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ORIGINAL ARTICLE

Design and Optimization of Gastroretentive Floating Microspheres for Remdesivir to Treat SARS-COV-2

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ABSTRACT
Remdesivir-loaded floating microspheres were formulated using the methacrylic polymer Eudragit S 100 to enhance oral bioavailability and therapeutic efficacy against SARS-CoV-2. A 3^2 factorial design was employed to study the influence of two independent variables—polymer concentration (50, 100, 150 mg) and stirring time (1, 2, 3 hours)—on key formulation parameters such as particle size, entrapment efficiency, drug release, and floating time. Nine formulations (RES1–RES9) were prepared using the emulsion solvent diffusion method. The optimized formulation (ORES) was identified with 50 mg of Eudragit S 100 and 3 hours of stirring, yielding microspheres with desirable particle size (0.278 µm), high entrapment efficiency (88.01%), prolonged floating time (23 hours), and sustained drug release (87.32%). Characterization studies including FTIR, DSC, XRD, SEM, PDI, and zeta potential confirmed drug-polymer compatibility and formulation stability. Kinetic modeling indicated zero-order drug release with a Higuchi diffusion mechanism. In vivo pharmacokinetic studies in rats demonstrated significant improvement in parameters such as Tmax, Cmax, AUC, MRT, and HVD for the microsphere formulation compared to pure drug. These results confirm that the optimized floating microspheres offer sustained drug delivery, enhanced gastric retention, and improved bioavailability, representing a promising oral delivery system for Remdesivir in the treatment of COVID-19.
KEYWORDS: Remdesivir, Floating microspheres, Factorial design, DoE, Controlled release, Eudragit L100.

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INTRODUCTION

Remdesivir is a nucleotide analog prodrug that inhibits viral replication by targeting RNA-dependent RNA polymerase (RdRp) in RNA viruses, particularly SARS-CoV-2 (1–3). Once inside the host cell, Remdesivir undergoes metabolic activation to form its active triphosphate metabolite, GS-441524 (Remdesivir-TP), which mimics ATP and acts as a substrate for RdRp (4,5). It was reported that hospitalized patients treated with intravenous (IV) Remdesivir for ten days showed a 68% clinical improvement. However, IV administration has been associated with adverse effects such as rash, diarrhea, hypotension, acute renal impairment, and septic shock (6–8). Remdesivir is classified as a Biopharmaceutical Classification System (BCS) Class II drug, characterized by high permeability and low solubility (9). Its poor aqueous solubility, short half-life (~1 hour), erratic absorption in the stomach, and dose-related toxicity particularly hepatotoxicity caused by fluctuating plasma concentrations necessitate the development of alternative delivery systems (10–12). These limitations call for the transformation of Remdesivir into a modified-release drug delivery system to enhance its therapeutic efficacy and safety profile (13,14).

Floating drug delivery systems (FDDS) offer a promising strategy to control drug release and improve absorption in the gastrointestinal tract (GIT) (15,16). Several gastroretentive drug delivery (GRDD) approaches have been explored over the years, including high-density systems, floating systems, mucoadhesive systems, swellable systems, superporous hydrogels, and magnetic systems (17,18).

Among these, multi-unit dosage forms such as microspheres offer significant advantages over single-unit floating systems, which may suffer from inconsistent release, unpredictable gastric retention, and the risk of dose dumping (19–21). Microspheres, with diameters ranging from 1 µm to 1000 µm, are widely used for controlled and sustained drug delivery (22,23). Eudragit polymers (methacrylate copolymers) are particularly favored in the design of modified-release systems due to their biocompatibility, variable solubility, low toxicity, and chemical inertness (24,25).

In the present study, multi-unit floating microspheres of Remdesivir were developed using Eudragit S 100 and an emulsion solvent diffusion technique (26). The floating nature of the microspheres prolongs gastric retention by reducing peristaltic clearance, while controlled drug release is achieved by manipulating the polymer-to-drug ratio.

The primary objective of this research was to formulate and optimize Remdesivir-loaded floating microspheres using Eudragit S 100 through a 3^2 factorial design approach. The impact of two independent variables—polymer concentration and stirring time—was investigated on critical formulation parameters: particle size (Y1), entrapment efficiency (Y2), percentage drug release (Y3), and floating time (Y4).

This study is the first to report the development of a gastroretentive floating microsphere system for Remdesivir. This novel formulation aims to improve patient compliance by reducing dosing frequency and minimizing the toxicity associated with plasma concentration fluctuations of conventional Remdesivir formulations.

MATERIAL AND METHODS

Materials

Remdesivir was procured from VAREN Life Sciences, located in Hyderabad, Telangana, India. Excipients such as hydroxypropyl methylcellulose K (HPMCK), ethyl cellulose, and Eudragit S 100 were sourced from Yarrow Chemicals Pvt. Ltd., Mumbai, Maharashtra, India. Additional reagents including methanol, polyvinyl alcohol (PVA), and dichloromethane were obtained from SD Fine Chemicals.

Experimental Design

To optimize the formulation of floating microspheres containing Remdesivir, a 3^2 full factorial design approach was employed using *Design Expert Software* (version 13.0.5.0) developed by Stat-Ease Inc., Minneapolis, MN, USA. This statistical method was used to design nine different formulations of Remdesivir-loaded floating microspheres. Two independent variables—Eudragit S 100 concentration and stirring time were investigated at three levels: low (-1), medium (0), and high (+1). The dependent variables included particle size, floating time, percentage drug release (%DR), and drug entrapment efficiency (%EE) responses were recorded and used to develop mathematical models to predict optimal formulation conditions. A response surface methodology (RSM) was applied through the software to evaluate and interpret the experimental outcomes (27). The full experimental matrix and levels of variables are presented in Table 1.

Table1: Variable scope and intensity in experiments

S. No	Independent Variables	Coded values with Actual values		
		-1	0	1
1	RES 100 (mg) (X1)	50	100	150
2	Stirring time (hrs) (X2)	1	2	3

Preparation of Remdesivir-Loaded Floating Microspheres

The floating microspheres containing Remdesivir were developed using a solvent diffusion technique, a method suitable for producing low-density microparticles capable of floating in gastric fluids, thereby ensuring extended gastric residence and sustained drug release. To begin, Eudragit S 100 was accurately measured and dissolved in a solvent mixture consisting of ethanol and dichloromethane in a 1:1 ratio. Once a clear polymeric solution was achieved, 200 mg of Remdesivir was incorporated into the solution and thoroughly mixed using a sonicator, ensuring homogeneous drug dispersion. Following this, a 1% w/v solution of polyvinyl alcohol (PVA) was added dropwise to the above mixture using a syringe fitted with a fine needle. The addition was carried out under continuous stirring at 1000 rpm using a magnetic stirrer. The stirring time varied based on the formulation requirements defined by the experimental design. This emulsification process facilitated the formation of microspheres by allowing the organic phase to diffuse into the aqueous medium. The resulting microspheres were collected by filtration, rinsed gently with distilled water to remove residual solvents or unreacted materials, and then air-dried at room temperature until a free-flowing powder was obtained. This preparation method was uniformly followed

to fabricate a total of nine formulations (designated as RES1 to RES9) as outlined in Table 2, with variations in polymer concentration and stirring time as dictated by the factorial design. Each batch was later subjected to physicochemical evaluation and characterization studies.

Table 2: Composition of Remdesivir Floating microspheres

Composition	RES 1	RES 2	RES 3	RES 4	RES 5	RES 6	RES 7	RES 8	RES 9
Stirring time (hrs)	**2**	**2**	**2**	**1**	**1**	**1**	**3**	**3**	**3**
REMDESIVIR [mg]	200	200	200	200	200	200	200	200	200
Eudragit S 100 [mg]	**50**	**150**	**100**	**50**	**150**	**100**	**50**	**150**	**100**
HPMC K 100 [mg]	100	100	100	100	100	100	100	100	100
Ethyl cellulose [mg]	750	750	750	750	750	750	750	750	750
Methanol [ml]	25	25	25	25	25	25	25	25	25
DCM [ml]	25	25	25	25	25	25	25	25	25
PVA 1%[ml]	100	100	100	100	100	100	100	100	100

Characterization of Remdesivir Floating Microspheres

Particle Size Determination (Y1)

The average size of the formulated microspheres was assessed using a Malvern Mastersizer 2000 particle size analyzer (Malvern Instruments Ltd., UK). For analysis, 5 mg of each formulation was accurately weighed and dispersed in 500 mL of double-distilled water. The suspension was subjected to gentle swirling at a constant speed of 600 rpm to ensure uniform dispersion without causing aggregation. Each sample was measured in triplicate, and the results were reported as mean particle size ± standard deviation (SD).

Drug Entrapment Efficiency (Y2)

To evaluate the efficiency of drug entrapment within the microspheres, 50 mg of each batch was precisely weighed and dissolved in 10 mL of ethanol. This solution was then diluted up to 100 mL using 0.1 N hydrochloric acid (HCl) in a standard volumetric flask. The resulting solution was filtered using Whatman filter paper No. 44 to remove undissolved particles. The absorbance of the filtrate was recorded at 245 nm using a UV-Visible spectrophotometer. The amount of drug present was quantified with reference to a calibration curve generated from known concentrations of pure Remdesivir. The entrapment efficiency (%) was calculated using the following formula:

Entrapment Efficiency (%) = (Actual drug content in microspheres Theoretical drug content) × 100

In Vitro Drug Release Study (Y3)

The release profile of Remdesivir from the floating microspheres was evaluated using a Franz diffusion cell equipped with a dialysis membrane, which was placed between the donor and receptor chambers. An accurately measured 10 mg of microspheres was placed in the donor compartment. The receptor compartment was filled with 200 mL of 0.1 N HCl, maintained at 37 ± 0.5°C, and stirred continuously at 100 rpm using a magnetic stirrer to simulate gastrointestinal motility. At predetermined time intervals, 1 mL samples were withdrawn from the receptor compartment and immediately replaced with an equal volume of fresh 0.1 N HCl to maintain constant volume and sink conditions. The withdrawn samples were appropriately diluted and analyzed spectrophotometrically at 238 nm to determine the percentage of drug released over time for each formulation.

In vitro Buoyancy and Floating Time (Y4)

The buoyancy behavior of the prepared microspheres was determined by evaluating their floating lag time and total floating duration in a simulated gastric environment. For this study, 100 mg of the formulated microspheres were gently introduced into a 300 mL solution of 0.1 N hydrochloric acid (HCl) maintained at 37 ± 0.5°C. The system was stirred continuously using a paddle apparatus set at 100 rpm to mimic stomach motility. The floating lag time was defined as the time taken by the microspheres to rise from the bottom of the vessel to the surface after administration. Once they reached the surface, their floating time—the total duration for which the microspheres remained buoyant without sinking—was carefully recorded. These two parameters were used to evaluate the gastro-retentive potential of each formulation.

Statistical Experimental Design and Optimization

To systematically study the influence of formulation and process variables, a 3^2 full factorial design was employed. This statistical approach was used to optimize the formulation by evaluating the effects of two independent variables: X_1: Concentration of the polymer (Eudragit S 100) and X_2: Stirring time during microsphere formation. Each factor was assessed at three levels: low (-1), medium (0), and high (+1),

resulting in the development of nine different formulations. The dependent variables (responses) selected for evaluation included: Particle size (Y1), Drug entrapment efficiency (Y2), Percentage drug release (Y3) and Floating time (Y4). A second-order polynomial equation was used to model the relationship between the independent and dependent variables. This equation incorporated the main effects, interaction effects, and quadratic terms to predict the formulation behavior accurately. The data was analyzed using Design Expert Software (version 13.0.5.0, Stat-Ease Inc., Minneapolis, MN, USA) employing response surface methodology (RSM). Overlay plots and other graphical optimization tools were generated to identify the most favorable levels of X_1 and X_2 for producing the desired microsphere characteristics.

Following the statistical analysis, the optimized formulation (ORES) was selected based on the best-fit model and response desirability. This formulation was then prepared under the optimized conditions and evaluated for its actual responses to validate the predictive accuracy of the model.

To further characterize the optimized microspheres, advanced physicochemical analyses were performed including Fourier-Transform Infrared Spectroscopy (FTIR) for drug-excipient compatibility, Differential Scanning Calorimetry (DSC) for thermal behavior, Zeta potential measurements for surface charge and stability, Scanning Electron Microscopy (SEM) for morphological assessment, X-ray Diffraction (XRD) for crystallinity analysis. These studies provided comprehensive insight into the stability, structural integrity, and overall performance of the optimized formulation.

Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR analysis was performed to examine the potential chemical interactions between Remdesivir and excipients in the optimized formulation (ORES). The spectra were recorded using the potassium bromide (KBr) pellet method on a Bruker FTIR spectrophotometer. Precisely 1 mg of the sample (either pure drug or formulation) was thoroughly blended with 100 mg of dry KBr powder and compressed into a thin, transparent disc using a hydraulic press. The prepared pellet was then placed in the sample holder, and IR spectra were captured in the range of 4000–400 cm^{-1}. Comparative analysis of the spectra for pure drug and formulation helped assess any potential chemical shifts or interactions (28–30).

Differential Scanning Calorimetry (DSC)

To investigate the thermal behavior and physical state of the drug in the optimized formulation, DSC thermograms were recorded for both pure Remdesivir and the ORES formulation. The analysis was conducted using a Mettler Toledo DSC 822e instrument. Accurately weighed samples were sealed in aluminum pans and subjected to a controlled heating rate of 10°C/min under a nitrogen purge to prevent oxidative degradation. The thermograms were analyzed to determine any shifts in melting point or disappearance of endothermic peaks, which would indicate interaction or encapsulation of the drug within the microspheres (31).

X-ray Diffraction (XRD) Analysis

XRD analysis was employed to study the crystalline nature of Remdesivir in the optimized microspheres. Both the pure drug and the ORES sample were analyzed using a Bruker AXS D8 Advance X-ray diffractometer. The samples were scanned over a suitable 2θ range at room temperature using Cu-Kα radiation as the source. The diffraction patterns were recorded and compared to determine any changes in crystallinity, such as peak broadening or reduction in intensity, which would imply drug amorphization or dispersion within the polymer matrix (28,29,32).

Zeta Potential Measurement

The surface charge and colloidal stability of the ORES microspheres were evaluated by determining the zeta potential using a Zetasizer Nano-ZS90 (Malvern Instruments, UK). The microspheres were dispersed in Milli-Q water and sonicated briefly to obtain a uniform suspension. Measurements were conducted at 25°C, and each sample was analyzed in triplicate to ensure reproducibility. The average zeta potential value was recorded to assess the surface charge, which is indicative of the stability of the microsphere suspension.

Micromeritic Properties

To evaluate the flow behavior of the optimized microspheres, several micromeritic parameters were calculated, including Bulk density, Tapped density, Hausner's ratio, Carr's index (compressibility index) and Angle of repose. Each measurement was conducted three times, and the mean values were reported. These parameters provide insight into the powder flowability, which is essential for subsequent handling, encapsulation, or tableting processes.

Scanning Electron Microscopy (SEM)

The surface morphology and shape of the ORES microspheres were observed using Scanning Electron Microscopy (SEM) on a JEOL JSM-50A microscope (Tokyo, Japan). A small quantity of microspheres was placed on an aluminum stub using double-sided adhesive tape and gently fractured to expose internal

structures. The sample was then coated with a thin layer of gold-palladium using a sputter coater under an argon atmosphere for 120 seconds at a current of 14 mA. SEM images were obtained at an acceleration voltage of 15 kV, allowing detailed visualization of surface texture, porosity, and particle uniformity.

***In Vivo* Study**

Animal Selection and Housing Conditions

To assess the sustained-release profile of the optimized Remdesivir-loaded floating microspheres (ORES), in vivo studies were conducted using male Wistar rats, in accordance with ethical guidelines (Protocol No: 15/IAEC-II/SLSRPL/2024). Healthy adult male rats were randomly selected and individually housed in polycarbonate cages equipped with stainless steel grid tops and lined with sterilized corn cob bedding. Environmental conditions were carefully maintained, with a controlled room temperature of 22 ± 3°C and relative humidity ranging from 30% to 70%. The animals were subjected to a 12-hour light/dark cycle. All rats were provided with standard laboratory diet and filtered drinking water ad libitum, unless otherwise specified during the experimental procedures. Prior to dosing, the animals were fasted for an appropriate period while ensuring continued access to water.

Pharmacokinetic Evaluation of Remdesivir-Loaded Floating Microspheres

An in vivo pharmacokinetic study was carried out to evaluate the performance of the optimized Remdesivir formulation. A total of 36 male Wistar rats were randomly divided into four groups, each consisting of six animals (n = 6): Group G1: Control group (received no treatment), Group G2: Administered pure Remdesivir (non-formulated drug), Group G3: Administered optimized Remdesivir-loaded floating microspheres (ORES) and Group G4: Administered Remdesivir-loaded microspheres containing barium sulfate along with Eudragit S100 polymer for real-time gastro-retentive tracking using X-ray imaging. The buoyancy behavior of the floating microspheres was monitored through radiographic imaging at time intervals of 0, 1, 3, 8, 12, 16, and 24 hours' post-oral administration for Group G4 to confirm the floating retention within the gastric environment. For pharmacokinetic analysis, blood samples were collected at predefined intervals: 0 (baseline), 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours after administration. Blood was withdrawn via retro-orbital puncture under mild anesthesia and transferred into heparinized microcentrifuge tubes to prevent clotting.

Samples were then centrifuged at 4000 rpm for 10 minutes, and the plasma fraction was carefully separated and stored for further analysis. Plasma drug concentrations were determined using a validated analytical method, and data were analyzed using PKSolver, an add-in tool for Microsoft Excel designed to calculate non-compartmental pharmacokinetic parameters.

The pharmacokinetic parameters including C_{max}: Maximum plasma concentration, T_{max}: Time to reach C_{max}, AUC_{0-t}: Area under the plasma concentration-time curve, MRT: Mean residence time, Vd: Apparent volume of distribution and CL: Clearance rate was analyzed. These parameters were compared across the treated groups to assess the effect of formulation on the bioavailability and sustained release behavior of Remdesivir.

Quantification of Plasma Samples via HPLC

To determine the concentration of Remdesivir in plasma, an extraction process followed by High-Performance Liquid Chromatography (HPLC) analysis was employed. Initially, 200 µL of rat plasma was transferred into microcentrifuge tubes and mixed thoroughly with 200 µL of acetonitrile, serving as the protein precipitating and extraction solvent. The mixture was vortexed for 30 seconds using a Barnstead Thermolyne vortex mixer (Dubuque, IA, USA) to ensure complete mixing and efficient extraction of the drug. Subsequently, the samples were centrifuged at 6000 rpm for 15 minutes, allowing for the separation of plasma proteins. The clear supernatant was carefully collected and subjected to chromatographic analysis. The analysis was carried out on an HPLC system (Shimadzu LC-20AD) integrated with a Photodiode Array (PDA) detector. Chromatographic separation was achieved using a C18 reverse-phase column (THERMOFISHER, 4.6 mm × 150 mm). The mobile phase consisted of methanol and 0.2% triethylamine in water in the ratio of 45:55 (v/v). The pH of the aqueous phase was adjusted to 4.0 using orthophosphoric acid. The system was operated at a constant column temperature of 25°C and a flow rate of 1 mL/min. The detection wavelength was set at 245 nm, optimal for Remdesivir, and 25 µL of each processed sample was injected into the system. The retention time for Remdesivir was observed to be approximately 5.23 minutes under the specified conditions (Taşkin, 2022). This method was validated previously for specificity, accuracy, precision, and reproducibility, and was used for the reliable quantification of Remdesivir in biological matrices.

Data Analysis and Pharmacokinetic Evaluation

Pharmacokinetic (PK) parameters of optimized formulation were determined using non-compartmental analysis (NCA), applied to the plasma concentration-time data following extravascular administration in

rats. All calculations were performed using PK Solver Software version 2.0, an add-in tool for Microsoft Excel.

The area under the concentration-time curve (AUC_0–t) was calculated using the linear trapezoidal rule, which involves summing the areas under the plasma drug concentration curve from time zero up to the last measurable time point. Key pharmacokinetic parameters such as: Maximum plasma concentration (Cmax), Time to reach Cmax (Tmax), Elimination half-life ($t_1/_2$), Clearance (CL), Volume of distribution (Vd), AUC from time zero to the last measurable time (AUC_0–t) and AUC extrapolated to infinity (AUC_0–∞) were derived directly from the plasma concentration data.

The elimination rate constant (Kel) was calculated using the formula Kel = 0.693 / $t_1/_2$. To compute the area under the first moment curve (AUMC), the average concentration over a time interval was multiplied by the corresponding time step. This reflects the drug's presence in the body over time. The mean residence time (MRT), which indicates the average time the drug molecules remain in the system, was then determined using the ratio MRT = AUMC / AUC. For estimation of the half-value duration (HVD), the time period during which the drug concentration stays above 50% of Cmax, two specific time points were identified: One when the plasma concentration first rises to 50% of Cmax and another when it declines to 50% of Cmax. The difference between these two time points represents the HVD. HVD values were computed separately for both the pure drug formulation and the optimized floating microsphere formulation (ORES). The HVD ratio was calculated by dividing the HVD of the pure drug by that of the ORES formulation, offering a comparative measure of the sustained release characteristics.

RESULTS AND DISCUSSION

Table 3 presents the experimental findings of the Remdesivir-loaded floating microspheres, formulated using varying concentrations of Eudragit S100 (Factor X_1) and different stirring durations (Factor X_2). The study measured four critical formulation responses: particle size (Y_1), entrapment efficiency (EE, Y_2), drug release (DR, Y_3), and floating duration (Y_4).

The results indicate that changes in both the polymer amount and the duration of stirring had a significant effect on all four evaluated parameters. For example, RES 7, prepared with the lowest polymer concentration (50 mg) and the longest stirring time (3 h), showed moderate particle size (0.278 µm) but high EE (88.01%) and prolonged floating time (23 h). In contrast, RES 4 and RES 6, which involved higher polymer concentrations (150 mg and 100 mg respectively) with shorter stirring durations (2 h), exhibited larger particle sizes (0.834 µm and 0.824 µm) and comparatively reduced floating durations. RES 2 (150 mg, 3 h stirring) demonstrated a smaller particle size (0.274 µm) and relatively high entrapment (87.13%), signifying that a longer stirring time aids in reducing particle size even at higher polymer concentrations.

Table 3: Results of the floating microspheres of Remdesivir

Formulation ID	Factor X 1: A	Factor X2 B:	Dependent Factors (Y)			
	ES 100 concentration mg	Stirring time hrs	Particle size [Y1] µm	EE[Y2] %	DR[Y3] %	Floating time [Y4] hrs
RES 2	150	1	0.251±0.03	74.34±1.15	92.02±1.15	18±1.31
RES 7	50	3	0.278±0.02	88.01±1.23	87.32±0.89	23±1.21
RES 4	150	2	0.834±0.05	83.54±1.22	86.86±0.98	12±1.14
RES 8	100	3	0.347±0.06	77.88±1.14	76.68±1.02	21±1.20
RES 1	100	1	0.261±0.03	69.45±1.16	85.25±0.88	19±1.19
RES 6	100	2	0.824±0.03	82.67±1.18	79.23±1.05	16±1.23
RES 2	150	3	0.274±0.04	87.13±1.29	78.62±0.88	19±1.03
RES 5	50	2	0.821±0.03	91.16±1.30	85.95±0.94	12±0.89
RES 3	50	1	0.344±0.02	86.43±1.36	91.55±1.12	17±0.96

* all the values are given in mean ± SD

These outcomes confirm that lower polymer content with longer stirring time generally enhances entrapment efficiency and floating capacity while maintaining smaller particle sizes. The relationship between formulation variables and the observed responses was further assessed using Design of Experiments (DoE) with Response Surface Methodology (RSM). This statistical approach enabled the generation of contour and 3D response surface plots (Figures 1 & 2), which visually represent how the independent variables influenced each response parameter.

The trends observed in these plots are further supported by polynomial regression models summarized in Table 4, which quantify the effects of formulation variables on the selected responses.

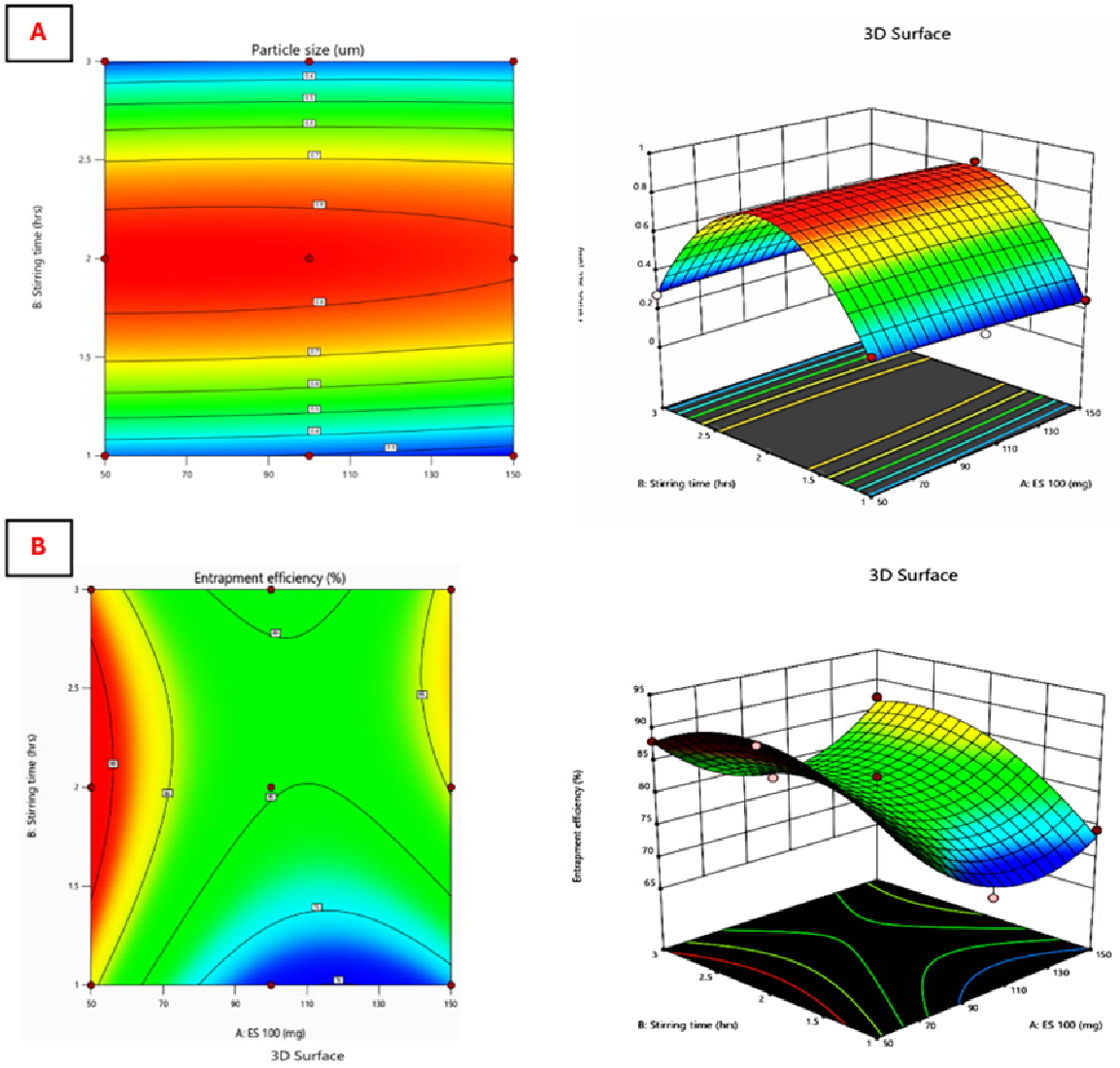

Figure 1: Contour plots for [A] PS (Y1), [B] EE% (Y2) with their corresponding response surface plots

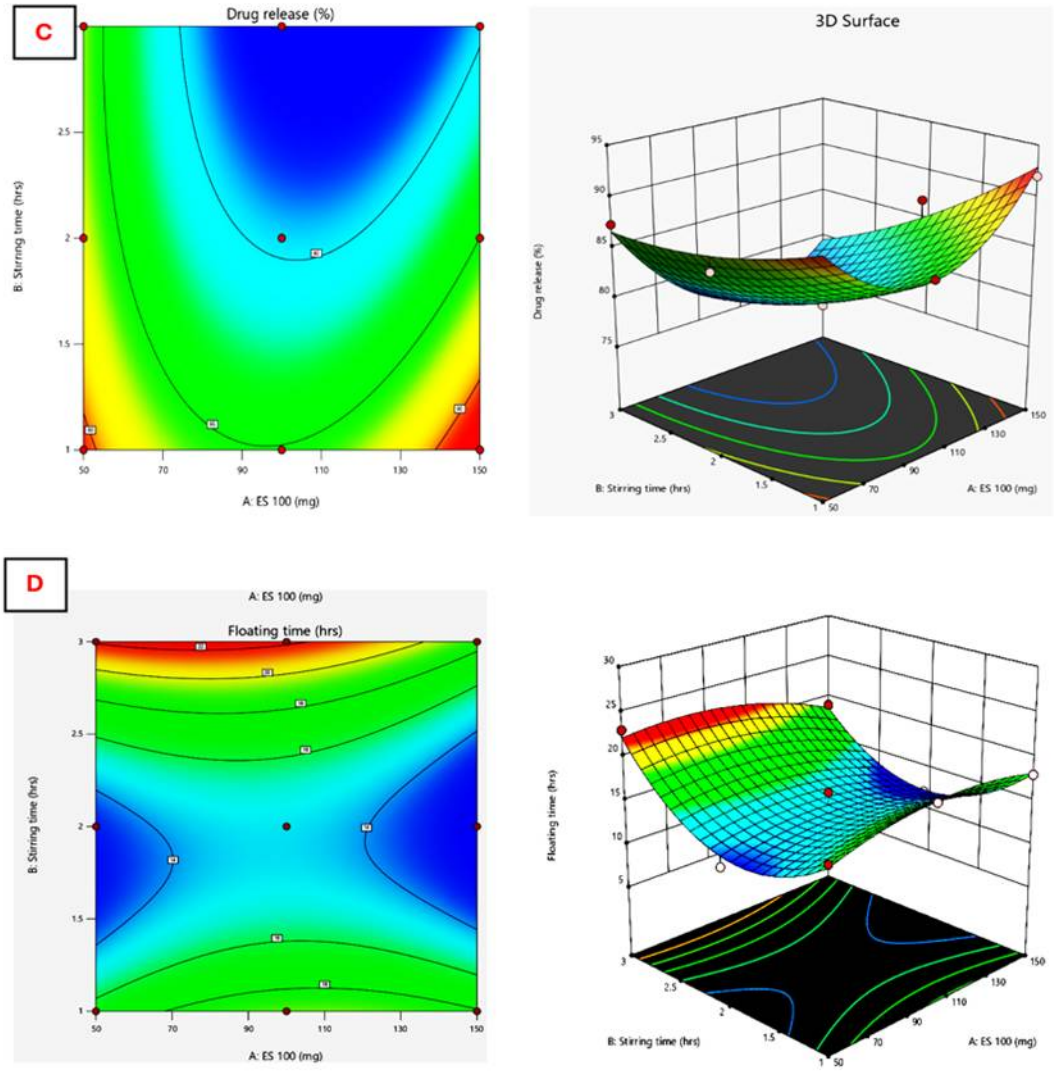

Figure 2: Contour plots for (C) % DR (Y3) and (D) (Y4) floating time with their corresponding response surface plots

Table 4: Factorial design proposed polynomial equations for responses

Particle Size [Y1]	= +0.8332 - 0.0140 X1 - 0.0072 X2 + 0.0222 X1X2 - 0.0103 $X1^2$ -0.5338 $X2^2$
Entrapment Efficiency% [Y2]	= +80.28 - 3.52 X1 + 3.80X2 - 3.88 X1X2 + 2.80 $X1^2$ + 8.52 $X2^2$
DR %[Y3]	= + 79.57 - 1.22 X1 - 4.37 X2 - 2.29 X1X2 + 6.67 $X1^2$ + 1.23 $X2^2$
Floating time [Y4]	= +14.56 - 0.5000 X1 + 1.50 X2 - 1.25 X1X2 - 1.83 $X1^2$ + 6.17 $X2^2$

Analysis of Particle Size Distribution (Y_1)

The particle size of the formulated Remdesivir-loaded floating microspheres was modeled by the polynomial equation:

Particle Size [Y1] = +0.8332 - 0.0140 X_1 - 0.0072 X_2 + 0.0222 X_1X_2 - 0.0103 X_1^2 - 0.5338 X_2^2

As presented in Table 4, the particle sizes of the formulations ranged from 0.251 ± 0.03 µm to 0.834 ± 0.05 µm. The results suggest that the concentration of Eudragit S100 (X_1) had a negative correlation with particle size, indicating that higher polymer content reduced the size of the microspheres. This reduction might be attributed to increased surface contraction or shrinkage during drying, which potentially enhances the buoyancy of the microspheres due to greater fluid uptake and structural compactness. This effect is further supported by the quadratic term (X_1^2) and interaction term (X_1X_2), although the influence of X_1^2 was less significant than that of X_2^2. Similarly, the stirring time (X_2) also showed a negative impact on particle size, implying that prolonged stirring resulted in finer microspheres. This outcome is likely due to enhanced shearing and continuous agitation, which prevents the formation of larger droplets and encourages the breakdown of larger particles through repeated collisions. Interestingly, the interaction between X_1 and X_2 (X_1X_2) demonstrated a positive relationship with particle size. This could be explained

by increased viscosity of the system and reduced coalescence of droplets, leading to improved stabilization and more uniform solidification of the microspheres. Overall, the data indicate that stirring time (particularly the quadratic term X_2^2) had a more dominant influence on particle size than polymer concentration, as reflected by the higher coefficient value of -0.5338 compared to -0.0103 for X_1^2. This suggests that optimizing stirring parameters is crucial for achieving desired microsphere size and morphology.

Evaluation of Entrapment Efficiency (Y2)

The following polynomial equation describes the entrapment efficiency of the Remdesivir-loaded floating microspheres:

Entrapment Efficiency % [Y2] = $+80.28 - 3.52 X_1 + 3.80 X_2 - 3.88 X_1X_2 + 2.80 X_1^2 + 8.52 X_2^2$

As shown in the results, the entrapment efficiency (%EE) of the formulations varied from 62.29 ± 1.23% to 84.12 ± 1.16%. The concentration of Eudragit L100 (X_1) demonstrated a negative correlation with %EE, indicating an antagonistic effect. As the polymer content increased, the viscosity of the system likely rose, reducing the ability of the drug molecules to diffuse efficiently into the polymeric matrix and resulting in lower entrapment. Similarly, the interaction between X_1 and X_2 (X_1X_2) also showed a negative effect on %EE, further confirming that simultaneous increases in both polymer concentration and stirring time can reduce encapsulation efficiency. In contrast, stirring time (X_2) exhibited a positive influence on %EE, suggesting a synergistic effect. This improvement in encapsulation may be attributed to better mixing, which facilitates the formation of uniformly sized microspheres with more efficient drug entrapment. Analyzing the coefficients, the squared term X_2^2 had a greater impact (+8.52) than X_1^2 (+2.80), highlighting that stirring duration played a more substantial role than polymer concentration in influencing drug encapsulation.

***In vitro* Drug Release Profile (Y_3)**

The drug release behavior of the formulations over time is described by the following quadratic model:

Drug Release % [Y3] = $+79.57 - 1.22 X_1 - 4.37 X_2 - 2.29 X_1X_2 + 6.67 X_1^2 + 1.23 X_2^2$

The cumulative drug release from the floating microspheres ranged from 76.68 ± 1.02% to 92.02 ± 1.15%, as observed over a 23-hour period (Figure 3). The minimum floating duration was 12 hours (as seen with formulation RES 8), while the drug release was extended up to 23 hours for other formulations. The concentration of Eudragit S100 (X_1) showed a negative relationship with drug release percentage, suggesting an antagonistic effect. This could be due to increased matrix density, decreased porosity, and a longer diffusion path, which slow down drug diffusion through the polymer network. Similarly, stirring time (X_2) also had a negative influence on drug release. Higher stirring rates may lead to smaller particle sizes but potentially reduce entrapment efficiency, ultimately lowering the amount of drug available for sustained release. Among the influencing factors, X_2 (stirring time) had a greater effect on drug release than X_1, based on their respective coefficient values (4.37 for X_2 vs. 1.22 for X_1). This indicates that adjusting stirring conditions can have a more pronounced impact on controlling the release profile of the drug.

Evaluation of Floating Time (Y_4)

The floating duration (Y_4) of the developed Remdesivir floating microsphere formulations ranged between 12 to 23 hours. The regression equation describing this response is:

Floating Time (Y_4) = $+14.56 - 0.50 X_1 + 1.50 X_2 - 1.25 X_1X_2 - 1.83 X_1^2 + 6.17 X_2^2$

An inverse relationship was observed between the concentration of Eudragit S100 (X_1) and floating time, suggesting that higher polymer concentrations negatively impact buoyancy. This may be attributed to increased density and reduced porosity of the microspheres at elevated polymer levels, resulting in a diminished ability to remain buoyant in the dissolution medium. Conversely, stirring time (X_2) showed a positive correlation with floating time, indicating a synergistic effect. Prolonged stirring likely contributed to the formation of smaller and more uniformly distributed microspheres, enhancing their surface-area-to-volume ratio and thus their floating capability. Interaction effects between X_1 and X_2 on floating time were minimal. Among the quadratic terms, the effect of X_2^2 (coefficient: +6.17) was more pronounced than X_1^2 (coefficient: -1.83), confirming that stirring time had a more substantial influence on floating duration than the polymer concentration.

Model Analysis Using Design-Expert Software

The experimental data from the factorial formulations were analyzed using Design-Expert software (v13.0.5.0, Stat-Ease Inc., Minneapolis, USA). A 3^2 full factorial design was implemented to study the effects of independent variables on the selected responses (Y_1 to Y_4).

Further statistical interpretation using Analysis of Variance (ANOVA) indicated that all response models were statistically significant ($p < 0.05$), as summarized in Table 5. The quadratic model was found to best describe the relationship between the variables and each response. Additionally, the F-values and

associated p-values revealed that both main effects and interactions had varying degrees of influence on the responses. Notably, stirring time (X_2) exhibited a more significant effect on the responses than polymer concentration (X_1), particularly in influencing floating behavior and particle characteristics.

Table 5: Model variation using factorial design expert

Particle size (Y1)						
Model type	**p-value**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	**Remarks**
Linear	0.9923	0.0026	-0.3299	-1.0884	1.21	
2FI	0.9008	0.0060	-0.5904	-2.8410	2.22	
Quadratic	**0.0009**	**0.9908**	**0.9756**	**0.8899**	**0.0638**	**Suggested**
Cubic	0.1899	0.9997	0.9974	0.9398	0.0349	Aliased
%Entrapment Efficiency (Y2)						
Modeltype	**p-value**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	**Remarks**
Linear	0.2249	0.3919	0.1891	-0.3028	534.75	
2FI	0.4349	0.4684	0.1494	-0.2571	515.97	
Quadratic	**0.0167**	**0.9652**	**0.9073**	**0.7249**	**112.90**	**Suggested**
Cubic	0.9490	0.9687	0.7495	-4.7065	2342.32	Aliased
% Drug release (Y3)						
Modeltype	**p-value**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	**Remarks**
Linear	0.1224	0.5035	0.3380	-0.1372	278.56	
2FI	0.3536	0.5893	0.3429	-0.7359	425.22	
Quadratic	**0.0254**	**0.9645**	**0.9053**	**0.5722**	**104.78**	**Suggested**
Cubic	0.1724	0.9989	0.9916	0.8077	47.09	Aliased
Floating Time (Y4)						
Model type	**p-value**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	**Remarks**
Linear	0.6448	0.1361	-0.1519	-0.8814	207.38	
2FI	0.5792	0.1928	-0.2915	-1.6155	288.28	
Quadratic	**0.0184**	**0.9438**	**0.8501**	**0.5173**	**53.21**	**Suggested**
Cubic	0.8705	0.9574	0.6593	-6.7622	855.56	Aliased

An optimized formulation of floating Remdesivir microspheres with desirable characteristics was successfully developed through the desirability function approach and graphical optimization using an overlay plot (refer to Figures 4 and 5). To validate the model's reliability, the observed values from the selected experimental batch were quantitatively compared with the values predicted by the design model, as presented in Table 6.

The comparison revealed a prediction error of less than 5% for all evaluated responses, indicating that the model accurately anticipated the experimental outcomes. These findings confirm that the optimization model is both robust and precise, with minimal deviation between experimental and predicted values, thereby validating its effectiveness for the formulation of Remdesivir-loaded floating microspheres.

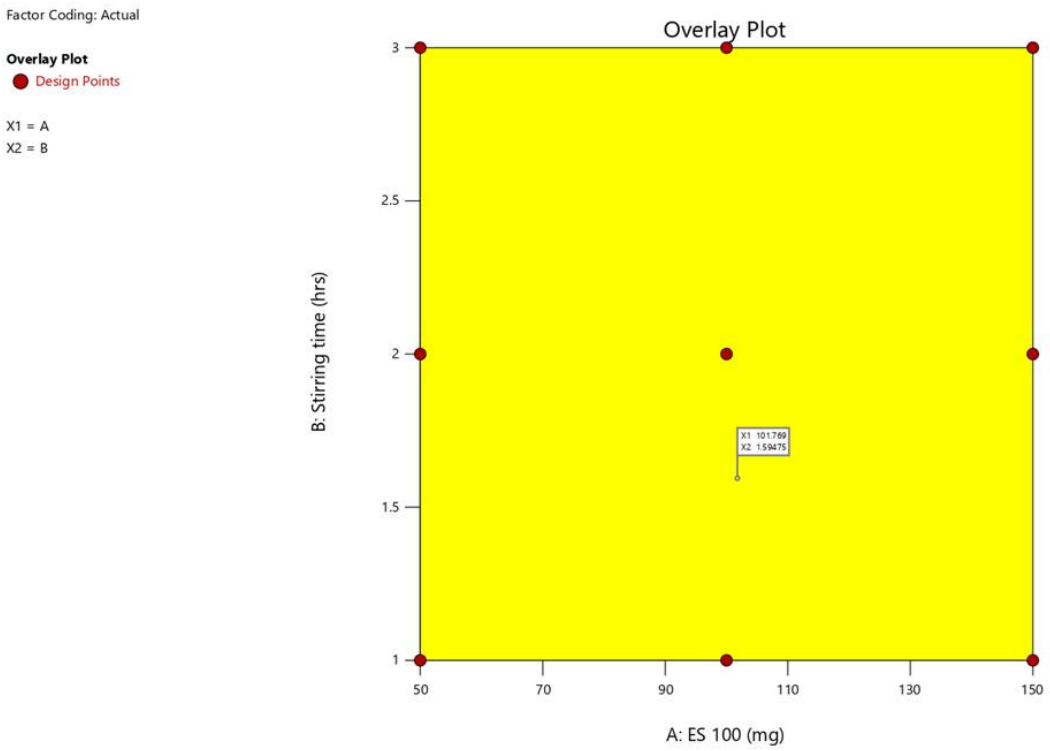

Figure 4: Overlay plot of RES showing optimized composition

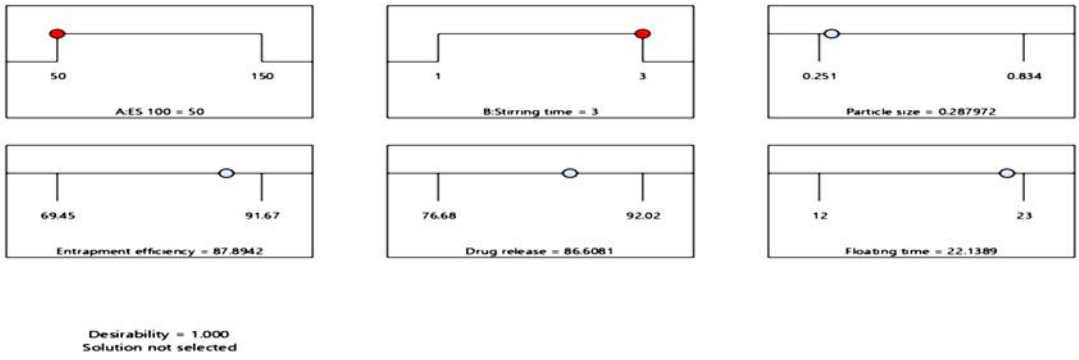

Figure 5: Desirability graphs

Table 6: Validation of Optimized formulation

Optimized formulation	X1: Eudragit S 100 (gm.)	X2; Stirring Time (hr.)	Responses	Predicted values	Experimental Values (Mean±SD)	Percent prediction error
ORES	50	3.00	Y1 Particle size (µm)	0.287±0.01	0.278±0.02	0.9
			Y2 EE (%)	87.89±1.12	88.01±1.23	0.136
			Y3DR (%)	86.60±0.69	87.32±0.89	0.824
			Y4 Floating time (hr)	22.13±0.89	23±1.21	3.782

Drug-Excipient Compatibility Study

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis was conducted to evaluate any potential chemical interactions between Remdesivir, and the excipients used in the formulation of floating microspheres. The spectrum of pure Remdesivir exhibited characteristic peaks at specific wavenumbers: a broad O–H stretching vibration was observed around 3451.96 cm^{-1}, N–H stretching near 2970.80 cm^{-1}, and C–H stretching at 2871.49 cm^{-1}. The carbonyl (C=O) stretching appeared distinctly at 1729.83 cm^{-1}, while C–N and C–O stretches were seen at

1267.00 cm^{-1} and 1375.00 cm^{-1}, respectively. Additionally, the P=O stretching vibration was identified at 1051.98 cm^{-1}.

In comparison, the FTIR spectrum of Remdesivir-loaded microspheres showed similar absorption bands with minor shifts: O–H appeared at 3382.53 cm^{-1}, N–H at 2958.27 cm^{-1}, and C–H at 2872.30 cm^{-1}. The C=O stretch slightly shifted to 1720.10 cm^{-1}, while C–N and C–O bands were detected at 1241.00 cm^{-1} and 1383.00 cm^{-1}, respectively. The P=O band also showed a slight shift to 1066.00 cm^{-1}. These minor changes in peak positions suggest possible hydrogen bonding or physical interactions between the drug and the polymer, rather than any chemical incompatibility. The preservation of all major functional group peaks indicates that there was no significant alteration in the chemical structure of Remdesivir, confirming its compatibility with the excipients used in the microsphere formulation.

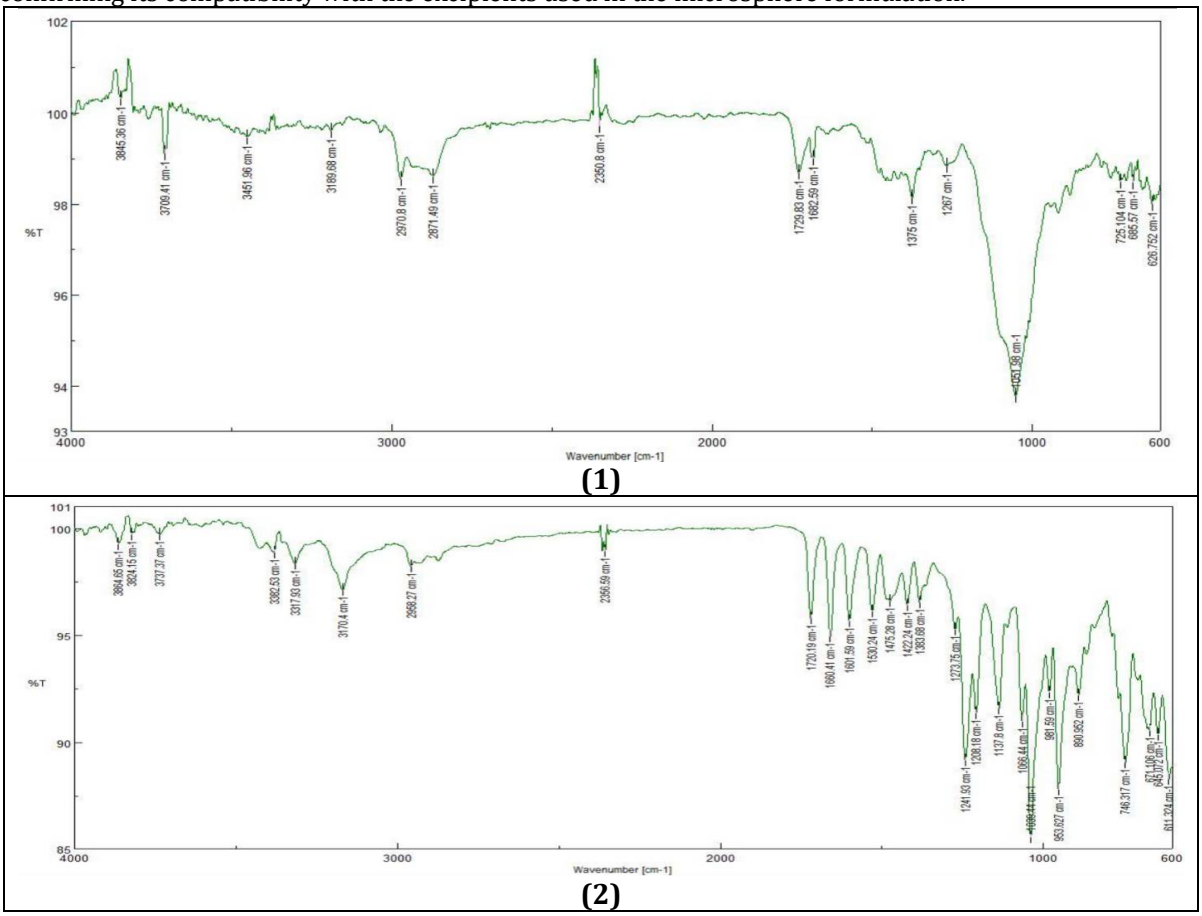

Figure 6: FT-IR Spectra of ORES (1) and Pure drug (2)

Differential Scanning Calorimetry (DSC) Study

The thermal behavior of Remdesivir and its formulated floating microspheres was assessed through DSC analysis, as shown in Figures 7 and 8. The thermogram of pure Remdesivir exhibited a sharp endothermic peak with an onset at approximately 133.0°C, reaching a maximum at 143°C, and concluding at around 147.07°C. This well-defined peak corresponds to the melting point of the pure drug, indicating its crystalline nature. In contrast, the DSC thermogram of the drug-loaded microsphere formulation revealed a broadened endothermic transition, beginning at around 60°C and extending up to 170°C. Notably, the distinct melting peak of Remdesivir was not clearly visible in the formulation, suggesting that the drug was molecularly dispersed or embedded within the polymer matrix. The absence of any new or shifted sharp peaks, along with the embedding of the drug's thermal signature within the formulation, indicates that there was no significant physicochemical interaction between Remdesivir and the excipients. This confirms the compatibility of the drug with the components used in the microsphere formulation.

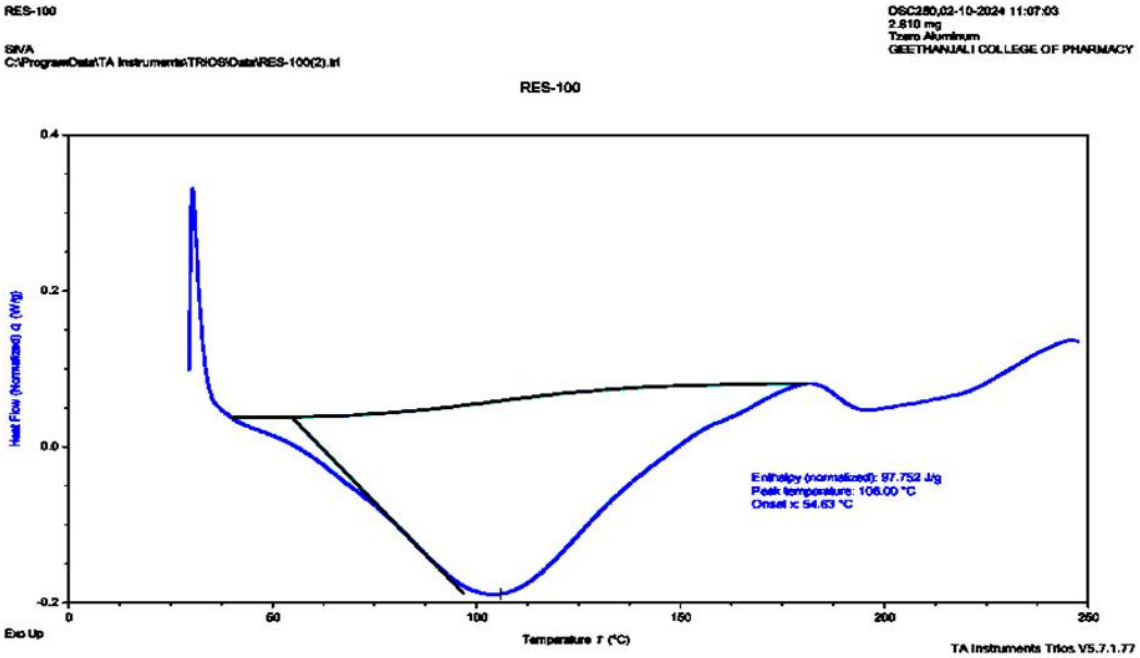

Figure 7: DSC thermogram of Remdesivir microspheres formulation (ORES)

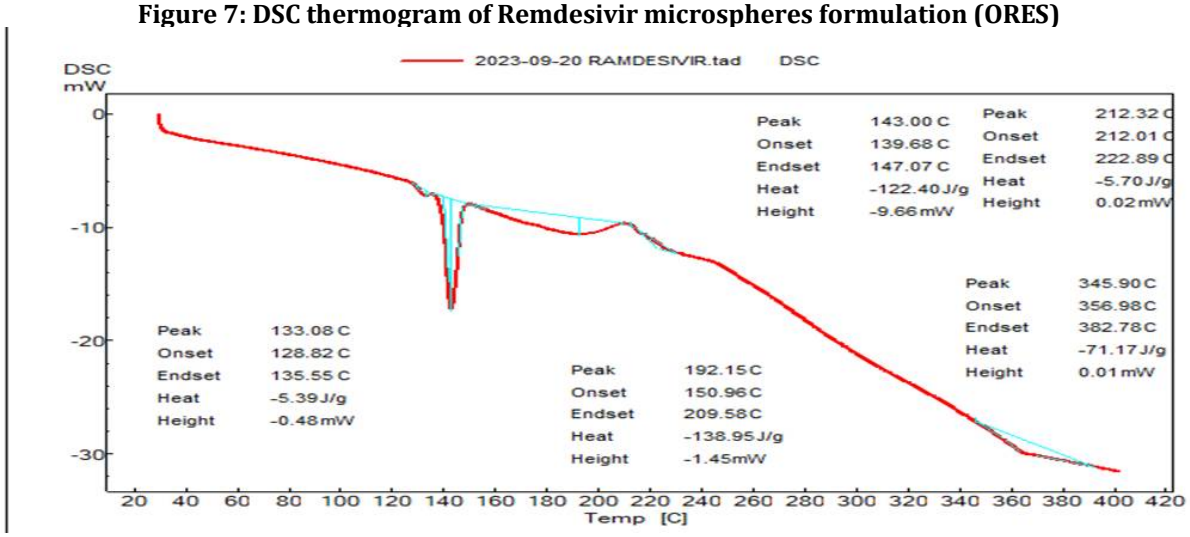

Figure 8: DSC of Remdesivir Pure Drug

X-Ray Diffraction (XRD) Study

The XRD analysis was performed to assess the physical state of Remdesivir before and after formulation into floating microspheres. The diffraction pattern of the pure drug displayed distinct and sharp peaks at 2θ values of 2.195°, 12.24°, and 16.0°, confirming its crystalline nature. In contrast, the XRD pattern of the optimized Remdesivir-loaded microspheres exhibited a noticeable reduction or complete absence of these characteristic peaks. This loss of distinct diffraction signals suggests that the crystalline structure of Remdesivir was disrupted during formulation, resulting in a transition to an amorphous or molecularly dispersed state within the microspheres. These observations, as represented in Figure 9, confirm that the drug has been successfully incorporated into the polymer matrix in an amorphous form, enhancing its potential for improved solubility and uniform dispersion.

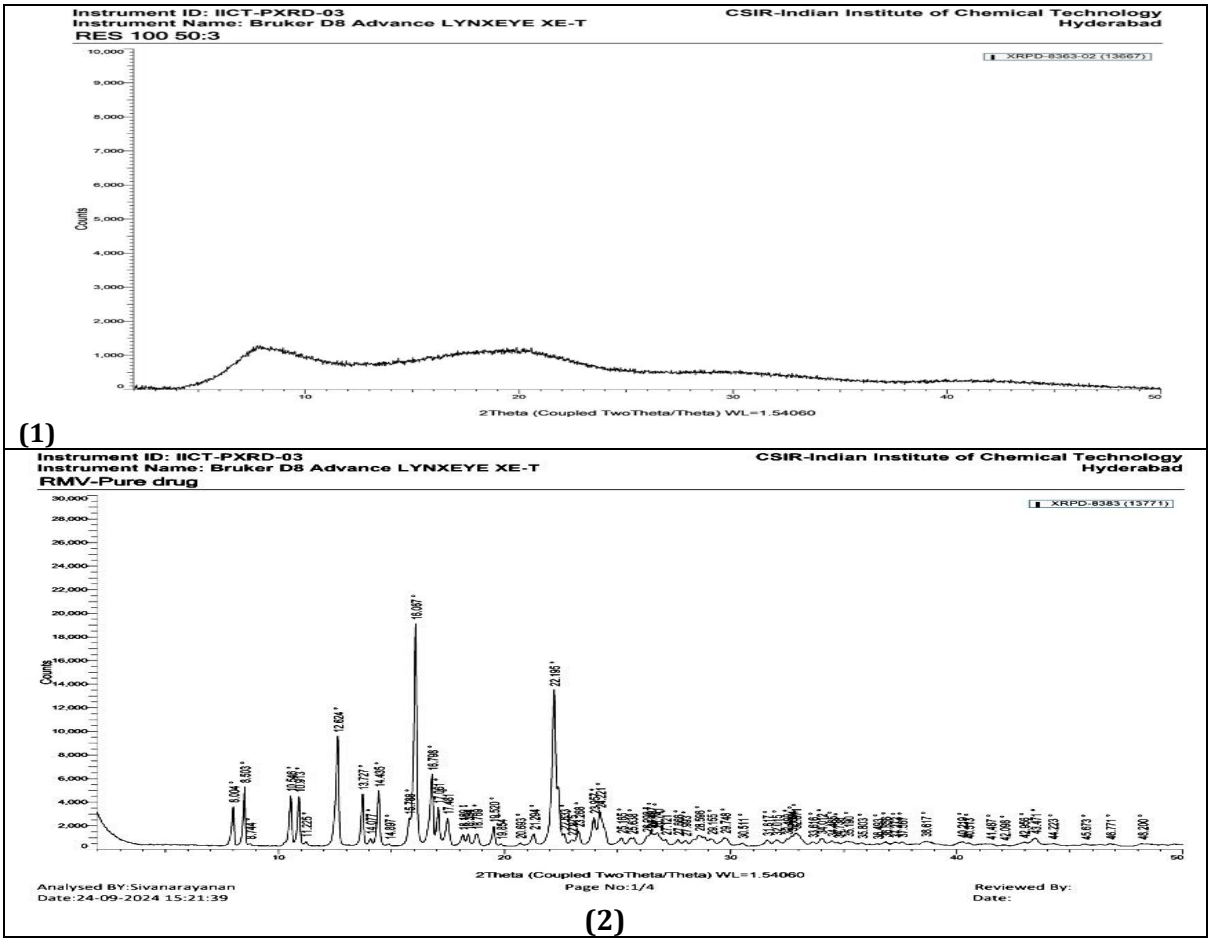

Figure 9: XRD of Remdesivir microspheres formulation (ORES) (1) and Pure drug (2)

Zeta Potential

The zeta potential analysis, as shown in Figure 10, along with the polydispersity index (PDI), revealed that the formulated microspheres exhibited a moderately charged surface. This moderate zeta potential value indicates sufficient electrostatic repulsion between particles, which contributes to the physical stability of the suspension. Such stability minimizes aggregation and ensures uniform dispersion, confirming that the floating microspheres are likely to remain stable over time.

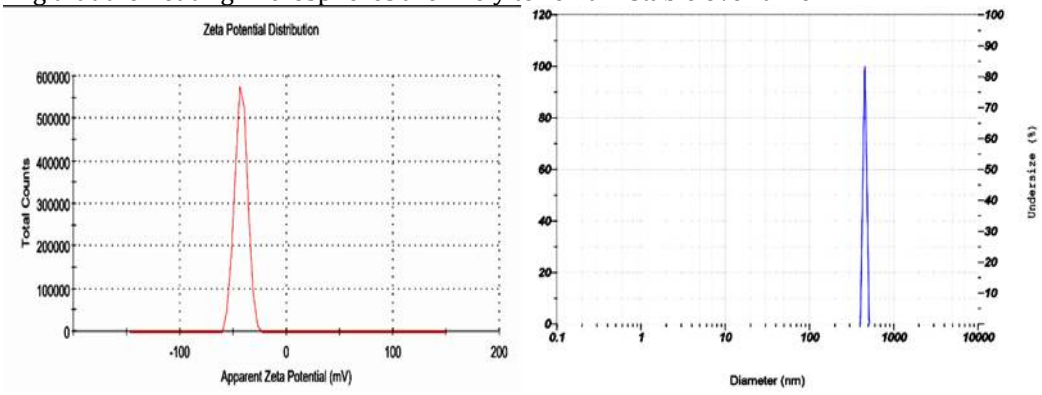

Figure 10: Zeta potential and PDI of ORES

SEM Analysis

The surface morphology of the optimized Remdesivir-loaded floating microspheres, as observed in Figure 11, confirmed that the particles were predominantly spherical in shape, well-separated from each other, and exhibited a rough, hollow, and porous texture. The presence of pores on the microsphere surfaces suggests enhanced buoyancy, which is likely to improve the floating efficiency of the formulation. This structural feature supports prolonged gastric retention by aiding better floatation in the gastrointestinal environment.

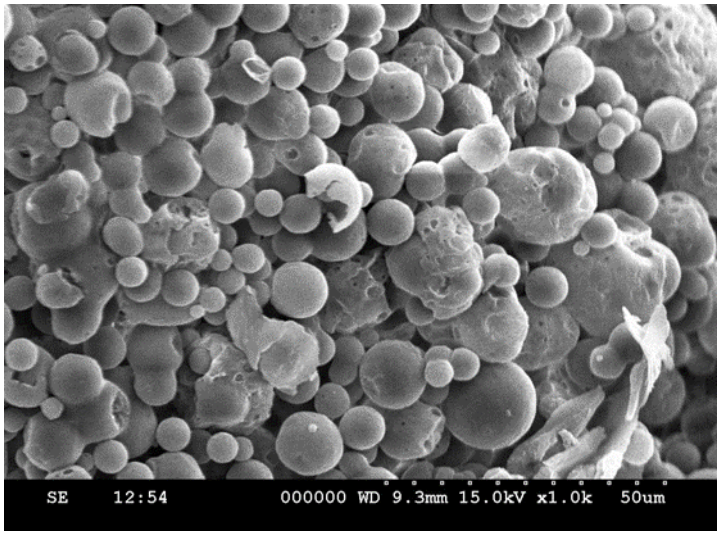

Figure 11: SEM images of ORES microspheres

Micromeritic Properties

The optimized Remdesivir-loaded floating microspheres (ORES) exhibited favorable micromeritic characteristics. The bulk density was recorded as 0.241 ± 0.012 g/cm^3, while the tapped density was 0.281 ± 0.009 g/cm^3. The calculated Carr's index (compressibility index) was 14.231 ± 0.694, and the Hausner's ratio was 1.165 ± 0.014, both indicating good flow behavior. The angle of repose was measured at 21.65 ± 0.733°, suggesting excellent flowability. The observed lower density of the microspheres compared to that of gastric fluids supports their ability to remain buoyant, thereby enhancing gastric retention.

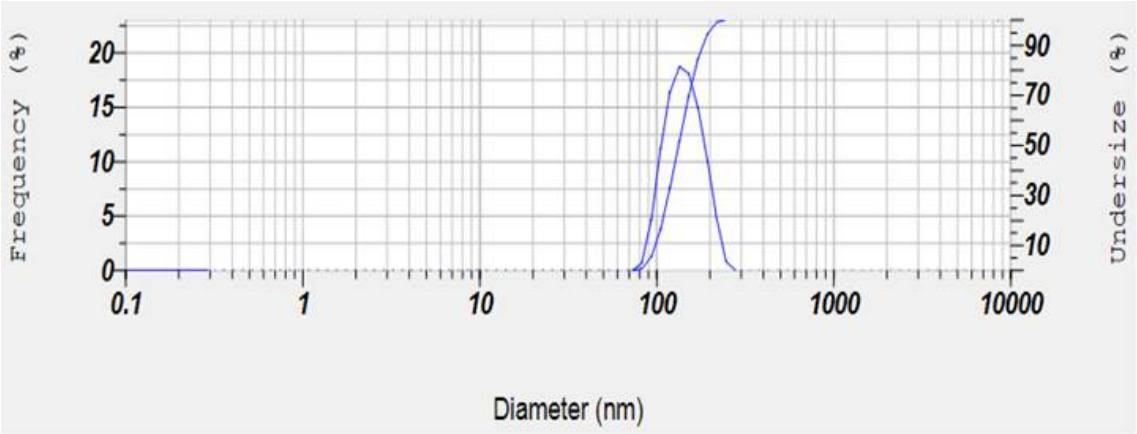

Figure 12: Particle size distribution Zeta potential and PDI of ORES

Release Kinetic Study

The in-vitro drug release behavior of the optimized Remdesivir-loaded microspheres (ORES) was evaluated using various kinetic models including zero-order, first-order, Higuchi, and Korsmeyer-Peppas. The kinetic parameters are summarized in Table 7, with graphical representations in Figure 13. Among all models, the zero-order kinetics exhibited the highest correlation coefficient (R^2 = 0.9896), indicating that the drug release followed a constant rate over time, independent of concentration. In contrast, the first-order model showed a lower R^2 value of 0.6928, suggesting it was less representative of the release pattern. The Higuchi model showed a strong linearity (R^2 = 0.919), indicating that diffusion was a significant mechanism in drug release. The data from the Korsmeyer-Peppas model also supported a diffusion-controlled release profile, confirming that the optimized formulation followed a sustained and controlled drug release behavior.

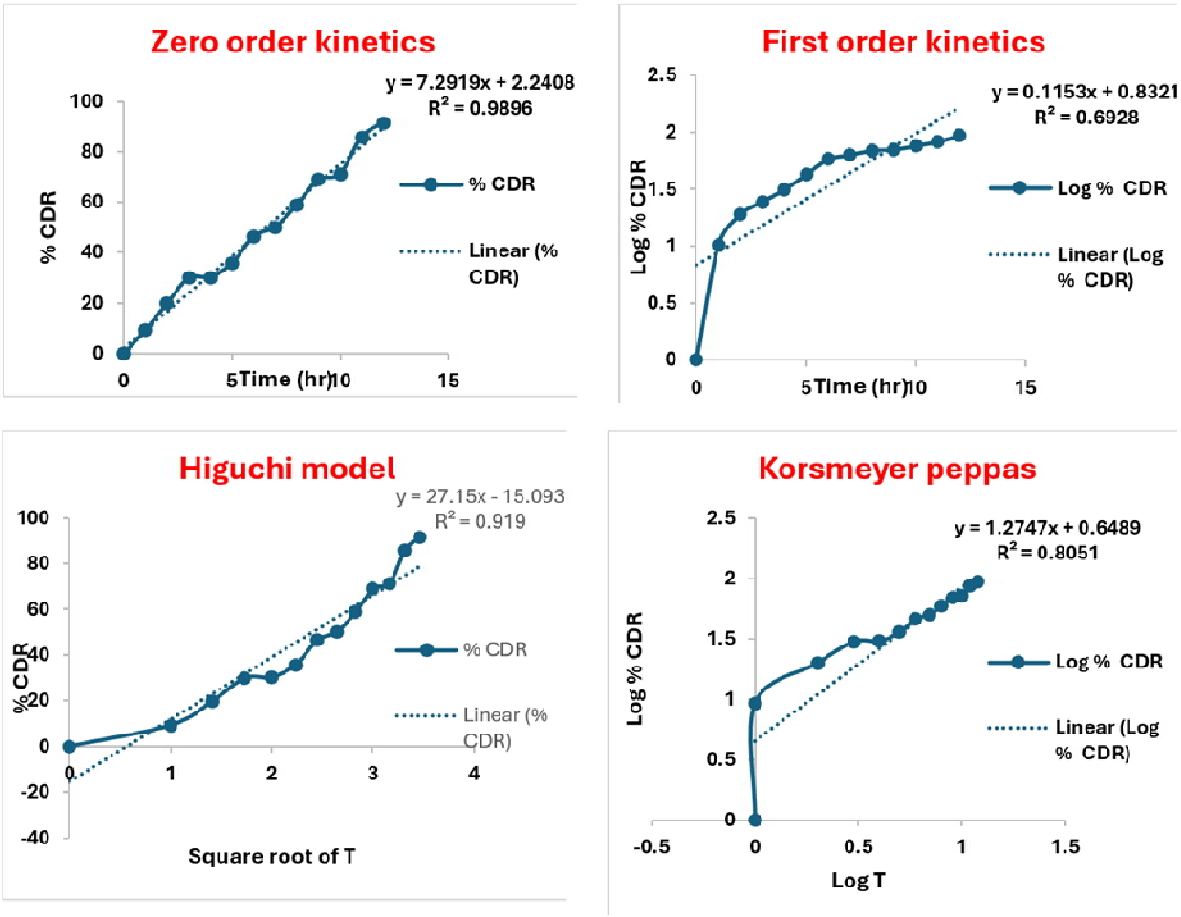

Figure 13*: In vitro* drug release kinetics

***IN VIVO* STUDIES**

The mean plasma concentration-time profiles following oral administration of either the plain drug or the optimized floating microsphere formulation (ORES) are depicted in Figure 13 and detailed in Table 8. A significant enhancement in plasma drug concentration over time was observed with the ORES formulation compared to the plain drug, indicating a sustained drug absorption pattern from the microspheres. Pharmacokinetic parameters derived from the study are outlined in Table 8. The peak plasma concentration (Cmax) achieved by the optimized formulation (RES 100) was 170 ± 8.47 ng/ml, which was notably higher than the Cmax of 136.4 ± 8.40 ng/ml seen with the plain drug. The time to reach peak concentration (Tmax) was prolonged for the microsphere formulation (1 ± 0.24 hr) compared to the plain drug (0.25 ± 0.07 hr), signifying a slower and sustained release from the floating microspheres.

The mean residence time (MRT), which plays a key role in determining absorption duration and overall drug exposure, was substantially higher for ORES (10.60 ± 1.23 hrs) compared to the plain drug (1.142 ± 0.02 hrs). This suggests enhanced gastric retention and sustained availability of the drug from the floating microsphere system. Additionally, the area under the plasma concentration-time curve ($AUC_{0-24}h$), a measure of the extent of drug absorption, was significantly greater for ORES (6167.53 ± 207.9) than for the plain drug (203.175 ± 56.14), confirming improved oral bioavailability with the microsphere formulation.

The half-value duration (HVD), or T50% Cmax, which reflects the time the plasma concentration remains above 50% of its maximum level, was also markedly extended for the ORES formulation (7.2 ± 0.04 hrs) compared to the plain drug (0.9 ± 0.13 hrs). The HVD ratio between the two formulations was 8, which exceeds the standard threshold of 1.25 proposed by Endrenyi et al. (2012) for extended-release systems. Overall, both MRT and HVD values strongly indicate that the ORES formulation offers prolonged drug release, improved absorption, and enhanced bioavailability over conventional Remdesivir administration.

Table 8: Pharmacokinetics parameters of Favipiravir oral administration of Favipiravir loaded floating microspheres (RES 100) and plain drug to rats

Parameter	Pure drug	ORES floating microspheres
$t_{1/2}$ (hrs)	0.7 ± 0.58	7.60 ± 0.64
Tmax (hrs)	0.25± 0.07	1 ± 0.24
Cmax (ng/ml)	170 ± 8.47	136.4 ± 8.40
HVD (hrs)	**0.9 ± 0.13**	**7.2 ± 0.04**
Kel	0.2092± 0.02	0.0911 ± 0.03
AUC $_{0\text{-}t}$ (ng/ml*hrs)	203.175± 56.14	929.435 ± 125.6
AUC $_{0\text{-}\infty}$(ng/ml*hrs)	208.77 ± 178.24	1080.83 ± 124.3
AUMC $_{0\text{-}\infty}$ (ng/ml*hrs^2)	238.53± 112.33	11467.02 ± 221.06
MRT $_{0\text{-}\infty}$(hrs)	1.142 **± 0.02**	10.60 **± 1.23**
Vz/F ((ng)/(ng/ml))	4.428959	8.374196
Cl/F (ng)/(ng/ml)/h)	3.951545	0.7632982
Relative bioavailability	-------	457.4
HVD Ratio		**8**

*All data are represented as mean ± SD. *(n=6)*

To assess the gastric retention capability of the optimized Remdesivir formulation, barium sulfate ($BaSO_4$) was incorporated as a radiopaque marker and administered to rats. X-ray radiographs were captured at various time intervals and are presented in Figure 13. Figure 13(a) depicts the pre-administration condition, showing no radiopaque material. Subsequent images—Figures 13(b), 13(c), and 13(d) taken at 3-, 6-, and 10-hours post-administration, respectively, clearly demonstrate the presence of the formulation within the stomach. The visibility of the radiopaque microspheres beyond 10 hours confirms their prolonged gastric retention and supports the formulation's effective gastro-retentive behavior.

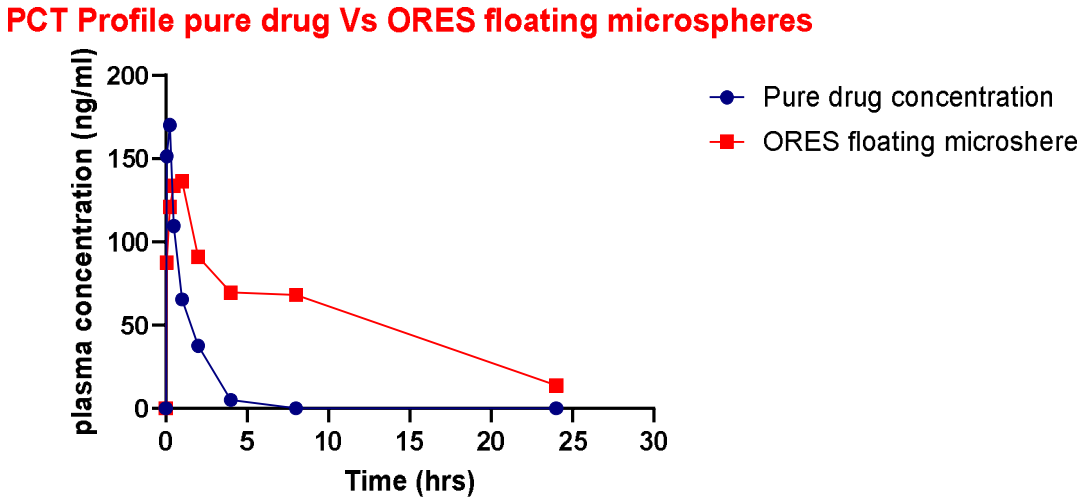

Figure 13*:* PCT profile of Remdesivir oral administration of Remdesivir loaded floating microspheres (RES 100) and plain drug to rats

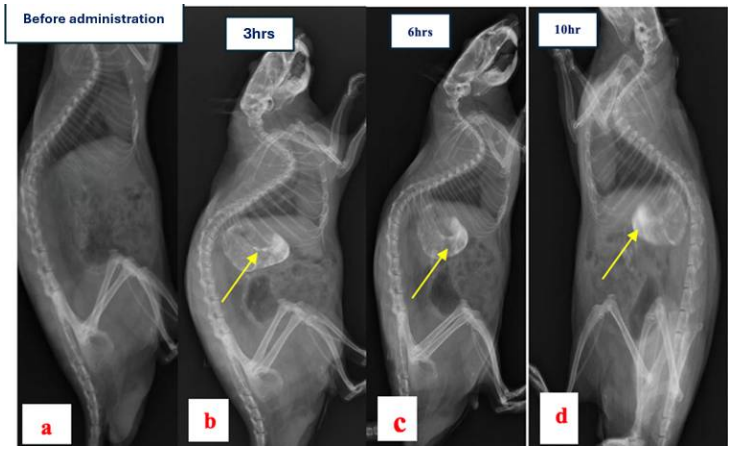

Figure 14. X-ray images of optimized formulation of ORES in the gastric region of albino rats at 1hr, 6 hr, 12hr that indicates floating buoyancy of ORES

CONCLUSION

This study focused on the formulation and evaluation of Remdesivir-loaded floating microspheres as a promising gastro-retentive drug delivery system, developed using a 3^2 factorial design. Microspheres were successfully prepared via the emulsion solvent diffusion method using Eudragit S 100 as the polymer. Among the formulations, the optimized batch (ORES) demonstrated superior performance, with an average particle size of 0.278 µm, high entrapment efficiency (88.01%), sustained drug release (87.32%), and an extended floating duration of up to 23 hours. The microspheres exhibited a polydispersity index of 0.285 and a zeta potential of +41.0 mV, indicating good stability, along with a spherical, rough, and porous surface morphology. Drug release followed both zero-order and Higuchi kinetics, suggesting a diffusion-controlled mechanism. FTIR and DSC analyses confirmed no significant interaction between Remdesivir and the excipients, supporting the chemical compatibility of the formulation components. This is the first reported development of Remdesivir-loaded floating microspheres aimed at achieving sustained drug delivery. The use of ES 100 effectively reduced the required dosing frequency, which could enhance patient compliance in managing viral infections. In vivo radiographic studies using $BaSO_4$ demonstrated prolonged gastric retention, with the microspheres remaining in the stomach for over 10 hours. Pharmacokinetic evaluations further revealed improved bioavailability, and an extended half-life compared to the plain drug formulation, affirming the potential of this delivery system for enhancing Remdesivir's therapeutic efficacy.

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