

QUALITY BY DESIGN-GUIDED DEVELOPMENT AND IN VITRO EVALUATION OF PACLITAXEL- AND NERATINIB-LOADED CUBOSOMES

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ABSTRACT

Paclitaxel and neratinib present formulation challenges associated with poor aqueous solubility and efficient drug delivery. The present study aimed to develop and independently optimize separate paclitaxel- and neratinib-loaded cubosomal dispersions using a Quality by Design approach and to characterize their physicochemical properties and in vitro release performance. Cubosomes were prepared by top-down emulsification using glyceryl monooleate and Poloxamer 407. Separate three-factor, three-level Box–Behnken designs were employed for paclitaxel and neratinib, generating 17 experimental runs for each drug. The formulations were evaluated for particle size, drug entrapment efficiency, cumulative drug release, zeta potential, morphology, and release kinetics. Particle size ranged from 149–221 nm for paclitaxel-loaded cubosomes and 129–207 nm for neratinib-loaded cubosomes, while entrapment efficiency ranged from 77.12–96.58% and 76.58–95.13%, respectively. In the independently optimized designs, **paclitaxel Run 11** exhibited a particle size of 165 nm and an 8-h cumulative drug release of 93.37%, whereas **neratinib Run 11** exhibited a particle size of 141 nm and an 8-h cumulative drug release of 92.58%. Thus, Run 11 was selected independently as the optimized formulation within each drug-specific experimental design. The QbD-guided approach successfully produced nanosized paclitaxel- and neratinib-loaded cubosomal dispersions with substantial drug entrapment and controlled in vitro release, supporting further biological evaluation.

Keywords: Box-Behnken design; cubosomes; glyceryl monooleate; neratinib; paclitaxel; Poloxamer 407.

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INTRODUCTION

HER2-positive breast cancer remains a clinically challenging molecular subtype, and formulation strategies that improve the handling of anticancer agents may complement existing therapeutic approaches. The two drugs investigated here act through different pharmacological mechanisms: neratinib irreversibly inhibits HER-family tyrosine kinases, whereas paclitaxel disrupts mitosis by stabilizing microtubules. Their physicochemical and biopharmaceutical limitations provide a practical basis for examining lipid-based nanoscale carriers as formulation tools.

Cubosomes are dispersed particles derived from bicontinuous cubic lipid phases.¹⁻³ In these systems, glyceryl monooleate (GMO) can provide the self-assembling lipid matrix and Poloxamer 407 can assist stabilization of the dispersed phase.⁴⁻⁷ Because the internal structure contains interconnected polar and non-polar regions, the carrier can accommodate molecules with different physicochemical characteristics and can modify the manner in which incorporated drug is released.⁸⁻¹²

Accordingly, this investigation focused on separate paclitaxel- and neratinib-loaded cubosomal formulations. A response-surface experimental design was used to examine formulation and processing variables, after which the dispersions were assessed by particle-size measurement, zeta-potential determination, electron microscopy, drug-entrapment estimation and in vitro release testing. Release profiles of the selected formulations were also fitted to established kinetic models. The work did not include cell-based efficacy, toxicity, pharmacokinetic or combination-synergy experiments; conclusions are therefore confined to formulation and in vitro physicochemical performance.

MATERIALS AND METHODS

Paclitaxel and neratinib were sourced from Hetero Labs, Hyderabad, India. GMO and Poloxamer 407 were supplied by Synpharma Research Labs, Hyderabad, India. Reagents used during formulation and analytical testing were of analytical grade.

METHODOLOGY

Box-behnken experimental design

Design-Expert was used to construct a three-factor Box-Behnken matrix for each drug formulation. The factors entered into the design were the quantity of GMO (X1), the quantity of Poloxamer 407 (X2) and probe-sonication time (X3). Their coded levels and actual settings are listed in Tables 1 and 2. Three experimental outputs were followed: mean particle size (Y1), percentage drug entrapment (Y2) and cumulative drug release (Y3). During numerical optimization, particle size was constrained to the studied range, whereas higher values of Y2 and Y3 were preferred. Response-surface plots were used to visualize changes in the measured outputs across the experimental domain. The analysis reported here is limited to the available design and response data; no additional inferential statistical tests were retrospectively added.

Preparation of cubosomes by top-down emulsification

The cubosomal dispersions were produced by a top-down procedure. GMO was first warmed to 40–45°C until a fluid lipid phase was obtained, and the required drug was dispersed into this phase with mixing. In a separate vessel, Poloxamer 407 was dissolved in distilled water and the solution was equilibrated close to the temperature of the lipid phase. The lipid mixture was then added progressively to the aqueous phase while stirring. Agitation was maintained for 30–45 min to obtain a preliminary coarse dispersion.^{4,6,7} This dispersion was subjected to probe sonication for the run-specific time prescribed by the Box-Behnken matrix, with intermittent cooling to limit heat accumulation. After processing, the preparation was allowed to equilibrate to room temperature and was stored in a closed container until characterization. Tables 3 and 4 give the compositions used for the individual experimental runs.

EVALUATION PARAMETERS

Scanning Electron Microscopy (SEM): Surface appearance of the selected cubosomal particles was investigated by SEM. A small portion of dispersion was placed on an aluminium stub, dried, secured with double-sided adhesive tape and coated with a thin gold layer before imaging.²

Zeta potential: An aliquot of each selected dispersion was diluted with distilled water to a concentration suitable for electrophoretic measurement. The prepared sample was transferred to the instrument cell and its electrophoretic response was recorded with a zeta-potential analyser. The resulting value was used as a descriptive measure of particle surface charge and was interpreted together with the other physicochemical observations.^{5,10}

Particle-size measurement: Dynamic light scattering was used to obtain the hydrodynamic size distribution and polydispersity index after dilution of the cubosomal dispersion with distilled water. Measurements were performed in clean cuvettes. Figures 1 and 2 show representative distributions for the selected paclitaxel and neratinib preparations.^{2,13}

In vitro release experiment: Drug diffusion from the formulations was followed with a Franz diffusion assembly. Before use, the dialysis membrane was conditioned overnight in pH 7.4 phosphate buffer. The receptor compartment was filled with pH 7.4 phosphate buffer containing 0.5% v/v Tween 80, maintained at $37 \pm 0.5^\circ\text{C}$ and stirred continuously. A measured amount of formulation was placed in the donor compartment. At each predefined time point, 5 mL of receptor fluid was withdrawn and the same volume of fresh, temperature-equilibrated medium was added. Samples were suitably diluted and quantified by UV-visible spectrophotometry at the analytical wavelength used for each drug. Cumulative release was calculated after accounting for the serial withdrawal and replacement of receptor medium.^{13,14}

Release-model assessment: The time-release data of the selected formulations were mathematically transformed as required for zero-order, first-order, Higuchi and Korsmeyer-Peppas analysis. The relative model fits were then compared to describe the release behaviour observed under the experimental conditions.^{15–17}

RESULTS AND DISCUSSION

Particle-size response

Across the design runs, paclitaxel dispersions measured 149–221 nm and neratinib dispersions measured 129–207 nm (Table 5). Thus, nanoscale particles were obtained throughout the tested formulation domain, although the magnitude of the response changed with factor settings. Figures 3 and 4 display these factor-response relationships. Run 11 gave particle sizes of 165 nm for the paclitaxel preparation and 141 nm for the neratinib preparation. The corresponding particle-size distributions are presented in Figures 1 and 2.

Electrokinetic Surface Characteristics

The zeta-potential profiles of the selected formulations are shown in Figures 5 and 6. These measurements characterize the electrokinetic surface behaviour of the dispersed particles. They are useful supportive physicochemical observations, but they do not by themselves demonstrate storage stability; a stability claim would require time-dependent measurements under defined storage conditions.

Particle morphology

The SEM images in Figures 7 and 8 show the appearance of the selected cubosomal preparations after sample drying and coating. The observed particles were generally discrete and approximately rounded, with no extensive agglomeration apparent in the fields examined.

Drug incorporation

The percentage of drug retained within the cubosomal preparations differed among experimental runs (Table 6), demonstrating that entrapment was responsive to the formulation/process settings.^{13,18} Paclitaxel values extended from 77.12% to 96.58%, while neratinib values extended from 76.58% to 95.13%. The corresponding design surfaces are provided in Figures 9 and 10. The highest paclitaxel value was observed for Run 11 (96.58%); the highest neratinib value occurred in Run 4 (95.13%). For Run 11 specifically, neratinib entrapment was 81.25%. These findings describe formulation performance only and should not be interpreted as evidence of a pharmacological interaction between paclitaxel and neratinib.

Release behaviour

End-point cumulative release varied markedly among the 17 experimental runs (Table 7). Paclitaxel values ranged from 62.98% to 95.58%, whereas neratinib values ranged from 66.59% to 97.89%. The influence of the experimental factors is visualized in Figures 11 and 12. Considering the response profile used for selection, Run 11 was carried forward for detailed time-course presentation. At 8 h, this run released 93.37% of paclitaxel and 92.58% of neratinib (Table 8; Figures 13 and 14). The similarity of these end-point percentages should be regarded as a formulation observation rather than proof of equivalent biological exposure.

Kinetic interpretation

The Run 11 release datasets were examined using zero-order, first-order, Higuchi and Korsmeyer-Peppas representations (Figures 15-22). Comparison of the reported fits supports a strong diffusion-related

contribution to release, while the Korsmeyer-Peppas treatment is compatible with anomalous transport. These mathematical descriptions apply to the in vitro system used in this study and cannot establish the release pattern that would occur in vivo.

CONCLUSION

Separate paclitaxel- and neratinib-loaded cubosomal dispersions were successfully prepared by the investigated top-down process and evaluated within a Box-Behnken experimental framework. For the selected Run 11 preparations, particle size was 165 nm for paclitaxel and 141 nm for neratinib; drug entrapment was 96.58% and 81.25%, respectively. After 8 h of in vitro testing, cumulative release reached 93.37% for paclitaxel and 92.58% for neratinib. Electron microscopy supported the presence of discrete, approximately rounded particles, and mathematical treatment of the release data indicated an important diffusion-associated component. The present findings establish the physicochemical performance of the investigated formulations but do not demonstrate anticancer efficacy, safety, pharmacokinetic improvement or drug synergy. Those questions require dedicated biological and in vivo studies together with formal stability assessment.

ACKNOWLEDGEMENT

The authors acknowledge the laboratory and technical resources that supported formulation preparation and physicochemical characterization.

ETHICS STATEMENT

No human participants, human-derived specimens, experimental animals or animal-derived disease models were used in this formulation and in vitro characterization work. Institutional ethics approval was therefore not applicable to the experiments reported here.

ABBREVIATIONS

CDR: Cumulative drug release; DEE: Drug entrapment efficiency; DLS: Dynamic light scattering; GMO: Glyceryl monooleate; HER2: Human epidermal growth factor receptor 2; PDI: Polydispersity index; QbD: Quality by Design; SEM: Scanning electron microscopy.

CONFLICT OF INTEREST

The authors report that they have no competing interests relevant to this work.

FINANCIAL SUPPORT AND SPONSORSHIP

The study was conducted without external grant or sponsorship support and was financed by the authors.

AUTHOR CONTRIBUTIONS

Ayesha Farhath Fatima and Kumaraswamy Gandla contributed equally to study conception, experimental planning, investigation, data interpretation, manuscript drafting and critical revision. Both authors approved the submitted version and accept responsibility for the integrity of the work.

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Table 1. Factor settings and optimization criteria used for the paclitaxel design

Parameter	Low (-1)	Medium (0)	High (+1)
X1: GMO (mg)	3	5	7
X2: Poloxamer 407 (mg)	0.5	1	1.5
X3: Sonication time (min)	10	12.5	15
Y1: Particle size	In range	In range	In range
Y2: DEE	Maximize	Maximize	Maximize
Y3: Drug release	Maximize	Maximize	Maximize

Table 2. Factor settings and optimization criteria used for the neratinib design

Parameter	Low (-1)	Medium (0)	High (+1)
X1: GMO (mg)	3	5	7
X2: Poloxamer 407 (mg)	5	7.75	15
X3: Sonication time (min)	5	10	15
Y1: Particle size	In range	In range	In range
Y2: DEE	Maximize	Maximize	Maximize
Y3: Drug release	Maximize	Maximize	Maximize

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Table 3. Composition of the 17 paclitaxel experimental runs

Sno.	Paclitaxel (mg)	GMO (mg)	Poloxamer 407 (mg)	Distilled water
1	10	5	0.5	q.s
2	10	5	1	q.s
3	10	7	1.5	q.s
4	10	5	1	q.s
5	10	3	1	q.s
6	10	7	1	q.s
7	10	3	1.5	q.s
8	10	3	0.5	q.s
9	10	5	1.5	q.s
10	10	5	0.5	q.s
11	10	7	0.5	q.s
12	10	5	1	q.s
13	10	7	1	q.s
14	10	3	1	q.s
15	10	5	1.5	q.s
16	10	5	1	q.s
17	10	5	1	q.s

Table 4. Composition of the 17 neratinib experimental runs

Run	Neratinib (mg)	GMO (mg)	Poloxamer 407 (mg)	Distilled water
1	10	3	7.75	q.s
2	10	7	7.75	q.s
3	10	7	5	q.s
4	10	7	7.75	q.s
5	10	7	15	q.s
6	10	5	7.75	q.s
7	10	5	7.75	q.s
8	10	5	15	q.s
9	10	3	5	q.s
10	10	5	0.5	q.s
11	10	5	7.75	q.s
12	10	3	15	q.s
13	10	5	5	q.s
14	10	5	7.75	q.s
15	10	5	15	q.s
16	10	3	7.75	q.s
17	10	5	7.75	q.s

Table 5. Measured particle sizes across paclitaxel and neratinib design runs

Runs	Paclitaxel Particle size (nm)	Neratinib cubosomes Particle size (nm)
1	198	135
2	173	166
3	188	183
4	164	207
5	204	178
6	149	180
7	183	162
8	156	129
9	175	149
10	221	159
11	165	141
12	180	156
13	151	154
14	163	149
15	168	181
16	170	192
17	172	196

Table 6. Entrapment percentages obtained across the two cubosome design set

Run	Paclitaxel DEE (%)	Neratinib DEE (%)
1	84.50	79.51
2	89.41	91.02
3	94.22	93.1
4	90.10	95.13
5	81.53	89.56
6	95.32	88.94
7	79.82	76.58
8	77.12	81.24
9	87.96	87.82
10	85.61	86.36
11	96.58	81.25
12	89.67	85.59
13	93.12	87.91
14	82.53	88.89
15	88.56	92.56
16	87.95	90.25
17	89.98	93.58

Table 7. End-point cumulative release recorded for the experimental runs

RUN	Paclitaxel CDR (%)	Neratinib CDR (%)
1	93.22	93.12
2	71.25	76.58
3	89.51	71.57
4	66.95	66.59
5	95.58	75.48
6	68.72	74.48
7	84.63	96.39
8	62.98	97.89
9	79.83	83.52
10	75.55	81.57
11	93.37	92.58
12	65.46	82.59
13	69.99	80.25
14	70.25	84.59
15	68.92	72.63
16	76.36	75.49
17	63.29	84.58

Table 8. Time-course release data for the selected Run 11 preparations

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Time (hr)	Paclitaxel Run 11 (%)	Neratinib Run 11 (%)
0	0	0
1	18.32	17.59
2	33.29	34.59
3	46.38	45.73
4	59.26	58.43
5	67.81	65.37
6	79.52	78.81
7	85.68	86.9
8	93.37	92.58

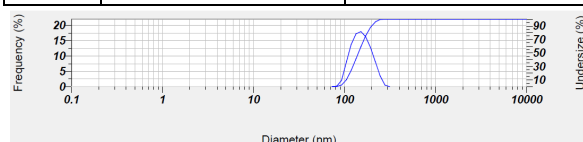


Figure 1. Particle-size distribution recorded for selected paclitaxel Run 11

Figure 2. Particle-size distribution recorded for selected neratinib Run 11

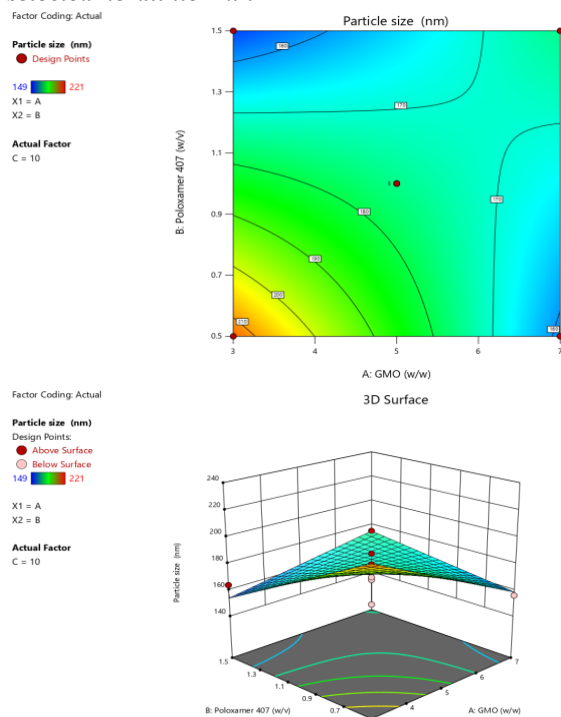


Figure 3. Paclitaxel particle-size response across the investigated factor space

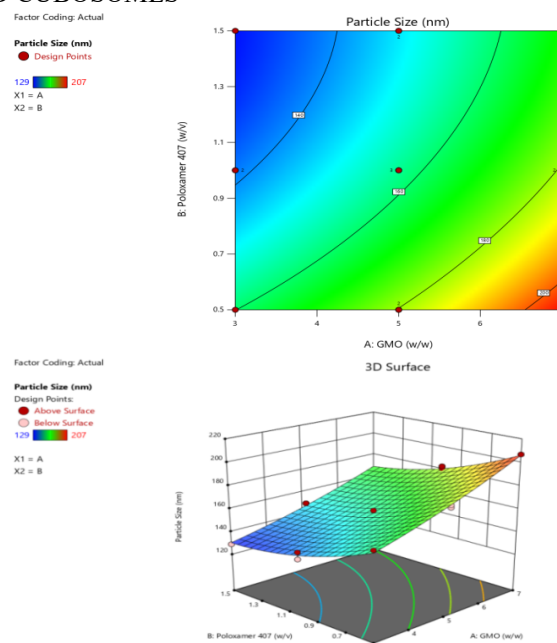


Figure 4. Neratinib particle-size response across the investigated factor space

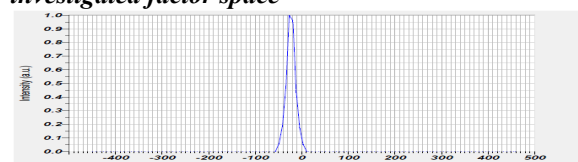


Figure 5. Zeta-potential profile of the selected paclitaxel dispersion

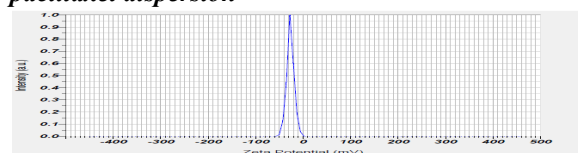


Figure 6. Zeta-potential profile of the selected neratinib dispersion

Electron-microscopy observations

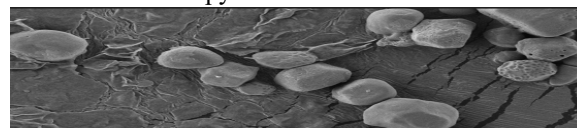


Figure 7. SEM micrograph of the selected paclitaxel cubosomal dispersion

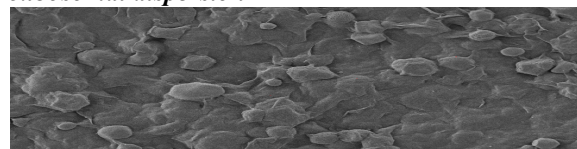


Figure 8. SEM micrograph of the selected neratinib cubosomal dispersion

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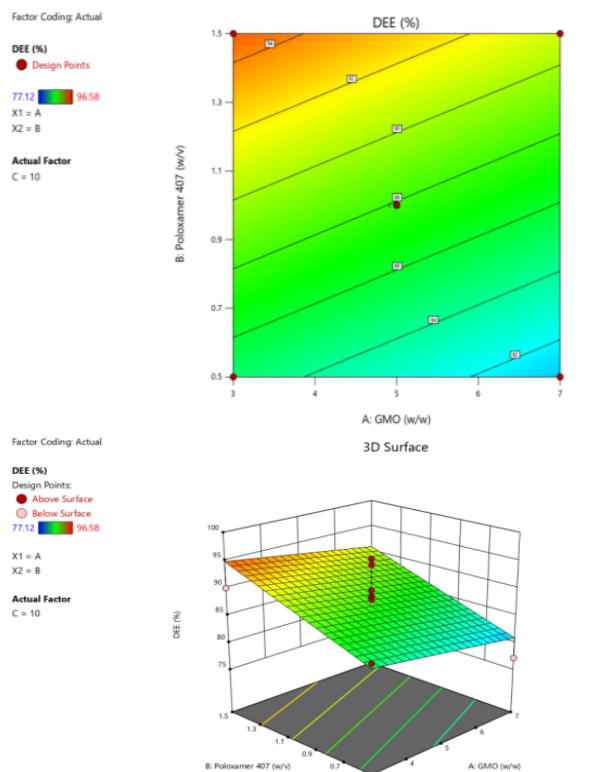


Figure 9. Factor-response surface for paclitaxel drug entrapment

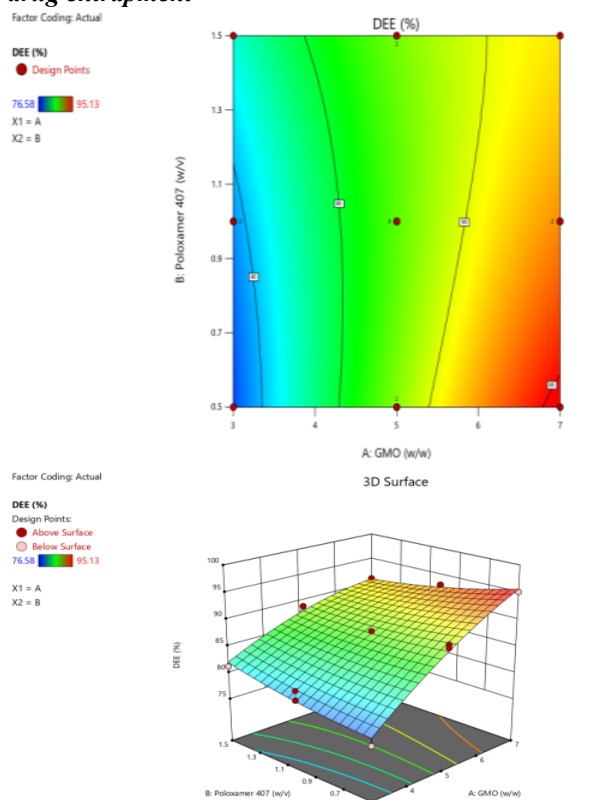


Figure 10. Factor-response surface for neratinib drug entrapment

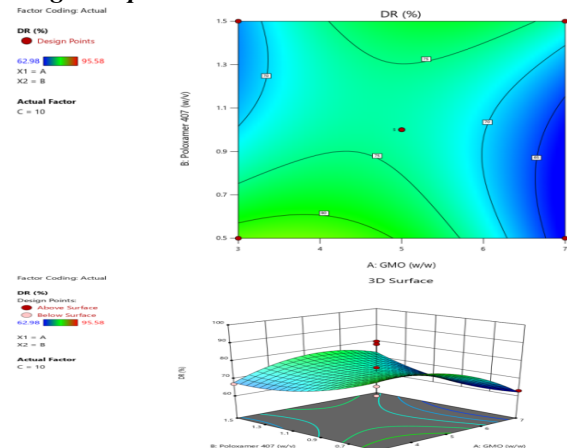


Figure 11. Factor-response surface for cumulative paclitaxel release

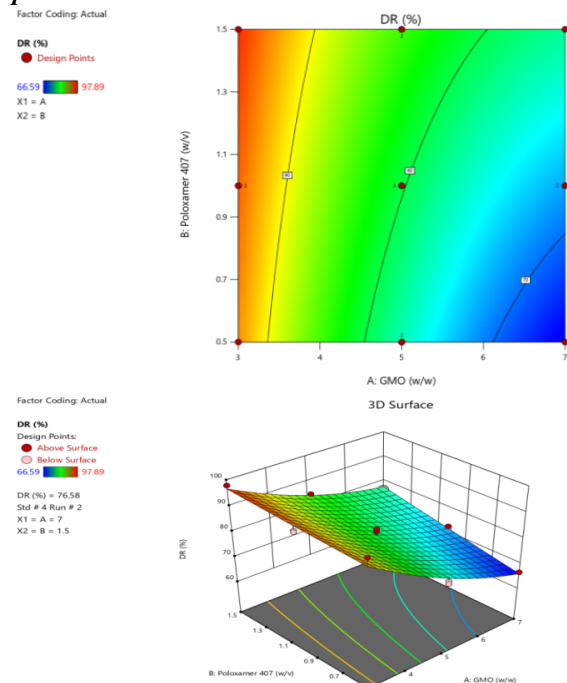


Figure 12. Factor-response surface for cumulative neratinib release

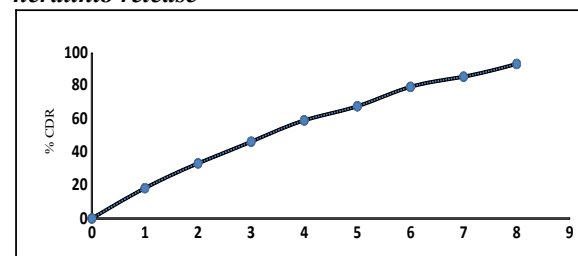


Figure 13. In vitro drug release of Paclitaxel cubosomes

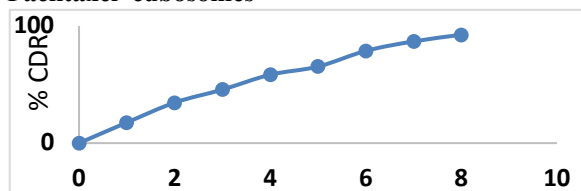


Figure 14. In vitro drug release of Neratinib cubosomes

Drug release kinetics of Paclitaxel cubosomes

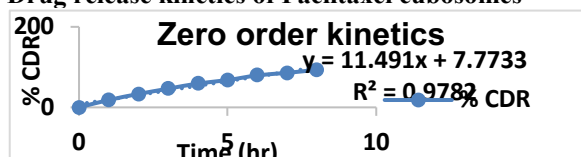


Fig-15: Zero order kinetics of Optimized formulation

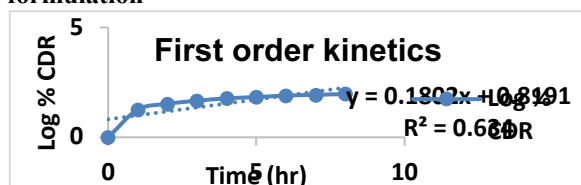


Fig-16: First order kinetics of Optimized formulation

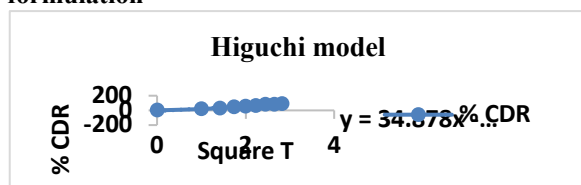


Fig-17: Higuchi model of Optimized formulation

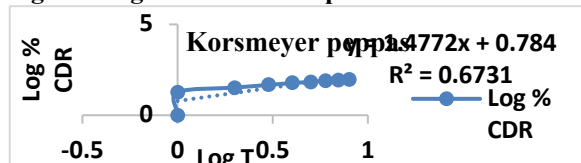


Fig-18: Korsmeyer peppas of Optimized formulation

Drug release kinetics of Neratinib cubosomes



Fig-19: Zero order kinetics of Optimized formulation



Fig-20: First order kinetics of Optimized Formulation

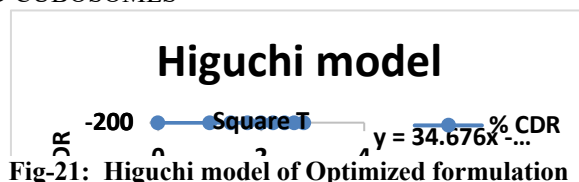


Fig-21: Higuchi model of Optimized formulation

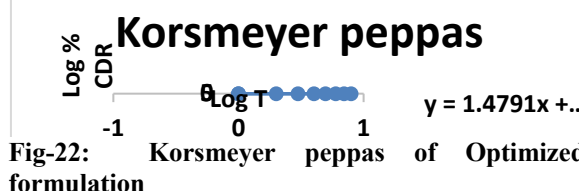


Fig-22: Korsmeyer peppas of Optimized formulation