RESEARCH PAPER

Revolutionizing 5-Fluorouracil through Nose to Brain Drug Delivery: A Nanosuspension Approach for Superior Dissolution and Performance

Sonali Baban Pawar[1*], Pratim Kumar Choudhury[1], Atul Arjun Baravkar[2]

[1*]Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India - 313024.

Email: sonalipawar241216@gmail.com (Corresponding Author)

[1]Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India - 313024

[2]Agricultural Development Trust's School of Pharmacy and Research Centre, Baramati, Maharashtra, India - 413115

ABSTRACT

The existing study aimed to advance and optimize a nanosuspension of 5-fluorouracil (5-FU) to expand its solubility, dissolution rate, and nasal permeation. Preformulation studies established the purity and compatibility of the drug using DSC, FTIR, and XRD. A UV spectrophotometric technique was established and validated at 264 nm with good linearity ($R^2 = 0.997$). Nanosuspension was prepared by nanoprecipitation, surveyed by high-speed homogenization and probe sonication. Stabilizers were screened, and PVP K30 was selected based on maximum solubility improvement. A 3^2 factorial design was employed to optimize stabilizer concentration and stirring speed. The optimized formulation exhibited a particle size of 215.7 nm, zeta potential of −17.5 mV, and enhanced solubility (85.18 µg/mL). Characterization studies established spherical morphology and amorphization of the drug in the nanosuspension. In vitro drug release displayed significantly improved dissolution (88.24%) associated with pure drug (53.82%). Ex vivo permeation studies via sheep nasal mucosa verified better-quality drug transport (80.14%). Stability studies designated not at all significant variations over 3 months. Thus, the developed nanosuspension could be a promising approach for enhancing the bioavailability of 5-FU via nasal delivery.

Keywords: 5-Fluorouracil, Nanosuspension, Nanoprecipitation, Factorial Design, Nasal Delivery, Solubility Enhancement

How to cite this article: Pawar SB, Choudhury PK, Baravkar AA. Revolutionizing 5-Fluorouracil through Nose to Brain Drug Delivery: A Nanosuspension Approach for Superior Dissolution and Performance. Int J Drug Deliv Technol. 2026;16(20s): 553-568. DOI: 10.25258/ijddt.16.20s.65

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Currently, cancer is observed as one of the most dangerous causes of morbidity and mortality universally, responsible for approximately 10 million deaths yearly [1]. The worldwide cancer load is expected to rise significantly, reaching roughly 28 million new cases by 2040 due to populace elderly and lifestyle variations [1]. Chemotherapy can be a keystone in cancer treatment; nevertheless, its efficiency is frequently compromised by poor pharmacokinetics, systemic toxicity, and lack of targeted delivery [2].

5-Fluorouracil (5-FU) is an extensively employed antimetabolite chemotherapeutic agent that prevents thymidylate synthase, thus interfering with DNA combination and cell proliferation [2]. It is widely utilized in the treatment of colorectal, breast, gastric, and head and neck cancers [3]. Conferring to the Biopharmaceutics Classification System, 5-FU is commonly classified as a BCS Class III drug, considered by high solubility but low permeability, which restricts its absorption and bioavailability [4]. The drug displays a pKa of almost 8.0 and a log P value of around −0.9, demonstrating its hydrophilic nature and poor membrane permeability [5]. Additionally, 5-FU experiences quick metabolism by dihydropyrimidine dehydrogenase in the liver, subsequent in a short half-life and demanding frequent dosing [3]. This manifested in systemic toxicities such as myelosuppression, gastrointestinal disturbances, and mucositis, which necessitated patient compliance [2].

Numerous conventional and progressive drug delivery systems, comprising liposomes, polymeric

nanoparticles, microspheres, and solid lipid nanoparticles, have been explored to overcome these restrictions [6]. While these schemes can improve targeting and diminish toxicity, they frequently include multifaceted industrial procedures, stability apprehensions, and high manufacturing costs [6]. Polymeric systems include complications connected to polymer toxicity, batch variability, and scalability [7]. Consequently, there is a requirement for a modest, well-organized, and mountable drug delivery system to advance the therapeutic presentation of 5-FU.

Nanosuspension technology has appeared as a hopeful method to improve the solubility and bioavailability of poorly soluble or poorly permeable drugs [8]. Nanosuspensions are submicron colloidal dispersions of pure drug particles stabilized by surfactants or polymers [8]. Decrease in particle size increases the surface area, foremost to improved dissolution rate and saturation solubility as enlightened by the Noyes–Whitney and Ostwald–Freundlich equations [9]. This eventually advances drug absorption and therapeutic efficacy [10]. Furthermore, nanosuspensions suggest advantages including high drug loading, negligible usage of excipients, comfort of scale-up, and adaptability in administration routes [8].

The nasal route of drug delivery offers numerous advantages, including non-invasive administration, rapid onset of action, evasion of first-pass metabolism, and possible shortest transportation to the brain via olfactory and trigeminal pathways [11]. This makes it an attractive route for transporting anticancer mediators necessitating quick systemic or localised action [11].

Regardless of these advantages, restricted studies have focused on the development of optimised 5-FU nanosuspensions for improved solubility and nasal permeation. Thus, a clear research gap occurs in designing a stable and effective nanosuspension system personalised for improved delivery of 5-FU.

Therefore, the existing study aims to develop, optimise, and characterise a 5-FU nanosuspension using nanoprecipitation and 3^2 factorial design. The study assesses important parameters such as particle size, solubility, in vitro drug release, and ex vivo nasal saturation to create its potential as a better-quality drug delivery system.

Associated with conventional delivery systems, nanosuspensions offer noteworthy advantages such as higher drug loading, better-quality dissolution without the need for complex carriers, lower toxicity, and better scalability [8]. Distinct liposomes and polymeric nanoparticles, nanosuspensions minimise formulation difficulty and excipient-related issues, although suggestively attractive drug solubility and permeation [6,8]. Henceforth, nanosuspension-based delivery signifies a greater and applied method for educating the therapeutic presentation of 5-FU.

MATERIALS AND METHODS

5-FU purchased from Shubham Chemicals, Mumbai, India, and other excipient including HPMC E5 LV, Poloxamer 188, and PVP K30, were purchased from Loba Chemicals, Mumbai. Analytical-grade organic solvents were procured from Loba Chemie (Mumbai, India)

Analytical Method development

The solution of (10 µg/mL) was scanned in the UV–Visible spectrophotometer over a wavelength range of 200–400 nm using the selected solvent as a blank. The spectrum was recorded, and the wavelength corresponding to maximum absorbance (λ_{max}) was recognized. For 5-FU, λ_{max} was observed at approximately 264 nm**,** which was selected for further analysis.

UV–Visible Calibration Curve of 5-Fluorouracil (5-FU)

A standard stock solution of 5-Fluorouracil (5-FU) was prepared by accurately dissolving an appropriate quantity of the drug in different solvents, namely distilled water, methanol, 0.1 N hydrochloric acid, phosphate buffer pH 6.8, and phosphate buffer pH 7.4, to obtain a final concentration of 100 µg/mL. From this stock solution, suitable aliquots were withdrawn and further diluted with the respective solvent to obtain working standard solutions in the concentration range of 2–14 µg/mL**.**

Screening of Surface Stabilisers

In the current study, diverse surface stabilisers, specifically HPMC E5 LV, Poloxamer 188, and PVP K30**,** were separated to categorise the most appropriate stabiliser for the preparation of 5-Fluorouracil (5-FU) nanosuspension. Choosing an appropriate stabiliser is a critical step in nanosuspension formulation, as it plays a key role in preventing particle aggregation. This supports improvement in wettability, enhancing solubility, and conserving the physical stability of the nanosized drug particles.

The screening was supported based on the capability of each stabiliser to improve the apparent solubility of 5-FU. Accurately weighed amounts of 5-FU were dispersed in aqueous solutions comprising individual stabilizers at a fixed concentration. The mixtures were subjected to unremitting stirring/sonication to confirm an appropriate interface between the drug and

stabilizer. After equilibration, the samples were filtered, and the solubility of 5-FU was determined spectrophotometrically. All experiments were achieved in triplicate (n = 3), and the results were articulated as mean ± standard deviation to confirm reproducibility and reliability.

Preparation of Nanosuspension

The nanosuspension of 5-fluorouracil (5-FU) was expressed retaining the nanoprecipitation technique surveyed by high-speed homogenization and probe sonication. Precisely weighed 5-FU (5 mg) was dissolved in 1 mL of ethanol to form the organic phase, confirming comprehensive solubilization of the drug. Distinctly, the essential quantity of stabilizer (PVP K30, HPMC E5 LV, or Poloxamer 188) was dissolved in Milli-Q water underneath incessant stirring to obtain a clear and homogeneous aqueous phase. The organic phase was subsequently added dropwise into the aqueous phase under constant stirring, permitting quick diffusion of ethanol into the aqueous medium, manifested in the immediate formation of fine drug particles due to supersaturation and precipitation. The subsequent coarse nanosuspension was further subjected to high-speed homogenization employing an Ultra-Turrax homogenizer at 14,000 rpm for 10–15 minutes to decrease particle size and accomplish unchanging dispersion. Later, the formulation was probe-sonicated for 15 minutes (in pulse mode to avoid overheating), which simplified additional size reduction and better-quality particle size distribution. The concluding nanosuspension attained was stable, consistently dispersed, and appropriate for additional characterization and evaluation studies [12,13,14].

Optimization Using a 3^2 factorial design

The present experiment applied a 3^2 factorial design to scientifically optimize the formulation variables, manipulating the features of 5-fluorouracil nanosuspension [15,16]. In this design, two independent variables (a) stabilizer concentration and (b) stirring speed were evaluated at three different levels (low, medium, and high), subsequently in nine experimental runs [15]. The selection of these independent variables was based on their critical impact on the formation, stability, and presentation of nanosuspensions [17]. The selection of appropriate stabilizer concentration is a crucial factor because it prevents aggregation and controls particle growth during nanoprecipitation [17,18]. A satisfactory concentration of stabilizer delivers steric or electrostatic stabilization, thus dropping particle size and cultivating physical stability [18]. Nevertheless, an inadequate stabilizer may manifest in particle agglomeration, whereas excessive stabilizer may increase viscosity and disturb drug diffusion [19]. Consequently, optimizing stabilizer concentration is indispensable to accomplish a stable nanosuspension with the necessary particle characteristics [17].

The stirring speed was designated as an additional significant independent variable because it directly affects the mixing efficiency between the organic and aqueous phases during nanoprecipitation [20]. Advanced stirring speeds endorse quick solvent diffusion and nucleation, important to the development of minor particles with uniform size distribution [20,21]. Conversely, minor stirring speeds may resulted in insufficient mixing, important to greater particle size and heterogeneity [21]. Therefore, stirring speed suggestively effects the particle formation dynamics and overall quality of the nanosuspension [20].

The dependent variables or responses designated for optimization were particle size (Y_1) and solubility (Y_2), as these are critical quality attributes of nanosuspensions [17,22]. Particle size is a chief response of dissolution rate, bioavailability, and stability; smaller particles provide a larger surface area, resulting in enhanced dissolution and better-quality drug performance [22,23]. Solubility was designated as a response because one of the major objectives of nanosuspension formulation is to improve the apparent solubility of poorly soluble drugs like 5-FU [22]. Decreasing particle size to the nanorange increases surface energy and saturation solubility, thus enhancing drug availability [23,9].

Thus, the application of a 3^2 factorial design permitted a systematic assessment of the separate and joint properties of stabilizer concentration and stirring speed on particle size and solubility, facilitating the documentation of an optimized formulation with enhanced physicochemical properties [15,16].

Characterization

Particle Size Analysis (Dynamic Light Scattering)

The particle size of the established nanosuspension was estimated using dynamic light scattering (DLS), which measures the Brownian motion of suspended particles and relates it to particle size via the Stokes–Einstein equation [24]. Before analysis, the nanosuspension was properly diluted with distilled water to circumvent numerous scattering effects. The extent delivers the hydrodynamic diameter, which comprises the particle core along with the adsorbed stabilizer layer [25]. Particle size is a serious parameter manipulating dissolution rate, bioavailability, and physical stability of nanosuspensions, where a smaller particle size leads

to improved surface area and enhanced drug performance [26].

Entrapment Efficiency (Centrifugation Method)

Entrapment efficiency (%EE) was carried out utilizing the centrifugation technique. The method included centrifuging at high speed (10,000 rpm for 20 min) to disperse free drug from the entrapped drug [27]. The supernatant containing unentrapped drug was collected, diluted appropriately, and analyzed using a UV-visible spectrophotometer. Entrapment efficiency reflects the ability of the formulation to retain drug within nanosized particles and is an important indicator of formulation efficiency and stability [28].

Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy was used to evaluate the compatibility between the drug and excipients [32]. The samples were prepared using the KBr pellet method and scanned over the range of 4000–400 cm^{-1}. FTIR detects functional groups and possible chemical interactions based on characteristic absorption peaks [32]. The presence of intact characteristic peaks of 5-FU in the formulation indicates the absence of chemical interaction and confirms formulation stability [33].

Scanning Electron Microscopy (SEM)

The surface morphology and particle features of the established nanosuspension were examined employing scanning electron microscopy (SEM). SEM is considered a commanding analytical technique that offers high-resolution images by scanning a intensive beam of electrons over the surface of a sample and detecting the emitted secondary or backscattered electrons [24]. This procedure empowers comprehensive visualization of particle shape, surface texture, and aggregation behavior at the micro- to nanoscale level.

For SEM examination, a minute amount of the lyophilized nanosuspension was kept on a clean aluminum stub utilizing double-sided adhesive carbon tape. The sample was then exposed to gold sputter coating under vacuum to concentrate the surface electrically conductive and to avoid charging throughout electron beam exposure [25]. The coated sample was inspected under SEM at a suitable accelerating voltage, classically reaching from 5 to 20 kV, and images were captured at different magnifications.

SEM examination delivers thoughtful data concerning particle morphology, size distribution, and surface characteristics. In nanosuspension systems, the presence of uniform, discrete, and spherical particles indicates fruitful formulation and active stabilization by the chosen polymer or surfactant [26]. Smooth surface morphology recommends correct coating of stabilizer around drug particles, which helps in preventing aggregation and improving stability [27]. In contrast, irregular shapes or aggregated structures may indicate inadequate stabilization or suboptimal formulation conditions.

Furthermore, SEM helps in confirming the nanoscale nature of particles and complements particle size analysis obtained from dynamic light scattering. While DLS provides hydrodynamic diameter in a dispersed state, SEM provides actual visualization of dried particles, thereby offering a comprehensive understanding of particle characteristics [28].

Thus, SEM serves as an essential tool in the characterization of nanosuspensions, enabling the evaluation of formulation quality, uniformity, and stability.

DSC Analysis

Differential scanning calorimetry (DSC) was performed to study the thermal behavior and physical state of 5-FU in the nanosuspension [27]. Precisely balanced samples of pure drug, physical mixture, and nanosuspension were sealed in aluminum pans and scanned over a temperature range of 30–350°C under a nitrogen atmosphere. DSC supports in recognizing melting transitions, crystallinity, and conceivable drug-excipient interactions [27]. The evaporation or shift of distinguishing melting peaks designates conversion of crystalline drug into amorphous form, which augments solubility and dissolution rate [29].

XRD Analysis

The crystalline nature of 5-FU and its nanosuspension was analyzed using X-ray diffraction (XRD) [26]. Samples were allowed to come in contact with Cu-Kα radiation and scanned over a 2θ range of 2°–60°. XRD patterns of crystalline drugs show sharp and intense peaks, whereas amorphous materials display broad and diffuse patterns [26]. Decrease or disappearance of characteristic peaks in nanosuspension indicates conversion of crystalline drug into amorphous form, which contributes to improved solubility and dissolution [30].

In-Vitro Drug Release Study

The in-vitro drug release profile of 5-fluorouracil (5-FU) nanosuspension and pure 5FU drug suspension using 3% CMC was assessed using a USP Type II (paddle) dissolution apparatus [34]. A pre-soaked dialysis membrane (molecular weight cut-off: 10,000–12,000 Da; HiMedia, India) was utilized as a diffusion barrier to simulate controlled drug release conditions [35]. The nanosuspension corresponding to a fixed

dose of 5-FU was placed inside the dialysis bag, which was firmly tied and secured to the paddle.

The dialysis bag was occupied with 250 mL of phosphate buffer (pH 7.4), maintained at 37 ± 0.5 °C to simulate physiological conditions [36]. The dissolution medium was continuously stirred at a paddle rotation speed of 50 rpm to ensure uniform mixing and maintenance of hydrodynamic conditions [34]. At programmed time intervals (0.5, 1, 2, 3, 4, 8, and 12 hours), 5 mL aliquots were withdrawn and replaced with an equal volume of fresh dissolution medium to maintain sink conditions throughout the study [34].

The collected samples were filtered, appropriately diluted, and analyzed using a UV–visible spectrophotometer at 266 nm to regulate the amount of drug released. The cumulative percentage drug release was premeditated and plotted as a function of time. A comparable process was conducted for the pure 5-FU suspension to permit comparative evaluation of dissolution behavior.

The dialysis bag technique confirms controlled diffusion of the drug into the dissolution medium and is extensively utilized for appraising nanosuspension systems, as it avoids interference from undissolved particles while permitting precise evaluation of drug release kinetics.

Ex-Vivo Permeation Study

The ex vivo permeation study of 5-fluorouracil (5-FU) nanosuspension was conducted using a Franz diffusion cell apparatus, an extensively accepted technique for assessing drug permeation across biological membranes [38]. Fresh sheep nasal mucosa, obtained from a local slaughterhouse, was designated due to its close anatomical and physiological likeness to human nasal mucosa [11]. The removed mucosa was prudently eviscerated with normal saline to remove adherent connective tissues and debris, confirming the integrity of the epithelial layer.

The organized nasal mucosa was mounted between the donor and receptor compartments of the Franz diffusion cell with the mucosal side facing the donor compartment. Both compartments were originally equilibrated using phosphate buffer (pH 7.4) to preserve physiological conditions [43]. The receptor compartment was filled with 50 mL of phosphate buffer (pH 7.4) and preserved at 37 ± 0.5 °C, simulating body temperature, while continuous stirring, employing a magnetic stirrer at 50 rpm to confirm uniform distribution of the drug and maintain sink conditions [44].

The 5FU nanosuspension formulation corresponding to a known amount of 5-FU was placed in the donor compartment. At predetermined time intervals (0.5, 1, 2, 3, 4, and 8 hours), aliquots were withdrawn from the receptor compartment and substituted with an equal volume of fresh buffer to preserve constant volume and sink conditions [35]. The collected samples were filtered, suitably diluted, and analyzed using a UV–visible spectrophotometer at 266 nm to enumerate the amount of drug permeated.

The Franz diffusion cell technique delivers a consistent and reproducible model for studying drug permeation across biological membranes and is widely utilized to assess nasal drug delivery systems [37]. The utilization of nasal mucosa permits assessment of formulation performance under conditions that thoroughly mimic in vivo nasal absorption, thus providing valued understandings into drug permeation behavior [11].

Short-term Stability Study

The stability of the optimized 5-fluorouracil (5-FU) nanosuspension was assessed in consideration of International Council for Harmonisation (ICH) guidelines for stability testing of pharmaceutical products [41]. The 5-FU formulation was stored under diverse temperature and humidity conditions, namely accelerated conditions (40 ± 2 °C / 75 ± 5% RH), intermediate/room temperature conditions (25 ± 2 °C / 60 ± 5% RH), and refrigerated conditions (8 ± 2 °C), for a period of three months [41,42].

Samples were reserved at programmed intermissions and analyzed for particle size (PS) and solubility, which are serious quality characteristics distressing the stability and performance of nanosuspensions [27]. Particle size analysis was achieved utilizing dynamic light scattering, while solubility was examined utilizing UV spectrophotometric analysis. These restrictions were designated to display any physical instability such as aggregation, crystal growth (Ostwald ripening), or variations in drug dissolution behaviour during storage [19].

Stability studies are essential to assess the robustness of nanosuspension formulations, as nanosized systems are prone to unpredictability due to high surface energy [26]. The assessment under dissimilar storage circumstances delivers understanding into the consequence of temperature and humidity on formulation integrity, which supports in envisaging shelf-life and appropriate storage conditions [42].

RESULTS AND DISCUSSION

UV–Visible Spectroscopic Analysis of 5-FU

The UV–Visible spectrum of 5-FU exhibited a characteristic absorption maximum at approximately 264 nm, corresponding to $\pi \rightarrow \pi^*$ transitions of the

pyrimidine ring. The distinct and sharp peak supports the purity of the drug and validates the designated wavelength for quantitative analysis. Hence, 264 nm was preferred as the analytical wavelength for additional spectrophotometric studies, as indicated in **Figure 1.**

For attaining optimum PS, %EE, and stability of nanosuspension, the formulation excipients play a significant role. In the current study, the stabilizer selected was PVPK30 based on the solubility studies.

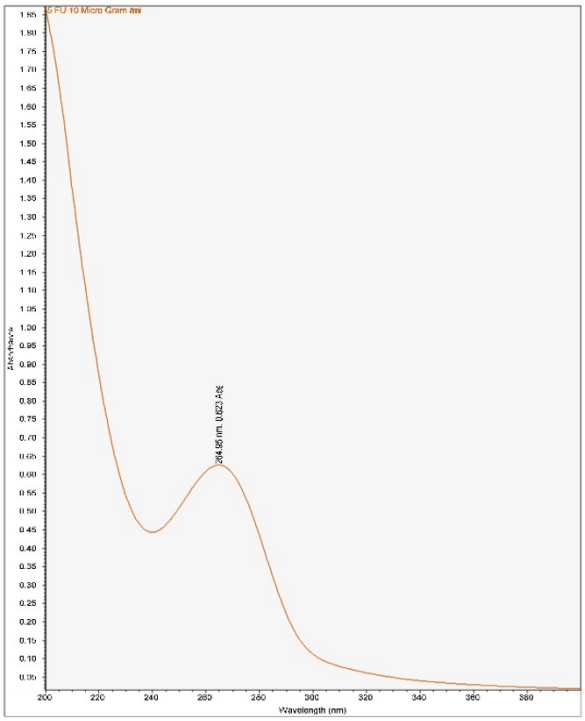

Figure 1: UV–Visible absorption spectrum of 5-Fluorouracil showing characteristic λ_{max} at ~264 nm.

UV–Visible Calibration Curve of 5-Fluorouracil (5-FU)

Each prepared solution absorbance was measured using a UV–Visible spectrophotometer at the selected analytical wavelength of 264 nm**,** using the corresponding solvent, water as blank. A calibration curve was created by plotting concentration (µg/mL) versus absorbance**,** which displayed a linear relationship within the studied range, as demonstated in **Figure 2.**

The regression equation gained was y = 0.048x – 0.001 with a correlation coefficient **(**R^2 = 0.997**),** demonstrating outstanding linearity and compliance with Beer–Lambert's law. The high value of the correlation coefficient authorizes the reliability and appropriateness of the advanced UV spectrophotometric method for quantitative approximation of 5-FU.

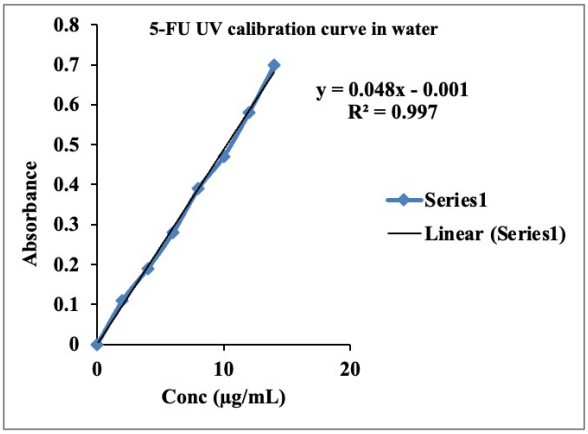

Figure 2. Calibration curve of 5-Fluorouracil in distilled water at 266 nm showing linear relationship between concentration (2–14 µg/mL) and absorbance (R^2 = 0.997).

Screening of Surface Stabilizers

The screening was accepted out built on the capability of each stabilizer to improve the apparent solubility of 5-FU**.** Precisely evaluated quantities of 5-FU were isolated in aqueous solutions comprising individual stabilizers at a fixed concentration. The mixtures were subjected to continuous stirring/sonication to confirm proper interaction between the drug and stabilizer. After equilibration, the samples were filtered, and the solubility of 5-FU was determined spectrophotometrically. All experiments were achieved in triplicate (n = 3)**,** and results were articulated as mean ± standard deviation to confirm reproducibility and reliability.

The outcomes of stabilizer screening confirmed that all designated polymers enhanced the solubility of 5-FU to variable amounts. Among them, PVP K30 displayed the highest solubility enhancement**,** followed by Poloxamer 188, while HPMC E5 LV presented moderately lower solubilization capacity**.** The improved presentation of PVP K30 can be attributed to its sturdy hydrophilic nature and capability to form hydrogen bonding interactions with the drug, thus cultivating drug dispersion and averting crystal aggregation. Poloxamer 188, a non-ionic surfactant, also improved solubility by plummeting interfacial tension and enhancing the wettability of drug particles. In distinction, HPMC E5 LV, although operative as a viscosity enhancer and stabilizer, displayed relatively lower solubilization efficiency.

The experiential trend in solubility enhancement can be represented as:

PVP K30 > Poloxamer 188 > HPMC E5 LV

Based on these conclusions, PVP K30 was designated as the maximum appropriate stabilizer for additional formulation development of 5-FU nanosuspension. The larger solubilizing and stabilizing properties of PVP K30 are predicted to lead to enhanced particle size decrease, improved dissolution rate, and improved total stability of the nanosuspension. **Figure 3.** Indicated screening of surface stabilizers showing the effect of HPMC E5 LV, Poloxamer 188, and PVP K30 on the solubility of 5-FU (mean ± SD, n = 3).

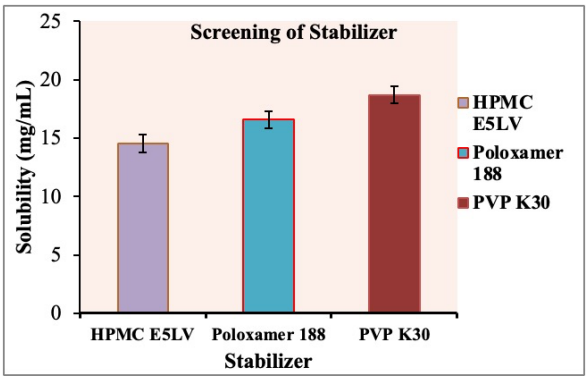

Figure 3. Screening of surface stabilizers showing the effect of HPMC E5 LV, Poloxamer 188, and PVP K30 on the solubility of 5-FU (mean ± SD, n = 3).

Bottom of Form

Particle Size, Entrapment Efficiency, and Zeta Potential

The formulated nanosuspension displayed a particle size in the range of 215.7 nm to 433 nm, confirming the effective development of nanosized particles utilizing the nanoprecipitation technique. Reduction of particle size to the nanorange is a critical factor, as it suggestively rises the surface area of the drug particles, thereby enhancing dissolution rate and saturation solubility according to the Noyes–Whitney principle.

The enhanced formulation presented a least particle size of 215.7 nm, as represented in **Figure 4,** which can be attributed to the collective effect of optimal stabilizer concentration and stirring speed. Acceptable stabilizer concentration avoids particle aggregation by providing steric stabilization, while higher stirring speed endorses rapid nucleation and establishment of smaller particles. These conclusions are consistent with preceding reports representative that procedure parameters and stabilizer concentration play a vital role in controlling particle size distribution in nanosuspensions.

Zeta potential analysis was used to assess the surface charge and stability of the nanosuspension. The optimized formulation displayed a zeta potential of −17.5 mV **(Figure 5),** a representative, reasonable electrostatic stabilization of particles. Zeta potential is a significant indicator of physical stability, as higher surface charge creates repulsive forces between particles, thereby avoiding aggregation and sedimentation [43]. Although values greater than ±30 mV are usually measured as ideal for electrostatic stabilization, nanosuspensions stabilized with polymers can display sufficient stability even at lower zeta potential values due to additional steric stabilization [44].

The solubility of the optimized nanosuspension was calculated to be 85.18 µg/mL, which is significantly higher compared to the pure drug. This enhancement in solubility can be attributed to the reduction in particle size, foremost to increased surface energy and dissolution pressure as enlightened by the Ostwald–Freundlich equation [45]. Besides, the attendance of stabilizers advances wettability and prevents particle agglomeration, thereby supporting improved apparent solubility.

Entrapment efficiency (%EE) is a vital restriction that reproduces the ability of the formulation to retain the drug within nanosized particles. High entrapment efficiency specifies effective combination of the drug into the nanosuspension and minimal drug loss during processing. The optimized formulation displayed satisfactory %EE, signifying efficient stabilization and uniform drug distribution within the nanosystem.

Overall, the combined effect of rigorous particle size, satisfactory zeta potential, and improved solubility establishes the successful advance of a steady and effectual 5-FU nanosuspension with enhanced physicochemical properties.

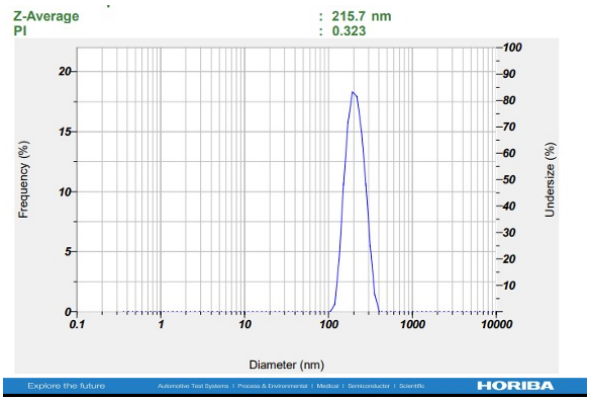

Figure 4. Particle size distribution curve of 5 FU nanosuspension

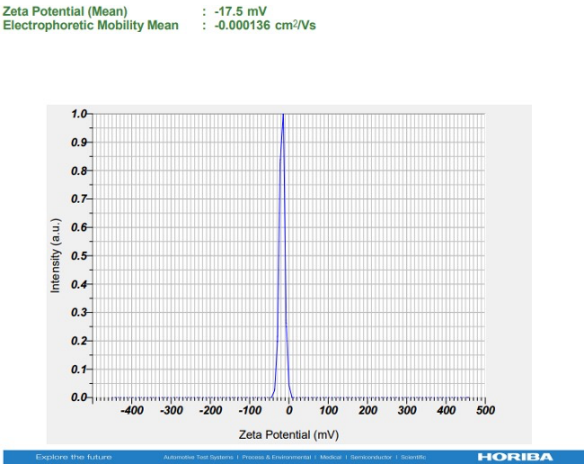

Figure 5. Zeta potential of 5-FU nanosuspension

Optimization Analysis

A 3^2 factorial design was employed to evaluate the effect of formulation and process variables on the characteristics of 5-fluorouracil (5-FU) nanosuspension. The independent variables selected were stabilizer concentration (A) and stirring speed (B), while particle size (Y_1) and solubility (Y_2) were considered as dependent responses. The experimental data were fitted to polynomial equations to understand the relationship between variables and responses.

The results demonstrated that both stabilizer concentration and stirring speed harmed particle size, as indicated by the polynomial equation:

Y_1 (Particle size) = 315.51 − 71.05A − 42.52B

The negative coefficients of A and B indicate that an increase in stabilizer concentration and stirring speed leads to a decrease in particle size. This can be explained by the role of stabilizers in providing steric stabilization, which prevents aggregation and controls particle growth during nanoprecipitation. At optimal concentration (0.6% w/v), the stabilizer effectively inhibits Ostwald ripening, a phenomenon in which smaller particles dissolve and redeposit onto larger ones, leading to particle growth. In contrast, lower stabilizer concentration (0.2% w/v) is insufficient to cover particle surfaces, resulting in agglomeration and increased particle size.

The effect of stirring speed on particle size is attributed to improved mixing efficiency between organic and aqueous phases. Higher stirring speed enhances turbulence, leading to rapid diffusion of solvent and faster nucleation, thereby producing smaller and more uniform particles. Conversely, lower stirring speeds result in inadequate mixing, leading to heterogeneous particle formation and larger particle size [46].

Additionally, increased drug concentration in the aqueous phase leads to rapid supersaturation and precipitation, which favors the formation of smaller particles due to instantaneous nucleation. This phenomenon is consistent with classical nucleation theory, where higher supersaturation results in increased nucleation rate and reduced particle size [47].

The influence of independent variables on solubility was represented by the polynomial equation:

Y_2 (Solubility) = 65.61 + 11.42A + 6.10B

The positive coefficients of A and B indicate that both stabilizer concentration and stirring speed positively influence solubility. The increase in solubility is primarily attributed to the reduction in particle size, which increases surface area and enhances dissolution rate according to the Noyes–Whitney equation. Furthermore, reduction in particle size leads to an increase in surface energy and saturation solubility, as explained by the Ostwald–Freundlich equation.

Stabilizers also contribute to improved wettability and dispersion of drug particles, which further enhances apparent solubility. Higher stirring speeds indirectly improve solubility by producing smaller particles with narrow size distribution and increased surface area [45].

The interaction effects of stabilizer concentration and stirring speed on particle size and solubility were further illustrated using contour plots and three-dimensional (3D) response surface plots **(Figure 6, and 7)**. These plots clearly demonstrate that increasing both variables simultaneously results in reduced particle size and enhanced solubility. Such graphical representations are essential for visualizing the design space and identifying optimal formulation conditions.

Overall, the factorial design approach enabled systematic optimization of formulation variables and demonstrated that appropriate control of stabilizer concentration and stirring speed is critical for achieving nanosuspensions with desirable physicochemical properties.

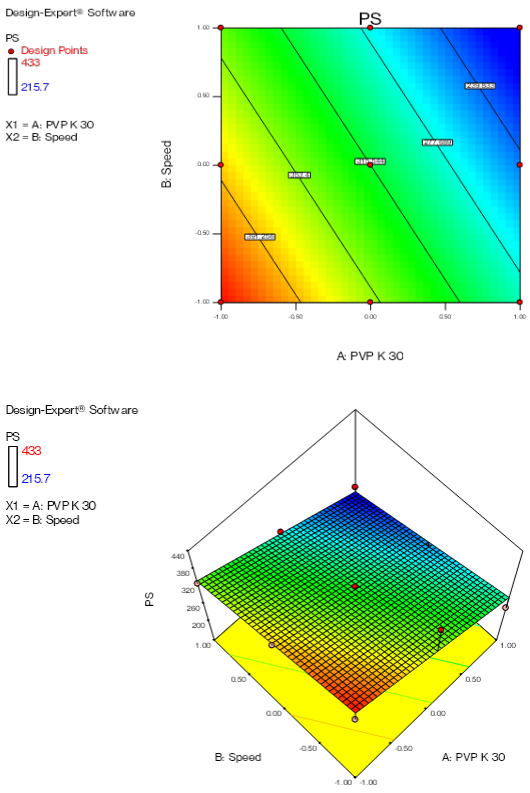

Figure 6. Effect of independent variables on particle size. A) Counter plot B) 3D response surface plot

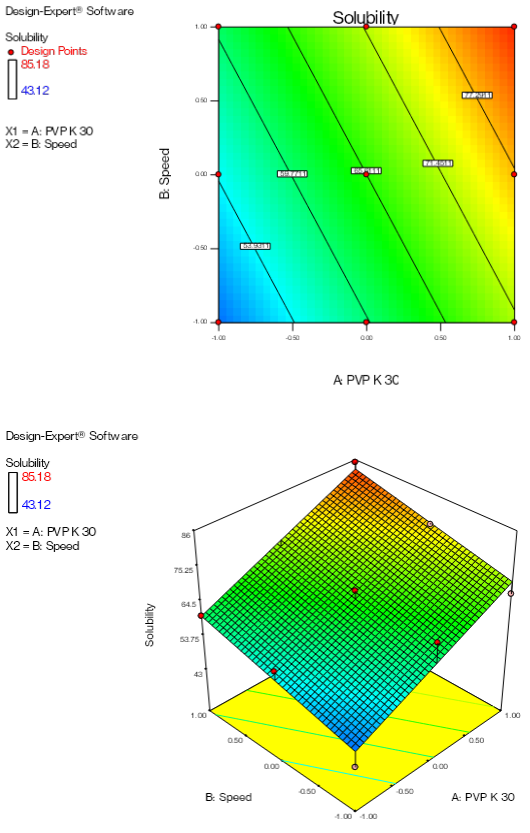

Figure 7. Effect of independent variables on solubility. A) Counter plot B) 3D response surface plot

FTIR Analysis

Fourier transform infrared (FTIR) spectroscopy was performed to evaluate the potential interactions between 5-fluorouracil (5-FU) and the excipients used in the nanosuspension formulation. The spectra of the pure drug, physical mixture, nanosuspension, and their overlay were comparatively analyzed to assess compatibility and structural integrity. The FTIR spectrum of pure 5-FU exhibited characteristic absorption bands at 3779.8 cm^{-1} corresponding to N–H stretching vibrations, a broad band in the region of 3400–3600 cm^{-1} attributed to hydrogen-bonded N–H/O–H groups, and a peak at 2935.13 cm^{-1} due to aromatic C–H stretching. A strong and distinct peak observed at 1770.33 cm^{-1} was assigned to C=O stretching of the pyrimidine ring, while the band at 1573.91 cm^{-1} corresponded to N–H bending vibrations. Additional peaks in the fingerprint region (1000–1300 cm^{-1}) were indicative of C–N stretching, confirming the structural identity of 5-FU and correlating well with reported spectral data [48,49].

In the physical mixture, all the characteristic peaks of 5-FU were retained without significant shifts, although slight broadening was observed in the hydrogen-bonding region around 3400 cm^{-1}. This broadening may be attributed to weak intermolecular interactions such as hydrogen bonding between the drug and excipients. However, the absence of peak disappearance or formation of new peaks indicates that no chemical interaction occurred during simple mixing, suggesting good compatibility between 5-FU and the formulation components [50].

Similarly, the FTIR spectrum of the nanosuspension formulation preserved all major characteristic peaks of 5-FU, including N–H stretching (~3779 cm^{-1}), C–H stretching (~2930 cm^{-1}), C=O stretching (~1770 cm^{-1}), and N–H bending (~1570 cm^{-1}). Minor changes such as peak broadening and reduction in intensity were observed, particularly in the hydrogen bonding region and fingerprint region. These variations can be attributed to physical encapsulation of the drug within the nanosuspension matrix, reduction in particle size, and possible weak intermolecular interactions such as hydrogen bonding. Importantly, no significant peak shifts or new peaks were detected, confirming that the drug remained chemically stable and no degradation or incompatibility occurred during formulation [51,52].

The overlay spectrum further substantiated these findings, as the characteristic peaks of 5-FU were clearly superimposed across all spectra with only minor variations in intensity. The preservation of functional group peaks without any substantial changes confirms that the formulation process does not alter the chemical structure of 5-FU. Overall, the FTIR analysis demonstrates that the drug and excipients are compatible, and the nanosuspension formulation involves only physical interactions rather than chemical modifications, thereby ensuring the stability and integrity of the drug within the developed system. **FTIR spectra of the 5-fluorouracil, Physical mixture, and nanosuspension formulation are illustrated in Figures 8, 9, 10, and 11, representing overlay spectra respectively**

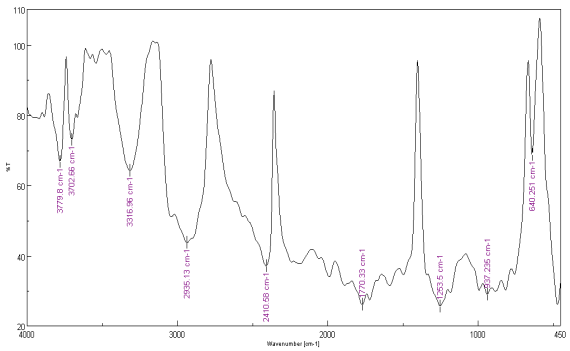

Figure 8. FTIR spectrum of pure 5-fluorouracil (5-FU) showing characteristic functional group peaks including N–H stretching (~3779.8 cm⁻¹), aromatic C–H stretching (~2935.13 cm⁻¹), C=O stretching (~1770.33 cm⁻¹), and N–H bending (~1573.91 cm⁻¹), confirming the structural identity of the drug.

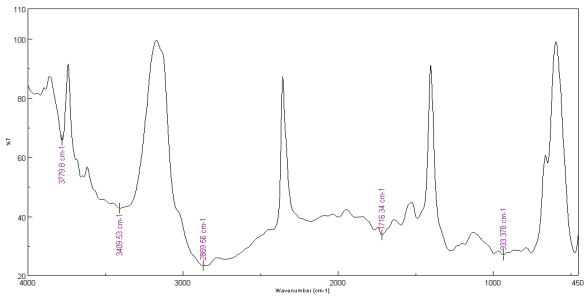

Figure 9. FTIR spectrum of the physical mixture of 5-fluorouracil with excipients showing retention of all characteristic peaks of the drug with slight broadening in the hydrogen bonding region, indicating absence of chemical interaction and confirming compatibility.

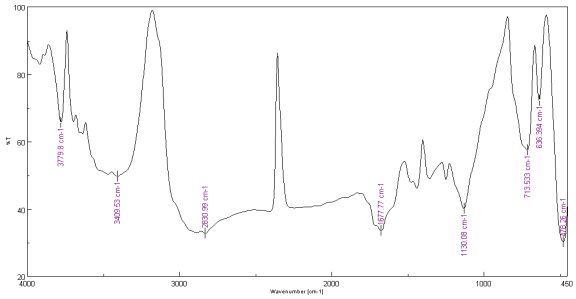

Figure 10. FTIR spectrum of the 5-fluorouracil nanosuspension formulation demonstrating preservation of characteristic drug peaks with minor changes in intensity and broadening, suggesting physical entrapment and weak intermolecular interactions without chemical modification.

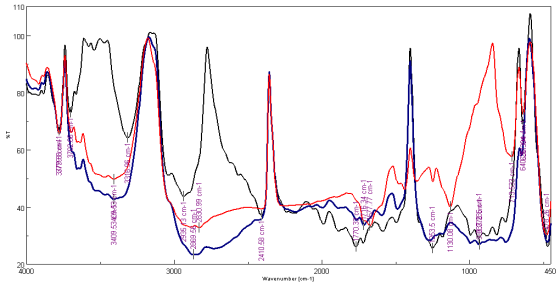

Figure 11. Overlay FTIR spectra of pure 5-fluorouracil, physical mixture, and nanosuspension illustrating superimposition of characteristic peaks with no significant shift or disappearance, confirming compatibility and absence of drug–excipient interaction.

SEM

The surface morphology of the developed 5-fluorouracil (5-FU) nanosuspension was examined using scanning electron microscopy (SEM), and the micrograph is presented in **Figure 12**. The SEM image clearly reveals that the particles are predominantly spherical in shape with a relatively smooth surface morphology. The spherical geometry indicates uniform nucleation and growth of particles during the nanoprecipitation process, which is essential for achieving a stable nanosuspension system.

The particles appear to be well-dispersed with minimal aggregation, suggesting effective stabilization by the selected polymer (PVP K30). The presence of discrete particles with clear boundaries indicates that the stabilizer provided sufficient steric hindrance, preventing particle agglomeration during and after formulation. However, a few localized clusters can be observed, which may be attributed to drying effects during sample preparation or slight aggregation due to the high surface energy of nanoparticles.

The observed particle size in SEM appears relatively larger compared to dynamic light scattering results, which can be attributed to the fact that SEM measures the dry particle **size**, whereas DLS measures the hydrodynamic diameter in dispersion, including the stabilizer layer. The particles exhibit a uniform size distribution, which supports the effectiveness of optimized formulation parameters such as stabilizer concentration and stirring speed.

The smooth surface morphology further indicates proper coating of stabilizer around the drug particles, which contributes to improved stability and prevents Ostwald ripening [44]. Additionally, the absence of irregular or crystalline structures suggests partial or complete transformation of the drug into an amorphous or less crystalline form, which is consistent with DSC and XRD findings [53].

Overall, SEM analysis confirms the successful formation of a **stable, nanosized, and uniformly distributed 5-FU nanosuspension**, which is expected to enhance solubility, dissolution rate, and drug performance.

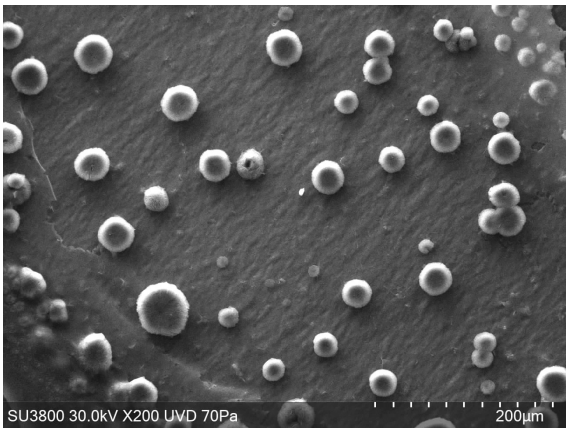

Figure 12. Scanning electron microscopy (SEM) image of optimized 5-FU nanosuspension showing spherical, uniformly distributed particles with smooth surface morphology.

DSC Analysis

The thermal behaviour of pure 5-fluorouracil (5-FU), physical mixture (PM), and optimized nanosuspension was evaluated using differential scanning calorimetry (DSC), and the thermograms are presented in Fig. X. DSC is a widely used technique for assessing the physical state, crystallinity, and possible drug–excipient interactions in pharmaceutical formulations.

The DSC thermogram of pure 5-FU exhibited a sharp and intense endothermic peak at 283.90 °C**,** corresponding to its melting point, which confirms the highly crystalline nature of the drug [54]. The sharpness and intensity of the peak indicate a well-defined crystalline structure with high purity. This finding is consistent with reported literature values for 5-FU, confirming its stable crystalline form [55],

In the case of the physical mixture (PM)**.** The characteristic endothermic peak of 5-FU was still present, although with slight broadening and reduced intensity. This suggests that the drug remained in its crystalline form and that there were no significant chemical interactions between the drug and excipients during simple physical mixing [56]. The minor changes observed may be attributed to dilution effects or partial mixing with excipients.

However, a significant change was observed in the DSC thermogram of the lyophilized nanosuspension, where the characteristic melting endotherm of 5-FU at 283.90 °C was completely absent**.** This disappearance of the melting peak indicates a **loss** of crystallinity and conversion of the drug into an amorphous or molecularly dispersed state within the nanosuspension. The amorphous form of a drug typically exhibits higher free energy and improved solubility compared to its crystalline counterpart, which contributes to enhanced dissolution and bioavailability [57], as indicated in **Figure 13.**

The transformation from crystalline to amorphous form can be attributed to the nanoprecipitation process, where rapid solvent diffusion and supersaturation lead to the formation of nanosized particles with reduced crystallinity. Additionally, the presence of stabilizer (PVP K30) may inhibit crystal growth and stabilize the drug in an amorphous state by forming a protective layer around the particles [44].

Overall, DSC analysis confirms that the developed nanosuspension successfully induced amorphization of 5-FU**,** which is a key factor responsible for the observed enhancement in solubility and dissolution rate. These findings are further supported by XRD results, which also indicate reduced crystallinity in the nanosuspension.

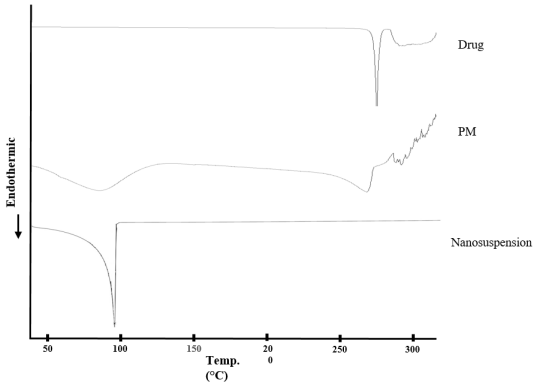

Figure 13. DSC thermograms of pure 5-FU, physical mixture (PM), and optimized nanosuspension showing the disappearance of the drug melting peak in nanosuspension, indicating amorphization.

3.6 XRD Analysis

X-ray diffraction (XRD) analysis was carried out to evaluate the crystalline nature of 5-fluorouracil (5-FU) and to investigate the physical state of the drug in the optimized nanosuspension formulation. The diffractogram of pure 5-FU exhibited multiple sharp and intense diffraction peaks at 2θ values of 13.5°, 15.3°, 18.2°, 22.1°, 28.7°, 31.5°, 32.2°, 33.5°, and 59.4°, which are characteristic of its highly crystalline structure. The presence of these well-defined peaks indicates a long-range ordered arrangement of molecules within the crystal lattice, confirming the crystalline purity of the drug [58,59].

In contrast, the diffractogram of the optimized nanosuspension showed a complete absence of sharp diffraction peaks, with only broad and diffused halos of significantly reduced intensity. This transformation from sharp peaks to a diffused pattern suggests a loss of crystallinity and conversion of the drug into an amorphous or partially amorphous state. Such a reduction in crystallinity can be attributed to the nanosizing process, where mechanical forces and stabilizer interactions disrupt the crystal lattice of the drug, leading to molecular dispersion within the nanosuspension matrix [59,53].

The amorphization of 5-FU in the nanosuspension is a desirable outcome, as amorphous forms generally exhibit higher free energy, enhanced solubility, and improved dissolution rate compared to their crystalline counterparts. Furthermore, the absence of new diffraction peaks indicates that no new crystalline phases or chemical interactions were formed during formulation, confirming the physical stability and compatibility of the drug within the nanosuspension system [60].

Overall, the XRD results clearly demonstrate that 5-FU, originally crystalline in nature, was successfully transformed into an amorphous form in the optimized nanosuspension, which is expected to enhance its biopharmaceutical performance. (**Figure 14)**

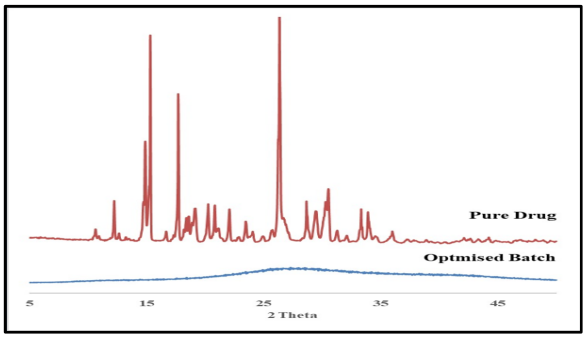

Figure 14. X-ray diffraction (XRD) patterns of pure 5-fluorouracil (5-FU) and optimized nanosuspension showing sharp and intense peaks in the pure drug indicative of crystalline nature, whereas the nanosuspension exhibits a diffused halo pattern with reduced intensity, confirming the conversion of 5-FU from crystalline to amorphous form and its successful incorporation into the nanosuspension matrix.

3.7 In-Vitro Drug Release Study

The in vitro dissolution study of the nanosuspension was performed using phosphate buffer (pH 7.4) at 37 ± 0.5 °C. The results demonstrated a significant enhancement in the dissolution profile of the nanosuspension compared to the pure drug suspension. The pure drug exhibited a relatively slow release, with only 23.42% of 5-fluorouracil (5-FU) released within the first 10 minutes. In contrast, the nanosuspension showed a markedly improved dissolution rate, achieving 37.12% drug release within the same time frame.

Furthermore, upon completion of the dissolution study (120 minutes), the nanosuspension exhibited a cumulative drug release of 88.24%, whereas the pure drug suspension showed a comparatively lower release of 53.82%. **Figure 15 illustrated,** *In –vitro* release of 5-FU nanosuspension and pure drug suspension. The results are expressed as Mean ± SD (n = 3)

This enhanced dissolution behaviour of the nanosuspension can be attributed to the reduced particle size, which increases the surface area available for dissolution, and the presence of stabilizers that improve the wettability and dispersibility of the drug particles. Collectively, these factors contribute to

enhanced solubility and a significantly improved dissolution rate of the nanosuspension formulation [61,62].

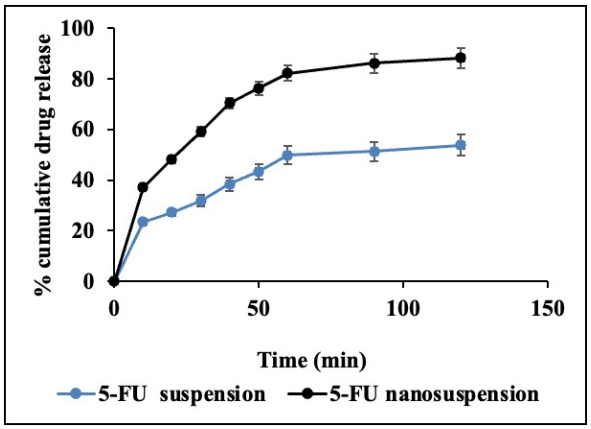

Figure 15. *In –vitro* release of 5-FU nanosuspension and pure drug suspension. The results are expressed as Mean ± SD (n = 3)

Ex-Vivo Permeation Study

Ex-vivo permeation studies were carried out using freshly excised sheep nasal mucosa, which is widely accepted as a suitable model due to its close structural and functional resemblance to human nasal epithelium, including comparable permeability characteristics and mucociliary architecture [63]. The permeation experiment was performed over a period of 8 hours, and the cumulative percentage of drug permeated was determined for both the plain drug solution and the optimized 5-FU nanosuspension.

The permeation profile demonstrated a time-dependent increase in drug diffusion across the nasal membrane for both formulations. However, a significantly higher permeation was observed in the case of the nanosuspension compared to the plain drug solution. The plain 5-FU solution exhibited a cumulative permeation of 44.27% at the end of 8 hours, whereas the nanosuspension formulation achieved 80.14% permeation under identical experimental conditions. This nearly two-fold enhancement in drug permeation clearly indicates the superiority of the nanosuspension system. **Figure 16**. illustrated ex-vivo permeation profile of 5-FU from nanosuspension and plain drug solution across sheep nasal mucosa over 8 hours (values expressed as mean ± SD, n = 3).

The enhanced permeation of the nanosuspension can be attributed to several factors. Firstly, the reduced particle size of the nanosuspension increases the surface area available for dissolution and absorption, thereby facilitating rapid drug diffusion across the mucosal barrier. Secondly, the presence of stabilizers and surfactants (e.g., Poloxamer 407) may act as permeation enhancers by transiently altering membrane fluidity and opening tight junctions, thus promoting paracellular transport [64,66]. Additionally, nanosuspensions can improve drug solubility and maintain a higher concentration gradient across the membrane, which serves as the driving force for diffusion according to Fick's law.

Furthermore, the intimate contact of nanosized particles with the mucosal surface enhances adhesion and residence time, thereby increasing the likelihood of drug absorption. The observed permeation trend (as shown in the figure) indicates a rapid initial release followed by a sustained permeation phase, which may be beneficial for prolonged therapeutic action via the nasal route.

Overall, the results confirm that the developed 5-FU nanosuspension significantly enhances nasal permeation compared to the conventional drug solution, suggesting its potential as an effective delivery system for improved bioavailability and therapeutic efficacy.

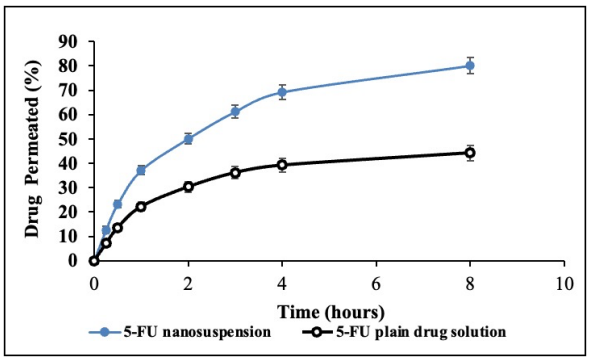

Figure 16. Ex-vivo permeation profile of 5-FU from nanosuspension and plain drug solution across sheep nasal mucosa over 8 h (values expressed as mean ± SD, n = 3).

3.9 Stability Study

Stability studies were carried out to evaluate whether the optimized formulation could retain its critical physicochemical properties during storage. In pharmaceutical development, stability testing is performed to confirm that a drug product remains within its predefined quality attributes under the influence of temperature, humidity, and time, so that its safety, performance, and shelf life can be justified. According to ICH guidance, stability studies are intended to establish how the quality of a drug

substance or drug product varies with environmental factors during storage.

The results obtained for the optimized formulation show only minimal variation in particle size (PS) and solubility under all storage conditions tested. The initial particle size of the formulation was 215.7 nm in the control sample. After storage, the particle size remained 214.2 nm at 8 ± 2°C, 213.97 nm at 25 ± 2°C/60 ± 5% RH, and 214.4 nm at 40 ± 2°C/60 ± 5% RH. **Table 1,** illustrated, stability study results of formulation for period of 90 days. These changes are very small and practically negligible, indicating that the nanosized system did not undergo significant aggregation, crystal growth, or particle fusion during storage. In nanosuspension systems, particle size is a key quality attribute because any major increase may indicate physical instability, especially aggregation or Ostwald ripening.

A similar trend was observed for solubility. The control formulation showed a solubility of 85.18 µg/mL, while the stored samples showed 84.72 µg/mL, 84.28 µg/mL, and 83.33 µg/mL under refrigerated, room temperature, and accelerated storage conditions, respectively. Although a slight decrease was observed at higher temperatures, the reduction was not substantial, suggesting that the formulation largely preserved its enhanced solubility even after storage. This observation is important because the improved solubility of nanosuspensions is closely related to their small particle size and high surface area; therefore, maintenance of solubility during storage supports preservation of the optimized nanoform.

Overall, the stability data indicate that the optimized formulation remained physically stable throughout the study period. The absence of any marked increase in particle size suggests that the stabilizer system was effective in preventing particle aggregation and maintaining uniform dispersion. Likewise, the near-constant solubility values indicate that the formulation did not undergo major recrystallization or loss of the nanosized advantage during storage. Thus, the developed formulation can be considered stable under the investigated storage conditions [66-67].

Table 1. Stability study results of formulation after a period of 90 days.

Storage condition	PS (nm)	Solubility (µg/mL)
Control	215.7	85.18
8±2°C	214.2	84.72
25±2°C/60±5% RH	213.97	84.28
40±2°C/60±5% RH	214.4	83.33

Conclusion

Overall, this study has been able to come up with a new stable nose to brain drug delivery system of 5-Fluorouracil. The systematic Preformulation studies established the purity and compatibility of the drug using DSC, FTIR, and XRD. A UV spectrophotometric technique was established and validated at 264 nm with good linearity (R^2 = 0.997). Nanosuspension was prepared by nanoprecipitation, surveyed by high-speed homogenization and probe sonication. A 3^2 factorial design was employed to optimize stabilizer concentration and stirring speed. Stabilizers were screened, and PVP K30 was selected based on maximum solubility improvement. Overall, SEM analysis confirms the successful formation of a stable, nanosized, and uniformly distributed 5-FU nanosuspension, which is expected to enhance solubility, dissolution rate, and drug performance. DSC analysis confirms that the developed nanosuspension successfully induced amorphization of 5-FU**,** which is a key factor responsible for the observed enhancement in solubility and dissolution rate. The Ex vivo permeation studies via sheep nasal mucosa verified better-quality drug transport (80.14%). The absence of any marked increase in particle size suggests that the stabilizer system was effective in preventing particle aggregation and maintaining uniform dispersion.

References

1. World Health Organization. Cancer fact sheet. 2024.
2. Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer. 2003;3(5):330–338.
3. Diasio RB, Harris BE. Clinical pharmacology of 5-fluorouracil. Clin Pharmacokinet. 1989;16:215–237.
4. Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification. Pharm Res. 1995;12:413–420.
5. Wishart DS, et al. DrugBank: 5-fluorouracil drug profile. Nucleic Acids Res. 2018.
6. Torchilin VP. Multifunctional nanocarriers. Adv Drug Deliv Rev. 2006;58:1532–1555.
7. Danhier F, et al. PLGA-based nanoparticles. J Control Release. 2012;161:505–522.

8. Rabinow BE. Nanosuspensions in drug delivery. Nat Rev Drug Discov. 2004;3:785–796.
9. Noyes AA, Whitney WR. The rate of solution of solid substances. J Am Chem Soc. 1897;19:930.
10. Möschwitzer JP. Drug nanocrystals in pharmaceutical development. Int J Pharm. 2013;453:142–156.
11. Illum L. Nasal drug delivery—possibilities, problems and solutions. J Control Release. 2003;87:187–198.
12. Al-Nasrawi H, et al. 5-FU chitosan nanoparticles. Ther Deliv. 2024;15:339–353.
13. Sun L, et al. 5-FU chitosan nanoparticles. Asian J Pharm Sci. 2017;12:418–423.
14. Gomathi T, et al. 5-FU nanoparticle cytotoxicity. Biomass Convers Biorefinery. 2025.
15. Montgomery DC. Design and analysis of experiments. Wiley; 2010.
16. Myers RH, Khuri AI, Carter WH. Response surface methodology. Technometrics. 1989;31:137–157.
17. Jacob S, et al. Role of nanosuspensions. Biomater Res. 2020;24:3.
18. Müller RH. Drug nanocrystals. 2004.
19. Patravale VB, Date AA, Kulkarni RM. Nanosuspensions. J Pharm Pharmacol. 2004;56:827–840.
20. Kesisoglou F, Panmai S, Wu Y. Nanosizing technologies. Adv Drug Deliv Rev. 2007;59:631–644.
21. Mondal PK, Mandal BK. Emulsified diesel study. Energy Rep. 2019;5:639–648.
22. Junghanns JU, Müller RH. Nanocrystal technology. Int J Nanomedicine. 2008;3:295–310.
23. Csicsák D, et al. Particle size and dissolution. Pharmaceutics. 2023;15:278.
24. Goldstein J, et al. Scanning electron microscopy. Springer; 2017.
25. Egerton RF. Electron microscopy principles. Springer; 2005.
26. Müller RH, Peters K. Nanosuspensions. Int J Pharm. 1998;160:229–237.
27. Keck CM, Müller RH. Drug nanocrystals. Eur J Pharm Biopharm. 2006;62:3–16.
28. Stetefeld J, et al. Dynamic light scattering. Biophys Rev. 2016;8:409–427.
29. Mohanraj VJ, Chen Y. Nanoparticles review. Trop J Pharm Res. 2006;5:561–573.
30. Danaei M, et al. Particle size impact. Pharmaceutics. 2018;10:57.
31. Williams DB, Carter CB. Transmission electron microscopy. Springer; 2009.
32. Giron D. DSC in pharmaceuticals. J Therm Anal Calorim. 2002;68:335–357.
33. Malvern Instruments. Dynamic light scattering. 2011.
34. United States Pharmacopeia. Dissolution <711>. USP; 2020.
35. Modi JD, et al. Dialysis bag method. Int J Pharm Sci Rev Res. 2013;21:20–26.
36. Dressman JB, et al. Dissolution testing. Pharm Res. 1998;15:11–22.
37. Sinko PJ. Martin's physical pharmacy. 6th ed. LWW; 2011.
38. Franz TJ. Percutaneous absorption. J Invest Dermatol. 1975;64:190–195.
39. Shah VP, et al. Permeation studies. Pharm Res. 1992;9:838–842.
40. Ugwoke MI, et al. Nasal drug delivery systems. Adv Drug Deliv Rev. 2005;57:1640–1665.
41. ICH Q1A(R2). Stability testing. 2003.
42. Waterman KC, Adami RC. Accelerated stability studies. Int J Pharm. 2005;293:101–125.
43. Honary S, Zahir F. Zeta potential. Trop J Pharm Res. 2013;12:255–264.
44. Bhakay A, et al. Stabilizers in nanosuspensions. Int J Pharm. 2011;413:106–115.
45. Ostwald W. Particle size and solubility. Z Phys Chem. 1900;34:495–503.
46. Verma S, et al. Homogenization techniques. Int J Pharm. 2009;379:229–238.
47. Kashchiev D. Nucleation theory. 2000.
48. Silverstein RM, et al. Spectrometric identification. Wiley; 2014.
49. Socrates G. Infrared spectroscopy tables. Wiley; 2004.
50. Stuart B. Infrared spectroscopy fundamentals. Wiley; 2004.
51. Pavia DL, et al. Introduction to spectroscopy. Cengage; 2015.
52. ICH Q8 (R2). Pharmaceutical development. 2009.
53. Hancock BC, Zografi G. Amorphous pharmaceuticals. J Pharm Sci. 1997;86:1–12.
54. Craig DQM, Reading M. Thermal analysis. Thermochim Acta. 2007;458:1–12.

55. Sweetman SC. Martindale drug reference. 2009.
56. Ford JL, Timmins P. Thermal analysis. 1989.
57. Yu L. Amorphous solids. Adv Drug Deliv Rev. 2001;48:27–42.
58. Cullity BD, Stock SR. X-ray diffraction. 2001.
59. Langford JI, Wilson AJC. Scherrer equation. J Appl Crystallogr. 1978;11:102–113.
60. Chieng N, et al. Polymorph analysis. J Pharm Biomed Anal. 2011;55:618–644.
61. Li Y, et al. Paclitaxel nanosuspension. Int J Pharm. 2015;490:324–333.
62. Salem HF, Kharshoum RM. Risperidone nanosuspension. Int J Pharm Pharm Sci. 2016;8:136–142.
63. Gizurarson S. Intranasal delivery factors. Curr Drug Deliv. 2012;9:566–582.
64. Patel A, et al. Nanosuspensions. J Drug Deliv Sci Technol. 2014;24:473–480.
65. Patel VR, Agrawal YK. Nanosuspension approach. J Adv Pharm Technol Res. 2011;2:81–87.
66. Yadav GV, Singh SR. Nanosuspension system. Pharmacophore. 2012;3:217–243.
67. Aghrbi I, et al. Nanosuspension dissolution. Drug Dev Ind Pharm. 2021.