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FORMULATION DESIGN OF EMPAGLIFLOZIN AND METFORMIN HYDROCHLORIDE EXTENDED RELEASE TABLETS: OPTIMIZATION OF FORMULATION USING STATISTICAL EXPERIMENTAL DESIGN

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Keywords:

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ABSTRACT: The objective of the present study was to develop Empagliflozin and Metformin hydrochloride extended release tablets, comprising immediate release empagliflozin and extended release metformin hydrochloride in a single tablet. Wet granulation process was employed to develop extended release part metformin hydrochloride. Impact of various formulation variables in extended release part was assessed using statistical interpretation such as analysis of variance. A 3^2 (two factor, three level) factorial design was employed to study the effect of independent variables (binder concentration [sodium CMC] and ER polymer [HPMC K100M CR/polyethylene oxide/carbopol] concentration)on dependent variables (drug release at 1, 4 and 10h). Optimization was done by fitting experimental data to the software program (design expert). The design space for formulation variables and its influence on drug release was developed. *In-vitro* release data observed from the optimized formulation was fitted into various kinetic equations. Extended release part drug release was extended for 12 h following zero order kinetics and non-fickian diffusion process. Extended drug delivery system of empagliflozin and metformin hydrochloride extended release tablets was successfully developed by employing granulation technology and formulation optimization facilitated by experimental design.

INTRODUCTION: Type 2 diabetes mellitus (T2DM) is a major and growing cause of morbidity and mortality worldwide. Despite continued advances in the treatments available for T2DM, glycemic control is frequently suboptimal. Because T2DM is characterized by multiple metabolic abnormalities, current treatment algorithms involving a stepwise approach to the use of combination therapy involving agents with complementary modes of action are frequently necessary.

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Metformin is generally prescribed as first-line pharmacologic therapy in T2DM and both the American Diabetes Association (ADA) and American College of Endocrinology (AACE) algorithms list sodium-glucose cotransporter-2 (SGLT2) inhibitors as a suggested option for dual combination therapy.

SGLT2 inhibitors have a unique mechanism of action which does not depend on the presence of insulin or the degree of insulin resistance and are suitable for coadministration with all classes of glucose-lowering agents. Empagliflozin is an SGLT2 inhibitor that, in common with other agents in this class, reduces elevated blood glucose levels by inhibiting SGLT2, the predominant transporter responsible for reabsorption of glucose from the

glomerular filtrate, thereby increasing urinary glucose excretion. Combination therapy with these two drugs has the potential to offer improved glucose control compared with that achieved with the individual agents. This approach can reduce the dosing frequency for patients, leading to a simplified dosing regimen, which may assist adherence to therapy [1, 2, 3].

Quality by Design (QbD) is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. It helps to assess the critical material attributes (CMAs) and critical process parameters (CPPs) that impacting the predefined critical quality attribute (CQAs). The design space concept is introduced as "the multidimensional combination and interaction of input variables (*e.g.*, materials attributes) and process parameters that have been demonstrated to provide assurance of quality". Using this approach, it is essential to define the relationship between critical formulation/process parameters and CQAs [4].

Response surface methodology (RSM) is one of the popular methods in the development and optimization of drug delivery systems. Based on the principles of design of experiments (DoE), the methodology involves the use of various types of experimental designs, generation of polynomial mathematical relationships, and mapping of the response over the experimental domain to select the optimum formulation. Central composite design, three level factorial design, Box-Behnken design and D-optimal design are the different types of RSM designs available for statistical optimization of the formulations. Factorial design is one type of RSM design enables, all factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interactions between them. Factorial design requires fewer experimental runs, less time and thus provides a cost-effective technique than the conventional processes of formulating and optimization of dosage forms. Hence, factorial design was selected as DoE [5].

The present investigation was aimed to fabricate empagliflozin and metformin hydrochloride extended release tablets, where immediate release (IR) empagliflozin part composition was maintained constant and extended release (ER) metformin hydrochloride part formulae was optimized. Lubricated blends of both IR part and ER part were compressed into bi-layer tablets. Preliminary trials were executed with various concentrations of binder (sodium CMC at 3-7% w/w) and various types and concentration of ER polymers (HPMC K100 M CR/polyethylene oxide/ carbopol at12-22% w/w). Optimization of the metformin hydrochloride part was done by employing factorial design as an optimization technique, with target on release of drug 15%-25% at 1 h, 40%-60% at 4 h and NLT 80% at 10 h. The independent variables for this study were concentration of binder (sodium CMC) and release retardant polymers (Hypromellose K100M CR/ polyethylene oxide/carbopol). The dependent variables studied were drug release at 1 h (15%-25%), 4 h (40%-60%) and 10 h (NLT 80%).

MATERIALS AND METHODS:

Materials: Empagliflozin and metformin hydrochloride were obtained as a gift sample from Aurobindo Pharma Ltd., Hyderabad. Sodium CMC7HF PH (Ashland), HPMC K100M CR (Dow chemicals), Polyethylene oxide Sentry Polyox WSR-303-LEO(Dow chemicals), Carbopol 971P (Lubrizol), Colloidal silicon dioxide aerosil 200 pharma (Evonik), Magnesium Stearate (Peter greven), Lactose spray dried Supertab 11SD (DFE), Microcrystalline cellulose PH112 (FMC), Croscarmellose sodium Ac-Di-Sol (FMC), Hydroxy propyl cellulose klucel LF (Nippon soda) and Iron oxide yellow (Neelikon) were used as received.

Methods:

Drug-Excipient Compatibility Studies: Both empagliflozin and metformin hydrochloride and selected excipients were subjected for drug excipient compatibility study. The drug and individual excipients were intimately mixed in equal parts by weight and filled in glass vials stoppered with teflon plugs and sealed with aluminium seals. These samples were kept in incubators at 40°C/75% RH for 4 weeks. Initial and 4 weeks (40°C/75% RH) samples were analysed for the solid-state property of the drug in the blended mixtures using differential scanning colorimeter (DSC).

Preparation of Empagliflozin and Metformin Hydrochloride Extended Release Tablets (Bilayer Tablets): IR part of Empagliflozin and ER part of metformin hydrochloride were prepared individually and compressed into Bi-layer tablets [6, 7]. Preparation of IR and ER part blends is described below.

Preparation of Metformin Hydrochloride Part (ER Part): Metformin hydrochloride part granules were prepared using wet granulation technology (rapid mixer granulator, make: Gansons). Dry mix material (metformin hydrochloride and sodium CMC) were granulated using purified water as a granulating fluid in RMG at impeller slow speed and chopper slow speed. Wet mass was dried in a rapid dryer for 45-60 min at the inlet temperature of 50 ± 5 °C. Dried granules (LOD at 105 °C: 0.5 to 1.5% w/w) were passed through ASTM mesh #20 and retains were milled using co-mill fitted with screen 1016 micron at slow to medium speed.

Extra granular materials (ER polymer and Aerosil) and milled granules were co-sifted through ASTM mesh #20 and blended in a low shear double cone blender for 10 min. Then lubricated with magnesium stearate for 5 min in the blender.

Preparation of Empagliflozin Part (IR Part): Empagliflozin part granules were prepared by direct blending approach. Empagliflozin, lactose spray dried, microcrystalline cellulose, hydroxy propylcellulose, croscarmellose sodium, colloidal silicon dioxide and iron oxide yellow were sifted together through ASTM mesh #30 and mixed in a low shear double cone blender for 20 min. Then lubricated with magnesium stearate for 5 min in the blender.

Compression: Both empagliflozin part and metformin hydrochloride part were compressed into bi-layer tablets using Eliza Press compression machine at a hardness of 22 to 28 KP.

Experimental Design: In preliminary trials, the formulation variables in each step of the manufacturing process were evaluated for their significance by analysis of variance (ANOVA). Finally, found that the type and concentrations of ER polymer and binder concentration had a significant impact on drug release of ER part. The factorial design was used to evaluate the effect of independent variables (binder concentration and ER polymer concentration) on responses/dependent variables (drug release at 1h [Y_1], 4 h [Y_2] and 10 h [Y_3]) of empagliflozin and metformin hydrochloride ER tablets. A two-factor, three-level factorial design is used for exploring quadratic response surfaces and constructing second order polynomial models with design expert (stat-ease, version-12). ANOVA (analysis of variance) is inevitably linked to experimental design, which was used to analyse the significance of the model and each selected response. It also generates polynomial equations. The response (Y_1) in each trial was estimated by carrying out a multiple factorial regression analysis using the generalized quadratic model:

$$Y_1 = b_0 + b_1X_1 + b_2X_2 + b_1b_2X_1X_2$$

Where, Y_1 is the measured response associated with each factor level combination; b_0 is an intercept; b_1 and b_2 are regression coefficients computed from the observed experimental values of Y_1 and X_1 and X_2 are the coded levels of independent variables.

After fitting the response data in experimental design as in **Table 1**, the experimental results were analysed by ANOVA. It demonstrated the various statistical parameters such as sum of squares, F values, P values of model terms and correlation coefficient (R^2) values. The suitability of model was authenticated by the predicted and adjusted R^2 values.

Results obtained from the preliminary studies, binder concentration (3, 5 and 7% w/w) and ER polymer type (HPMC K100 M CR, polyethylene oxide, carbopol) and concentration (12, 17 and 22% w/w) were identified as high-risk variables, have a potential impact on drug release. Hence these factors were studied by a two factor, three-level factorial experimental design, individually.

Optimization of ER Part (Metformin Hydrochloride): The independent variables in ER part were concentration of binder (sodium CMC) and concentration of ER polymer (HPMC K100M CR/polyethylene oxide/carbopol). Both variables were studied at three levels (−1, 0, +1). Percentage of drug release at 1 h (Y_1), percentage of drug release at 4 h (Y_2) and percent of drug release at 10

h (Y_3) were selected as responses. The impact of each selected binder and ER polymer concentration on responses were studied and optimized individually.

TABLE 1: VARIABLES IN FACTORIAL DESIGN

Factors	Levels used, actual (coded)		
	Low (-1)	Medium (0)	High (+)
Binder concentration (mg/tablet) (X_1)	39.00	65.00	91.00
ER polymer concentration (mg/tablet) (X_2)	156.00	221.00	286.00
Dependant variables		Target	
Y_1= % drug release at 1 h		15 -25	
Y_2= % drug release at 4 h		40 – 60	
Y_3= % drug release at 10h		NLT 80	

Evaluation of Metformin Hydrochloride Part Granules:

Micromeritic Properties: Bulk density (BD), tapped density (TD) and Hausner's ratio (HR) of metformin hydrochloride part granules were determined individually. BD and TD were determined by USP method I using a TD tester.

BD = Weight of the sample (g)/untapped volume (ml),

TD = Weight of the sample (g)/tapped volume (ml)

HR was calculated using following formulae:

$$HR = TD/BD$$

Where, TD and BD are tapped and bulk densities.

Assay-Metformin Hydrochloride Part: Finely grinded powder of 10 tablets was taken and transferred an accurately weighed portion of the powder equivalent to the average tablet weight, to a homogenization vessel and accurately added 500 ml of 10% acetonitrile solution. Homogenized and allowed to soak the sample till fully homogenized. Stock preparation was passed through a 0.45µ filter having, discarded the first 3 ml of filtrate. Transferred 25 ml of the filtrate to a 200 ml volumetric flask and diluted with water to volume [8].

The following chromatographic conditions were employed for analysis

Column	:	3.9-mm × 30-cm; 10µm packing L1
Column temperature	:	30°C
Injection volume	:	10 µl
Flow rate	:	1.0 ml/min
Detector	:	UV 218 nm
Runtime	:	20 min

Calculations:

Assay of metformin hydrochloride:

$$\% \text{ labelled amount} = (r_u / r_s) \times (C_s/C_u) \times 100$$

Where,

r_u= peak response from sample solution

r_s= peak response from standard solution

C_s = Concentration of USP metformin hydrochloride RS in the standard solution (mg/ml)

C_u = Nominal concentration of metformin hydrochloride in the sample solution

***In-vitro* Drug Release Studies-Metformin Hydrochloride Part:** Empagliflozin and metformin hydrochloride ER tablets were evaluated for *in vitro* drug release studies, which were performed using USP Type-I (Basket) dissolution test apparatus. The volume of the dissolution medium was 900 ml with a stirring speed of 100 rpm and the temperature was maintained at 37 ± 0.5 °C. These conditions were kept constant for all dissolution studies. The study was carried out in pH 6.8 phosphate buffer at 1, 2, 4, 6, 8, 10 and 12 h [9].

10 ml of sample was withdrawn periodically and replaced with equal volume of fresh dissolution medium. The collected samples were filtered through 0.45 µ filter by discarding initial 4 ml of solution. Further diluted 2 ml of filtrate to 100 ml with dissolution medium and analysed to assess the % drug dissolved by employing same chromatographic conditions as that of assay.

The % labeled amount of Metformin hydrochloride dissolved at respective time intervals (Dn) was estimated from following formulae:

$$\% \text{ Labeled amount} = A_T / A_S \times D_S / D_T \times P / 100 \times 100 / L$$

Where,

A_T = Peak area of Metformin hydrochloride obtained from the sample solution,

A_S = Average peak area of Metformin hydrochloride obtained from the standard solution,

D_S = Dilution factor of standard solution,

D_T = Dilution factor of sample solution,
L = Label claim of Metformin hydrochloride in mg, per tablet.

Calculate the correction factor (CFn) at each time point by using the following formula:

$$CFn = Dn \times 10 / V$$

Drug Release Kinetics: The drug release kinetics and mechanism of the formulations were studied by fitting the data obtained from the *in-vitro* release study into several mathematical equations [10, 11].

Zero Order Model: Drug dissolution from formulations that do not disaggregate and release the drug at a constant rate (slowly) can be represented by zero order equation. To evaluate the release kinetics, *in-vitro* data were constructed as cumulative percent drug released *vs*. time. It describes the rate of drug release is independent of the concentration of dissolved substance.

$$C = K_0 t$$

Where, 'K_0' is zero order rate constant (concentration / time) and 't' is time.

First Order Model: In this model drug release is depending on the concentration.

$$LogC = LogC_0 - kt / 2.303$$

Where, 'C_0' is the initial concentration of drug and 'K' is first order constant.

Erosion Model: This equation defines the drug release based on erosion alone.

$$Q = 1-(1-k_3 t)^3$$

Where, 'Q' is fraction of drug released at time t and 'k_3' is release rate constant.

Thus, a plot between [1-(1-Q)1/3] *vs*. time will be linear, if the release follows erosion equation.

Korsmeyer-Peppas Model: To evaluate release kinetics, in vitro drug release data was constructed as log cumulative % drug release vs. log time.

$$Mt / M\infty = Ktn$$

Where,
Mt / M∞ -a fraction of drug released at time t,
K - Release rate constant and n - Release exponent.

The 'n' value is used to describe various drug release mechanism for cylindrical shaped matrices.

Higuchi's Model: The mechanism of the drug release described by following equation.

$$Q = KH \times T_{1/2}$$

KH = The Higuchi dissolution constant

The values of cumulative percent drug release vs. square root of time.

Drug release data were built-in according to the below cited Equation:

$$Mt / Ma = ktn$$

Where,
Mt/Ma - Fractional drug released at time t;
k i- constant and 'n' - kinetic constant (used to evaluate the transport mechanism).

If, n=0.45 for Case I or Fickian diffusion, n=0.89 for Case II transport, 0.45<n<0.89 for anomalous behaviour or non-Fickian transport, and n>1.0 for Super Case II transport.

RESULTS AND DISCUSSION:

Drug-Excipient Compatibility Studies: DSC thermograms are presented in **Fig. 1**. There are no significant differences in onset melting points and peak melting points of initial and 40°C/75% RH, 4 weeks samples. Corresponding data represented in **Table 2**.

TABLE 2: ONSET AND PEAK MELTING POINTS OF DRUG-EXCIPIENT COMPATIBILITY SAMPLES

Sample		Condition	Onset melting point (°C)	Peak melting point (°C)
Empagliflozin		Initial	148.34	152.22
		40°C/75%RH, 4 weeks	149.21	154.24
Metformin hydrochloride		Initial	214.48	218.12
		40°C/75%RH, 4 weeks	209.24	215.21
Composite blend	Empagliflozin	Initial	153.00	157.58
	Metformin		214.52	216.24
	Empagliflozin	40°C/75%RH, 4 weeks	160.21	162.34
	Metformin		218.67	221.54

Hence, it was concluded that there was no interaction between the drug substances and the chosen excipients. Hence, these excipients were considered for the use in the development of the formulation.

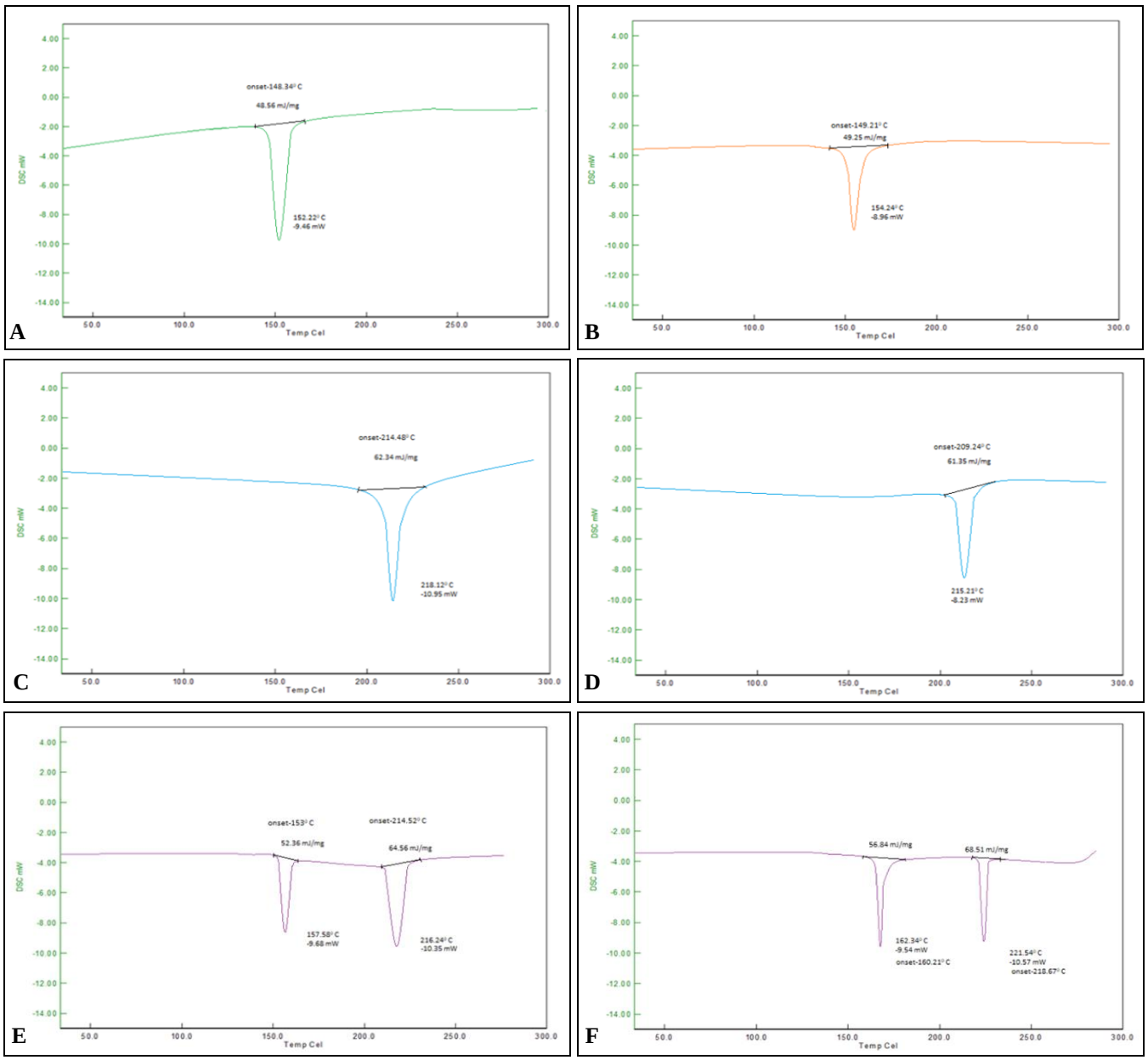


FIG. 1: DSC THERMOGRAMS:(A) EMPAGLIFLOZIN-INITIAL; (B) EMPAGLIFLOZIN-4 WEEKS AT 40°C/75% RH; (C) METFORMIN HYDROCHLORIDE-INITIAL; (D) METFORMIN HYDROCHLORIDE-4 WEEKS AT 40°C/75% RH; (E) COMPOSITE BLEND – INITIAL; (F) COMPOSITE BLEND-4 WEEKS AT 40°C/75% RH

Data Analysis and Model Validation:

Fitting of Data to the Model: Two factors with three levels factorial experimental design for triplicates require 27 experiments, the independent variables and responses for all experimental runs are given in **Table 3**. Models of various responses were obtained using design expert (stat-ease). The values of R^2, adjusted R^2 and predicted R^2 were shown in **Table 4-6**, for each response along with their ANOVA results. Values of probability $P<0.05$ represent significant model terms. After elimination of non-significant ($P > 0.05$) coefficients in **Table 4-6**, following correlations for response variables were obtained in terms of coded factors. The regression equations carry factors along with coefficients (positive/negative) which quantify response values. A positive sign of coefficient indicates synergistic effects, whereas negative sign represents an antagonistic effect.

TABLE 3: OBSERVED RESPONSES IN FACTORIAL DESIGN FOR EMPAGLIFLOZIN AND METFORMIN HYDROCHLORIDE ER TABLETS

Independent variables		Dependent variables/responses								
Binder con. (mg) (X_1)	ER polymer con. (mg) (X_2)	% Drug release at 1h (Y_1)			% Drug release at 4h (Y_2)			% Drug release at 10h (Y_3)		
		HPMC K100M CR	Polyethy-lene oxide	Carbopol	HPMC K100M CR	Polyeth-ylene oxide	Carbopol	HPMC K100M CR	Polyeth-ylene oxide	Carbopol
39	156	28±1.6	31±1.9	20±1.1	64±1.1	73±0.3	42±1.2	95±1.0	95±0.5	69±0.7
65	156	23±1.0	26±1.5	17±0.5	61±0.8	71±0.5	45±1.3	92±1.1	92±0.5	66±2.1
91	156	18±0.6	22±1.3	17±0.2	60±0.5	70±0.3	44±0.5	90±1.3	90±0.8	65±2.4
39	221	27±0.8	29±1.5	17±0.5	56±1.4	69±1.2	39±0.4	86±1.4	88±1.3	58±1.5
65	221	21±0.7	24±0.5	16±0.4	52±1.2	67±1.1	37±0.5	83±0.5	87±1.4	57±1.6
91	221	16±1.0	20±0.8	15±0.8	50±1.0	65±0.8	35±0.3	80±0.7	86±1.7	55±0.7
39	286	26±0.6	27±0.7	15±0.6	46±0.5	63±1.2	33±0.5	77±0.9	83±2.0	49±0.4
65	286	20±0.7	24±1.3	14±0.4	43±0.5	62±1.6	30±0.8	75±1.0	83±1.6	46±1.3
91	286	14±1.0	19±1.4	13±0.8	41±0.6	59±1.1	28±0.7	71±1.3	82±2.4	44±1.4
39	156	27±1.0	30±1.2	20±0.7	65±0.5	73±1.4	43±1.2	94±1.2	95±1.8	68±1.5
65	156	24±1.3	26±1.6	18±0.5	62±0.4	70±1.3	43±1.1	91±1.4	92±2.1	67±0.5
91	156	19±0.8	22±1.4	18±0.7	60±0.7	71±1.4	44±1.0	91±1.0	89±2.2	65±0.7
39	221	26±0.7	28±0.8	17±0.9	57±0.8	68±1.0	39±1.3	87±1.3	89±1.8	59±0.9
65	221	21±1.5	26±0.4	15±1.0	53±0.9	67±1.2	36±1.2	82±1.2	86±0.5	58±0.4
91	221	15±1.0	21±1.0	15±1.1	51±1.2	67±1.5	38±0.5	81±0.4	86±1.3	57±1.2
39	286	26±0.5	27±0.6	14±1.2	45±1.3	61±1.6	31±0.8	78±0.7	85±2.4	48±1.3
65	286	23±0.8	23±1.2	14±0.5	43±1.4	59±1.4	34±0.4	74±0.6	84±1.3	47±1.4
91	286	14±0.4	18±1.5	13±0.5	41±1.5	58±1.3	30±0.6	72±1.2	82±1.8	46±1.5
39	156	28±0.9	31±0.5	20±0.6	66±1.2	73±1.4	44±0.7	94±1.4	95±1.7	68±0.5
65	156	24±0.4	26±0.4	19±0.4	63±0.6	71±1.5	44±0.6	93±1.3	91±1.6	66±0.7
91	156	19±1.3	22±0.8	17±0.7	60±0.5	71±1.6	43±0.4	90±1.2	90±2.1	64±0.6
39	221	27±1.1	29±0.7	17±0.5	56±1.2	69±1.4	38±0.8	86±1.1	88±2.5	57±1.2
65	221	22±1.0	24±1.5	16±0.4	53±1.1	68±1.4	37±1.0	83±1.2	87±0.5	57±0.5
91	221	17±0.4	21±1.4	15±0.8	51±1.2	65±1.0	34±1.2	81±1.4	86±0.4	55±1.3
39	286	26±0.6	27±1.0	15±0.6	45±1.3	62±0.5	31±1.3	77±0.6	85±0.6	48±0.5
65	286	20±0.4	25±1.1	15±0.7	44±1.2	61±0.6	30±1.2	73±0.4	83±1.2	45±0.5
91	286	13±0.8	18±1.6	13±0.4	40±0.2	60±0.7	29±1.1	70±0.8	82±1.1	45±1.2

All the responses observed for various formulations were fitted simultaneously to first order, second order and quadratic models using design expert. Responses Y_1, Y_2 andY_3 were found to follow second order, linear model and second order model respectively, for the formulations prepared employing HPMC K100M CR as ER polymer. Response Y_1 was found to follow linear order, Y_2 and Y_3 were found to follow Quadratic model for the formulations prepared employing polyethylene oxide as ER polymer. Responses Y_1, Y_2 andY_3 were found to follow linear order, second order and linear order model respectively for the formulations prepared employing carbopol as ER polymer.

TABLE 4: ANOVA RESULTS FOR PREDICTING % DRUG RELEASE AT 1, 4 AND 10 h EMPLOYING HPMC K100 M CR AS ER POLYMER

Source	Sum of Squares	Df	Mean Square	F-value	P value, P>F
Y_1 (%)					
Model	576.19	3	192.06	248.1	< 0.0001
X_1	522.72	1	522.72	675.22	< 0.0001
X_2	46.72	1	46.72	60.35	< 0.0001
X_1X_2	6.75	1	6.75	8.72	0.0071
Residual	17.81	23	0.7742		
Lack of Fit	4.47	5	0.8944	1.21	0.3453
Pure Error	13.33	18	0.7407		
Cor Total	594.00	26			
R^2=0.9700; adjusted R^2=0.9661; predicted R^2=0.9609					
Y_2 (%)					
Model	1780.28	2	890.14	1724.39	< 0.0001
X_1	117.56	1	117.56	227.73	< 0.0001
X_2	1662.72	1	1662.72	3221.06	< 0.0001
Residual	12.39	24	0.5162		

Lack of Fit	4.39	6	0.7315	1.65	0.1920
Pure Error	8.00	18	0.4444		
Cor Total	1792.67	26			
R^2=0.9931; adjusted R^2=0.9925; predicted R^2=0.9913					
Y_3 (%)					
Model	1608.14	3	536.05	1033.00	< 0.0001
X_1	128.00	1	128.00	246.67	< 0.0001
X_2	1476.06	1	1476.06	2844.47	< 0.0001
X_1X_2	4.08	1	4.08	7.87	0.0101
Residual	11.94	23	0.5189		
Lack of Fit	1.94	5	0.3870	0.6967	0.6328
Pure Error	10.00	18	0.5556		
Cor Total	1620.07	26			
R^2=0.9926; adjusted R^2=0.9917; predicted R^2=0.9900					

Regression equation of the fitted model[#]:

Y_1 (%) =34.24167−0.109188*X_1+0.004060*X_2+0.000444*X_1X_2;

Y_2 (%) =91.95556−0.098291*X_1−0.147863*X_2;

Y_3 (%) =115.68241−0.026282*X_1−0.116880*X_2−0.000345*X_1X_2

[*]P<0.05 considered as significant. [#]Only the terms with statistical significance are included. X_1: Sodium CMC concentration, X_2: HPMC K 100M CR concentration,

Y_1: % drug release at 1 h, Y_2: % drug release at 4h, Y_3: % drug release at 10 h.

TABLE 5: ANOVA RESULTS FOR PREDICTING % DRUG RELEASE AT 1, 4 AND 10 h EMPLOYING POLYETHYLENE OXIDE AS ER POLYMER

Source	Sum of Squares	df	Mean Square	F-value	P value, P>F
Y_1 (%)					
Model	364.44	2	182.22	378.46	< 0.0001
X_1	320.89	1	320.89	666.46	< 0.0001
X_2	43.56	1	43.56	90.46	< 0.0001
Residual	11.56	24	0.4815		
Lack of Fit	4.22	6	0.7037	1.73	0.1719
Pure Error	7.33	18	0.4074		
Cor Total	376.00	26			
R^2=0.9693; adjusted R^2=0.9667; predicted R^2=0.9624					
Y_2 (%)					
Model	578.04	5	115.61	147.30	< 0.0001
X_1	34.72	1	34.72	44.24	< 0.0001
X_2	533.56	1	533.56	679.83	< 0.0001
X_2^2	8.96	1	8.96	11.42	0.0028
Residual	16.48	21	0.7848		
Lack of Fit	2.48	3	0.8272	1.06	0.3892
Pure Error	14.00	18	0.7778		
Cor Total	594.52	26			
R^2=0.9723; adjusted R^2=0.9657; predicted R^2=0.9550					
Y_3 (%)					
Model	415.64	5	83.13	193.37	< 0.0001
X_1	50.00	1	50.00	116.31	< 0.0001
X_2	355.56	1	355.56	827.08	< 0.0001
X_1X_2	6.75	1	6.75	15.70	0.0007
X_2^2	2.67	1	2.67	6.20	0.0212
Residual	9.03	21	0.4299		
Lack of Fit	3.03	3	1.01	3.03	0.0564
Pure Error	6.00	18	0.3333		
Cor Total	424.67	26			
R^2=0.9787; adjusted R^2=0.9737; predicted R^2=0.9643					

Regression equation of the fitted model[#]:

Y_1 (%) =40.51111−0.162393*X_1+0.023932*X_2;

Y_2 (%) =75.21093−0.085043*X_1+0.050513*X_2−000289*X_2^2;

Y_3 (%) =122.22056−0.226282*X_1−0.166966*X_2+0.000444*X_1X_2+000158*X_2^2

[*]P<0.05 considered as significant. [#]only the terms with statistical significance are included. X_1: Sodium CMC concentration, X_2: Polyethylene oxide concentration,

Y_1: % drug release at 1 h, Y_2: % drug release at 4 h, Y_3: % drug release at 10 h.

From the obtained ANOVA results **Table 4-6**, in all the cases, main factors binder (sodium CMC) concentration and ER polymer (HPMC K100M CR/polyethylene oxide/carbopol) concentration caused variation on drug release. The model shows that the binder concentration and ER polymer concentration had a negative impact on drug release. The both interaction terms have combined negative impact on response Y_1 from all formulations. Binder concentration had not shown any impact on responses Y_2 and Y_3.ER polymer concentration showed negative impact on responses Y_2 and Y_3. Responses (Y_2 and Y_3) were decreased with increasing the ER polymer concentration from all formulations (HPMC K 100M CR/polyethylene oxide/carbopol).

TABLE 6: ANOVA RESULTS FOR PREDICTING % DRUG RELEASE AT 1, 4 AND 10 h EMPLOYING CARBOPOL AS ER POLYMER

Source	Sum of Squares	df	Mean Square	F-value	P value, P>F
			Y_1 (%)		
Model	108.94	2	54.47	169.29	< 0.0001
X_1	20.06	1	20.06	62.33	< 0.0001
X_2	88.89	1	88.89	276.26	< 0.0001
Residual	7.72	24	0.3218		
Lack of Fit	3.06	6	0.5093	1.96	0.1248
Pure Error	4.67	18	0.2593		
Cor Total	116.67	26			
			R^2=0.9338; adjusted R^2=0.9283; predicted R^2=0.9172		
			Y_2 (%)		
Model	768.39	3	256.13	157.25	< 0.0001
X_1	12.50	1	12.50	7.67	0.0109
X_2	747.56	1	747.56	458.95	< 0.0001
X_1X_2	8.33	1	8.33	5.12	0.0335
Residual	37.46	23	1.63		
Lack of Fit	7.46	5	1.49	0.8956	0.5049
Pure Error	30.00	18	1.67		
Cor Total	805.85	26			
			R^2=0.9535; adjusted R^2=0.9474; predicted R^2=0.9382		
			Y_3 (%)		
Model	1843.56	2	921.78	1321.49	< 0.0001
X_1	43.56	1	43.56	62.44	< 0.0001
X_2	1800.00	1	1800.00	2580.53	< 0.0001
Residual	16.74	24	0.6975		
Lack of Fit	4.74	6	0.7901	1.19	0.3576
Pure Error	12.00	18	0.6667		
Cor Total	1860.30	26			
			R^2=0.9910; adjusted R^2=0.9903; predicted R^2=0.9888		
			Regression equation of the fitted model[#]:		
			Y_1 (%) =26.30556−0.040598*X_1−0.034188*X_2;		
			Y_2 (%) =53.98519+0.076923*X_1−0.067094*X_2−0.000493*X_1X_2;		
			Y_3 (%) =94.51852−0.059829*X_1−0.153846*X_2		
			[*]P<0.05 considered as significant. [#]Only the terms with statistical significance are included.		
			X_1: Sodium CMC concentration, X_2: Carbopol concentration,		
			Y_1: % drug release at 1 h, Y_2: % drug release at 4 h, Y_3: % drug release at 10 h.		

Contour and Three-Dimensional (3D) Response Surface Plot Analysis: The design expert software (stat-ease) generated the contour and 3D surface plots are presented in **Fig. 2-4**, which are very useful to study the interaction effects of the factors on responses. This type of the plot visualizes the effects of two factors on the response at a time. **Fig. 2** exhibits a curvilinear relationship with Y_1 and nonlinear relationship with Y_2 and Y_3. **Fig. 3** shows a linear relationship with Y_1 and curvilinear relationship with Y_2 and Y_3. **Fig. 4** shown curvilinear relationship with Y_1, Y_2 and Y_3.

The data of % drug release at 1, 4and 10 h from all batches executed with HPMC K 100M CR as ER polymer ranges from 13-28%, 40-66% and 70-95%, respectively. The drug release from these formulations was well around the predetermined

specifications. The data of % drug release at 1, 4 and 10 h from all batches executed with polyethylene oxide as ER polymer ranges from 18-31%, 58-73% and 82-95% respectively. At 22% w/w concentration also polyethylene oxide fails to provide a controlled release. The data of % drug release at 1, 4 and 10 h from all batches executed with carbopol as ER polymer ranges from 13-20%, 28-45% and 44-69% respectively. Drug release at 4 h and 10 h was retarded at low concentration of carbopol (12% w/w) and release further retarded with higher concentrations.

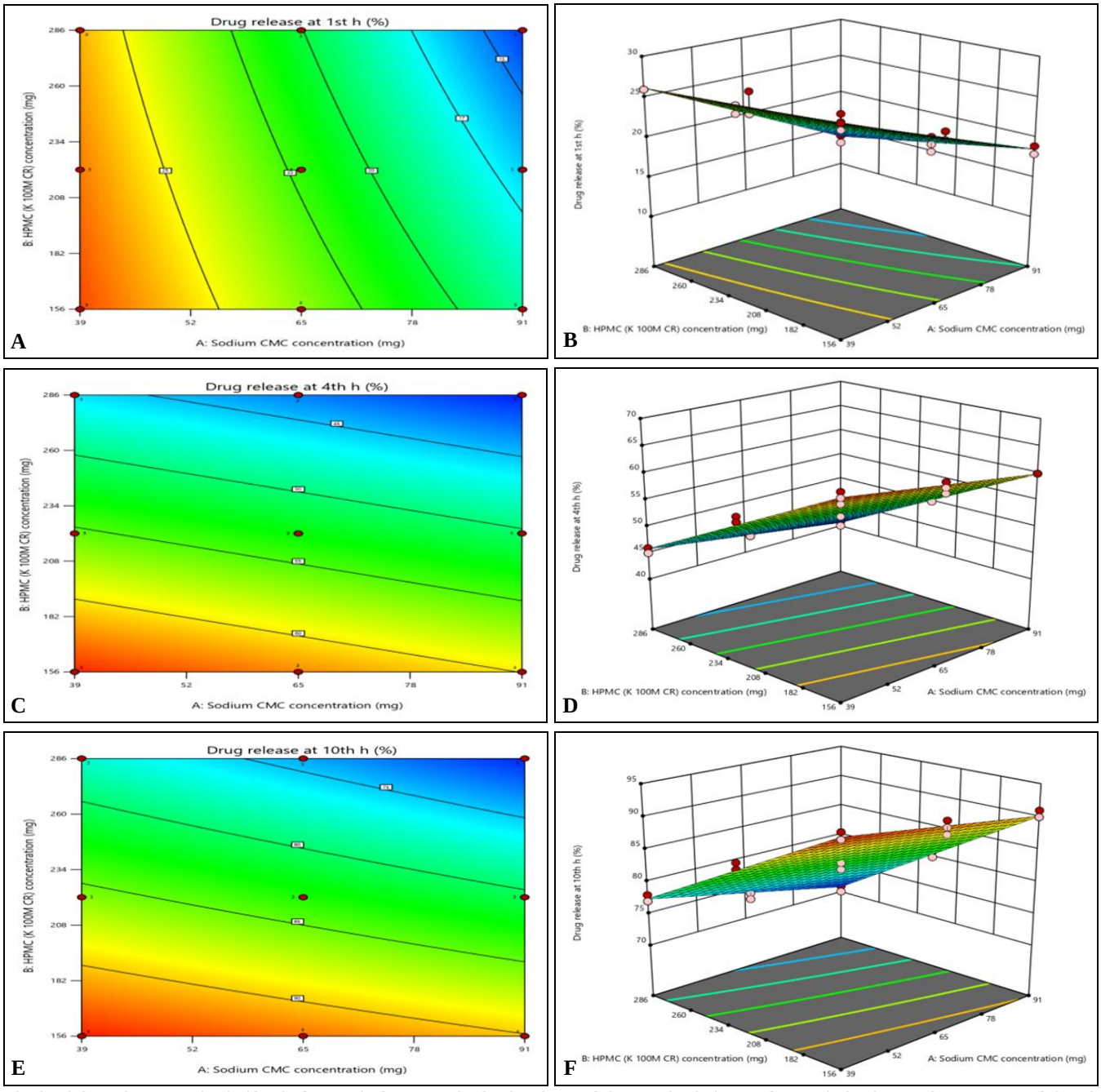


FIG. 2: CONTOUR PLOTS (A, C AND E) AND RESPONSE SURFACE PLOTS (B, D AND F) SHOWING THE IMPACT OF FACTORS (CONCENTRATION OF SODIUM CMC AND HPMC K 100M CR) ON % DRUG RELEASE AT 1, 4 AND 10 H

Among the studied range, the concentration of 5% w/w Sodium CMC and 17% w/w HPMC K 100M CR have shown drug release at 1, 4 and 10 h well within the predetermined specifications **Fig. 5**. Composition of optimized formulation of empagliflozin and metformin hydrochloride extended release tablets is presented in **Table 7**. The drug release profile of optimized formulation is presented in **Fig. 6**.

Evaluation of Lubricated Blend:

Micromeritic Properties: The bulk and tapped density of batches (ER part blend) range from 0.55-0.57 g/cc and 0.61-0.64 g/cc respectively. The Hausner's ratio values (1.11-1.12) indicated good flow properties according to USP limits.

Evaluation Tablets:

Assay: The assay (ER part) of the all formulations was tested, and results were found in the range of 98.5-101.2%. Assay of the optimized formulation was observed to be 99.6%.

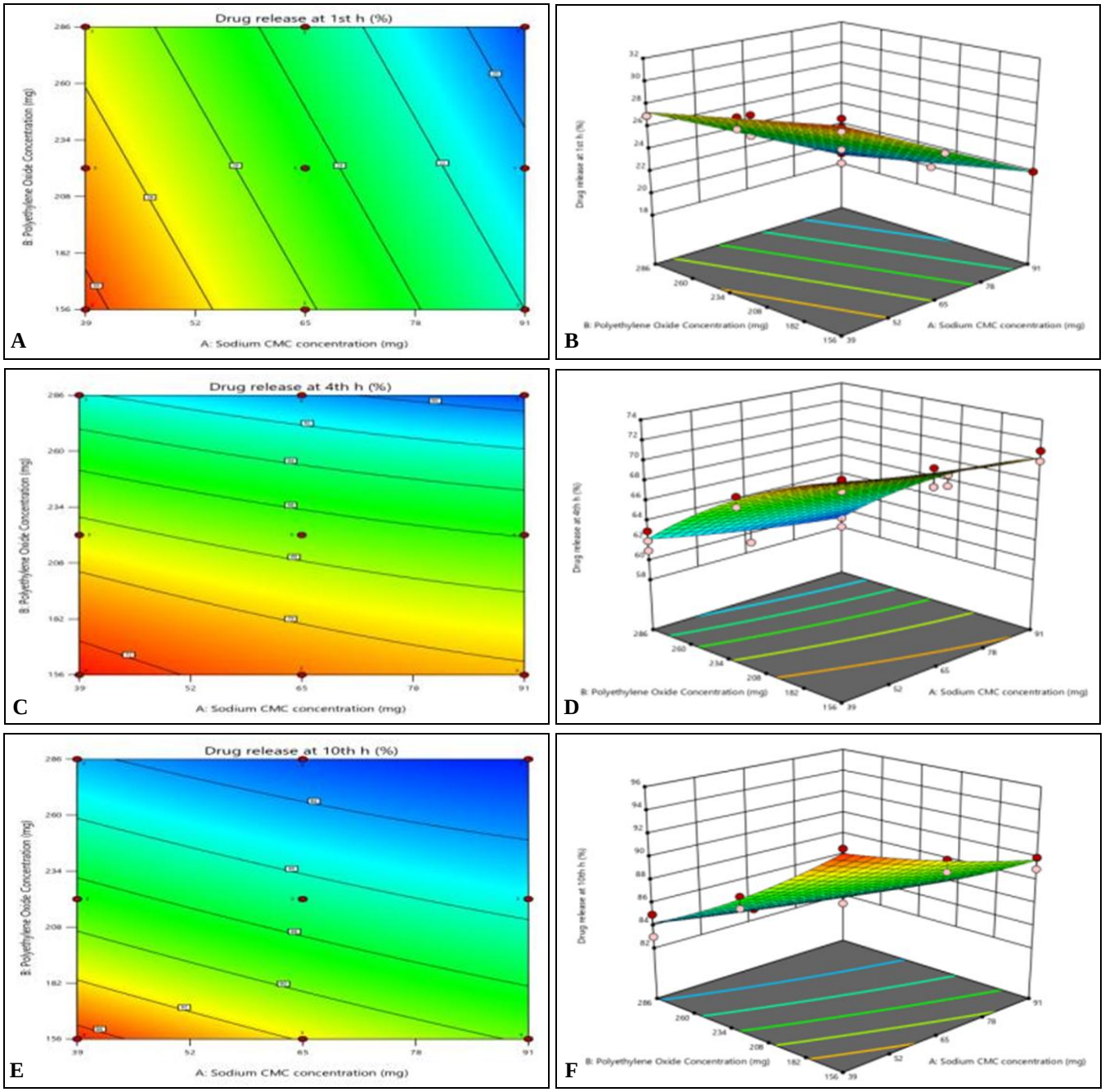


FIG. 3: CONTOUR PLOTS (A, C AND E) AND RESPONSE SURFACE PLOTS (B, D AND F) SHOWING THE IMPACT OF FACTORS (CONCENTRATION OF SODIUM CMC AND POLYETHYLENE OXIDE) ON % DRUG RELEASE AT 1, 4 AND 10 H

Drug Release Kinetics: The dissolution data of optimized formulation (ER part) fitted into kinetic models and obtained results concluded that the drug release followed the zero order kinetics as R^2 values were higher for zero order model (0.946) than first order model (0.861). The n value is >0.45 (0.576); hence, the mechanism of drug release was non-fickian diffusion.

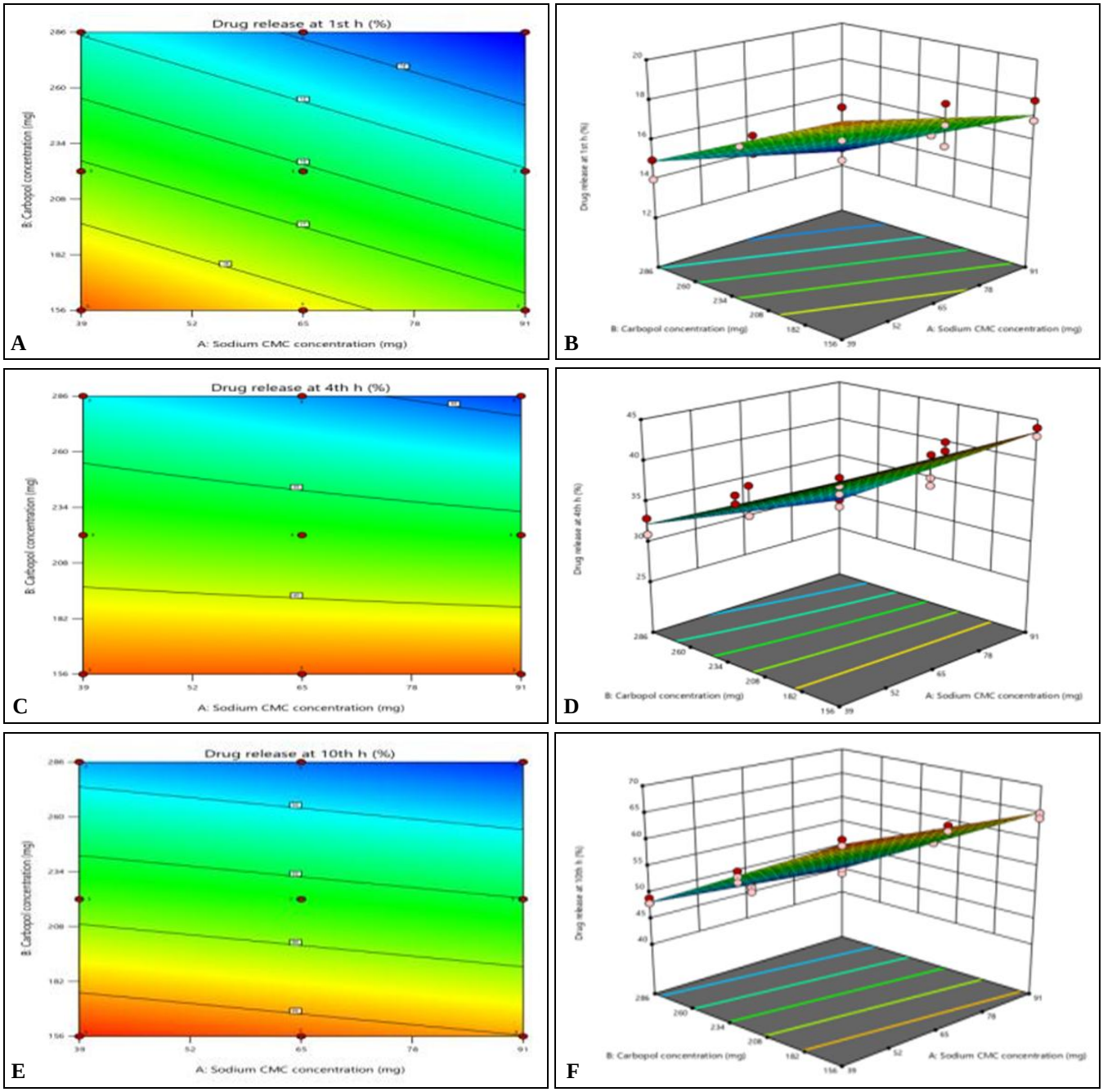
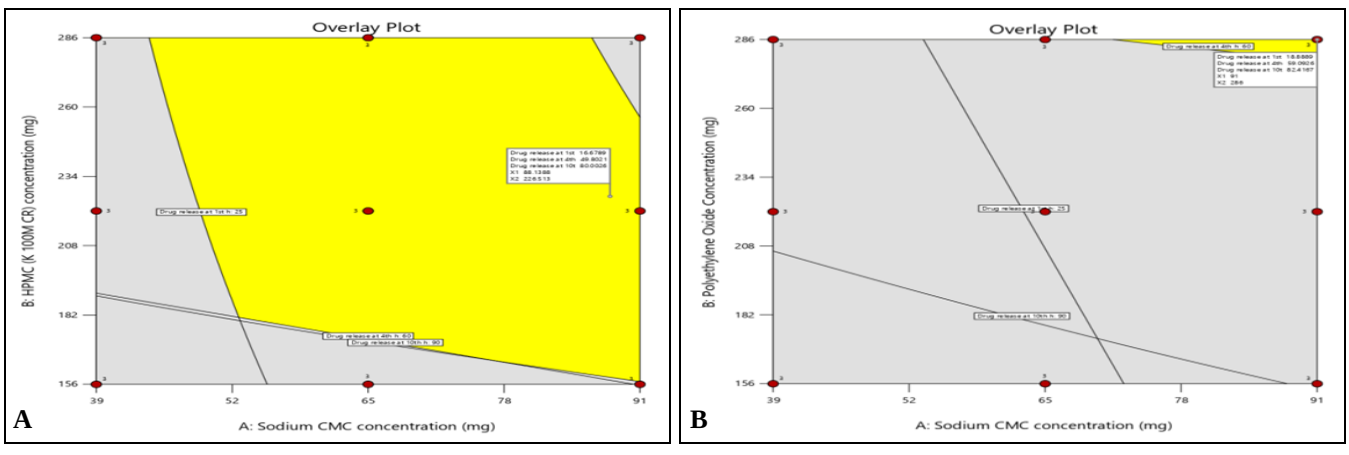


FIG. 4: CONTOUR PLOTS (A, C AND E) AND RESPONSE SURFACE PLOTS (B, D AND F) SHOWING THE IMPACT OF FACTORS (CONCENTRATION OF SODIUM CMC AND CARBOPOL) ON % DRUG RELEASE AT 1, 4 AND 10 H



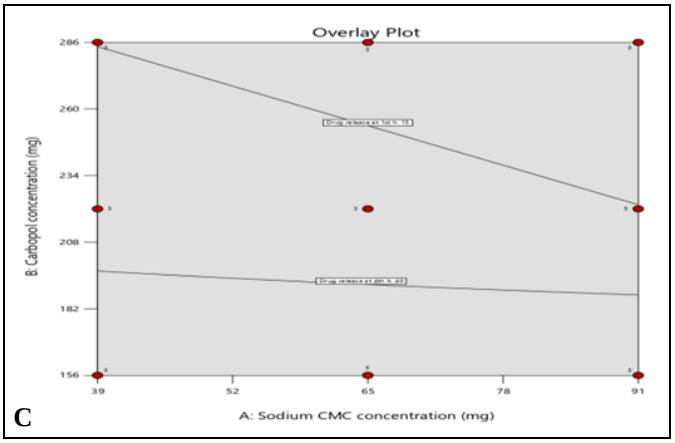


FIG. 5: OVERLAY PLOTS OF: (A) HPMC K 100M CR; (B) POLYETHYLENE OXIDE AND (C) CARBOPOL

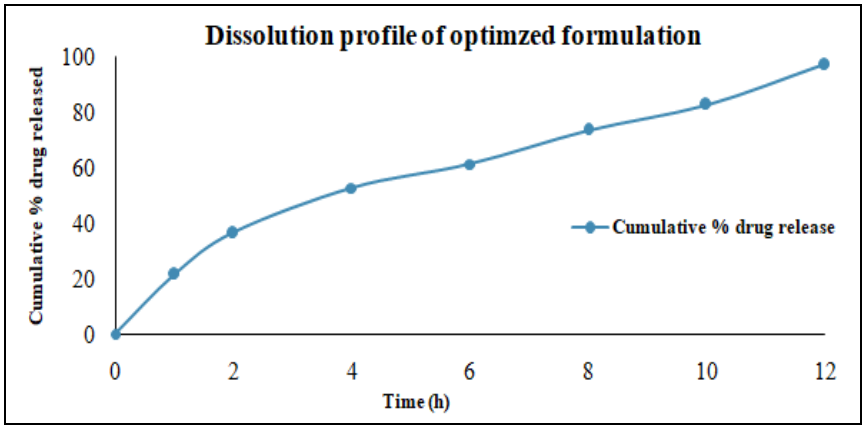


FIG. 6: DISSOLUTION PROFILE OF THE OPTIMIZED FORMULATION (METFORMIN PART)

TABLE 7: OPTIMIZED FORMULATION COMPOSITION OF EMPAGLIFLOZIN AND METFORMIN HYDROCHLORIDE EXTENDED RELEASE TABLETS

S. no.	Ingredients	Qty. (mg/tablet)
I	**Empagliflozin part (IR part)**	
	Pre-lubrication	
1	Empagliflozin	25.00
2	Lactose spray dried (Supertab 11SD)	59.00
3	Microcrystalline cellulose (PH112)	90.00
4	Hydroxy propyl cellulose (Klucel LF)	19.80
5	Croscarmellose sodium (Ac-Di-Sol)	22.00
6	Colloidal silicon dioxide (Aerosil 200 pharma)	2.00
7	Iron oxide yellow	0.20
	Lubrication	
8	Magnesium Stearate	2.00
	Empagliflozin part weight	220.00
II	**Metformin hydrochloride part (ER part)**	
	Intragranular	
9	Metformin hydrochloride	1000.00
10	Sodium CMC (7HF PH)	65.00
	Binder	
11	Purified water [&%]	213.00
	Extra granular	
12	HPMC K 100M CR	221.00
13	Colloidal silicon dioxide (Aerosil 200 pharma)	7.50
14	Magnesium Stearate	6.50
	Metformin hydrochloride part weight	1300.00
	Total tablet weight	1520.00

[&] 20% w/w to intragranular weight (water uptake)
[%] It is not present in the final product, except in traces.

CONCLUSION: Empagliflozin and metformin hydrochloride ER tablets consisting of immediate release empagliflozin and extended release metformin hydrochloride were successfully fabricated by granulation technology. DSC studies evidenced that there was no interaction between selected drugs and excipients. The effect of two independent variables (binder concentration and ER polymer concentration) on three responses were studied and optimized systematically using RSM.

This investigation revealed that independent variables had a significant impact on the measured responses. The quantitative effect of these factors at different levels on drug release could be predicted by polynomial equations. Linearity observed between the actual and predicted values of the response variables indicated that analytical ability of the selected design. The optimized batch showed 99.6% assay and drug release was well within the predetermined specifications. Micromeritic properties of lubricated blend exhibited excellent flow properties, which are crucial to attain the uniformity of dosage units during compression. The optimized formulation can be used as an alternative to the marketed formulation.

Hence, the applicability of RSM to optimize the formulation variables in the fabrication of empagliflozin and metformin hydrochloride ER tablets is apt enough.

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