Stability**
  - FTIR confirmed no chemical interactions between luteolin and excipients—functional groups remained intact.
  - Out of 9 batches, only 2 passed freeze-thaw and flocculation tests, resulting in stable nanosuspension formulations (F2, F3).
- **Particle Characteristics**
  - Both optimized batches showed sub-micron sizes (300–322 nm) with moderate PDI—F2 (322 nm, PDI 0.271) is more homogeneous than F3 (300 nm, PDI 0.409).
  - Zeta potentials: F2 = –33 mV (good stability), F3 = –4 mV (marginal).

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- **Solid-State & Morphology**
  - SEM reveals mostly spherical, amorphous particles in the optimized formulation.
  - XRD shows a shift from crystalline luteolin to an amorphous form, which typically correlates with enhanced solubility.
- **Drug Content & Release Profile**
  - Drug loading in the nanosuspension is high (97.2%), within BP standards (95–110%).
  - In vitro dissolution shows ~95.7% release over 24 hours, with significantly faster release than pure drug—consistent with zero-order kinetics, thanks to reduced particle size and better wetting.
- **Antibacterial Activity**
- With a 15 mm inhibitory zone, the nanosuspension demonstrated significant antibacterial effectiveness against E. coli.

Overall Summary

The optimized luteolin nanosuspension (particularly Batch F2) demonstrates:

- Excellent **physical stability**
- **Amorphization** (confirmed by XRD/SEM)
- High **drug content**
- **Enhanced dissolution** with zero-order release
- **Effective antibacterial activity**

This formulation shows strong promise for improving luteolin's bioavailability and therapeutic performance.

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