Quality by Design Enabled Formulation Development, and Optimization of Floating Microballoons of Sildenafil Citrate: In Vitro and In Vivo Characterization

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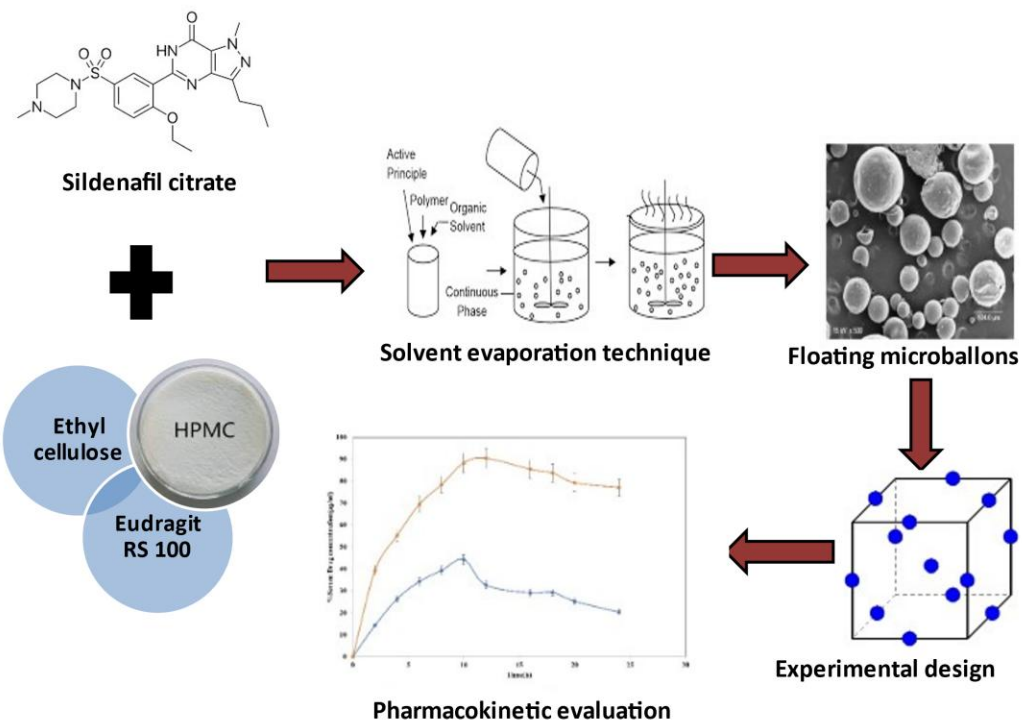

ABSTRACT

The current study envisages an experimental design to develop sildenafil citrate floating microballoons for improved oral bioavailability. The product profile was established based on the intended product quality of floating microballoons. Several significant quality criteria have been defined based on the target product profile. Using the proper concentrations of influencing variables, HPMC K4M (mg) (X1), Carbopol 940P (X2), and Eudragit RS 100 (X3), formulations with the best levels were to be produced. The Box-Behnken designs with 33 are encoded in experimental formulations to calculate the dependent variables to maximize the identified critical components. Based on their particle size (µm), cumulative drug release (%), and % entrapment efficiency, floating microballoons were selected for the study. ANOVA was used to evaluate these traits for the dependent variables. Drug release was favorable and promising for up to 24h in formulation 14, with the highest % drug content and smallest particle size. This formulation contained the optimal amounts of the enteric-coated polymers, HPMC K4M, Carbopol 940P, and Eudragit RS100. Optimized floating microballoons of the drug demonstrated the desired formulation features, including a bioavailability increase of up to 3 times compared to pure drug. Research supports the development of sildenafil citrate microballoons for treating pulmonary arterial hypertension.

KEYWORDS

Bioavailability, In-vitro Buoyancy, Experimental Design, Cumulative Drug Release, Target Product Profile, Critical Material Attributes

1. INTRODUCTION

The release of drugs from oral drug delivery systems (ODDS) is evaluated by how long the outline stays in the digestive tract and how quickly it releases its contents. Formulation scientists have invented many methodologies and injections for this specific purpose [1]. Faster GI transit, for instance, could lead to insufficient drug transport from the drug delivery device to the window of absorption, which would lessen the efficiency of the given dose. Prolonged gastric retention is necessary to create a controlled gastric residency length because it supports the controlled release mechanism in the stomach for more time while still operating normally [2]. Low-density systems are hydrodynamically regulated systems that are impermeable, as opposed to digesting fluids. The fact that these delivery systems are sufficiently resilient to float over belly fills and remain buoyant or resilient in the gastrointestinal cavity for an extended period raises concerns about the gastric emptying rate [3].Furthermore, multiple-unit dosage forms (microballoons) are superior to single-unit dosage forms for more prolonged oral controlled release. Compared to single-unit dosage forms, the advantages of multiple-unit dosage forms include remarkable drug release consistently laterally towards the GI tract, resulting in an increased replicable absorption rate of the drug, fewer dosage removals or dumping, and a lower risk of local irritation [4,5]. The spherical, devoid-of-a-core, rigid, non-effervescent floating micro balloons are frequently formed of free-flowing powders, including synthetic polymers. When taken slowly, they work best at 224.5m [6,7]. Sildenafil citrate is a class I, BCS (high solubility and high permeability) drug with a molecular weight of 474.6 g/mol, a half-life of 3-4 h, and a melting point of 192 °C. It is a cyclic guanosine monophosphate-specific, selective inhibitor of phosphodiesterase type 5 [8]. The significant solubility profile of the model drug reveals that it is soluble in water (3.5 mg/ml), DMSO (23 mg/ml at 25°C), ethanol (1 mg/ml at 25°C), methanol, and DMF (14 mg/ml), and that it has poor oral bioavailability (41%), an did not pass through the absorption site in the intestine and wasn't absorbed in the gastrointestinal system. It doesn't take long for VIAGRA to permeate into the body. Maximum plasma concentrations are recorded between 30 and 120 minutes after oral dosing in the fasted condition (median 60 minutes). The absorption rate is slowed when VIAGRA is taken with a high-fat meal, with a mean delay in Tmax of 60 minutes and a mean drop in Cmax of 29 % [9,10].Additionally, loaded floating microballoons have been developed and optimized using one of the most effective tools: response surface methodology (RSM). Experimental design, regression analysis, constraint optimization, and validation are a few of the phases that make up RSM. The approach also lends itself to studying quadrate polynomial response surfaces and constructing second-order polynomial models. The QbD (Quality by Design) approach was used in the current research to overcome the constraints of conventional optimization techniques. This strategy not only addresses the shortcomings of traditional optimization techniques but also has additional benefits, such as considering how several independent elements interact and how this affects important quality features and critical quality attributes (CQAs). The QbD strategy starts by identifying the QTPPs (Quality target product profiles), which wholly depend on the target product quality. Then, using the design of experiments, this method effectively examined the several components and how they interact to affect the result (DoE). Finally, box-Behnken Design (BBD) was used to reach the critical quality attributes (CQAs), such as mean particle size in m (R1), percent entrapment efficiency (R2), and percent drug released at 12 h (R3) [11]. Thus, as previously noted, the floating micro balloons technique may greatly aid in resolving the problems related to sildenafil citrate. Men with erectile dysfunction (commonly known as sexual impotence) and pulmonary arterial hypertension are treated with floating microballoons with improved drug delivery activity. The fundamental objective of the current research relies on the concept of formulation and optimizing sildenafil citrate floating microballoons for enhanced oral bioavailability incorporating the design of the experiment. The main aims of the work thus detail the manufacture, statistical optimization, in vitro and in vivo characterization of floating microballoons, and formulations of sildenafil citrate for erectile dysfunction with better dissolving rate, solubility, and bioavailability potential [12].

2. METHODOLOGY

2.1. Materials

The active pharmaceutical ingredient (sildenafil citrate) was purchased from Ranbaxy Private Limited (Delhi, India). as a sample gift. Loba Chem Pvt. Ltd., Mumbai, India, supplied Ethylcellulose, Hydroxypropyl methylcellulose (HPMC K4M), Carbopol 940P, Eudragit-(RS 100), and Tween-80. All analytical (AR) grade reagents and solvents were acquired from SD Fine-Chem Ltd. (Mumbai, India).

2.2. Method

2.2.1. Sildenafil citrate maximal concentrations in a phosphate buffer at pH 7.4

To create a stock solution with a 1000 µg/ml concentration, 100 mg of sildenafil citrate (SIL) was precisely measured and fully dissolved in 10 ml of methanol. The volume was then raised to 100 ml using pH 7.4 phosphate buffer (ppm). A pH 7.4 phosphate buffer was then used to dilute the standard working solution from 10 to 100 ml, yielding a concentrated solution with a 100 µg/ml concentration. Using phosphate buffer pH 7.4, adjust 10 ml of the first dilution step to 100 ml to produce a concentrated solution with a 10 µg/ml concentration (10 ppm). The wavelengths of 200-400nm were thoroughly scanned over these solutions. The UV corresponding scan spectrum curve with the maximum absorbance noted for additional dilutions of 10, 20, 30, 40, 50, 60, and 70 µg/ml concentrated solutions was recorded down the relevant wavelength. Max had a maximum wavelength of 278 nm [13].

2.2.2 The quality target product profile and critical quality attributes

In a general context, QTPP refers to the anticipated expected characteristics of drug required to establish the product's planned performance in terms of safety and efficacy and to identify product CQAs. The regulatory and scientific specifications stated in (Table1). were used to determine the QTPP. CQAs are produced by QTPPs, which control how products and processes are developed. In the synthesis of micro balloons, they are additionally related to Critical Material Attributes (CMAs), and Critical Process Parameters (CPPs) pertain to in-process materials and process parameters, respectively [14,15].

Table 1. Identified quality target product profiles (QTPPs) and critical quality attributes (CQAs) for developing microballoons of sildenafil citrate (SIL).

QTTPs	Target	CQAs	Pre-determined target	Justification
Dosage type	Sustained release dosage forms	% Cumulative drug release	≥ 95%	Sustained release of drug is the objective of the study and it is important for better drug absorption.
Dosage form	Better entrapment	% Entrapment efficiency	≥ 80%	Highly critical factor for developing optimized dosage form.
Drug release	C_{max} and AUC higher compared to pure drug	Mean particle size (µm)	10-50µm	Particle size in these ranges is highly critical and important for better absorption of drug.

2.2.3. Preparation of Floating microballoons

Using the solvent evaporation technique, the microballoons were formed. At room temperature, 1000 mg of sildenafil citrate, 100 mg of HPMC K4M, and 100 mg of ethylcellulose were dissolved in a mixture of ethanol and acetone of each of 10mL. The resulting mixture was transferred to 200 ml of pre-mixed distilled water with 0.01% v/v of Tween 80, allowed to stand at room temperature for 30 minutes and then agitated at 2000 rpm using mechanicalstirrer to allow the volatile solvent to evaporate. The tiny microballoons that resulted were filtered, rinsed with distilled water, and dried. The formulations of sildenafil citrate floating microballoons were created using an ethylcellulose-based solvent evaporation technique.

2.2.4. Statistical optimization of formulations

Box-Behnken Design (BBD) and Design-Expert (version 13.0, M/s Stat-Ease, Minneapolis, USA) were used together to enhance the most essential parts in a planned way. HPMC K4M (X1), carbopol 940P (X2), and Eudragit-RS 100) (X3) are the excipients of interest in the formulation. The mean particle size in µm (R1), the percentage of drug released (R2), and the percentage entrapment efficiency (R3) are the independent variables (R3). Table2 depicts the values for independent variables that are both coded and not coded [16,17].

Table 2. Experimental formulations obtained as per BBD along with the selected CQAs responses and their coded values for the prepared microballoons of sildenafil citrate.

Runs	Factor 1 EC: HPMC K4M	Factor 2 EC: Carbopol 940P	Factor 3 EC: Eudragit-RS 100	Response 1 Mean particle size (µm)	Response 2 Cumulative drug release (%)	Response 3 Entrapment efficiency (%)
1	-1	0	0	36.98	79.64	79.65
2	0	0	0	37.29	75.11	75.97
3	1	1	-1	48.5	22.69	67.26
4	0	0	0	39.28	72.64	73.9
5	1	-1	1	30.21	83.61	83.22
6	0	0	0	40.14	70.97	73.01
7	0	-1	0	33.65	80.2	80.25
8	0	0	1	43.33	69.95	70.297
9	-1	-1	-1	29.32	89.28	86.2

10	0	0	0	44.54	69.36	69.208
11	1	0	0	46.32	55.98	68.36
12	0	1	0	46.69	45.02	68.79
13	0	0	0	47.29	37.89	67.93
14	-1	1	1	26.2	98.29	89.2
15	0	0	-1	48.1	24.56	67.55

Independent variables	**Levels**		
	Low (-1)	**Medium (0)**	**High (+1)**
X1: EC: HPMC K4M (mg)	250	625	1000
X2: EC: Carbopol 940P (mg)	200	500	800
X3: EC: Eudragit-RS 100 (mg)	250	625	1000

2.2.5. Forecast of optimization and design space

The Box-Behnken prediction plots confirmed the design space. Based on the needed answer values, the computer offered a batch. The software's proposed algorithm was developed and tested to get the intended outcomes. Every anticipated consequence matched the observed effect or results [16].

3. CHARACTERIZATION

3.1. FT-IR spectroscopy

The FT-IR (Thermo Scientific Nicolet 6700, USA) is used to investigate drug-excipient compatibility. It is carried out to detect any substantial interactions and shifting of the prominent drug peaks in the spectrum of the drug's physical mixture with the specified active ingredients [17].

3.2. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed on microballoons containing pure drug. Mettler Toledo SC 822c was used for DSC measurements. The thermograms were acquired at a scanning rate of 10oC/min over a 20ml/min temperature range [18].

3.3. Particle size distribution and mean particle size

Optical microscopy with an Olympus Micro image LITE-microscope was used to measure the size of the particles in drug-loaded microballoons. Using a calibrated ocular micrometer, the size of the particles in the micro balloons was looked at under a microscope. First, it was worked out what the ocular micrometer's most minor count was. Each time, about 100 particles per formulation were found, and the sizes of those particles were written down [19].

3.4. Morphological examination

Using scanning electron microscopy, the microballoons morphology was investigated. A modest amount of powder was distributed over an aluminum stub placed in the SEM chamber after gold sputtering. Photographs were taken at an electron beam acceleration voltage of 20KV [20].

3.5. % Yield

The FT-IR (Thermo Scientific Nicolet 6700, USA) is used for the inquiry into whether or not the drug and the excipient are compatible with one another. It is carried out to identify any significant interactions and shifts of the primary drug peaks in the spectrum of the drug's physical mixture with the predetermined active ingredients [21].

$$Production\ yield = \frac{Total\ weight\ of\ microballoons}{Total\ weight\ of\ drug\ and\ polymers} \times 100 ------(1)$$

3.6. Calculated drug content

Quantifying the amount of drug: 50 mg of dried micro balloons carrying a drug were dissolved in 0.1N HCl, and then the polymer and drug were extracted for 6 h by stirring with a magnetic stirrer. UV spectrophotometry was utilized to assess the amount of dissolved drug[22].The following equation was utilized to determine how much drug was included in each microballoon:

$$\% \, Drug \, content \; = \frac{Weight \, of \, microballoons \, recovered}{Weight \, of \, drug \, in \, micro \, balloons} \times 100 - - - - - - -(2)$$

3.7.Percentage of entrapment efficiency

The FT-IR (Thermo Scientific Nicolet 6700, USA) is used for the inquiry into whether or not the drug and the excipient are compatible with one another. It is carried out to identify any significant interactions and shifts of the primary drug peaks in the spectrum of the drug's physical mixture with the predetermined active ingredients [23].

$$\% \, Entrapment \, efficiency = \frac{Actual \, drug \, content}{Theoretical \, drug \, content} \times 100 - - - - - - -(3)$$

3.8. In-vitro buoyancy

Microballoon buoyancy was evaluated using the USP dissolving test apparatus II. This tiny balloon is filled with 0.1N HCl (100 mg). The container was then placed in a water bath at 37^0C while being agitated with a paddle turning at 100 revolutions per minute. After floating for 2h, the little balloons were recovered while still in the air and again after they had settled. After exposure to air for some time, the microballoons were weighed [24]. This equation was used to calculate the buoyancy fraction.

$$\% \, Buoyancy = \frac{Wf}{Wf + Ws} \times 100 - - - - - (4)$$

Where, Wf and Ws are the weights of the microballoons when they are floating and when they are at rest.

3.9. In vitro drug release studies

The type-II USP dissolving device was used to study the release of drugs in a lab setting. In a tank with a lid and about 900 ml of dissolution medium, the temperature was kept at 37°C and 0.5°C (0.1N HCl). The paddle was set to move at 50 rpm. Every so often, a sample was taken

(30 min, 1h, 2h, 4h, 6h, 8h, 10h, 12h, and 24h). For each sample, 5 ml of the dissolving medium was taken out and replaced with the same amount of 37°C dissolving medium. The extracted material was combined with a buffer solution with a pH of 7.4 and analyzed with an ultraviolet spectrophotometer at a wavelength of λ_{max}278 nm [25].

3.10. Kinetics of drug release

The findings of the in vitro release studies can be utilized to generate various kinetic equations. The zero-order model (log cumulative percentage of drug left versus time), the first-order model (cumulative log rate of drug left versus time), the Higuchi model (cumulative proportion of drug release versus square root of time), and the Korsmeyer-Peppa model (cumulative percentage of drug release versus square root of time) were all used (Log cumulative percent drug release versus the log of time). The linear arcs of the regression analysis were used to obtain the coefficient of determination [26].

3.11. In-vivo pharmacokinetic investigations

A rapid and efficient validated RP-UFLC method was used to assess the pharmacokinetic profile characteristics of sildenafil citrate in rabbit serum following an oral dose of 4.66 mg of sildenafil citrate microballoons and 9.33 mg of the active component in suspension form. The herd at the animal shelter was selected for twelve male albino rabbits weighing 1.5 kg. They were split into two groups, one receiving micro balloons containing sildenafil citrate as the test and the other receiving the standard (control) (aqueous suspension of Sildenafil citrate). Additionally, they got fresh water twice a day and sterile food. All animals underwent a 15-day washout period before the trial. On the other hand, the assay showed enough specificity and sensitivity to quantify Sildenafil in rabbit serum samples accurately. At the SIMS College of Pharmacy in Andhra Pradesh, India, the Institutional All methodologies were investigated and approved by the

Animal Ethical Committee, with registration number 05/IAEC/SIMS/2019. The retro-orbital venous plexus was punctured at 0 h, 1 h, 3 h, and 6 h after administering the dose. Approximately 0.5 ml of blood was removed from the Eppendorf tube and spun at 3000 rpm for 30 minutes. [27,28]. The plasma was transferred into a second sterile Eppendorf tube and stored at -20°C until analysis. The established RP-UFLC method and non-compartment modeling techniques were used for additional pharmacokinetic data analysis. Numerous relevant characteristics, including the peak plasma drug concentration (C_{max}), the area under the curve (AUC), and the corresponding time (T_{max}), were identified for each sample. The parameters, as mentioned earlier, recorded values were statistically distinguished or compared using an ANOVA and a post hoc t-test with a 5% significance threshold [29].

The dose for rabbits was calculated as follows.

$$Total\ dose\ (in\ humans) X\ 0.07\ (factor\ for\ each\ rabbit) X\ 2kg\ weight\ of\ rabbit/1.5$$

$$= \frac{50mg\ X\ 0.07X2}{1.5} = \ 4.66mg\ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(5)$$

3.12. Accelerated stability analysis

The best formulation was subjected to stability studies following ICH recommendations. The sildenafil citrate microballoons were stored in the stability chamber for three months after being correctly packaged in high-density plastic bottles at 4ºC, 25ºC, 60^0C, and 40ºC, 65±5% RH. The physicochemical characteristics, drug content, drug release percentage, entrapment efficiency, and particle size of SIL floating microballoons were measured at zero, one, two, and three months [30].

4. RESULTS

4.1. Linearity curve for sildenafil citrate in pH 7.4 phosphate buffer

A UV spectrophotometer measured the solution's absorbance at λ_{max}278 nm at pH 7.4phosphate. The graph of absorbance vs. concentration created, as shown in (figure 1) indicates that Beer's law was observed in the 10 to 80 µg/ml (a-b) concentration range.

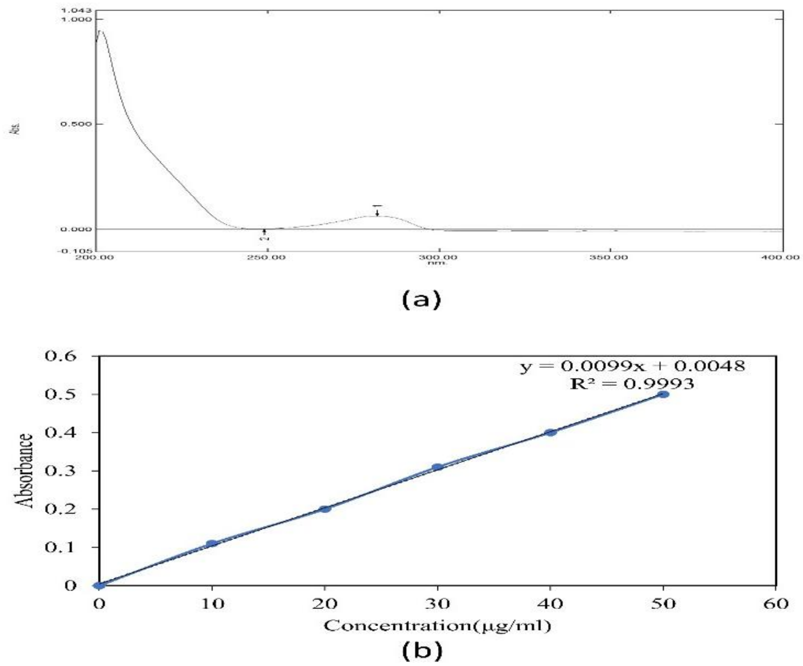

Figure 1. UV spectra of sildenafil citrate (a) and standard calibration curve of sildenafil citrate (b)

4.2. Design of Experiments enabled optimization and analysis

To investigate critical quality attributes (CQAs) and improve process variables, a three-factor, three-level (3^3) experimental design was used. In addition, the RSM methodology used statistical analysis and ANOVA to analyze predicted, experimental, and PRESS (predicted residual error sum of squares) data for optimization. Last but not least, it was discovered that the predicted R^2 and modified R^2 could provide statistical information on whether the model is significant.

4.3. 2D and 3D plots enabled response surface illustration

4.3.1. RSM-based studyof mean particle size

Figure 2 is a contourand response surface assessment of the desired response variables, particlesize (µm) (2D and 3D). (a-b). Mean particle size (Y) was shown to be affectedby the concentrations of HPMC K4M (X1), Carbopol 940P (X2), and Eudragit-RS100 (X3) in the graph (R1). Particle size (µm) increased with HPMC K4Mconcentration in a contour plot analysis (X1). Their variables showed variationin a linear and rising fashion concerning the response variances. The 3Dresponse arch demonstrated that the mean particle size (m) rose as HPMC K4M(X1) and Carbopol 940P concentrations increased (X2). It was believed thatincreasing values of both variables would result in bigger particle sizes (µm)[31].

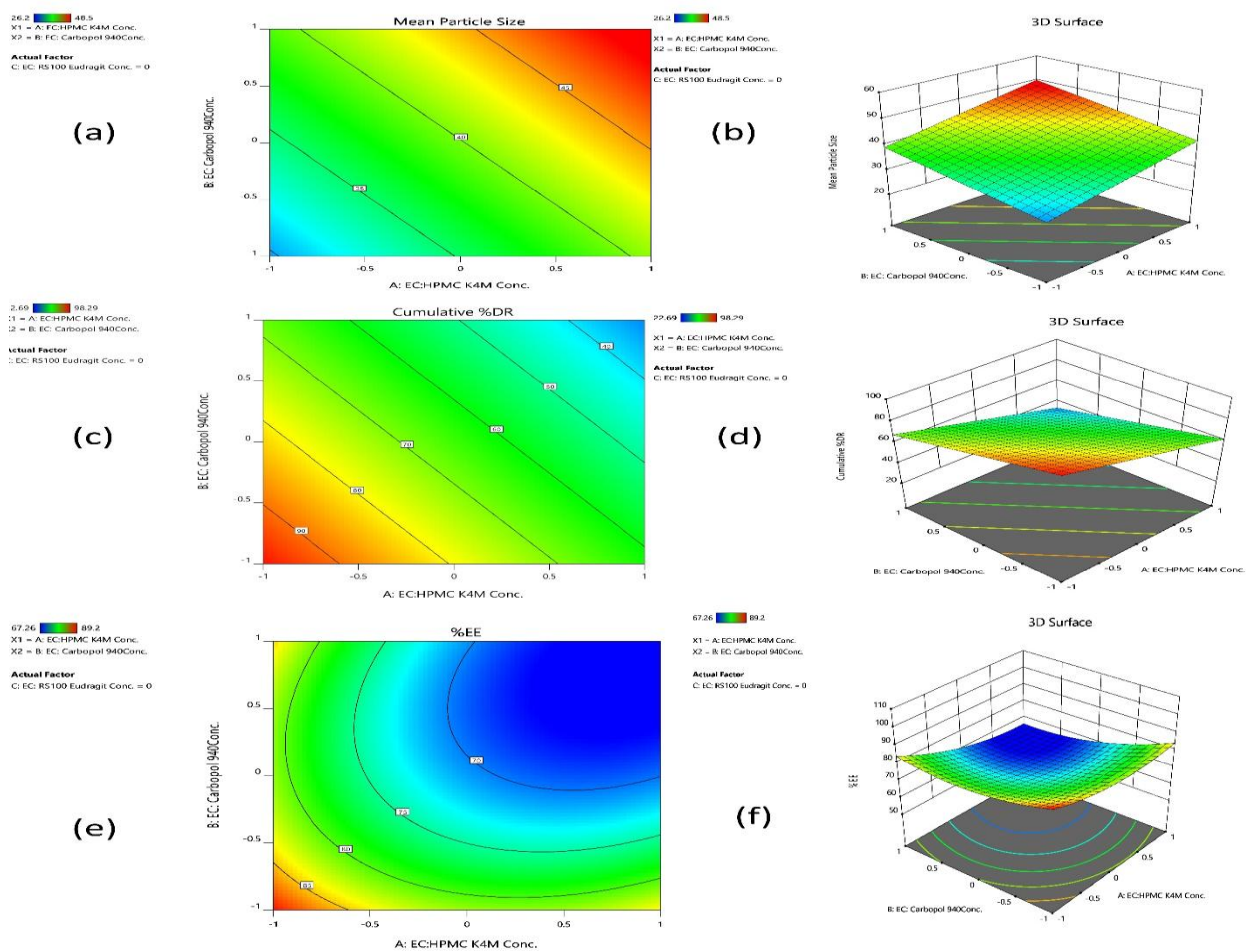

Figure 2. 2D and 3D surface optimization graphs for particle size (µ)m [R1], % drug release [R2] and % entrapment efficiency [R3] (a–f)

4.3.2. RSM-based studyof cumulative percentage in vitro drug release

The percentage of entrapmentefficiency was the response variable studied, depicted in a contour plot (2Dand 3D) in figure 2. (c-d). The graphs show that the response variable, thetotal percentage of drug released after 12 h, was significantly affectedby the two independent factors, HPMC K4M concentration (X1) and Eudragit-(RS100) (X3). The contour plots summary shows that at 12 h, the cumulative drugrelease rate decreases as HPMC K4M (X1) concentration rises, while theproportion of drug released increases. A linear increase or decrease in thevariables was predicted due to the observed reaction. From what can be seen inthe three-dimensional graph [32]; the cumulative drug releasedecreases as HPMC K4M (X1) concentration rises.

4.3.3. RSM-based studyof % entrapment efficiency

(Figure 2) examines theinvestigated response variable, percent entrapment efficiency, using responsesurface plots in two and three dimensions (e-f). The graphs showed how themeasured response variable, or percentage entrapment efficiency, was changed bythe concentrations of HPMC K4M (X1), Carbopol 940P (X2), and Eudragit-(RS 100)(X3) (R3).The contour plot demonstrated that the % entrapment efficiencyincreased as Eudragit-RS 100 concentration grew (X3). The 3D graph showed thatthe percentage of entrapment efficiency increased when carbopol 940P andEudragit-RS 100 concentrations did. It was discovered that the drug entrapmentefficiency increased in tandem with increases in the values of both inputvariables [33]. Figure 3 displays the interactiongraphs for the perturbation of the answers and the expected v/s actual values(a-f). Based on the outcomes of the key variables, Eudragit-RS 100 is chosen asthe rate-controlling polymer with the inclination for oral sustained-releasedrug delivery since it has the most reliable formulations and is the easiest tomanufacture the polymers under consideration.

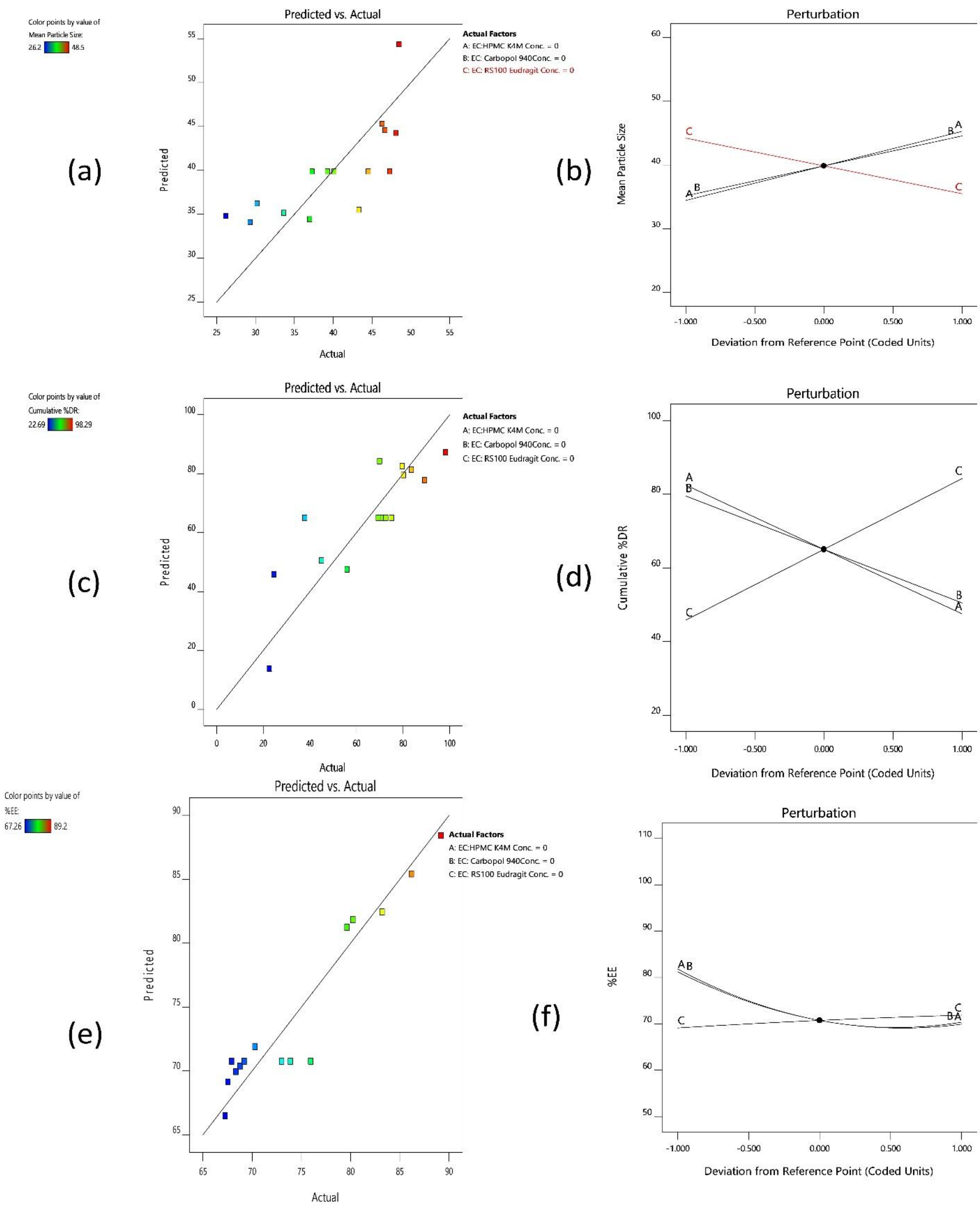

Figure 3. Predicted versus actual values for the observed responses of R1, R2 and R3 (a),

(c), and (e) and perturbation plots for the observed responses of R1, R2 and R3 (b), (d), and (f).

4.4. FT-IR aided theinvestigation

According to thefindings of the FT-IR research, the drug did not have any significantinteractions with the polymer utilized in the production of the floatingmicroballoons composition. Furthermore, the FT-IR analysis showed noalterations in the major peaks of the drug, which is evidence that the sample used was of very high purity and was also stable. Figure 4, displays the Fourier transform infrared spectroscopy examination results of the optimized Formulation. The OH stretching of a pure sildenafil citrate pill reaches its maximum at 3616 cm^{-1}, whereas the NH stretching reaches its maximum at 3299 cm^{-1}, and the CH (aromatic) stretching reaches its maximum at 3028 cm^{-1}. The IR investigation of Formulation, F14 likewise generates comparable results, with peaks at 3452 cm^{-1}, for the OH stretching, 2924 cm^{-1}, for the CH stretching, and 1727 cm^{-1}, for the C=O stretching [34,35].

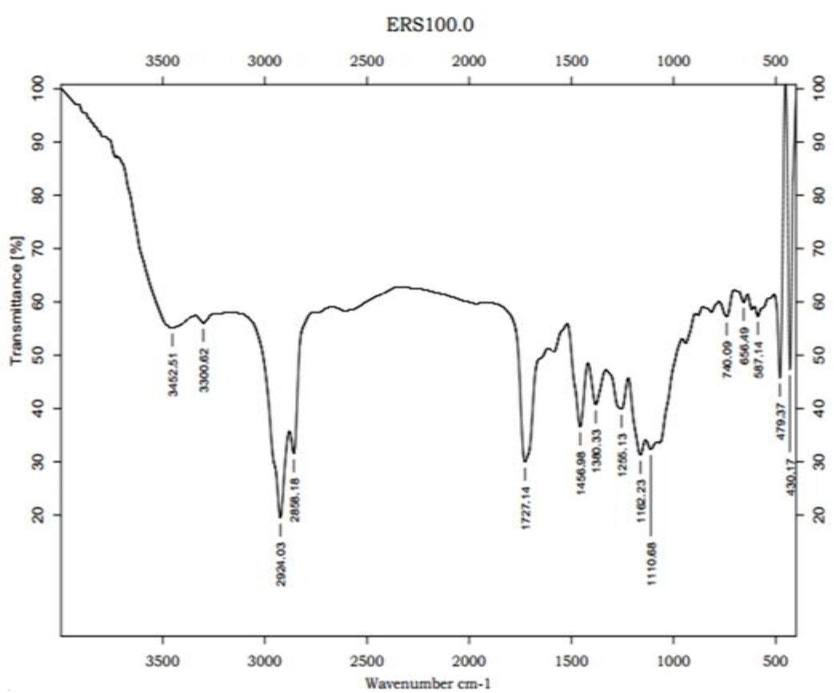

Figure 4. FT-IR spectrum of optimized formulation of floating microballons

4.5. DSC analysis

Optimized formulationdifferential scanning calorimetry findings indicated sildenafil citrate peak temperature to be 196.34^{0}C. Figure 5shows that the improvedformulation F14 peaked at 195.76^{0}C when differential scanningcalorimetry was conducted.

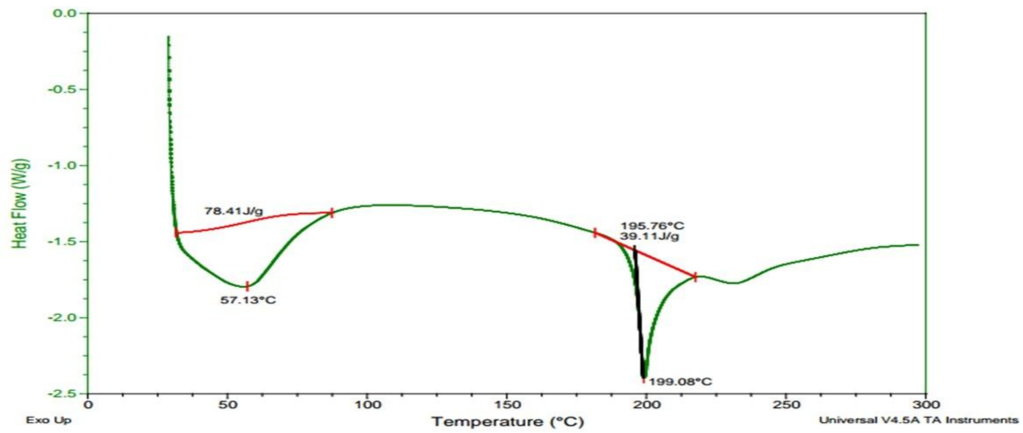

Figure 5. DSC thermogram of optimized formulation of floating microballons

4.6. Mean particle size

After manufacturing, it was found that the floating microballoonformulations F1-F15 had mean particle sizes ranging from 26.20 to 48.5 µm (Table3). When the HPMC K4M polymer's concentration was raised, the average particle size also increased. Thishappens due to the solution's increased viscosity and the diminishedeffectiveness of the stirring. As a result, the time needed for the microballoons to become rigid was shortened as the polymer content graduallyincreased. This saves time that droplets would otherwise wasteseparating from one another, allowing more enormous micro balloons to form[36].

Table 3. Calculated data for the different formulations (F1 to F15) related to particle size (µm), yield (%), drug entrapment efficiency (%) and *in vitro* buoyancy (%).

Formulation code	Particle size (µm) Mean ± SD	Yield (%) Mean ± SD	Drug content (%) Mean ± SD	Drug entrapment efficiency (%) Mean ± SD	In vitro buoyancy (%) Mean ± SD
F1	36.98±0.02	44±0.029	45.79±0.36	79.65±0.02	55±0.050
F2	37.29±0.06	41.6±0.035	62.14±001	75.97±0.04	62±0.023
F3	48.5±0.22	48±0.055	48.01±0.02	67.26±0.15	71±0.032
F4	39.28±0.15	88±0.036	66.9±0.26	73.9±0.002	66±0.0142
F5	30.21±0.22	86.6±0.014	75.11±0.69	83.22±0.05	68±0.069
F6	40.14±0.36	88.6±0.002	72.54±0.45	73.01±0.014	70±0.023
F7	33.65±0.05	46.1±0.06	63.25±0.015	80.25±0.22	64±0.055
F8	43.33±0.15	39.7±0.032	49.89±0.021	70.297±0.02	63±0.078
F9	29.32±0.023	45.6±0.012	53.77±0.009	86.2±0.06	70±0.056
F10	44.54±0.06	26.2±0.024	68.22±0.036	69.208±0.35	69±0.026
F11	46.32±0.03	29.3±0.032	40.25±0.008	68.36±0.08	73±0.029
F12	46.69±0.22	42.6±0.028	59.14±0.045	68.79±0.04	59±0.033
F13	47.29±0.04	49.2±0.034	65.98±0.025	67.93±0.01	69±0.0214
F14	26.2±0.012	90.39±0.045	79.25±0.022	89.2±0.02	78±0.098
F15	48.9±0.15	49.5±0.027	65.22±0.456	67.55±0.05	69±0.020

4.7. % Yield, contentuniformity, and entrapment efficiency

The percentage yield,drug content and drug entrapment effectiveness of microballoons varied from26.2to 90.39%, 40.25 to 79.25%, and 67.26 to 89.2%, correspondingly(Table 3). This suggests that the formulationF14 containing sildenafil citrate has lower HPMC K4M concentrations and higherdrug content and integration effectiveness [37].

4.8. Floating ability

For the GRT of the drugto be increased, floating microballoons have to be created first. Therefore, aninvestigation into the microballoons' buoyancy was carried out so that it wouldbe possible tocalculate the amount of time that the micro balloons that hadbeen created would remain airborne as a group. Microballoons were equallydispersed across the top of 0.1N HCl, and the rate at which they settled wasmeasured. Figure 6, reveals that formulation F14 has a remarkable floating capacity. However, circumstances in vivo can be verydifferent, and the amount of time a chemical spends in the stomach may varydramatically depending on the phase of gastric motility [38].This is because in vivo conditions might be more complex.

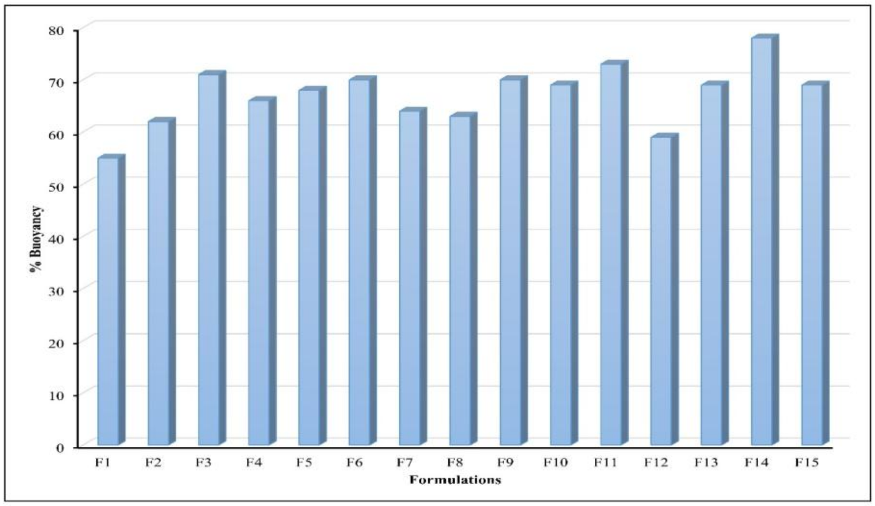

Figure 6. Bar diagram indicating in vitro buoyancy (%) for the floating microballoons of formulations F1-F15

4.9. Microballoonsmicrometric properties estimation

According to reports, tappeddensity ranged from 0.326 to 0.654 grams per cubic centimeter, while bulkdensity ranged from 0.289 to 0.492 grams per cubic centimeter. The flowparameters of the created micro balloons, which demonstrate good behavior, areshown in Table 4. The angle of repose goes from 16.36°to 29.32°, and Carr's index is between 6.3 to 15.02.

Table 4. Flow characteristic parameters for the prepared formulations of F1 to F15.

Formulation Code	Angle of repose (ɵ) Mean ± SD	Bulk density (g/cm^3) (%) Mean ± SD	Tapped density (g/cm3) (%) Mean ± SD	Carr's index (%) Mean ± SD	Porosity (%) Mean ± SD	Hausner's ratio
F1	25.23°±0.01	0.338±0.03	0.326±0.02	12.72±0.20	19±0.26	0.645
F2	29.32°±0.02	0.466±0.02	0.425±0.031	13.39±0.12	14.3±0.320	0.794
F3	23.12°±0.03	0.381±0.01	0.568±0.002	11.44±0.18	57±0.115	1.133
F4	22.02°±0.001	0.466±0.021	0.654±0.03	11.20±0.15	20±0.005	0.706
F5	25.66°±0.005	0.438±0.023	0.495±0.014	12.60±0.11	48±0.206	0.604

F6	27.45°±0.031	0.352±0.028	0.472±0.024	13.06±0.15	34±0.114	0.120
F7	27.36°±0.014	0.361±0.029	0.528±0.014	12.54±0.02	23±0.025	0.165
F8	25.34°±0.012	0.397±0.032	0.543±0.021	14.25±0.14	25±0.008	0.354
F9	22.13°±0.022	0.456±0.012	0.539±0.034	15.02±0.04	45±0.032	0.929
F10	16.36°±0.031	0.362±0.024	0.503±0.026	9.78±0.05	33±0.004	0.726
F11	19.78°±0.003	0.293±0.032	0.512±0.031	10.66±0.04	29±0.098	0.445
F12	23.93°±0.013	0.426±0.028	0.468±0.022	13.66±0.14	36±0.004	0.954
F13	21.89°±0.004	0.492±0.034	0.493±0.011	14.36±0.13	32±0.008	0.629
F14	26.55°±0.015	0.289±0.045	0.452±0.041	6.30±0.008	40±0.002	0.726
F15	23.02°±0.019	0.295±0.027	0.492±0.022	12.58±0.07	52±0.204	0.145

4.10. Morphologyof surface characteristics

The optimized floatingmicro balloons' scanning electron micrograph (SEM) shows different shapes andparticles at different magnification levels. These shapes and particles showthat when the floating micro balloons go through this change, they go fromcrystalline to amorphous [39].

4.11. Release kineticmechanism and in-vitro drug releaseanalysis

Equipment for performingUSP dissolution tests to test the dissolving ability of sildenafil citrate in-vitro wereused to insert micro balloons holding the drug in 0.1 N HCl for24 h. Tabular data for drug release kinetics are shown in (Table 5) for formulations F1 through F15. The data suggests a disintegration value between-22.69% and +98.29%. Regression coefficient ('R2') values for analysingmicroballoon release data using the equations of different kinetic models as shown inFigures7 and 8. From the simplest (zero) to the most complex(F15)(a-d). Based on the collected information, it can be concluded that thenew and improved formulation of F14 follows the kinetics established by Higuchi[40,41] The value of its regressioncoefficient was found to be R2, is 0.9595.

Formulation code	Zero order	First order	Higuchi's model	KoresmeyerPeppas model

Table 5. In vitro drug release kinetics data of formulations F1-F15.

	Slope	**R^2**	**K_0**	**Slope**	**R^2**	**K_1**	**Slope**	**R^2**	**K_h**	**Slope (n)**
F1	7.139	0.9403	16.42	0.0598	0.919	0.137	27.09	0.984	62.39	1.0254
F2	10.279	0.937	23.67	0.0763	0.952	0.175	33.3	0.916	76.78	1.658
F3	7.424	0.9634	17.09	0.0695	0.970	0.1600	27.7	0.9816	64.00	0.9587
F4	7.582	0.9641	17.46	0.0589	0.957	0.1356	28.5	0.9932	57.98	1.0254
F5	6.472	0.8728	14.90	0.0585	0.925	0.1347	25.17	0.9965	57.96	1.0413
F6	9.087	0.9823	20.927	0.0586	0.953	0.134	33.08	0.9663	76.18	1.3419
F7	5.026	0.8596	18.02	0.0568	0.965	0.1395	22.38	0.9658	63.69	0.9632
F8	7.005	0.9878	25.14	0.0698	0.986	0.1455	26.55	0.9635	64.12	1.0363
F9	6.965	0.9683	22.96	0.0759	0.961	0.1376	28.69	0.9962	63.88	0.9458
F10	8.015	0.9365	21.02	0.0936	0.915	0.1458	36.58	0.9632	79.21	1.0453
F11	10.024	0.8547	20.36	0.0796	0.978	0.1792	35.66	0.9147	70.65	1.0265
F12	7.014	0.9874	18.09	0.0597	0.939	0.1723	28.97	0.9326	69.12	0.9965
F13	6.339	0.8966	16.24	0.0647	0.925	0.1693	33.69	0.9932	59.21	0.9869
F14	6.986	0.9974	23.36	0.0596	0.969	0.1589	32.01	0.9595	72.63	1.0154
F15	8.015	0.8695	16.14	0.0796	0.947	0.1693	29.36	0.9367	59.69	0.9631

R^2: Regression coefficient; K_0: Zero order rate constant; K_1: First order rate constant; K_h: Higuchi's order rate constant

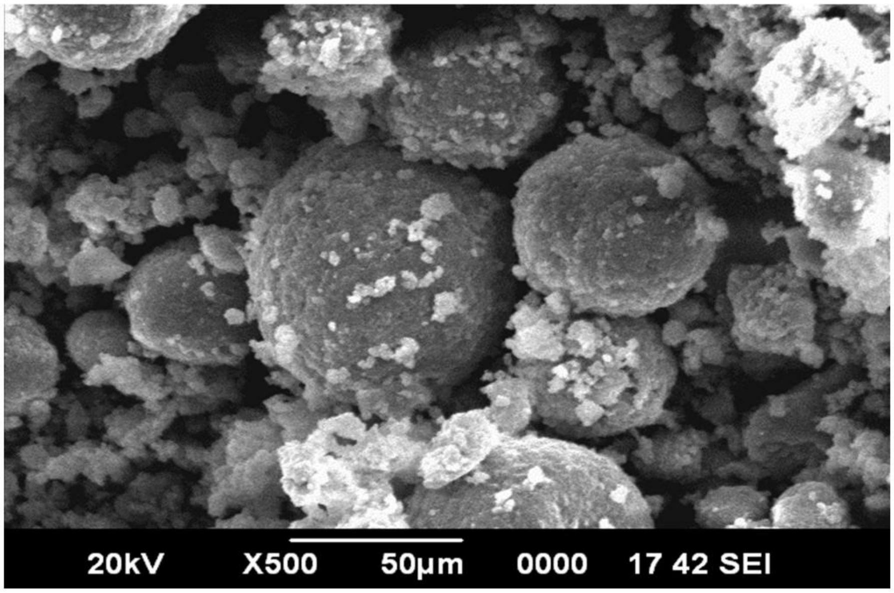

Figure 7. SEM image of the optimized batch of floating microballoons using high magnification (500X)

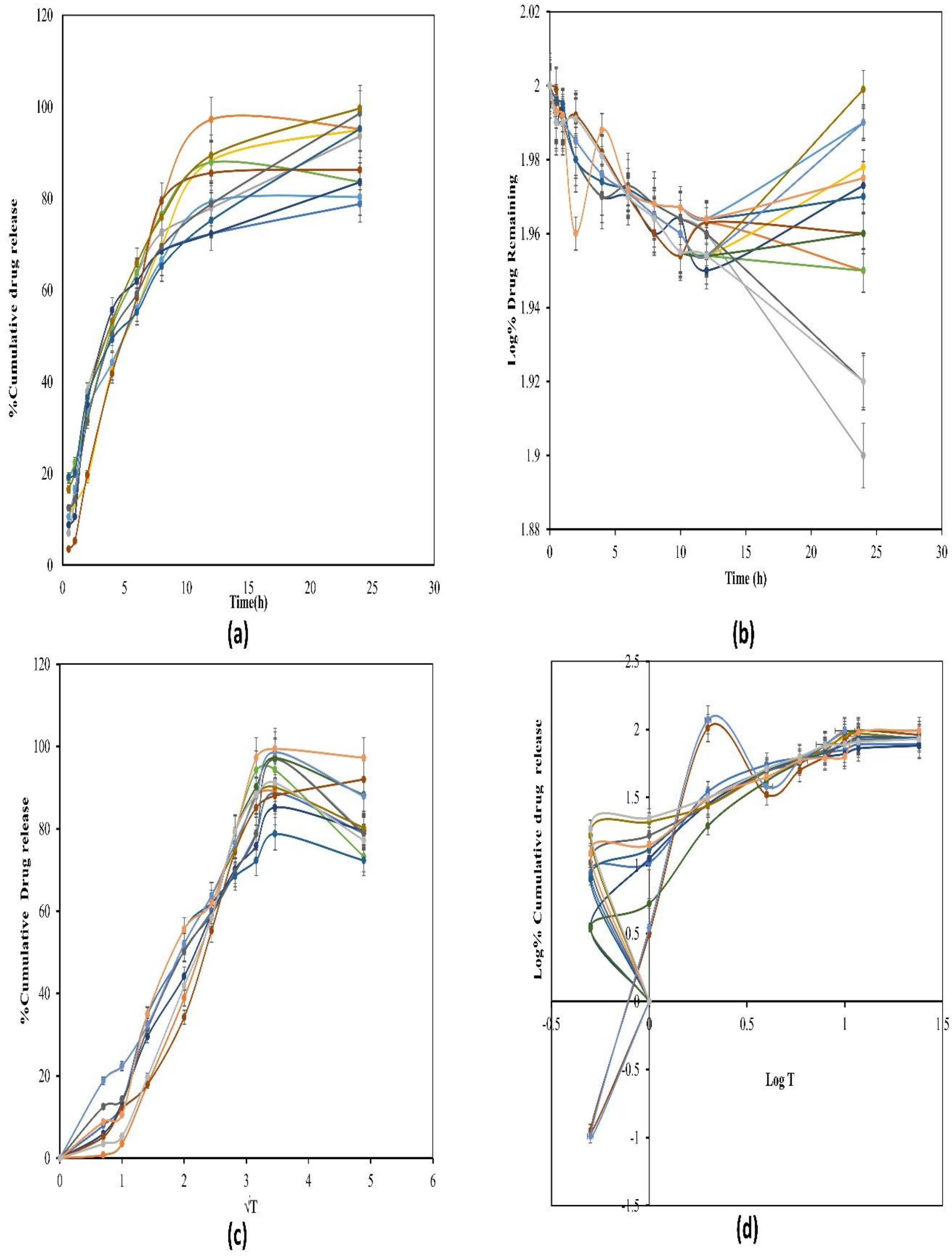

Figure 8. In vitro drug release profile (Zero, first, Higuchi's and Korsemeyer Peppa's plot) of formulations F1 to F15 (a-d)

4.12. Analyses overlay plotsto establish the design space

Table 6 describes theoptimization strategy and the predicted and experimental values of the formulation's responses based on the point prediction. Using the Box-Behnkendesign, optimized micro-balloons of sildenafil citrate were manufactured.

Table 6. Constraints of point prediction for the process of optimization of microballoons of sildenafil citrate using DoE.

Run 14 Response	Predicted Mean	Predicted Median	Observed	Std Dev	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
Mean particle size (µm)	34.7727	34.7727	26.2	5.57209	4.19452	25.5406	44.0047	4.98389	64.5614
Cumulative drug release (%)	87.206	87.206	98.29	13.6668	10.288	64.5622	109.85	14.1422	160.27
Entrapment efficiency (%)	88.4129	88.4129	89.2	3.74245	3.6963	78.9112	97.9145	60.8244	116.001

4.13. In-vivo pharmacokinetic studies

Figure 9depicts thelink between the mean plasma concentration of sildenafil citrate and the timeplot of the optimized microballoons of sildenafil citrate due to thepharmacokinetic experiment. This relationship was determined to exist as aconsequence of the investigation. The outcomes of the simulations performed forvarious pharmacokinetic parameters are detailed in Table 7, which may be foundhere. It was observed that the T_{max} for the newly developed formulation was 12h, the same amount of time as the SIL for the drug in its purest form.This leads one to believe that the drug was administered frequently. Themaximum concentration of active ingredient (C_{max}) in improved microballoonscontaining sildenafil citrate was 90.3 µg/ml. This is a substantial increasefrom the C_{max} of the pure drug, which is 32.66 µg/ml. It was found thatthe AUC of the improved formulation was 2905.69 µg/h/ml, which is significantly higher than the AUC of the unadulterated drug, which was 958.25 (micrograms per hour) per milliliter. Thisdifference is more than three times greater than the difference between the twovalues. Because of the revised formulation's increased efficiency, thepharmacokinetic profile was significantly improved. In addition to this, theAUC of the optimized batch was considerably higher when compared to the drug in its most unadulterated form (Area under the curve).On the other hand, therewas no detectable change in the time at peak plasma concentration (T_{max}), andit did not change at any point during the investigation. A statisticallysignificant improvement (p 0.001) was observed in the patients as a direct consequenceof the drug's high systemic bioavailability. Clear evidence of the outcome waspresented to us in the form of the values of AUC and Cmax for the enhancedfloating microballoonsformulation compared to pure drug[42,43]. These values show that the improved formulation is moreeffective than the pure drug.

Table 7. *In vivo* pharmacokinetic parameters values of pure drug and optimized formulation batch

Formulations			Pharmacokinetic parameters		
	Cmax (µg/ml) Mean ± SD	**T_{max} (h)**	**Ke Mean ± SD**	**MRT (h) Mean ± SD**	**AUC0-∞ (µg/h)/mL Mean ± SD**
Pure drug aqueous suspension of SIL	32.66±0.003	8	0.22±0.06	4.06 ± 0.058	958.25±0.004
Optimized Microballoon formulation (F14)	90.365±0.006	12	0.78±0.011	7.069 ± 0.0253	2905.69±0.020

Ke: Elimination rate constant; MRT: Mean residence time; AUC: Area under the curve

4.14. Accelerated stability analysis

Table8, illustrates theANOVA design p-values for the accelerated stability investigation.All CQAs have p-values greater than 0.05, indicating nostatistically significant change. As a result, it was concluded that theoptimized microballoons of sildenafil citrate passed the stability criteriabecause their CQAs did not vary significantly over time, indicating the formulation'sstability under accelerated conditions [44].

Table 8. Short term stability studies data for the F14 formulation of microballoons of sildenafil citrate.

Stability studies as per ICH	4±1°C			25±2°C (60±5% RH)			40±2°C (65 ±5% RH)		
No. of months of studies									
Parameters	**1M**	**2M**	**3M**	**1M**	**2M**	**3M**	**1M**	**2M**	**3M**
Drug content (%)	79.32	69.29	62.98	75.74	60.85	65.232	70.25	69.20	63.65
Entrapment efficiency (%)	80.22	79.69	75.22	79.48	73.22	70.47	71.25	69.27	62.54
Drug release (%)	95.58	79.21	71.25	90.25	79.58	76.24	79.22	68.23	65.25
Particle size (µm)	26.2	40.27	43.12	32.25	42.12	42.25	40.53	46.22	49.35
p-value ≤0.05 indicates significant difference	**0.115**	**0.065**	**0.062**	**0.089**	**0.135**	**0.061**	**0.075**	**0.069**	**0.125**

M: Month; RH: Relative humidity

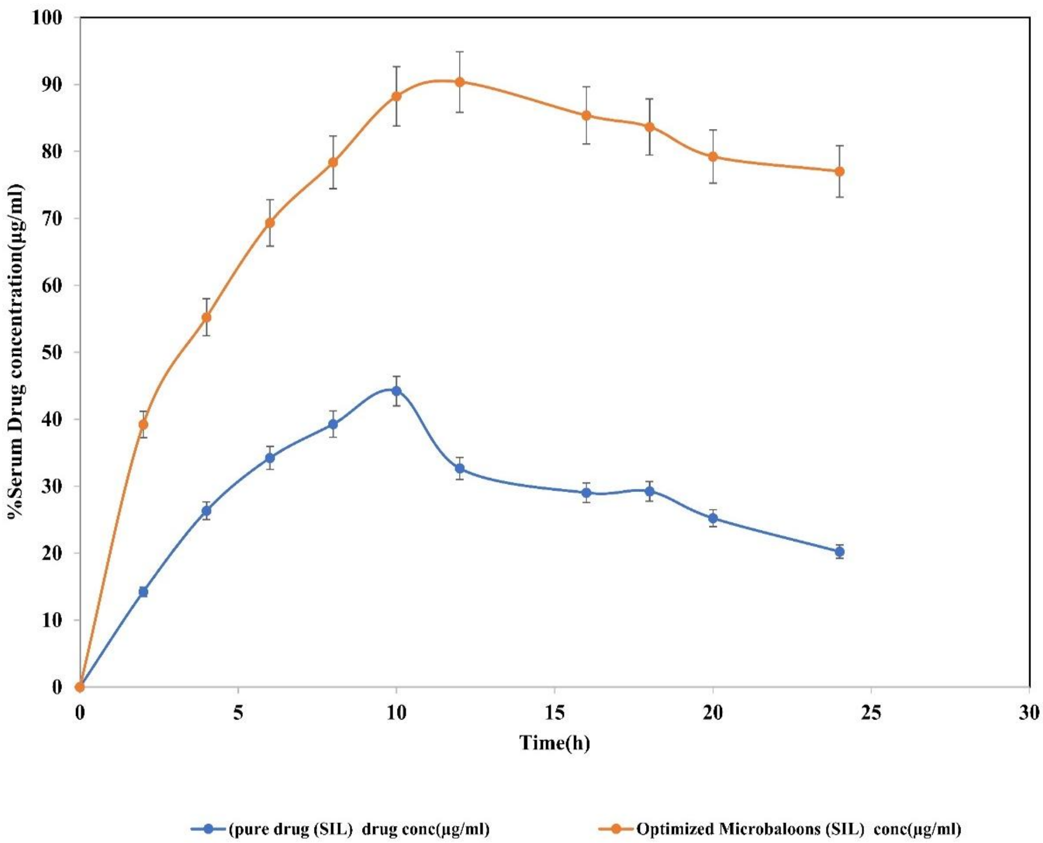

Figure 9. *In vivo* bioavailability curve of pure drug vs optimized formulation

5. DISCUSSION

When optimizing and confirming a pharmaceutical process's stability, the Box-Behnken design is a reliable instrument. The BBD is a relevant design for response surface analysis. It primarily comprises a cube, center, and axial points. Because of these differences, the examinations were divided up into several blocks. An exothermic peak was observed at 199.08 º Celsius on the optimized floating microballoons SIL DSC thermogram, with a temperature of 195.76º Celsius as the beginning or end temperature. It was determined that the latent heat of fusion, denoted by the symbol H, is 39.11 millijoules. Therefore, it has been demonstrated that SIL does not refer to a tangible idea. The addition of optimized microballoons to material combinations of HPMC K4M, Eudragit RS100, or Carbopol 940P did not significantly impact the exothermic peak of SIL. Because the drug and excipients used in the study are compatible, it is possible to generalize these results. Microballoons suspended in water and containing a mixture of HPMC K4M at a lower concentration. Eudragit RS100 and Carbopol 940P at a greater concentration had an appropriate release profile after 24 h. Equations in zero order, first order, Higuchi order, and Korsmeyer order Peppa's was applied to the results of in vitro dissolution for each of the 15 different formulations. Because the regression coefficient (R^2) value is better for Higuchi-order kinetics, each of the 15 different microballoon compositions followed this kinetics. The microballoon particles' size and the cumulative drug release tended to increase with increasing concentrations of HPMC K4M, suggesting a non-Fickian diffusion-controlled release mechanism for SIL in all formulations. Out of the 15 different formulations examined, F14 had the release kinetics that was the quickest and most predictable. When the pure drug of SIL was suspended in water, an elimination half-life of 8 h was observed; however, an elimination half-life of 12 h was recorded when the drug was encapsulated in the optimized floating micro balloons. The area under the concentration curve (AUC) for the optimized floating microballoons was 3.75 times higher when compared to an aqueous suspension of pure sildenafil citrate. The enhanced formulation exhibited a higher MRT value because the absorption process was drawn out for extended periods before being eliminated from the system. The pharmacokinetic profile of the selected micro balloon had significantly improved characteristics compared to the aqueous suspension of the SIL pure drug, indicating that it may have both therapeutic and commercial applications. Last but not least, the bioavailability of the sildenafil citrate was considerably improved when it was encapsulated in floating micro balloons with lower levels of HPMC K4M and more significant quantities of Eudragit RS100 and carbopol 940P.

6. CONCLUSIONS

The study's results indicate that a moderate dosage of HPMC K4M, Eudragit RS 100, and Carbopol 940P was advantageous for producing sildenafil citrate microballoons with enhanced bioavailability. Adopting the Box-Behnken design allowed for developing a potent formulation with a targeted profile of delayed drug release, hence increasing release rates and, more importantly, entrapment efficiency. According to the FT-IR and DSC investigations, there does not appear to be any physiochemical interference between the drug and the polymers. Microballoons were stable and spherical, as determined by SEM examination. The pharmacokinetic studies revealed that the enhanced bioavailability function led to a threefold increase in the C_{max} and AUC (Area under Curve). According to accelerated stability testing, the optimizedformulation was stable for three months, as advised by the ICH, and could be used to treat patients with pulmonary hypertension in a different dose form.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The data that support the findings of this study are included in the article.

Competing interests

Authors declare no competing interests

Funding

None

Authors' contributions

K Sasikanth performed the experiments, analyzed and interpreted the data and drafted the manuscript. S Swain designed the study and critically revised the manuscript for additional valuable content. MEB Rao, D Ghoshe and B Jena all other authors are applied the Design of experiment, drawn the experimental data and also validated the final data associated to the manuscript. All authors read and approved the final manuscript.

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LIST OF ABBREVIATIONS

AUC: Area under the curve

BBD: Box–Behnken design

C_{max}: Maximum concentration

h: Hour

QbD: Quality by design

SIL: Sildenafil citrate

T_{max}: Time to drug peak concentration

UFLC: Ultra-Fast liquid chromatography

UV: Ultra-violet spectroscopy

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