Quality By Design Driven Development of Enteric-Coated Resveratrol Micropellets For Colitis Management

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KEYWORDS
Ulcerative colitis, resveratrol, quality by design, micropellets, targeted colon delivery

ABSTRACT

Background:
Ulcerative colitis (UC) is a chronic condition requiring targeted drug delivery for effective management. Resveratrol, with its anti-inflammatory properties, faces bioavailability challenges in the colon. This study focuses on formulating resveratrol-loaded micropellets for targeted colonic delivery using Eudragit S-100 and an HPMC as enteric coating polymer.

Aim:
The study aimed to develop and optimize resveratrol-loaded micropellets using Quality by Design (QbD) principles to ensure controlled release in the colon and assess their physicochemical and biological properties for UC management.

Method:
The micropellets were optimized using a Box-Behnken Design with three factors at three levels and were formulated via the ionotropic gelation method. Their stability and morphology were confirmed through characterization techniques such as FTIR, SEM, DSC, and XRD. Micrometric properties, including the angle of repose, bulk density, and compressibility index, were assessed. Biological evaluations involved cell viability assays, ROS generation studies, cytokine profiling using CBA, and NF-kB expression analysis. The drug release profile was examined under different pH conditions to mimic the colonic environment, and stability studies were also performed.

Result:
The optimized micropellets formulation exhibited several key characteristics, including a particle size of 552 ± 7.42 µm, an entrapment efficiency of 86.97 ± 0.34%, and a drug release of 96.14 ± 4.38% over 16 hours, with negligible drug release occurring in the stomach. The formulation demonstrated excellent flow properties, with an angle of repose of 24.271° ± 0.417, a bulk density of 0.783 ± 0.006 (g/cm^3), and a tapped density of 0.785 ± 0.019 (g/cm^3). Stability testing confirmed the preservation of resveratrol within the micropellets. Biological assessments revealed significant anti-inflammatory activity, coated micropellets reducing colon inflammation, as indicated by a drop in MFI values to 8736. Controlled release, particularly from HPMC-coated micropellets, was successfully achieved in colonic conditions.

Conclusion:
HPMC coated Resveratrol-loaded micropellets offer a promising approach for targeted Management, providing controlled drug release, reduced inflammation, and potential for improved therapeutic outcomes.

1. Introduction

The chronic inflammatory bowel illness ulcerative colitis (UC) is typified by persistent mucosal inflammation and ulceration(1). Usually starting in the rectum, the illness spreads proximally and may affect the entire colon. Symptoms of UC include diarrhoea, tenesmus, abdominal pain, and rectal bleeding(2, 3). The prevalence of UC varies around the world, with wealthier nations showing higher rates. About 10–20 people per 100,000 live in the United States and Europe who have UC; in Asia and Africa, the frequency is much lower. The incidence and prevalence of the condition have been increasing, which is indicative of alterations in environmental variables and lifestyle(4).

The mainstay of conventional UC treatment approaches is the use of pharmaceuticals to induce and sustain remission(5). Amino salicylates, corticosteroids, immunomodulators, and biologic therapy are some of these treatments. The anti-inflammatory properties of amino salicylates, like mesalamine, are frequently employed, but corticosteroids offer quick relief from inflammation(6). Targeting particular inflammatory pathways, immunomodulators and biologics can have major adverse effects such as increased susceptibility to infections, gastrointestinal problems, and long-term hazards including cancer(7). Notwithstanding their effectiveness, these medications frequently have drawbacks, such as systemic side effects and partial response in certain individuals, underscoring the need for more focused and potent interventions(8).

Because of its strong anti-inflammatory and antioxidant qualities, resveratrol, a polyphenolic chemical present in many plants, including berries and grapes, has become a viable option for the treatment of ulcerative colitis(9). Resveratrol has been shown in studies to improve mucosal healing in colitis models, lower oxidative stress, and regulate inflammatory pathways(10). It is a promising therapeutic agent due to its capacity to target particular cellular pathways implicated in ulcerative colitis(11). However, resveratrol's poor solubility and stability in the gastrointestinal tract limit its therapeutic utilization, requiring creative drug administration strategies(12).

Therefore, a special drug delivery system is highly required which have the ability to protect the drug from the harsh acidic pH. of stomach, as well as ensure the drug release in the colonic site. The pH-sensitive polymer Eudragit S-100 is made to withstand the acidic conditions of the stomach and small intestine(13). It serves as an efficient carrier for targeted colonic drug administration since it assurances the controlled release of drug specifically in the alkaline environment of the colon(14).

Using micropellets to deliver drugs specifically to the colon is an innovative approach to improve treatment effectiveness for UC(15). Enteric coatings of micropellets, which prevent the medication from being released too soon in the stomach and small intestine(16). Rather, they precisely release their payload where it is most needed in the colonic environment. This tailored delivery method reduces systemic side effects and increases the absorption of medications like resveratrol, making ulcerative colitis (UC) treatment more efficient and patient-friendly(17).

For a variety of reasons, the use of micropellets in targeted administration of drugs is especially beneficial(18). First off, the drug is protected from the stomach's acidic environment and the small intestine's enzymatic activity by the enteric coatings of micropellets(19). The integrity and effectiveness of the medication must be preserved until it reaches the colon, and this protection is essential(20). Micropellets offer a targeted therapy that directly targets the inflammatory tissues obstructed by (UC) by releasing the drug within the colon, resulting in enhanced therapeutic results(21).

Additionally, a greater amount of the drug reaches the desired area in the colon due to the improved bioavailability of drug administered by micropellets(18). Because of the drug's improved bioavailability, treatment may be more successful at lower dosages, which lowers the risk of side effects that are frequently linked to higher systemic concentrations of the medication(22). These results improved overall disease management, and a safer and more comfortable treatment experience for patients(23-26).

The study aims to develop and evaluate Eudragit S-100 micropellets loaded with resveratrol that are coated with hydroxypropyl methylcellulose (HPMC) and are intended to be delivered specifically to the colon. The research aims to optimize these formulations for precise and regulated release of resveratrol in the colonic environment by applying the principles of Quality by Design (QbD). The goal of the research is to determine how well various enteric coatings work to transport resveratrol to areas of inflammation, evaluate the stability of the formulations, and investigate the possibility of using them for UC management.

2. Material and methods

2.1 Materials

Resveratrol was sourced from Thermo Fisher Scientific. Sodium Alginate and Eudragit S100 were obtained from Sigma-Aldrich, while methacrylic acid was procured from Central Drug House (P) Ltd. Hydroxypropyl Methylcellulose (HPMC), Dibutyl Phthalate, and Triethyl Citrate were supplied by HiMedia Laboratories Ltd., Mumbai, India. Additionally, Calcium Chloride, Talc, and Isopropyl Alcohol were purchased from Thermo Fisher Scientific, and Calcium Carbonate was acquired from Sigma-Aldrich. All chemicals used were of analytical grade.

2.2 Methods

2.2.1 Method of preparation of micro pellets

The ionotropic gelation method was utilized for the preparation of micropellets, considering Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) to optimize the formulation. The independent variables selected were Eudragit S100 concentration (X1), stirring speed (X2), and sodium alginate concentration (X3), each studied at three levels as shown in Table 1;

Firstly, a homogenous mucilage of sodium alginate was prepared by dissolving required quantity sodium alginate in 50 mL of distilled water using an electric mixer. Mixing was continued at a slow pace until a uniform solution was obtained. Then, mucilage was blended with Eudragit S100, a controlled-release polymer, in different proportions. The dispersion was stirred for 30 minutes to ensure uniform polymer dispersion. The water-insoluble drug resveratrol was gradually added to the polymeric solution. Stirring was maintained at required rpm using an electric stirrer to achieve a uniform distribution of the drug. The dispersion was mixed for 15 minutes to ensure even drug incorporation. The air-bubble-free dispersion was loaded into a glass syringe and extruded through a 17-gauge needle into 100 mL of a gently stirred 5% w/v calcium chloride solution. The beaker was pre-graduated to ensure that the needle tip remained 2 cm above the solution level, allowing uniform droplet formation. As soon as the droplets entered the solution, they formed dense, moist calcium alginate micropellets, which rapidly gelled and settled at the bottom. The micropellets were left in the calcium chloride solution for 30 minutes to allow complete gelation and hardening. Calcium ions from the solution acted as crosslinking agents, stabilizing the micropellets. The micropellets were filtered using standard filter paper and washed with distilled water to remove any residual calcium ions. The micropellets were then transferred to a petri dish lined with dry filter paper for air-drying, allowing surface moisture absorption. Finally, to obtain completely dried, non-adhering micropellets, the samples were subjected to drying at 60°C for 6 hours in a hot air oven (33).

2.2.2 Quality by design

2.2.2.1 Establishing Critical quality attributes and defining Quality Target Product Profile

A Quality by Design approach involves identifying the Quality Target Product Profile (QTPP), which outlines the desired quality characteristics of the product being developed. For the current formulation, the goals included sustained release profile with targeted to the colonic region of resveratrol within the micropellet formulation. This approach lays the groundwork for creating a formulation that prioritizes quality, safety, and efficacy (27, 28).

From the established QTPP, the critical quality attributes (CQAs) were identified. These CQAs include key factors related to patient acceptability and the physical properties of the formulation. Specifically, critical features such as particle size, entrapment efficiency, and drug release of the resveratrol micropellets were selected. These attributes were determined based on a comprehensive review of existing literature, previous experience, and expert knowledge, along with well-founded reasoning (29).

2.2.2.2 Optimization of resveratrol micropellets

To optimize various critical material attributes (CMAs) and critical process parameters that influence the response variables or critical quality attributes (CQAs), a three-factor, three-level Box-Behnken Design (BBD) was employed using a response surface methodology with Design-Expert software (version 13.0). The selected CMAs, which served as independent variables, included Eudragit S100 (X_1), Stirring Speed (X_2), and Sodium Alginate (X_3), as shown in Table 1. The dependent variables, representing the CQAs, were particle size in micrometers (Y_1), % Entrapment Efficiency (Y_2), and % Drug Release (Y_3). The design included an additional five center points for each block, resulting in a total of 17 experimental runs, each performed in triplicate. All other material and process parameters were kept constant throughout the study (30, 31).

Linear regression was used to develop polynomial models that examined the effects of independent factors on the response variables. These models were validated using ANOVA, and the software-generated equations were analyzed to assess the influence of each CMA and CPP. The combination with the highest desirability was selected, and actual outcomes were compared to the predicted results (32).

Table 1: Independent and dependent variables

Critical Material Attributes and Critical Process Parameter	**Levels**		
	-1	0	+1
Eudragit S100, % (X_1)	2	4	6
Stirring speed, rpm (X_2)	400	500	600
Sodium Alginate, % (X_3)	1	2	3

2.2.3 Coating of micropellets

The optimized micropellets, coated by using an Accela-Cota coater. Isopropyl alcohol was utilized as the solvent for preparing the coating dispersions. Hydroxypropyl Methylcellulose (HPMC E5) act as a coating agent. The process began by gradually adding isopropyl alcohol to ethyl cellulose N50, which contained Triethyl Citrate (TEC) as a plasticizer, while continuously stirring to achieve a uniform dispersion. Separately, a homogeneous dispersion of HPMC E5 was prepared using purified water. These two dispersions were combined and stirred continuously for approximately 15 minutes to ensure uniformity.

Talc was incorporated into the mixture as an anti-sticking agent, calculated based on the dry weight of the polymers, and the mixture was stirred for an additional 10 minutes. The final coating dispersions were applied to the drug-loaded micropellets using the Accela-Cota coater via a spray system, with optimized parameters to ensure uniform coating. The micropellets were tumbled within the rotating pan of the coater, while the coating solution was sprayed onto them, ensuring an even application and avoiding clumping.

The coating level was monitored and controlled throughout the process, ensuring consistency in the coating thickness. The compositions coating material as shown in Table 2.(34).

Table 2: Composition of coating material used for coating

Ingredients	**Amount**
HPMC	1.5%
Dibutyl phthalate	8%
Triethyl citrate	15%
Talc	20%
Iso propyl alcohol	150ml

2.3 Characterization of developed micropellets and coated microparticles

2.3.1 Size

The size of the micropellets was assessed using an optical microscope. In this process, 50 micropellets were placed on a slide, and their particle sizes were determined using a calibrated optical micrometres(38).

2.3.2 Entrapment efficiency

The entrapment efficiency of micropellets was identified by indirect method. Briefly resveratrol microparticles was centrifuged using centrifuge at 5000 rpm for 10 minutes and unentrapped drug was separated as supernatant. To quantify resveratrol in the samples, further dilutions were prepared. treated in a sonication bath (40050, Sheryl Medi Equip System, Chennai) for 10-15 minutes(36). Using the calibration curve and the following equation, the amount of drug in the solution was determined.

$$\% \text{ Entrapment} = \frac{\text{Entrapped drug}}{\text{Total drug}} \times 100$$

2.3.3 Drug release of microparticles

By using USP Type II (Paddle) apparatus at 50 rpm. Maintain temperature at 37 ± 0.5°C with 900 mL of dissolution medium (pH 7.4). Added a pre-weighed quantity of micropellets. Withdraw 5 mL samples at specific time intervals and replace with fresh medium. Filter samples using 0.45 µm membrane filter. Measure drug concentration using UV-Vis spectrophotometry at 303 nm (35).

$$\% \text{ Drug Release} = \frac{Ct \times V}{D} \times 100$$

Ct = Drug concentration at time "t", V = Volume of dissolution medium, D = Total drug content.

2.3.4 Angle of repose

The angle of repose for micropellets is determined using a funnel method. A funnel is positioned above a flat surface, and micropellets are allowed to flow through the funnel to form a cone-shaped pile. After the funnel is removed, the height of the cone and the diameter of its base are measured by using given formula. This method provides insights into the flow properties of the micropellets, indicating their flowability and handling characteristics(39).

$$\text{Angle of repose} = \frac{h}{r}$$

h: height of the heap (mm), r: radius of the heap (mm)

2.3.5 Bulk Density

It is determined by dividing the micropellets volume by the weight of the micropellets. Particle form has a major impact on bulk density; bulk density increases as particles become more spherical in shape. Moreover, granule size improves bulk density. A defined volume of powder was added to a measuring cylinder, and it was mechanically tapped either by hand or with the use of a tapping device until a consistent volume was obtained(40).

$$\text{Bulk Density} = \frac{\text{Weight of micropellets}}{\text{Volume of micropellets}}$$

2.3.6 Tapped Density

A measuring cylinder filled with a predetermined mass of mixtures is set up on a mechanical tap and tapped for the number of taps required to reduce the volume of the powder bed to its smallest extent(41).

$$\% \text{ Tapped density} = \frac{\text{Weight of micopellets (W)}}{\text{Tapped volume of micopelletes (Vt)}}$$

2.3.7 Carr's Compressibility Index

Carr's formula was used to calculate the micropellets compressibility index(42).

$$\% \text{ Compressibility Index} = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Bulk Density}} \times 100$$

2.3.8 Friability

To determine the friability of micropellets, weigh a sample (e.g., 10 grams) and record its initial weight. Place the micropellets in a friabilator and rotate at 25–50 rpm for 10–15 minutes. After rotation, sieve and weigh the remaining intact micropellets(43). Calculate the friability percentage using the formula:

$$\% \text{ Friability} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

2.3.9 Hausner's Ratio

The calculation of Hausner's ratio was done by dividing the bulk density by the tapped density(44).

$$\text{Hausner Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

2.3.10 Fourier Transformation Infra-Red spectroscopy

FTIR spectroscopy was utilized to identify and analyse the structure of the samples. FTIR spectra for drug micropellets and without drug loaded micropellets were recorded using an Agilent FTIR spectrophotometer. The potassium bromide (KBr) pellet method was employed, where a small quantity of the sample powder was mixed with spectroscopic grade KBr and compressed under vacuum to form a pellet. The infrared spectra were then captured by scanning across the wavenumber range of 400-4000 cm^{-1}, using Empower software for data acquisition and analysis(36).

2.3.11 Shape and surface morphology

The optimized micropellets have been analysed and surface morphology using scanning electron microscopy (SEM). By applying a dried powdered microparticle sample on two pieces of double-stick tape that were secured to an aluminium stub, the process was finished. SEM was used to acquire samples images at a magnification of 1300X after coating stubs with gold to a thickness of about 300 Å (JSM-840; Joel, Tokyo, Japan)(42).

2.3.12 Differential scanning calorimeter

The drug's nature has been determined by DSC, along with thermal and polymorphic transitions involved in energy fluctuation during the formulation process. Resveratrol, Eudragit S100, and formulation DSC curves were evaluated using DSC (Mettler Toledo stare DS822, Germany) in perforated aluminium-sealed pans heated at a rate of 5°C/min from 10 to 340°C while in the presence of nitrogen gas (50 mL/s)(45).

2.3.13 X-ray diffractometry

Optimized formulations of coated and uncoated microparticles were subjected to XRD examination. The diffractometer used for the measurements was a wide-angle (D8 Advance from BRUKER Germany). The measurements involved measuring the X-ray scattering angle using a copper anode fixed at 45 kV and 40 mA(46)**.**

2.3.14 *In-vitro* drug release study

Drug release experiments were carried out *in vitro* on plain drug, coated and uncoated pellets using a USP dissolving test apparatus I (dissolving Test apparatus, Lab India DS 8000 instruments) in a 900 ml medium at 37°C and 100 rpm. The dissolution basket medium was filled with verified pellet quantities, each equalling 50 mg of Resveratrol. Two hours were spent on the dissolution experiments in SGF (pH 1.2, 900 ml), 3 hours in SIF (pH 6.8, 900 ml), and about 19 hours (total of 24 hours) in SCF (pH 7.4, 900 ml).
Samples were taken at different times, diluted correspondingly, and measured at 305 nm using a UV spectrophotometer. To maintain the volume in the dissolving medium after the sample collection, 5 mL of new media were added(47).

2.3.15 Cell viability

HCT116 cells were cultured in high-glucose DMEM with 10% fetal bovine serum at 37°C in a 5% carbon dioxide atmosphere. The cytotoxic effects of varying concentrations of resveratrol, uncoated drug-loaded micro pellets, and coated drug-loaded micro pellets on HCT116 cells were assessed using the MTT assay. 9×10^4 HCT116 cells were plated into 96-well plates and incubated at 37°C with 5% CO_2 for 24 hours. After 24 hours of incubation, the cells were treated with 5, 10, 20, 40, 60, and 80 µM of resveratrol, as well as uncoated and coated drug-loaded micro pellets for an additional 24 hours. After 24 hours, 10 µL of 5 mg/mL MTT was added to each well, and the samples were incubated at 37°C for 2 hours. MTT converts the viable cell converts MTT into formazan crystals, which are then solubilized in DMSO. Subsequently, the optical density at 570 nm was measured using the iMark™ Microplate Absorbance Reader(48, 49).

2.3.16 ROS detection

To evaluate the impact of formulation on DSS-induced total ROS production, 1×10^5 HCT116 cells were cultured in 12-well plates. The pre-treatment involved applying formulation (80µM) to the plates for 1 hour following a 23-hour 2% DSS treatment. Following 24-hour incubation, a final concentration of 10 µM of the

sensitive fluorescent probe DCFH-DA was introduced to each well, and the mixture was incubated for 30 minutes at 37°C. Following staining, washing was performed, and flow cytometry was utilized to analyse the cells(50).

2.3.17 NF-kB expression assay

1×10^5 HCT116 cells were seeded and cultured in each well of 12-well plates. Pre-treatment was conducted with 80 µM of formulations for 1 hour, followed by treatment with DSS (2%) for 23 hours. After 24 hours, collect the cells, centrifuge to pellet them, and discard the supernatant. Stabilize the cell pellets in 100 µL of 4% formaldehyde for duration of 15 minutes. Centrifuge the pellet and wash it with 1X PBS to eliminate excess formaldehyde. Introduce 100% cooled methanol to the cells, followed by centrifugation and two washes of the pellet with 1X PBS. Re-suspend the cells in 100 µL of diluted primary antibodies: NF-κB (Invitrogen, 51-0500). Incubate for 30 minutes. Samples underwent a second centrifugation for 5 minutes at 1500×g and were washed three times with 1X PBS. The cells underwent centrifugation and were washed twice with 1X PBS prior to incubation with 100 µL (1:25) of diluted fluorochrome-conjugated secondary antibodies, Alexa Fluor 488 for 30 minutes at room temperature. The cells were centrifuged, rinsed with 1X PBS, and the supernatant was discarded. Re-suspended in 200-500 µL of 1X PBS and analysed using a flow cytometer; the resulting data were analysed with Flowjo V10 software(53)**.**

2.3.18 CBA based extracellular screening of Interleukins

The concentrations of TH1 (IL-2, TNF-α, and IFN-γ), TH2 (IL-4, IL-6, and IL-10), and TH17 (IL-17 A) cytokines were quantified utilizing a BD CBA human TH1/TH2/TH17 cytokine kit according to the manufacturer's guidelines. Fifty microliters of cell lysate and fifty microliters of capture beads were added to the assay tubes, which were subsequently incubated in the dark for 150 minutes. The samples underwent analysis using a BD Accuri™ C6 Plus flow cytometer following three washes with 300 µL of wash buffer(51, 52).

2.3.19 Storage stability studies

The optimized coated micropellets formulation were subjected to stability testing in compliance with ICH recommendations. micropellets containing drugs were kept at two different temperatures: 25°C ± 2°C and 40°C ± 2°C, with relative humidity levels of 60% ± 5% and 75% ± 5%, respectively. Aluminium foil was used to seal the coated micropellets compositions. The stability was observed over a six-month period, and any noteworthy changes were evaluated. The percentage change in drug entrapment and percentage cumulative drug release were all measured as part of the examination(54).

3. Results and Discussion

3.1 Design of Experiment (DoE)

As presented in Table 1, the defined Quality Target Product Profile (QTPP) and related characteristics for the development of resveratrol micropellets are outlined, focusing on an enhanced sustained-release formulation designed for colonic site drug release. The Box-Behnken design was utilized to assess the impact of the chosen independent variables on the final responses simultaneously. Table 3 provides a comprehensive overview of the experimental setup, detailing the 17 runs conducted according to the Box-Behnken Design, with the corresponding observations documented.

Table 3: Design Summary

Response	**Name**	**Unit**	**Observation**	**Analysis**	**Minimum**	**Maximum**	**Mean**	**S.D.**
Y_1	Particle Size	µm	17	Polynomial	480	954	670.76	64.65
Y_2	Entrapment Efficiency	%	17	Polynomial	53	92	75.47	8.98
Y_3	Drug Release	%	17	Polynomial	65	96	85.29	7.72
Independent Variables				**Responses**				
Run	X_1: Eudragit S100, %	X_2: Stirring speed, rpm	X_3: Sodium Alginate, %	Particle Size (µm) (Y_1)		% Entrapment Efficiency (Y_2)		% Drug Release (Y_3)
1	-1	0	1	750		75		84
2	-1	1	0	510		75		83

3	-1	0	-1	783	82	92
4	-1	-1	0	739	70	81
5	0	0	0	615	82	94
6	0	-1	-1	740	77	83
7	0	0	0	676	84	95
8	0	0	0	598	81	91
9	0	1	1	510	82	92
10	0	0	0	610	81	93
11	0	0	0	684	73	85
12	0	1	-1	490	84	91
13	0	-1	1	690	72	84
14	1	1	0	480	92	96
15	1	0	-1	904	53	65
16	1	-1	0	670	65	75
17	1	0	1	954	55	66

3.2 Response analysis

3.2.1 Effect on particle size (Y_1)

The resveratrol micropellets particle size is major parameter which affect drug release and entrapment efficiency, if particle size is less more entrapment and show sustained release action. The polynomial equation to analyze the impact of the variables X_1, X_2, and X_3 on the response variable Y_1.

$$\text{Particle Size } (Y_1) = +636.60 + 28.25X_1 - 106.13X_2 - 1.63X_3 + 9.75X_1X_2 + 20.75X_1X_3 + 17.50X_2X_3 + 101.70X_1^2 - 138.55X_2^2 + 109.45X_3^2 \quad (1)$$

As indicated by polynomial equation (1) and the response surface analysis, the interaction between X_1 and X_2 exhibits a synergistic effect, reducing particle size (Y_1) due to the strong compatibility interactions. Additionally, the interaction showed a central tendency and positive curvature, indicating that no transformation was needed. The interaction involving X_3 significantly reduced particle size in the quadratic models, as illustrated in Figure 1. This observation also supports the increased entrapment efficiency of the drug in the micropellets, leading to smaller particle size and enhanced stability. Conversely, a decrease in X1 and X2, with X_3 held constant, negatively impacted micropellets size by increasing it. The interaction between X_2 and X_3 demonstrated a transient effect in stabilizing micropellets size, highlighting the compatibility between the drug and polymers. The quadratic relationship was visualized in the 3D contour plot, and the polynomial equation revealed a blend dependency graph, as shown in Figure 2.

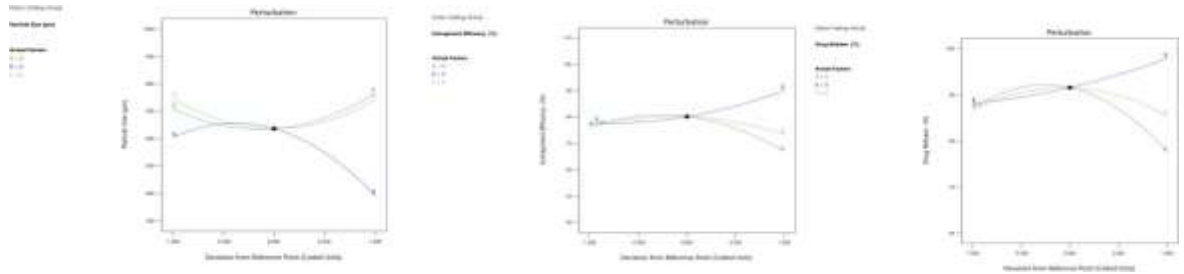


Figure 1: Perturbation plot

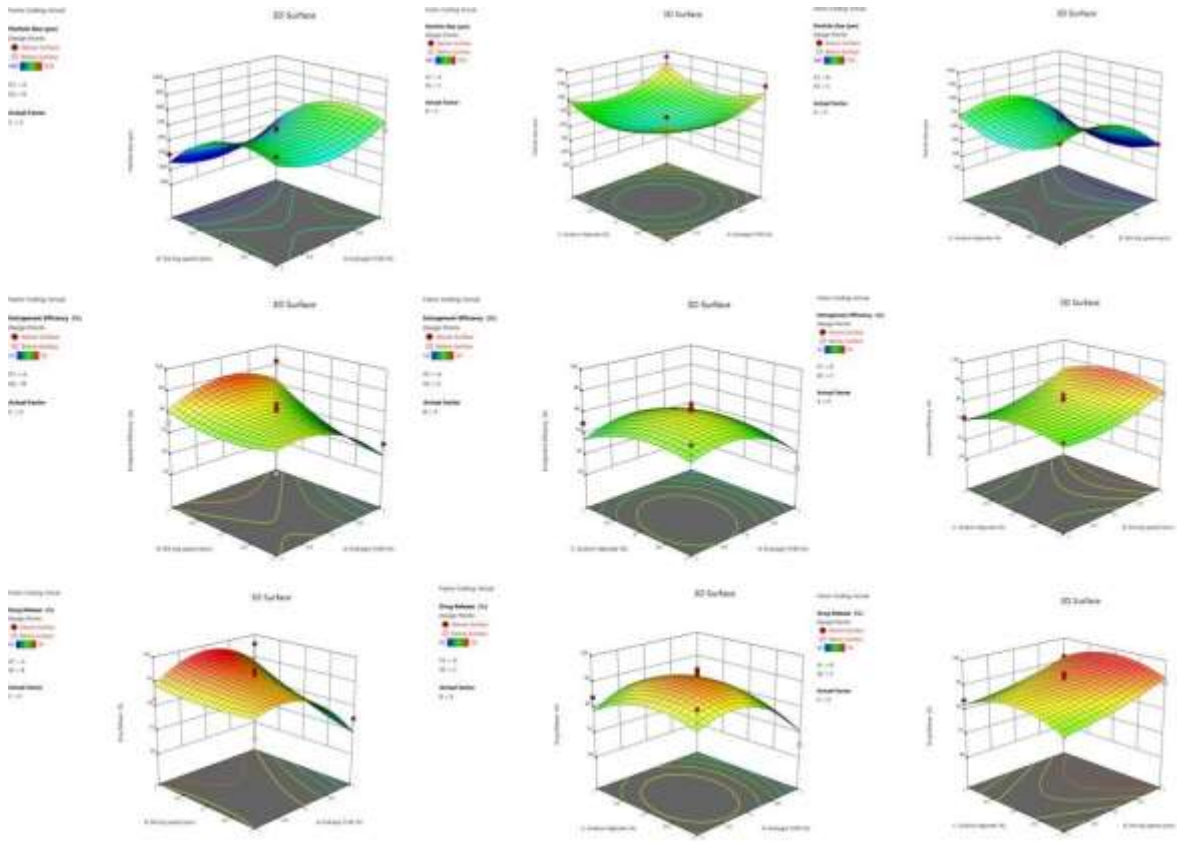


Figure 2: 3D Plots

3.2.2 Effect on Entrapment Efficiency (Y_2)

Increased efficiency enhances drug delivery and treatment outcomes, while higher drug loading in micropellets reduces dosing frequency for better patient convenience; polynomial equation 2 illustrates the interaction effects of Eudragit S 100 (X_1), Stirring speed (X_2), and Sodium alginate (X3) on Y_2.

$$\text{Entrapment Efficiency } (Y_2) = +80.20 - 4.62X_1 + 6.13X_2 - 1.50X_3 + 5000X_1X_2 + 2.25X_1X_3 - 0.75X_2X_3 - 8.60X_1^2 + 3.90X_2^2 - 5.35X_3^2 \quad (2)$$

When the concentrations of X_1 and X_3 were increased to +1, there was a notable improvement in Entrapment Efficiency (Y_2). This finding aligns with plots shown in Figures 1 & 2, where the quadratic model highlighted the significant impact of the independent variables on the predicted response. Further observations indicated that decreasing X_1 while increasing X_2 led to a slight reduction in Entrapment Efficiency, likely due to the lower concentration of Eudragit S100. These results are consistent with polynomial equation 2, as well as the perturbation plots in Figure 1 &2., which displayed positive curvature and suggested no need for further transformation. The model also indicates that the combined interaction terms X_1^2, X_2^2, and X_3^2 contribute to therapeutic synergism in the formulation by enhancing Entrapment Efficiency.

3.2.3 Effect on Drug Release (Y_3)

The impact of Critical Material Attributes (CMAs) on the drug release from micropellets is detailed through the contour plot and polynomial equation 3. According to equation 3, the individual variables X_1, X_2, and X_3 do not exhibit significant transient effects on each other's inherent properties. However, the combined interaction terms $X_1.X_2$ and $X_2.X_3$ significantly influence the drug release of the formulation.

$$\text{Drug release } (Y_3) = +91.60 - 4.75X_1 + 4.88X_2 - 0.6250X_3 + 4.75X_1X_2 + 2.25X_1X_3 - 0.005X_2X_3 - 9.30X_1^2 + 1.45X_2^2 - 5.55X_3^2 \quad (3)$$

The contour graph indicates that the interaction term $X_1.X_2$ positively affects drug release, while increasing X_3 has a neutral impact on the release. Ultimately, the combined interaction term $X_1.X_2.X_3$ significantly influences drug release, providing an optimal value for the micropellet formulation.

3.2.4 Statical analysis

The statistical results in Table 4 show significant model values for particle size ($p < 0.0003$), entrapment efficiency ($p = 0.0001$), and drug release ($p < 0.0004$), indicating a strong fit of the model. R^2 values for particle

size and entrapment efficiency were high (0.9818 and 0.9981), while drug release had a lower R^2 of 0.7063. Lack of fit was not significant for all parameters.

Table 4: ANOVA results

Characteristics	Particle Size (Y_1)	% Entrapment Efficiency (Y_2)	% Drug Release (Y_3)	Remarks
Source	p-value	p-value	p-value	
Model	< 0.0003	0.0001	< 0.0004	Significant
R^2	0.9818	0.9981	0.7063	
Mean	670.76	75.47	85.29	
Std. Dev.	64.62	8.98	7.72	
Adequate Precision	8.8684	3.8293	3.9888	
Lack of Fit	0.0846	0.0282	0.0407	Not significant

3.3 Characterization of uncoated micropellets and coated micropellets

3.3.1 Particle Size

From the observed results, the particle size of the uncoated and coated micropellets 552 ± 7.42 µm 628 ± 11.48 µm were observed respectively, which is optimal for colon-targeted drug delivery. This size range ensures effective retention and localized release of the drug within the colonic environment, enhancing therapeutic efficacy.

3.3.2 Entrapment efficiency of drug

The entrapment efficiency of drug-loaded micropellets was ascertained and verified three times. The uncoated and coated micropellets were found to have an entrapment efficiency of 86.97%±0.34 and 84.44±0.92 respectively. The loss of drug-related coating polymer would cause a little decrease in entrapment efficiency in coated micropellets, which would remain on the coating surface throughout the coating procedure.

3.3.3 Drug content

The drug content of the uncoated micropellets and coated micropellets were 98.34% ± 0.58 and 96.22% ± 0.75 respectively. This minor reduction in drug content after coating could be attributed to the additional polymer layer, which may have led to slight drug loss during the coating process.

3.3.4 Micromeritic and other properties of coated optimized micropellets formulation

We have evaluated several key physicochemical properties to ensure their suitability for pharmaceutical applications. The angle of repose was found to be 24.271° ± 0.417°, indicating excellent flowability of the micropellets. The bulk density was measured at 0.783 ± 0.006 g/cm³, and the tapped density was 0.785 ± 0.019 g/cm³, reflecting consistent packing characteristics. The Carr's Compressibility Index was 8.63% ± 0.74%, which suggests good flowability with minimal compressibility. Friability was observed to be 0.48% ± 0.52%, demonstrating that the micropellets possess robust mechanical strength during handling. Lastly, the Hausner's Ratio was calculated to be 1.082 ± 0.04, confirming the stability and uniformity of the micropellets as shown in table 4. All these parameters fall within standard ranges, indicating that the resveratrol-loaded micropellets have desirable physical characteristics for effective drug delivery.

Table 4: Micromeritic properties of coated optimized micropellets formulation.

Angle of repose(θ)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Carr's index (%)	Friability%	Hausner's ratio
24.271±0.417	0.783±0.006	0.785±0.019	8.627±0.738	0.48±0.521	1.082±0.04

3.3.5 Final optimized process variables for coated micropellets

The concentration of hydroxypropyl methylcellulose (HPMC) at 1.5% was found to be the optimal level for achieving efficient coating and a desirable drug release profile. Increasing the HPMC concentration beyond

1.5% resulted in a further rise in viscosity, leading to a reduction in droplet size. This negatively impacted the atomization process, causing irregular coating formation. Conversely, reducing the HPMC concentration below 1.5% led to a significant decrease in viscosity, resulting in poor film formation and inconsistent drug release. The optimized process variables for coated micropellets as shown in Table 5.

Table 5. Optimization of process variables for coated micropellets

Flow rate (ml/min)	Drum speed (rpm)	Temperature (°C)	Air pressure (kg/cm^2)
1.5	20	55	10

3.3.6 FTIR data of Optimized Formulation

The FTIR analysis, both with and without resveratrol, underscores the successful encapsulation of the drug within the Eudragit S-100 polymer matrix. The presence of distinct peaks in the encapsulated sample, such as the aromatic C-H stretching at 3745.56 cm^{-1} and 3425.73 cm^{-1}, as well as the O-H stretching at 3670.58 cm^{-1}, confirms that the resveratrol retained its chemical integrity. Furthermore, the absence of any significant shifts or new peaks in the polymer-only sample suggests that there were no interactions or chemical alterations between the polymer and resveratrol during the encapsulation process. The consistent C=O stretching at 1729.88 cm^{-1} and C-H bending at 2923.51 cm^{-1} and 1154.20 cm^{-1} in both analyses demonstrate that the polymer matrix successfully incorporated resveratrol without compromising its structural properties as shown in figure 3(a. FTIR Spectra of Optimized Formulation) (b. FTIR Spectra of Blank Formulation), ensuring the effectiveness of the controlled release system.

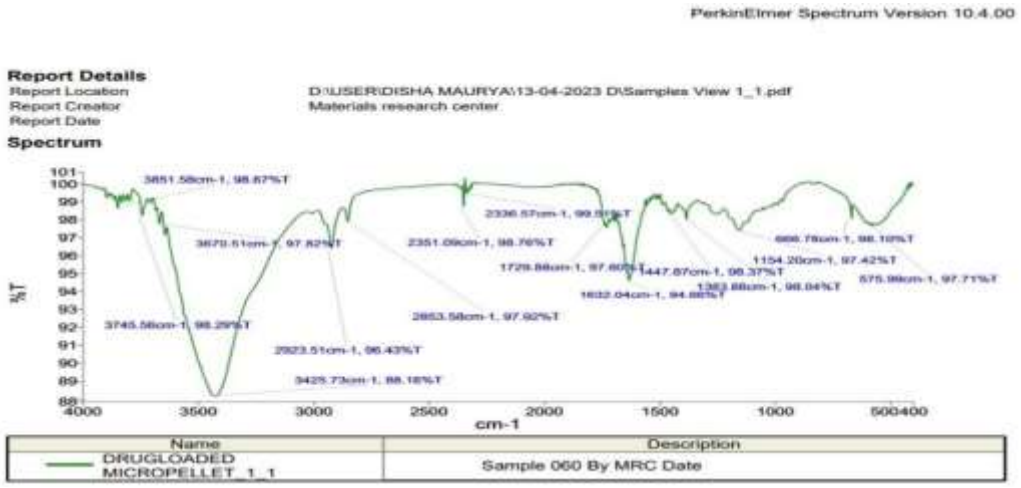


Figure 3(a): FTIR Spectra of Optimized Formulation

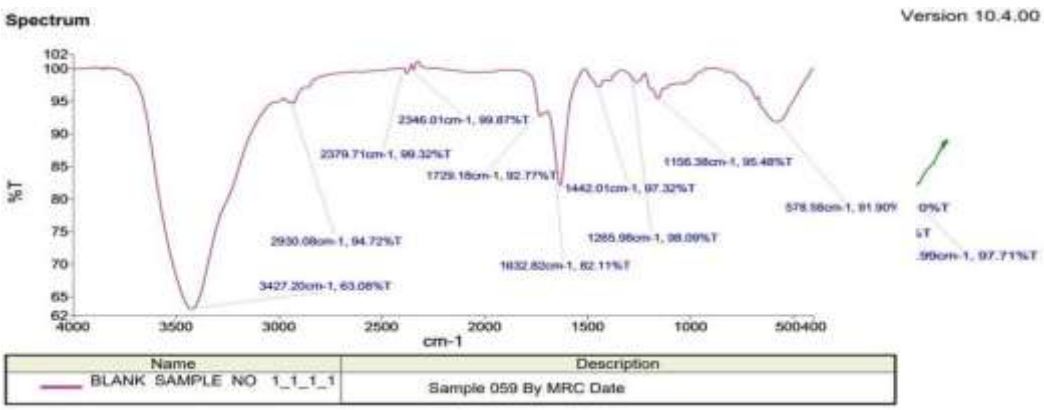


Figure 3(b): FTIR Spectra of Blank Formulation

3.3.7 Morphology

The scale bar at the bottom of the image indicates that the image is magnified 30 times (X30), with a scale of 500 µm. This magnification level reveals the surface morphology and texture of the micropellets.
The image appears to capture spherical micropellets, possibly indicating the encapsulation of resveratrol within a polymeric matrix as shown in figure 4.

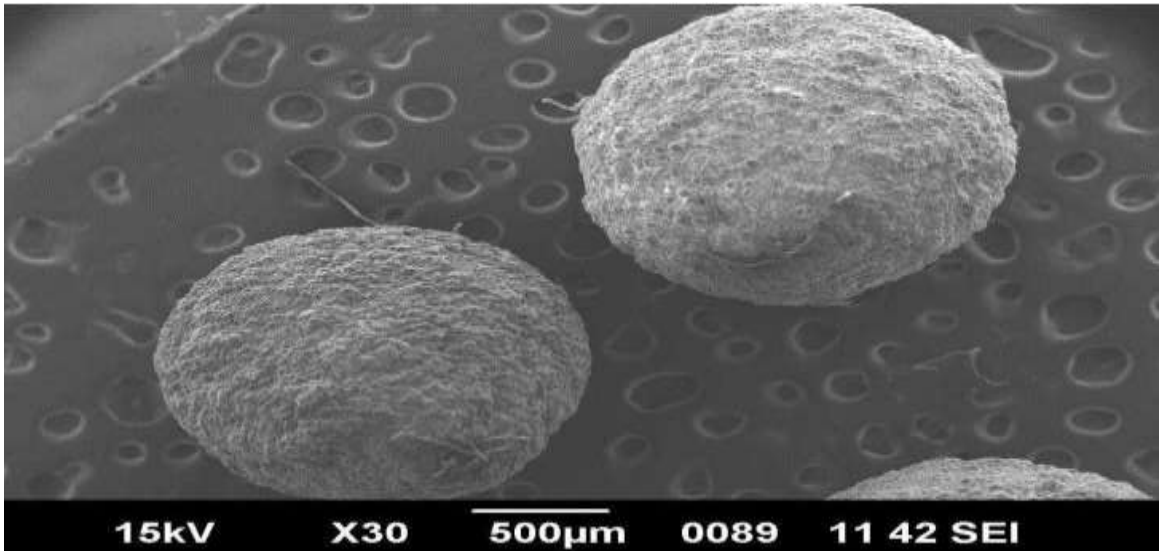


Figure 4: SEM of optimized coated formulation

3.3.8 Differential scanning calorimeter

We successfully prepared resveratrol-loaded micropellets using Eudragit S 100, as confirmed by Differential Scanning Calorimetry (DSC). The DSC analysis revealed a distinct endothermic peak corresponding to the melting point of resveratrol, indicating that the drug was successfully encapsulated and maintained its crystalline nature within the polymer matrix as shown in the given figure 5.

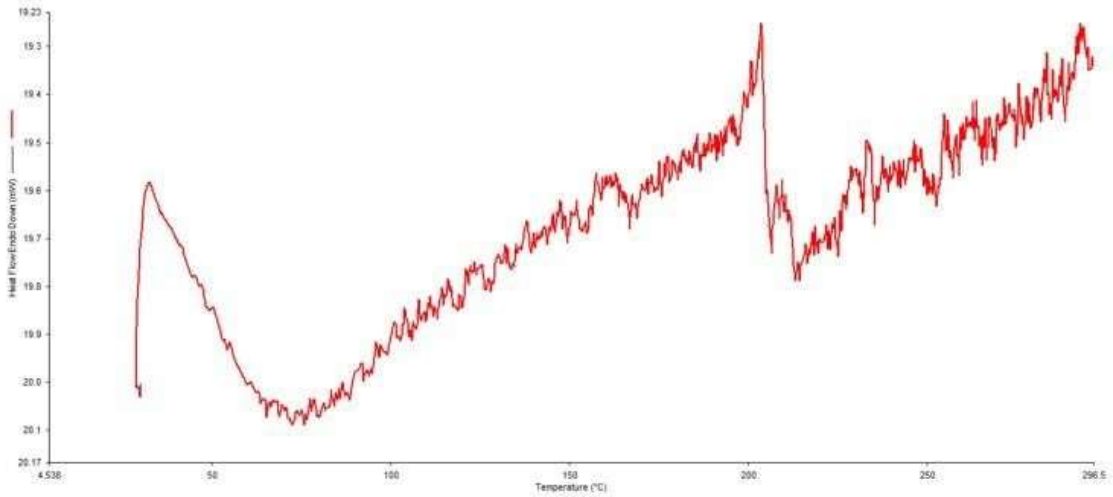


Figure 5: DSC of optimized micropellets formulation

3.3.9 X-ray diffractometry (XRD)

The X-ray diffraction (XRD) analysis provided valuable insights into the crystalline nature of both the blank Eudragit micropellets and the resveratrol-loaded micropellets. The blank micropellets demonstrated clear diffraction peaks, particularly in the 2θ range of 10° to 40°, with a notable peak around 30° 2θ. This indicates that the Eudragit polymer retains a partially crystalline structure even without the presence of an active pharmaceutical ingredient, contributing to the stability of the micropellets.
When comparing this with the resveratrol-loaded micropellets, similar diffraction peaks were observed, though with differences in peak intensities. This similarity in the XRD patterns suggests that the inclusion of resveratrol

within the Eudragit matrix did not significantly disrupt the crystalline properties of the polymer. The encapsulation process effectively preserved the structural characteristics of both the resveratrol and the Eudragit matrix, indicating that these micropellets have strong potential for stable and efficient drug delivery applications as shown in figures 6 (a) optimized micropellets Formulation (b) Blank Formulation.

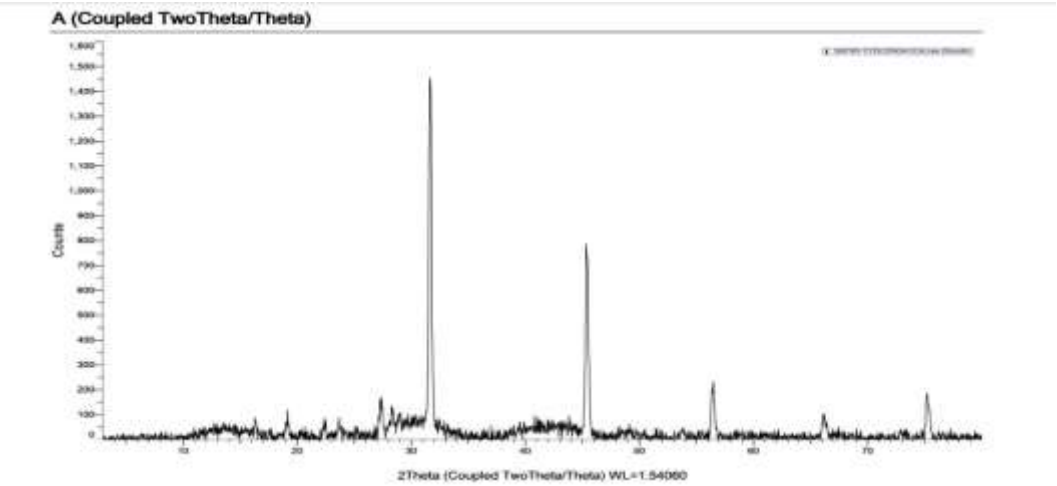


Figure 6(a): XRD of optimized micropellets Formulation

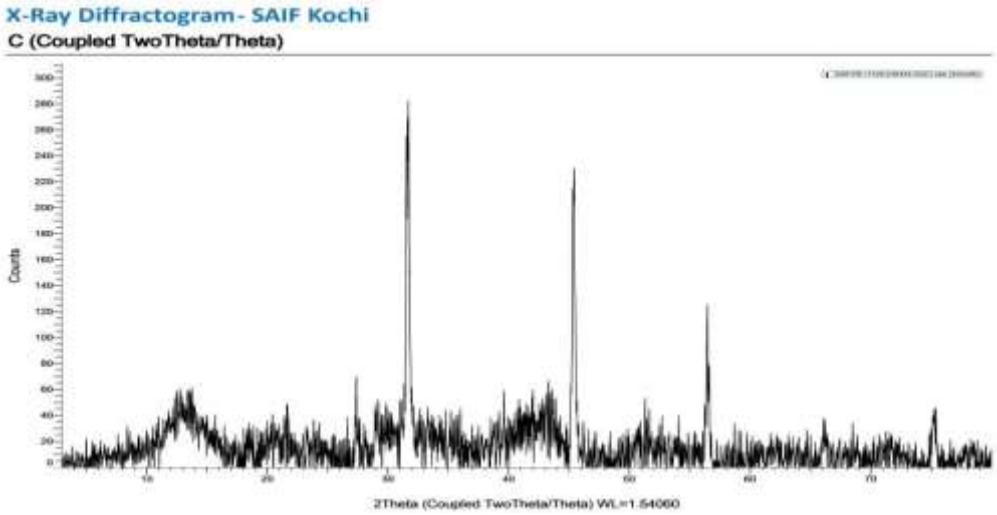


Figure 6(b): Blank Formulation

3.3.10 *In-vitro* drug release

The release profiles of Resveratrol (plain drug), uncoated micropellets, and coated micropellets were evaluated. The plain Resveratrol showed an initial burst release of 65.21% within the first two hours in the stomach, with complete release occurring within 6 hours. *In-vitro* studies of uncoated micropellets revealed an initial release of 40.51% within 2 hours, followed by complete release between 10 and 12 hours. In contrast, *in-vitro* analysis of coated micropellets showed no drug release within the first 2 hours, 21.41% release within 6 hours, and 96.14% release by 16 hours as shown in figure 7. These findings confirm that the polymer coating (HPMC) effectively sustained the drug release, preventing premature release in the stomach.

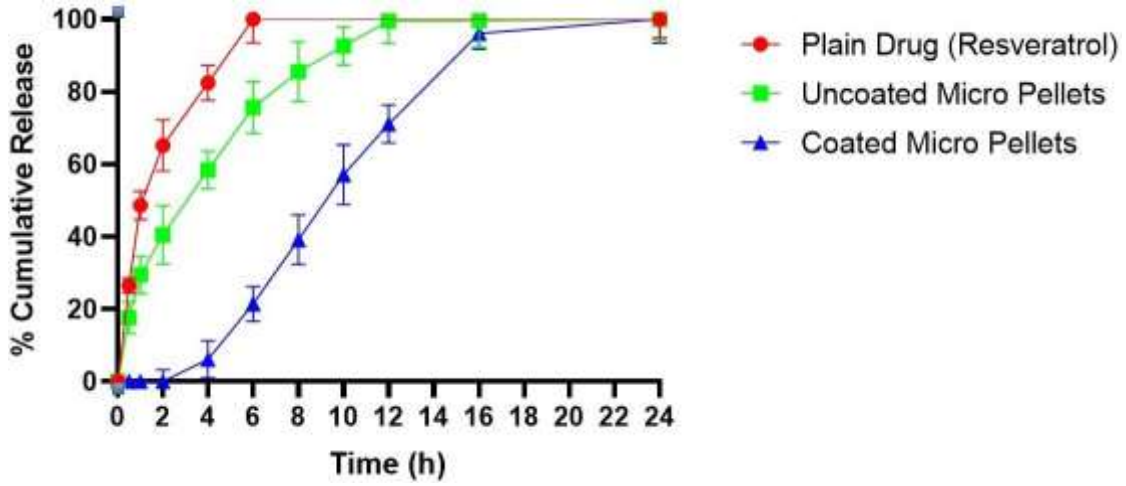


Figure 7.: *In-vitro* drug release

3.3.11 Cell viability assay

HCT116 cells were treated with varying concentrations of plain drug and formulations (of 5, 10, 20, 40, 60, 80, and 100µM) for a duration of 24 hours. The MTT assay was used to assess cytotoxicity, and the results are shown in Figure 8. After the formulations were exposed at different concentrations, it was noted that there were no significant changes in the viability of the cells. The formulations did not exhibit cytotoxicity on HCT116 cells, according to the cell viability experiment.

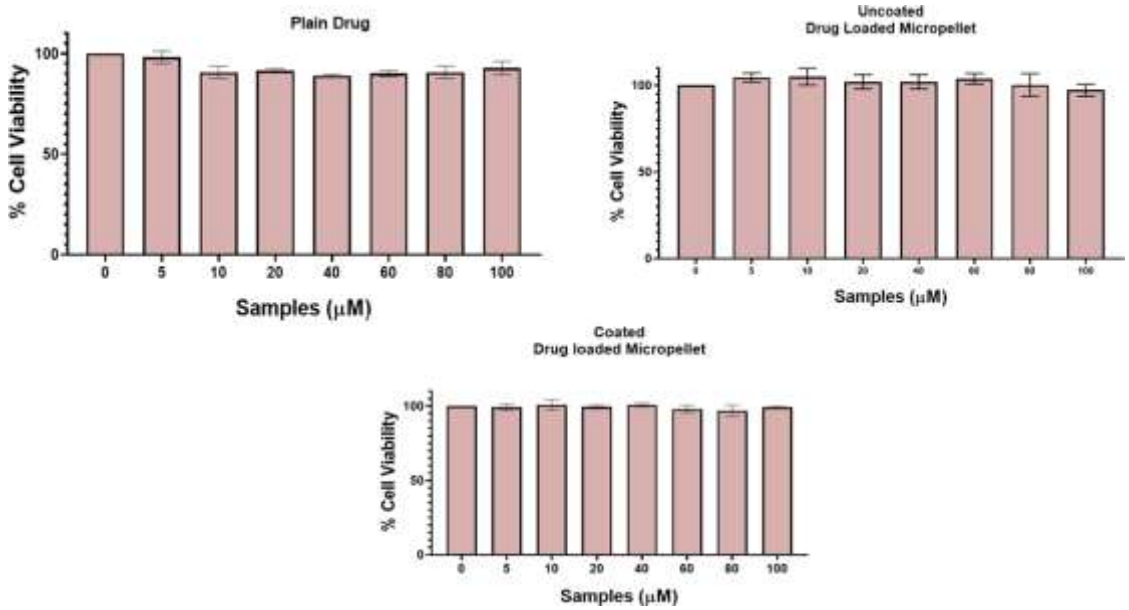
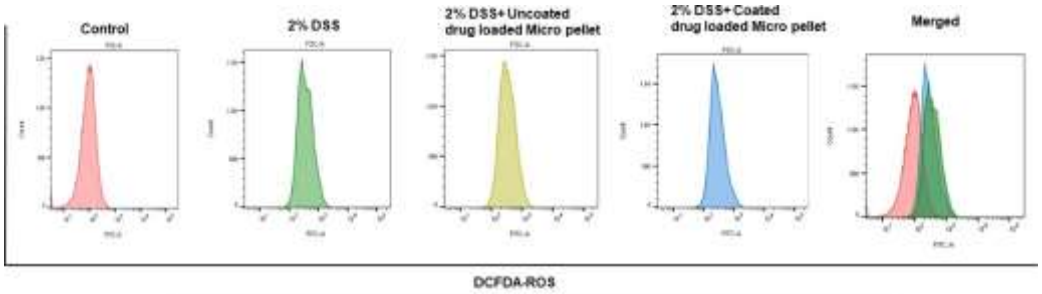


Figure 8: MTT based cell viability assay of plain drug, uncoated Drug Loaded Microparticle and Coated Drug Loaded Microparticle. Experiments are performed in triplicate, n=3.

3.3.12 Reactive oxygen species assay

Reactive oxygen species (ROS) are essential to toxicity caused by inflammation. In this work, we looked at how the formulations affected the production of ROS in HCT116 cells. After a day, flow cytometric analysis was used to carry out the ROS experiment. The sensitive dye 2'7'-dichlorofluorescein diacetate (DCFDA) was used to stain the cells. We also investigated the possibility of uncoated Drug Loaded Microparticle and Coated Drug Loaded Microparticles to lessen the production of ROS in HCT116 cells caused by DSS. The mean fluorescence intensity (MFI) for the control group was found to be 4362, as shown in Figure 9. The DSS-

induced group experienced a considerable increase in colon inflammation with MFI, reaching 17465. Nevertheless, the pre-treatment of Uncoated and coated drug loaded micro pellet with 5µM decreased the colon inflammation with MFI values dropped to 11367 and 8736, respectively.



Samples	DCFDA (MFI)
Control	4362
2%DSS	17465
2%DSS+Uncoated Drug loaded Micro Pellet	11367
2%DSS+coated Drug loaded Micro Pellet	8736

Figure 9: HCT116 cells were pre-treated with Uncoated and Coated Drug Loaded Micropellets (5µM) for 1 hour, followed by induction with 2% DSS for 23 hours. Showed that Uncoated and Coated Drug Loaded Micro pellet attenuates the DSS induced ROS production.

3.3.13 NF-kB Expression assay

The primary mediator of inflammation is NF-kB; colon inflammation is caused by an increase in NF-kB expression, which is known to be enhanced by DSS. Antigen-antibody assay has been used to analyse the impact of formulation on the DSS-induced inflammation and ulcerative colitis via NF-kB. Figure 10 shows that the DSS-induced NF-kB up-regulation was reduced by the coated and uncoated drug-loaded micro pellet. When compared to an uncoated drug-loaded micro pellet, the coated micro pellet demonstrated superior NF-kB inhibition. In the context of DSS-induced colitis, our results highlight the potential of coated medication loaded micro pellet in regulating oxidative stress and inflammatory responses.

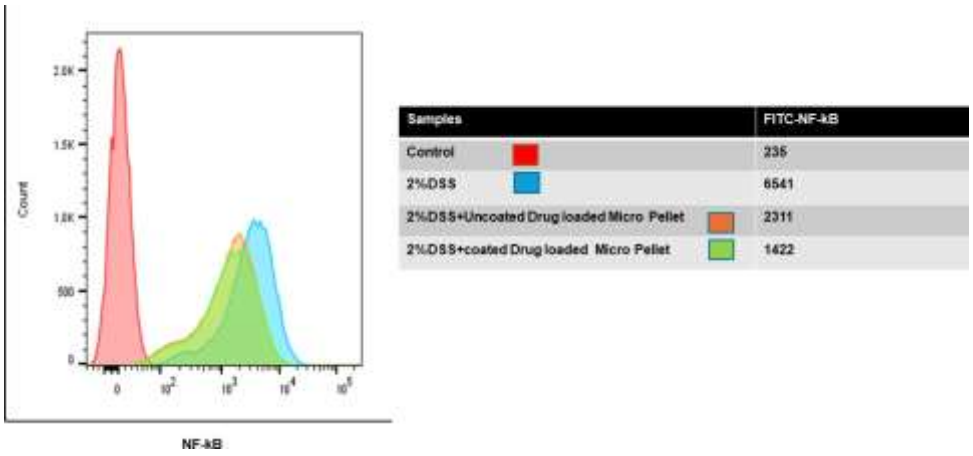
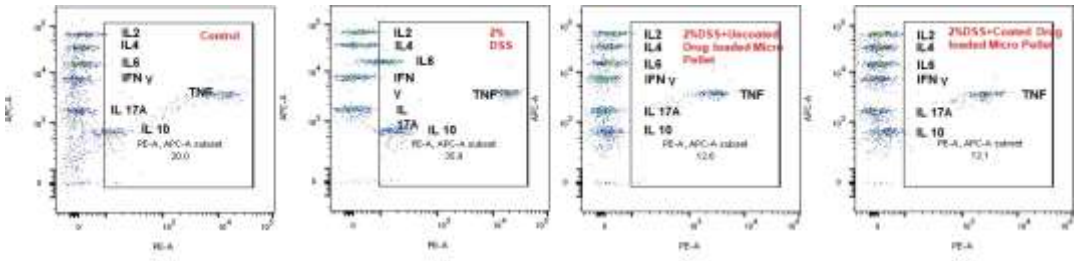


Figure 10: HCT116 cells were pre-treated with Uncoated and Coated Drug Loaded Micro pellet (5µM) for 1 hour, followed by induction with 2% DSS for 23 hours. Showed that Uncoated and Coated Drug Loaded Micro pellet attenuates the DSS induced NF-kB up-regulation.

3.3.14 Coated Drug Loaded Micro pellet attenuates the DSS induced interleukins

The extracellular interleukin screening of T_H1, T_H2, and T_H17 in cell lysate was performed by Cytometric bead array (CBA) assay in Flow cytometry; treatment with DSS increased the levels of T_H1, T_H2, and T_H17 interleukins, suggesting robust differentiation of interleukins. DSS treatment significantly increased TNF-α, IFN-γ, IL-4, IL-6, and IL-17A by compared to control. The elevated T_H1, T_H2, and T_H17 interleukins were quenched after pretreatment of uncoated and Coated Drug Loaded micro pellet, the levels of interleukins decreased levels of TNF-α, IFN-γ, IL-4 IL-6, IL-10, and IL-17A compared to DSS treated groups as shown in figure 11.



Sample	TNF	IL6	IL10	IL4	IL2	IL17A	IFN-γ
Control	2531	214	232	323	221	114	332
2%DSS	6895	2565	1156	656	1411	1696	552
2%DSS+Uncoated Drug loaded Micro Pellet	3564	564	654	411	214	665	236
2%DSS+coated Drug loaded Micro Pellet	2745	211	221	310	119	289	114

Figure 11: The effect of pretreatment of Uncoated and Coated Drug Loaded Micropellets followed by DSS (Interleukins screening with CBA kit, BD bioscience), DSS induced interleukins expression, Coated Drug Loaded Micropellets decreased the expression of interleukins.

3.3.15 Storage stability studies

A six-month stability study was carried out in controlled conditions, with relative humidity kept at 60 ± 5% and 75 ± 5%, and temperatures kept at 25 ± 2°C and 40 ± 2°C, respectively. As can be seen from Table 6, the results show that the coated micropellets formulations did not significantly alter in terms of drug entrapment % and cumulative drug release. The micropellets formulations seemed to have excellent stability under the investigated environments.

Table 6: Stability of optimized micropellets after 6 months.

Parameters	**Stability at 25±2 °C**				**Stability at 40±2 °C**			
Coated Micropellets	0th day	60th day	120th day	180th day	0th day	60th day	120th day	180th day
% drug entrapment	86.97 ±0.34	85.28±0.08	83.61±0.06	82.65±0.11	86.97 ±0.34	84.31±0.04	82.78±0.12	81.69±0.15
% cumulative drug release	96.73±4.5	95.37±5.2	93.62±4.8	91.39±6.1	96.14±5.4	94.14±6.2	92.34±3.7	91.56±5.6

4. Discussion

This detailed study on resveratrol-loaded micropellets highlighted their potential for targeted colonic delivery through thorough physicochemical characterization and biological evaluation. Using a Box-Behnken design, the research optimized the micropellets by analysing the effects of Eudragit S-100, stirring speed, and sodium alginate on particle size, entrapment efficiency, and drug release. FTIR confirmed successful encapsulation of resveratrol without any chemical interactions, while SEM showed the formation of smooth, spherical micropellets. Additionally, DSC and XRD analyses validated that the crystalline structure of both the polymer and resveratrol was maintained, ensuring stability and controlled drug release.

The micropellets exhibited exceptional flowability, with an angle of repose of 24.27° ± 0.47°, and consistent packing properties as reflected by bulk and tapped densities of 0.783 ± 0.006 g/cm³ and 0.785 ± 0.019 g/cm³, respectively. The Carr's Compressibility Index of 8.63% ± 0.74% and a Hausner's Ratio of 1.082 ± 0.04 confirm their optimal flow characteristics and stability. Additionally, the friability percentage of 0.48% ± 0.52% further underscores their mechanical strength, ensuring durability during handling and processing

The particle size, of coated and uncoated micropellets found to be 552 µm and 628 µm, ensures effective colonic retention and targeted drug release. In cell viability assays using HCT116 cells, no significant cytotoxicity was observed across varying concentrations of both plain drug and micropellets formulations, confirming their safety. The formulations also attenuated DSS-induced oxidative stress, as indicated by the reduction in ROS production, with coated micropellets showing superior efficacy over uncoated ones.

Further supporting their anti-inflammatory potential, the NF-kB expression assay showed a pronounced reduction in DSS-induced NF-kB upregulation, especially with coated micropellets. These results underscore the efficacy of the coated micropellets in regulating colon inflammation. The modulation of immune responses was evident as well, as coated micropellets significantly reduced elevated levels of interleukins (TNF-α, IFN-γ, IL-4, IL-6, IL-17A) induced by DSS, as shown through the Cytometric Bead Array (CBA) assay. Stability studies over six months demonstrated excellent robustness of the micropellets, with no significant changes in drug entrapment and cumulative release.

The resveratrol-loaded micropellets present a stable and efficient formulation with excellent physicochemical properties, controlled release profiles, and significant therapeutic potential for managing ulcerative colitis. These *in vitro* results set the stage for future *in vivo* studies and clinical applications, with the study adhering to Quality by Design (QbD) principles for optimal formulation development.

5. Conclusion

The developed resveratrol-loaded micropellets, utilizing Eudragit S-100, demonstrated strong potential for targeted colonic delivery, as validated by FTIR, SEM, DSC, and XRD analyses. The micropellets exhibited superior flowability, mechanical integrity, and stability and suitable particle sizes for colonic retention. *In vitro* assays confirmed the absence of cytotoxicity, significant reduction in DSS-induced ROS levels, and notable anti-inflammatory properties, particularly in coated micropellets. Stability assessments over six months revealed minimal variations in drug entrapment and cumulative release.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

Conflicts of interest.

The writers disclose no competing interests. The paper's writing and content are solely the authors' responsibility.

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