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Formulation development and evaluation of modified oral drug delivery system of tolterodine tartrate microsponges

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ABSTRACT: **ABSTRACT**: The present study was carried out with the objective of formulation and evaluation of a multi particulate drug delivery system of Tolterodine Tartrate microsponges by Quashi emulsion solvent diffusion method. The response surface methodology (RSM) with central composite design (CCD) was employed to study the effect of 5 independent variables including amount of rate retardant polymers (mg), internal phase volume (ml), emulsifier (W/V%) and Rotational speed (RPM) on the dependant variables production yield, drug entrapment, particle size, and % drug release at time interval of 1 st , 2 nd , 4 th and 8 th h . The results showed that the optimized quantities of formulation components for extraction of microsponges included rate retardants (Eudragit RSPO : 209.55 mg, HPMCK4M: 121.07 mg), internal phase volume(in equal ratio) that is(Dichloromethane : ethanol = 9.21 ml), emulsifier(Dibutyl phthalate:1.44 w/v%) and the process variable rotations per minute (RPM): 750. In vitro release data obtained from the optimized formulation was fitted into various kinetic models. The optimized formulation showed desired % yield (94.90%), Drug entrapment (97.56%),Particle size(194.00 µm), cumulative % drug release at 1st, 2 nd, 4th and 8 th hr with 20.74%, 40.85%, 71.02%, and 91.02% respectively. Tolterodine Tartrate microsponge Tablets showed first order rate kinetics with Higuchi mechanism of diffusion process. Conclusion: Extended release tablets of Tolterodine tartrate microsponges were successfully developed by employing Quasi emulsion solvent diffusion technique. The response surface method with central composite design facilitated the formulation and optimization of modified oral drug delivery system of Tolterodine tartrate.

KEYWORDS: response surface method -1; central composite design -2; Tolterodine tartrate -3; modified drug delivery systems -4; microsponges -5.

1. INTRODUCTION

Microsponge drug delivery systems are gaining importance over single unit dosage forms since they do not risk fluctuations in the plasma concentration of drugs, results in minimal risk to local irritations, irritation at applied sight, have less intra and inter subject variability, can incorporate into various dosage forms and due to their increased bioavailability [1]. Microsponges are extremely cross-linked, non-collapsible, porous, polymeric microspheres which have particle size ranging from 5 to 300 µm. This can help to entrap a wide range of active ingredients and then release those over extended time [2]. The unique dissolution and compression properties of microsponges can be attributed to their sponge-like texture [3].

Tolterodine tartrate is an anti-muscarinic agent with targeted activity on muscarinic acetyl choline receptors (M1, M2, M3, M4 and M5) of urinary bladder. It has more affinity on M2 receptors to treat overactive and unstable bladder. However, it has a lower affinity for M3 receptors and causes dry mouth. Tolterodine tartrate is well absorbed from the intestine due to its maximum solubility at pH 6.8 and above [4]. Oral tolterodine tartrate immediate-release dosage forms reach maximum plasma concentration within 2 hours due to its short half-life (1.9 to 3.7 hours). These reasons led to the development of modified extended-

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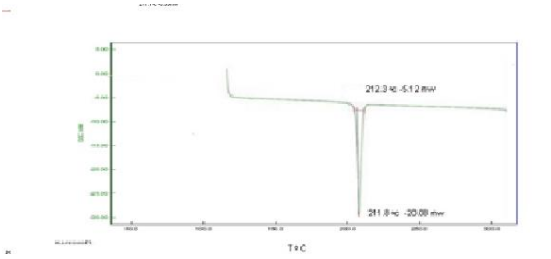
release oral dosage forms that provide patient compliance, stability, and consistent plasma concentrations [5]. Quality by design is a potential approach in the development of pharmaceuticals in a more scientific, risk-based manner, by restricting the flexibility in the manufacturing process to ensure predetermined product specifications [6]. Response surface methodology (RSM) is one of the important methods in the formulation development and the optimization of formulation in drug delivery systems. RSM involves the use of various types of experimental designs, generation of polynomial mathematical relationships, and mapping of the response over the experimental domain to find out the optimum formulation. Central composite design, Box-Behnken design, one factor and D-optimal design are the different types of RSM designs available for statistical optimization of the formulations. Central composite design (CCD) facilitates assessment of all factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interactions between them. Hence Central composite design was selected for the study to reduce cost, time and resources [7].

Many formulations of once daily sustained /extended-release capsules or tablets are available in the market with film coating or pellet form. However, they have their own disadvantages in terms of stability and manufacturing. The present study was carried out with the aim of determining the optimum conditions for various experimental factors such as the amount of release inhibiting polymers (Eudragit RSPO, HPMC K4 M), volume of internal phase (dichloromethane: Ethanol), plasticizer (di-butyl phthalate), and stirrer revolutions per minute (RPM) during the preparation of tolterodine tartrate microsponges using RSM with CCD, including limitations on percent product yield, percent drug entrapment efficiency, particle size, and percent drug release at the first, second, fourth, and eighth hours. The independent variables for this study were the amount of rate retarding polymers (mg), volume of internal phase (ml), emulsifier (W/V%), and rotation speed (RPM). The dependent variables studied were percent yield, percent drug entrapment, particle size, percent drug release in the first hour (up to 30%), second hour (30-50%), fourth hour (65-90%), and eighth hour (80-100%).

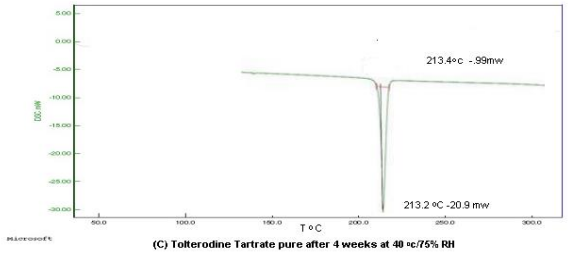
2. RESULTS AND DISCUSSION

2.1. Drug Excipient Compatibility Studies

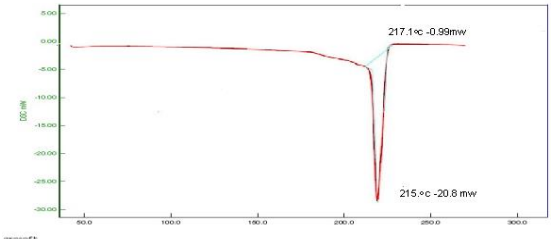
DSC thermograms of Tolterodine Tartrate API (active pharmaceutical ingredient) and formulation blend initially revealed endothermic peaks at 211.8 °C and 215.4 °C, respectively. At the end of 4 weeks, DSC thermo grams of Tolterodine tartrate and formulation blend showed endothermic peaks at 213.2 °C and 220 °C (at 40 °c/75% RH) respectively. It was found that there were no interactions between the excipients and the drug and the components were stable.



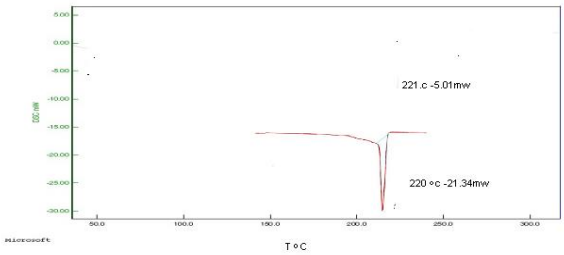
(a) Tolterodine Tartrate pure initial



(C) Tolterodine Tartrate pure after 4 weeks at 40 °c/75% RH



(b) Optimized formulation blend initial



(d) Optimized formulation blend after 4 weeks at 40°c /75% RH

Figure 1. (a) DSC thermogram of Tolterodine tartrate API and (b) formulation blend initial (c) DSC thermogram of Tolterodine tartrate API (d) formulation blend after 4 weeks at 40 ^{o}C/75 % RH

2.2. Preparation and evaluation of Tolterodine Tartrate ER microsponges by central composite design

In the present study Tolterodine tartrate microsponges were prepared by Quashi-emulsion diffusion method as it suited best for highly water-soluble drugs. The external phase (PVA solution) and internal phase (DM: ETH solution) were selected based on dielectric constant values as per previous studies. The micrometrics of prepared microsponges included bulk density (0.66 to 0.69) g/ml, tapped density (0.70 to 0.79) g/ml and Hausner's ratio (1.14 to 1.16) were within the range. Total intrusion volume, total pore area and average pore diameter of microsponges were in the range of 0.8 to 1.02 ml/g 20.28 to 60.12 m^2/g 0.2 to o.53 µm Table 2 showed 50 formulation runs as well as the responses include % production yield, % drug entrapment efficiency, particle size and % drug release at various time intervals (1 st h, 2 nd h, 4 th h and 8 th h).

2.3. The evaluation parameters of directly compressed tablets

The prepared tablets were tested for thickness (4.89 ± 0.01 to 5.02 ± 0.01) mm, average weight of tablet (200±0.01to 200±0.04) mg, hardness (4.48±0.01 to 5±0.01) Kg/cm^2, % friability (0.06±0.001 to 0.08±0.001) % and % drug content (92.89±0.2 to 95.89±0.2% were within the range.

From Table -2, it was observed that percent production yield ranges from 35.23%to 96.34 %, percent drug entrapment ranges from 38.21% to 97.55%, particle size ranges from 190.23 µm to 214.22 µm. Total intrusion volume, total surface area and average pore diameter of microsponges were 0.5 to 3.5 ml/g, 20.02 to 60.12 m^2/g and 0.09 to 0.23 µm Percent drug release at first h 8.76% to 30.56 %, % drug release at 2nd h 28.56 % to 49.34%, % drug release at 4 th h 58.89% to 79.89 % and % drug release at 8 th h 78.89% to 100.33%. Lower amounts of independent variables drastically affecting the responses with low production rates and higher drug release rates.

Table 2. Formulation composition of independent variables and responses as dependent variables

Trail	A: ERSPO mg	B: HPMC K4M mg	C: DM:ETH ml	D: Di.but. phthalate %w/v	E: RPM	% YIELD	% DE	P.SIZE (um)	%DR 1st h	% DR 2 nd h	% DR 4 th h	% DR 8 th h
F1	100	50	5	1	500	43.34	54.86	201.45	19.67	39.45	69.02	89.12
F2	250	50	5	1	500	83.42	88.68	205.23	15.45	35.47	65.32	85.32
F3	100	150	5	1	500	67.45	74.76	203	17.12	37.45	67.67	87.54
F4	250	150	5	1	500	88.12	90.56	207.45	15.12	30.01	60.45	80.45
F5	100	50	10	1	500	59.21	64.32	200	22.37	42.23	73.67	93.21
F6	250	50	10	1	500	88.34	89.01	204.34	16.68	36.04	67.12	87.44
F7	100	150	10	1	500	81.67	82.97	201.21	19.12	39.34	69.22	89.12
F8	250	150	10	1	500	94.89	95.87	206.56	15.53	32.88	62.56	82.12
F9	100	50	5	1.5	500	49.34	58.32	198.5	20.27	40.35	70.86	90.78
F10	250	50	5	1.5	500	85.78	88.98	204.00	16.62	36.12	66.23	86.34
F11	100	150	5	1.5	500	68.32	75.34	202.57	18.22	38.56	68.34	88.24
F12	250	150	5	1.5	500	90.45	97.55	207.22	15.42	31.02	61.34	81.25
F13	100	50	10	1.5	500	59.56	66.34	200.32	24.21	44.02	74.67	94.36
F14	250	50	10	1.5	500	88.43	89.12	207.01	17.87	37.78	67.89	87.98
F15	100	150	10	1.5	500	84.54	80.68	206.56	22.35	42.46	72.45	92.54
F16	250	150	10	1.5	500	95.78	97.45	206.44	15.78	31.02	61.56	81.74

F17	100	50	5	1	750	54.34	62.45	196.02	22.87	44.23	74.24	94.31
F18	250	50	5	1	750	87.23	90.12	201.21	17.05	37.86	67.34	87.25
F19	100	150	5	1	750	73.45	77.56	199.22	20.43	40.83	71.02	91.12
F20	250	150	5	1	750	90.12	93.22	200.56	15.67	35.67	65.89	85.02
F21	100	50	10	1	750	63.75	66.34	191.23	28.22	48.32	78.76	98.79
F22	250	50	10	1	750	90.12	93.73	197.67	19.01	39.78	69.24	89.23
F23	100	150	10	1	750	84.45	85.45	194.23	26.45	46.78	76.56	96.78
F24	250	150	10	1	750	95.43	95.45	197.12	17.11	37.11	67.12	87.17
F25	100	50	5	1.5	750	63.33	66.54	194.34	23.68	43.78	73.54	93.23
F26	250	50	5	1.5	750	91.67	95.78	198.78	18.45	38.78	68.23	88.34
F27	100	150	5	1.5	750	76.67	80.01	196.54	21.34	41.24	71.67	91.34
F28	250	150	5	1.5	750	93.34	94.23	198.22	16.78	36.34	66.78	86.77
F29	100	50	10	1.5	750	64.53	70.35	190.23	29.98	49.23	79.89	99.34
F30	250	50	10	1.5	750	91.34	95.45	195.98	20.12	40.89	71.67	91.22
F31	100	150	10	1.5	750	85.21	85.45	193.34	28.32	48.13	78.78	98.32
F32	250	150	10	1.5	750	96.34	95.78	196.21	18.78	38.34	68.77	88.65
F33	-3.381	100	7.5	1.25	625	35.23	38.21	193.78	25.73	45.56	75.21	95.34
F34	353.4	100	7.5	1.25	625	93.98	91.78	205.23	8.76	28.56	58.89	78.89
F35	175	-18.92071	7.5	1.25	625	65.56	69.34	196.24	22.21	42.22	72.34	92.56
F36	175	218.92071	7.5	1.25	625	90.77	92.08	202.21	16.23	36.34	66.45	84.76
F37	175	100	1.55396	1.25	625	76.67	79.56	204.22	22.45	42.67	72.32	92.45
F38	175	100	13.44604	1.25	625	93.32	94.23	201.22	30.56	49.34	79.22	100.33
F39	175	100	7.5	0.655396	625	90.23	90.23	201.67	19.23	39.45	69.45	87.42
F40	175	100	7.5	1.844604	625	89.34	92.22	198.12	18.45	38.33	68.55	88.45
F41	175	100	7.5	1.25	328	65.87	76.89	214.22	15.56	34.67	64.43	84.34
F42	175	100	7.5	1.25	922	83	91.57	194	22.02	42.89	72.25	92.78
F43	175	100	7.5	1.25	625	88.56	91.11	196.12	19.21	39.24	69.19	89.02
F44	175	100	7.5	1.25	625	88.03	91.34	196.41	19.54	39.57	69.72	90.34
F45	175	100	7.5	1.25	625	89.12	92.97	197.7	18.21	38.18	68.02	88.47
F46	175	100	7.5	1.25	625	86.97	90.35	195.34	20.65	40.74	70.93	90.02
F47	175	100	7.5	1.25	625	86.56	89.68	194.42	21.79	41.98	71.98	91.57
F48	175	100	7.5	1.25	625	89.56	92.84	197.52	18.85	38.89	69.84	90.09
F49	175	100	7.5	1.25	625	88.56	91.04	196.12	18.87	38.98	68.02	88.45
F50	175	100	7.5	1.25	625	87.16	90.23	195.02	20.21	40.34	70.44	90.08

The significance of the coefficients of the formulation runs were analyzed using the P values ($P \leq 0.0001$ to $P \leq 0.05$) of the analysis of variance (ANOVA), and the obtained R-squared values (0.900799831 to 0.991826) were statistically accepted, as shown in Table 3. In the regression equations, the factors together with the coefficients (positive/negative) quantify the response values. A positive sign of coefficient indicates an additive effect whereas negative sign represents an opposite effect. Factors A, B, C, D and E were found to follow second order quadratic polynomial model for all the dependent variables with a linear interaction and quadratic pattern among the independent variables.

The results showed that the amounts of the polymers (Eudragit RSPO and HPMCK4M) affected the dependent variables to the desired predicted values in all formulation runs. Hence the results were mentioned with the formulations containing (250 mg of Eudragit RSPO and 150 mg of HPMCK4M) and compared with maximum and minimum values of responses. Effect of independent variables on responses were explained by the following second order quadratic polynomial equation.

$$Y = \beta_0 + \beta_1 X_1 + \beta_1 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{15} X_1 X_5 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{25} X_2 X_5 + \beta_{34} X_3 X_4 + \beta_{35} X_3 X_5 + \beta_{45} X_4 X_5 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{55} X_5^2.$$

Table 3. β coefficient values of independent variables based on ANOVA results for predicting the responses

Source	% Yield	% DE	Particle size	% DR 1st h	% DR 2 nd h	% DR 4 th h	% DR8 th h
Y-Intercept	88.0051	91.176852	196.02596	19.641362	39.668084	69.731769	89.726087
X_1-E RSPO	11.80624***	10.773763***	2.1349556***	-3.0831276***	-3.5028874***	-3.501564***	-3.485615***
X_2-HPMC K4M	6.059509***	4.9874543***	1.0236744***	-0.9974421***	-1.4114487***	-1.4203092***	-1.5385806***
X_3-DM:ETH	3.620577***	2.3016117***	-0.7617737***	1.5475225***	1.4557521***	1.5792011***	1.6237793***
X_4-Di butyl phthalate	0.858463*	0.8485315**	-0.4313501*	0.4263047*	0.2758059	0.3539163*	0.4363461**
X_5-RPM	2.618622***	2.0320384***	-3.9029569***	1.5658912***	2.1392896***	2.071612***	2.0640998***
$X_1 X_2$	-3.94438***	-3.233125***	-0.594375*	0.214375	-0.4221875*	-0.3009375	-0.3690625*
$X_1 X_3$	-1.75438***	-1.22875**	0.099375	-0.846875***	-0.6890625*	-0.7278125**	-0.6865625**
$X_1 X_4$	-0.26188	0.105625	-0.0725	-0.1225	-0.1146875	-0.0815625	0.0040625
$X_1 X_5$	-0.9975*	-0.625625	-0.12625	-0.733125***	-0.1328125	-0.1871875	-0.2284375
$X_2 X_3$	0.73625*	0.216875	-0.01125	-0.033125	0.0840625	-0.1334375	-0.0678125
$X_2 X_4$	-0.28625	-0.335	0.179375	0.0175	-0.0165625	0.0290625	0.0815625
$X_2 X_5$	-0.78437*	-0.91125*	-0.318125	-0.000625	0.3215625	0.4646875*	0.4690625*
$X_3 X_4$	-0.73625*	-0.533125	0.553125**	0.1725	0.1303125	0.1684375	0.1290625
$X_3 X_5$	-1.09938**	-0.269375	-0.996875***	0.490625*	0.3909375	0.3803125	0.3953125*
$X_4 X_5$	0.243125	0.20375	-0.53125*	0.03	-0.0721875	0.0265625	-0.0428125
X_1^2	-4.20108***	-4.647878***	0.5554961**	-0.4504133**	-0.5384661**	-0.5125387*	-0.4927044**
X_2^2	-1.80399***	-1.869832***	0.5059986**	-0.1012794	-0.1460219	-0.0979973	-0.2195844
X_3^2	-0.5966*	-0.776468*	1.1238332**	1.1865389***	1.0428014***	1.0289541***	1.1468994***
X_4^2	0.250159	-0.011025	0.624439***	-0.1684545	-0.2149648	-0.1678241	-0.3477475*
X_5^2	-2.46336***	-1.247578***	1.3695528***	-0.1772933	-0.2344102	-0.2844968	-0.2372621
R-Squared	0.991826	0.9893783	0.9706109	0.9695412	0.9679162	0.9670491	0.978306
Adj R-Squared	0.986189	0.9820529	0.9503425	0.9485351	0.9457894	0.9443243	0.9633446
Pred R-Squared	0.968428	0.9616859	0.9100364	0.9031216	0.9007998	0.904209	0.9391152

*P≤.05 * P≤0.01** P≤0.0001****

Regression equation of fitted model for % production yield (Y_1) = 88.005096+ 11.80624* X_1+ 6.0595094* X_2+ 3.6205765* X_3+0.8584629* X_4+ 2.618622* X_5 -3.944375* X_1* X_2-1.754375* X_1* X_3-1.3975* X_1 * X_5 0.73625 * X_2* X_3 -0.78437* X_2* X_5 -0.73625* X_3 * X_4-1.099375* X_3 * X_5+ 0.8-4.20108*X_1^2 -1.80399*X_2^2 -0.5966*X_3^2 -2.46336*X_5^2.

Increase in amount of polymer increases the production yield at a constant amount of active ingredient as in formulation F8, because the diffusion of the bridging dichloromethane from the concentrated dispersed phase into the dispersion medium is slower. Further increase in the amount of polymer is not beneficial due to the high viscosity differences between the inner and outer media. Increasing the inner phase volume, surfactant concentration (at a constant retardation rate), and rotational speed increases the production yield by reducing the viscosity of the polymer solution in F8 compared to F4. However, the volume of the internal phase must be proportional to the retardants to produce microsponges with high mechanical strength, otherwise microsponge production is not possible.

Regression equation of fitted model for %Drug entrapment (Y_2) = +91.176852+10.773763* X_1 + 4.9874543 * X_2+ 2.3016117* X_3+0.8485315* X_4+ 2.0320384* X_5 -3.233125* X_1 * X_2 -1.22875* X_1* X_3 -0.91125* X_2 * X_5 -4.6478775 * X_1^2-1.8698318*X_2^2-0.7764679*X_3^2-1.2475778*X_5^2.

The percent drug entrapment increases with the increase of retarders at constant amount of drug as in F14, since the high ratio of drug to polymer facilitates slow diffusion of the internal phase into the dispersion medium and availability of the polymer to each drug particle. Thus, the largest possible amount of the dispersed phase is converted into microsponges. Increasing the volume of the internal phase, surfactant concentration, and rotational speed balanced the viscosity of the internal phase and controlled the particle size at high polymer rates.

Regression equation of fitted model for particle size(μm) (Y_3) =+ 196.02596 +2.1349556* X_1+1.0236744* X_2-0.7617737* X_3-0.4313501* X_4-3.9029569* X_5-0.594375* X_1* X_2 0.553125* X_3 * X_4 -0.996875* X_3 * X_5 -0.53125* X_4 * X_5+0.5554961 * X_1^2+ 0.505998648 * X_2^2+1.123833198 * X_3^2+0.624439034 +0.624439034 *X_4^2+ 1.369552805 * X_5^2.

Increse in amounts of internal phase volume (DM:Eth), plastisizer (Dibutyl phthalate) and RPM decreasing the particle size by reducing the viscosity of polymer soluton, preventing flocculation and increasing the dispersion capcity of internal phase (drug and rate retardants) in external phase as in F31 where as in F32 partcle size incresed with increse in rate retardants due to high viscosity of dispersed phase. However, a slight increase in particle size was observed at maximum plasticizer amount in F32 compared to F24, which could be the reason for porosity. The increase in pore volume on the surface of the microsponges increases with plasticizer concentration, as shown in the SEM photos of the microsponges.
High concentration of plasticizer reduces the intermolecular forces in polymer chains and increases the mobility which leads to pore formation.

Regression equation of fitted model for % drug relaese at first hour (Y_4) = 19.64136163 -3.083127585 * X_1 - 0.997442117 * X_2 +1.547522522 * X_3 +0.426304686 * X_4+ 1.565891222 * X_5-0.846875* X_1 * X_3-0.733125 *X_1 * X_5 + 0.490625 * X_3* X_5 -0.4504133* X_1^2+1.1865389* X_3^2.

Percent drug release was decreased with higher amount of polymers as in F34. % Drug release at first hour increases with increase in volume of internal phase due to low viscosity by keeping other independents at constant level for F2 and F24 formulations. Increase in Plasticizer concentration and RPM fastens the drug release in F24 when compared to F8 due to decrease in particle size.

Regression equation of fitted model for % drug relaese at 2 nd hour (Y_5) = + 39.668084-3.5028874* X_1 - 1.4114487* X_2 + 1.4557521* X_3 +2.1392896* X_5 -0.4221875 * X_1 * X_2 -0.6890625 * X_1 * X_3 -0.53846613 * X_1^2 + 1.04280141*X_3^2

Percent drug release at 2 nd hr was decreased with higher polymer amounts as in F34. Slight increase in release was observed with increase in RPM for F24 when compared to F8 due to decreased particle size.
Regression equation of fitted model for % drug relaese at 4 th hour (Y_6) = +69.73176918 -3.50156395 * X_1 -1.420309226 *X_2+ 1.579201148 *X_3 + 0.3539163* X_4 + 2.071612 * X_5-0.7278125* X_1* X3 + 0.4646875* X_2* X_5 - 0.512538698* X_1^2 + 1.028954085* X_3^2.

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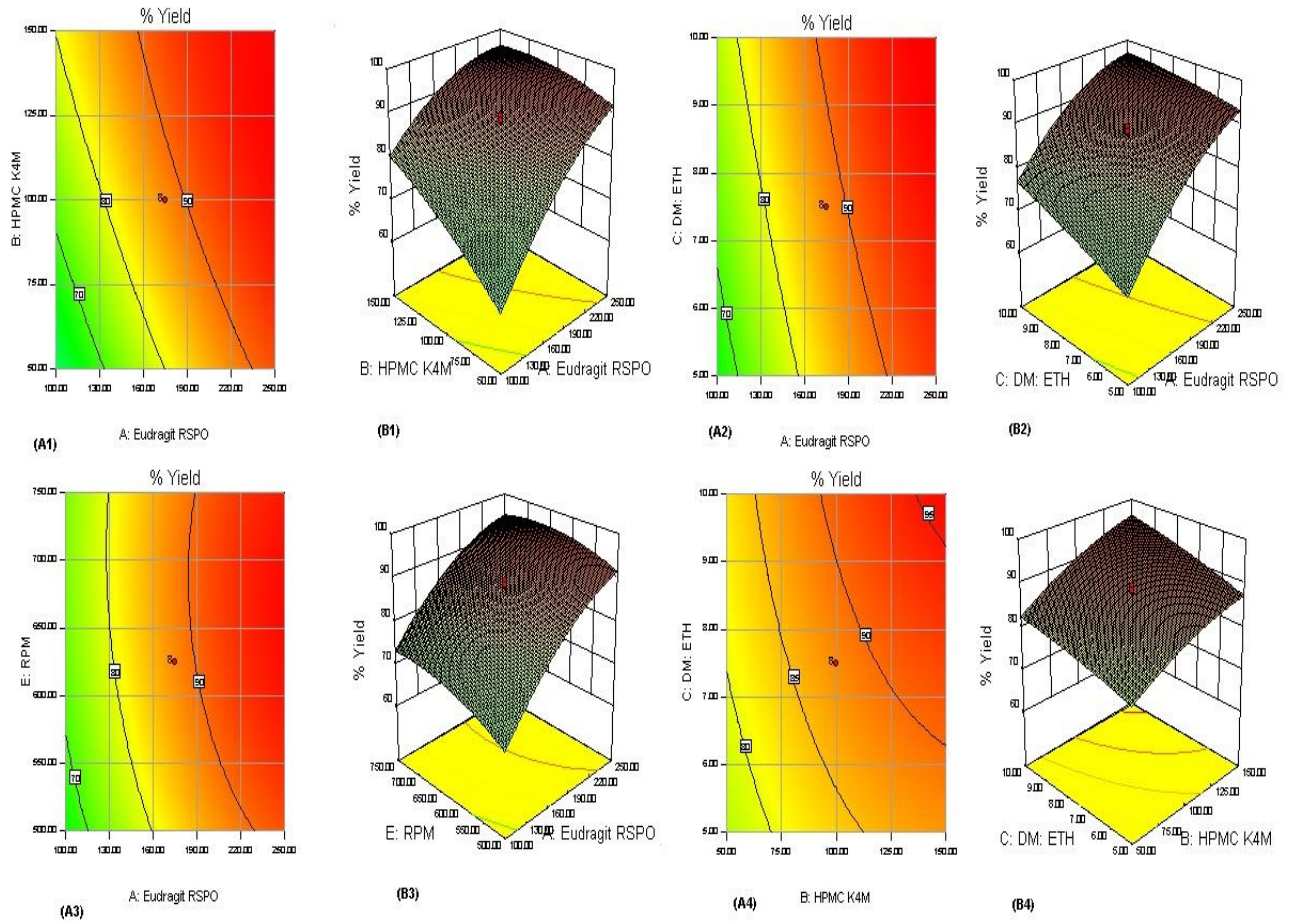
The percentage of active ingredient released after 4 hours decreased at high polymer volumes as in F34. F32 showed increased drug release at high levels of internal phase volume and plasticizer (di-butyl phthalate) compared to F24. This was due to the surfactant property and increased pore volume of the microsponges. Increasing the rotational speed reduces the particle size and thus increases the percent active ingredient release.

Regression equation of fitted model for % drug relaese at 8 th hour (Y_7) = 89.726087-3.485615*X_1-1.5385806 * X_2+ 1.6237793* X_3 +0.4363461* X_4 +2.0640998* X_5 -0.3690625* X_1* X_2 -0.6865625* X_1 * X_3 + 0.4690625* X_2* X_5+ 0.3953125* X_3* X_5-0.4927044* X_1^2 +1.1468994* X_3^2 -0.3477475* X_4^2

At 8th hour, drug release was siginificantley affected by the amount of drug and polymer ratio. F34 released lowest amout of drug. When compared to F24, F21 it showed faster drug release due to low amount of HPMCK4M which gave additive effect to viscosity of polymer network and high crosslinking ability due to aqueous swellability behaviour. The increase in internal phase volume, plasticizer concentration and rotational speed decreased the particle size and viscosity of the polymers and increased the pore opening interaction and consequently the drug release. The quadratic terms also showed significant effects in all formulation runs, as shown in Table 3.

2.4 . Contour and three-dimensional (3D) response surface plot analysis



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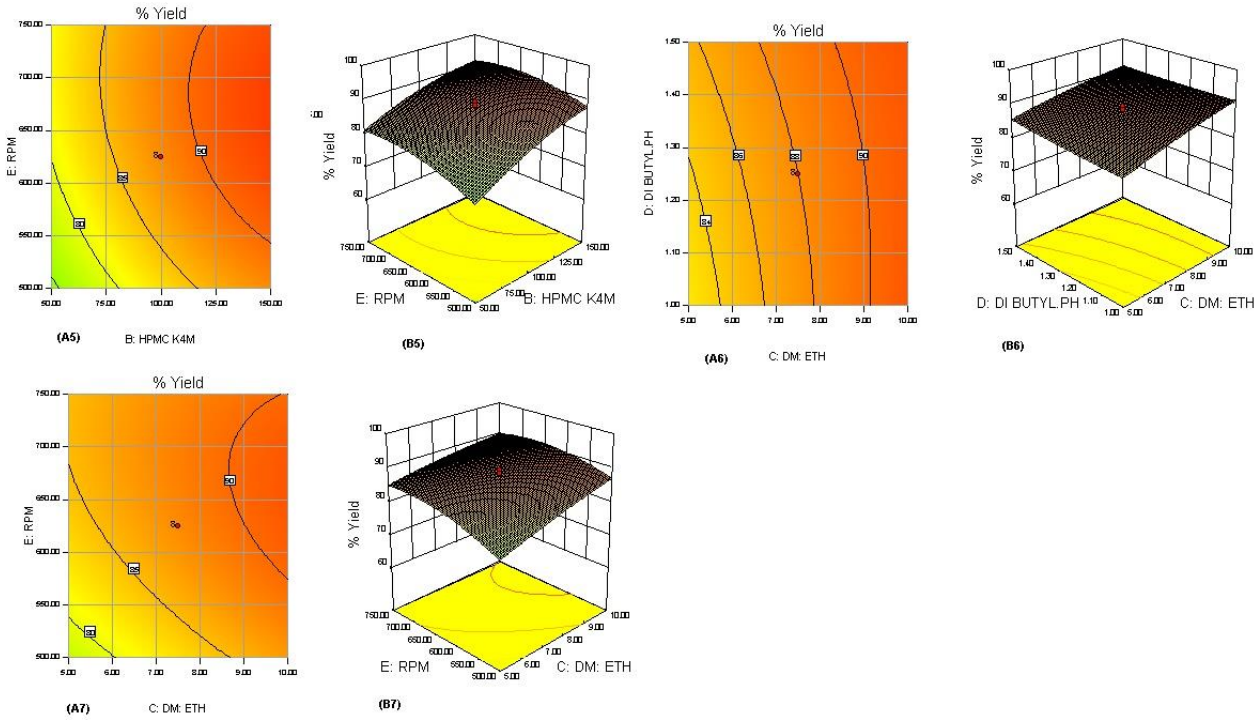

Figure 2. Contour plots (A1,A2,A3,A4,A5,A6 and A7) and response surface plots (B1,B2,B3,B4,B5,B6 and B7) showing interaction effect of ERSPO, HPMCK4M, (DM:ETH) , Dibutyl phthalate and RPM on % yield .

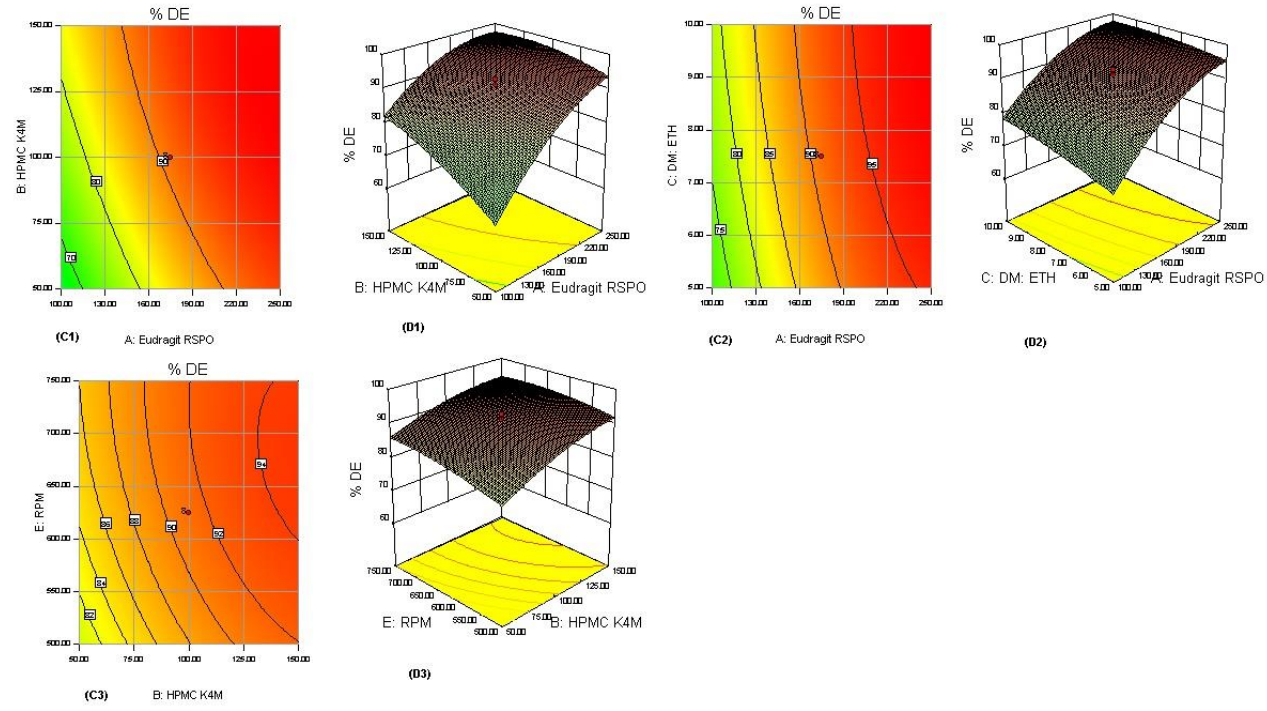

Figure 3. Contour plots (C1,C2 and C3) and response surface plots (D1,D2 and D3) showing interaction effect of ERSPO, HPMCK4M,(DM:ETH) and RPM on % DE.

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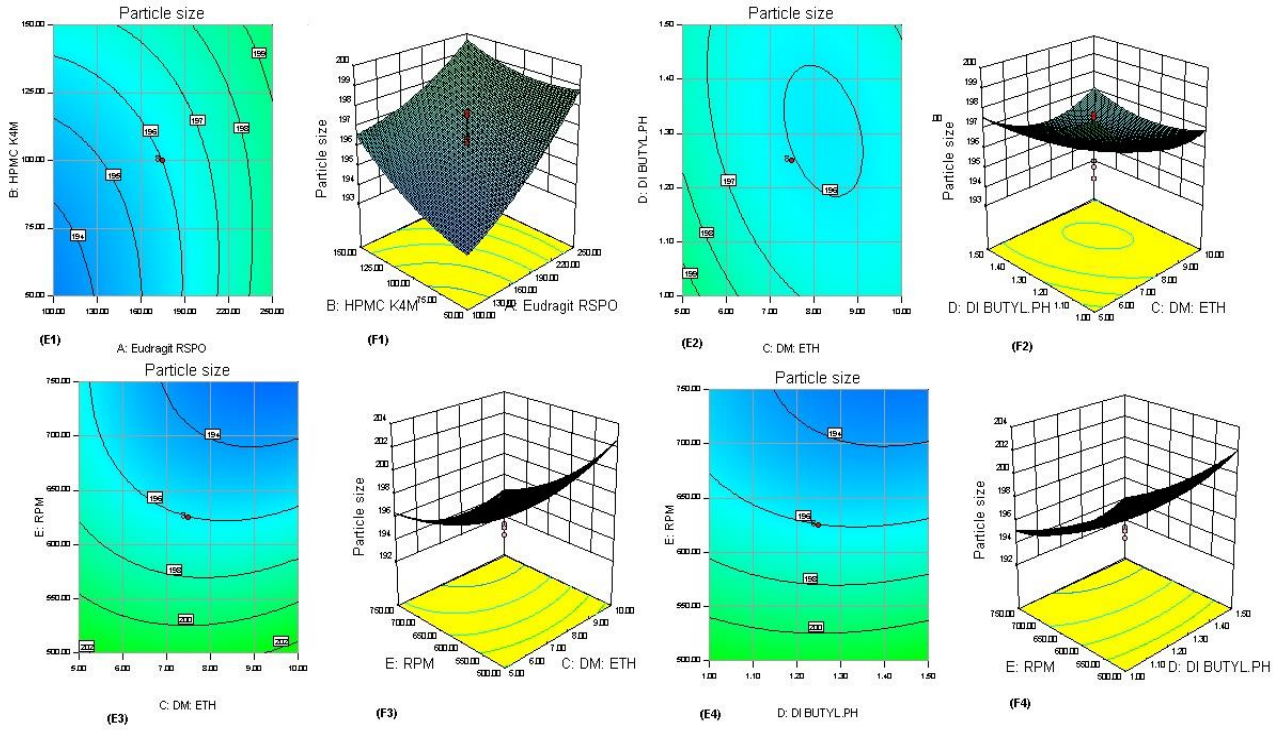
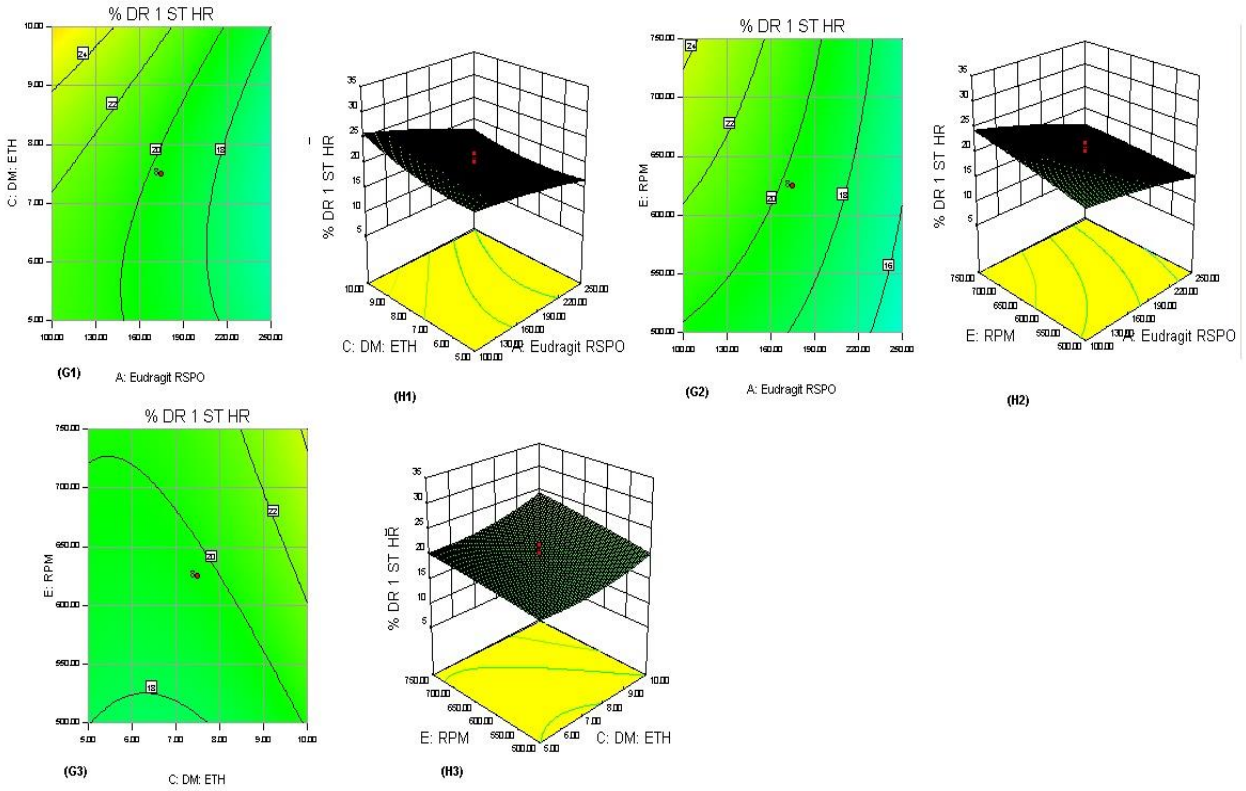


Figure 4. Contour plots (E1,E2, E3 and E4) and response surface plots (F1,F2, F3 and F4) showing interaction effect of Eudragit RSPO, HPMC K4M ,internal phase volume (DM:ETH),plasticizer (Dibutyl pthalate) and RPM on particle size



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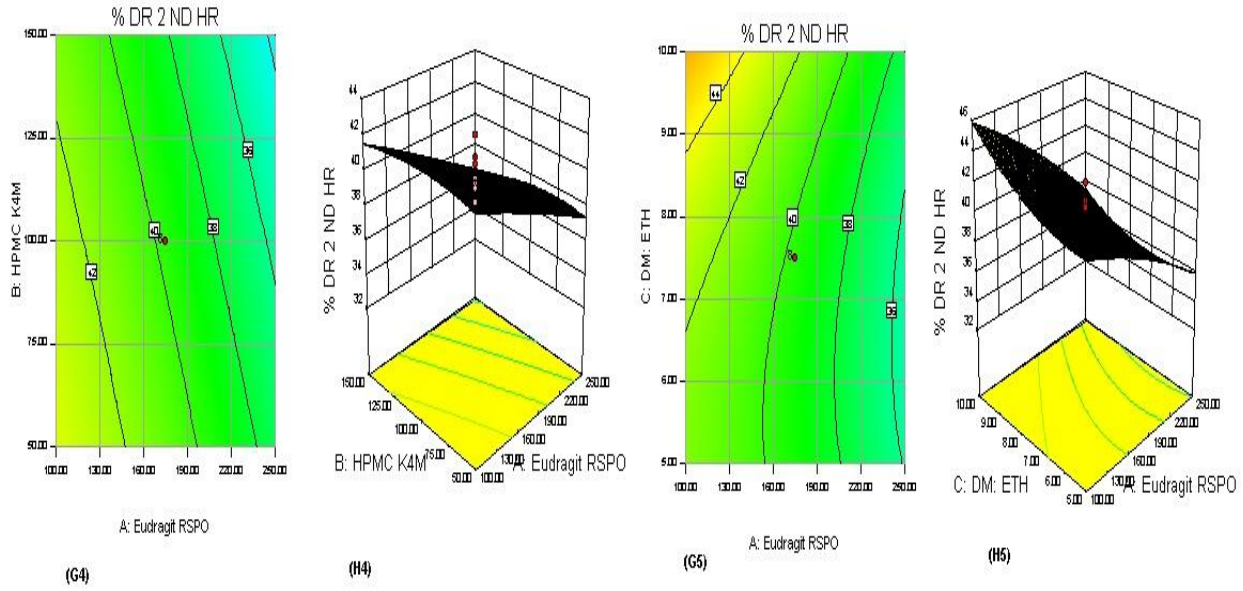

Figure 5. Contour plots (G1,G2,G3,G4 and G5) and response surface plots (H1,H2,H3,H4 and H5) showing interaction effect of ERSPO , HPMCK4M, internal phase volume (DM:ETH) and RPM on % drug release at 1st h and 2 nd h.

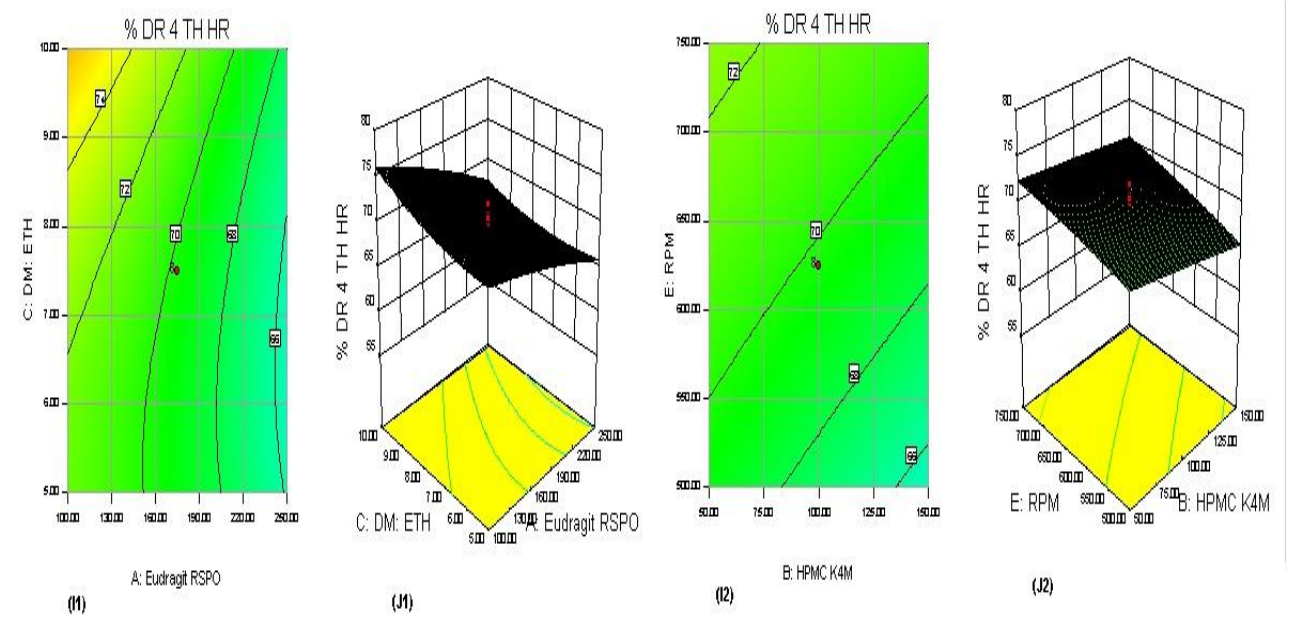

Figure 6. Contour plots (I1 and I2) and response surface plots (J1 and J2) showing interaction effect of ERSPO , HPMCK4M, internal phase volume (DM:Eth) and RPM on % drug release at 4 th h.

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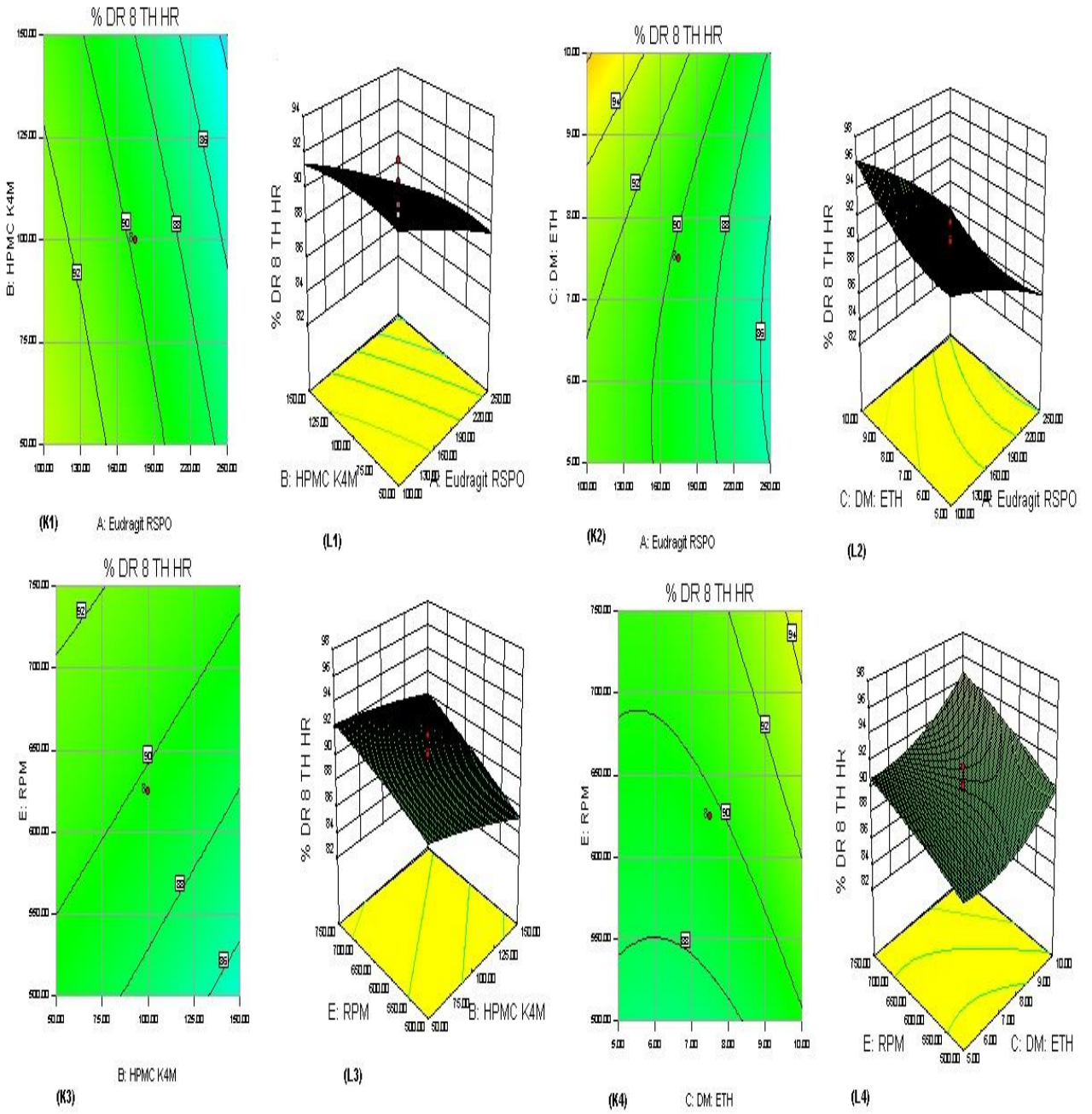

Figure 7. Contour plots (K1,K2,K3 and K4) and response surface plots (L1,L2, L3and L4) showing interaction effect of ERSPO , HPMCK4M, Internal phase volume (DM:ETH) and RPM on % drug release at 8 th h.

The optimized Tolterodine tartrate microsponges were developed and the tablets were prepared by direct compression technique. The observed results of optimized formulation were comparable with predicted values of DOE as in Table 4. The % cumulative drug release profiles of marketed brand (Kytolt 4 Psycho care Health Pvt. Ltd) and optimized formulation were shown in Table 5. The drug release kinetics was best fitted with first order Higuchi diffusion mechanism as shown in Table 6.

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Table 4. Validation of optimised formulation of Tolterodine tartrate microsponges

Independent variable	Name	Level	Response	Predicted value	Observed value	% Error
A	E RSPO (mg)	209.55	% Yield	94.89	94.90	0.01
B	HPMC K4M(mg)	121.07	% DE	97.54	97.56	0.02
C	DM:ETH (ml)	9.21	Particle size(um)	194.10	194.00	0.05
D	DI BUTYL PHTHALATE (%w/v)	1.44	% DR 1st h	20.76	20.74	
E	RPM	750	% DR 2 nd h	40.84	40.85	0.02
			% DR 4 th h	71.05	71.02	0.04
			% DR 8 th h	91.06	91.02	0.04

DM: ETH: Dichloromethane: ethanol, ERSPO: Eudragit RSPO, RPM: Rotations per minute, Desirability: 0.936

Table 5. Cumulative %drug release profiles of optimized and marketed formulation of extended release tablets of Tolterodine tartrate microsponges

Time (h)	Optimized Formulation	Marketed brand (Kytolt 4)	Desirability
1	20.74	20.31	1 to 30%
2	40.85	40.19	30 to 50%
3	52.86	52. 14	
4	71.02	71.47	65 to 90 %
5	75.12	75.56	
6	78.49	78.12	
7	88.24	89.12	
8	91.02	93.24	80 to 100 %
9	94.45	94.47	
10	95.34	95.12	
12	99.27	97.45	

Table 6. Drug relaese kinetics of optimised formulation of Tolterodine Tartrate microsponge tablets

					Korsemeyer- peppas constants		
Formulation	**zero order**	**First order**	**Higuchi**	**Hixon-crowell**	**Peppas**	**n**	**k**
Optimized	0.8676	0.9419	0.969	0.6153	0.9588	0.7032	1.3329
marketed	0.8597	0.9862	0.9638	0.6083	0.9590	0.696	1.338

Table 7. Stability study data of optimized formulation

	Cumulative % drug release at 40±2 °c and 75±5 % Relative humidity			
Time (h)	0 days	30 days	90 days	180 days
0	0	0	0	0
0.5	10.62	10.95	12.49	13.67
1	20.74	21.01	21.45	21.34
2	40.85	40.04	42.96	43.04
3	52.86	52.76	55.45	54.23
4	71.02	71.11	72.34	73.32
5	75.12	75.08	76.21	76.55
6	78.49	78.34	79.12	78.53
7	88.24	89.12	89.24	87.45
8	91.02	91.12	91.23	92.45
9	94.45	93.21	93.12	94.45
10	95.34	97.02	96.88	97.12
12	99.27	99.87	101.01	100.34

2.5. Scanning Electron Microscopy

Scanning electron microscopy of optimized formulation of tolterodine tartrate microsponges as in Figure: 8 revealed porous nature of polymer coat on microsponge surface due to the diffusion of internal phase into the polymer in presence of plasticizer Dibutyl phthalate with better control on drug release.

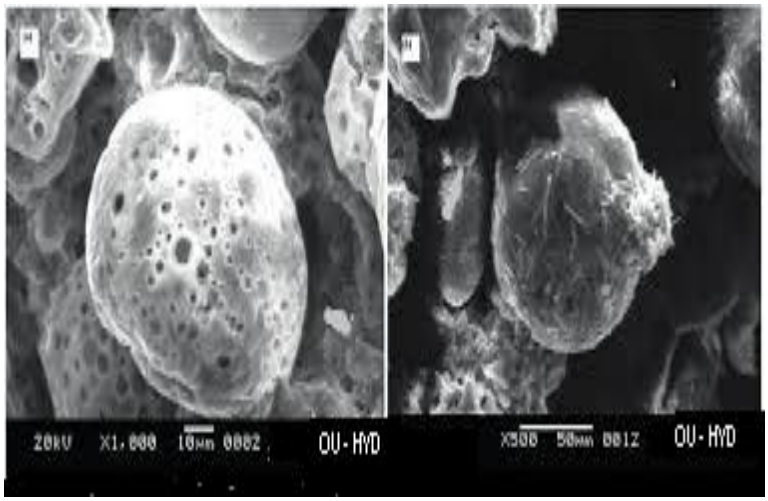

Figure 8. SEM analysis optimized Tolterodine microsponges with 1000 µm and 500 µm magnification showing porous nature on the surface.

3. CONCLUSION

Drug-excipient compatibility studies revealed no interaction between drug and polymer. Tolterodine tartrarte extended release microsponge tablets were successfully developed by Quashi-emulsion solvent diffusion method. The effect of independent variables on responses (dependent variables) were controlled by Response surface method with central composite design to obtain desired results of production yield with high drug entrapment and desired particle size. The drug release profiles of optimized formulation were comparable to marketed formulation. So, Response surface method with central composite design was selected as one of the best methods to optimize formulation and process variables at each level to reach the desired response values especially in case of multiple factors affecting the responses.

4. MATERIALS AND METHODS

4.1. Materials

Tolterodine tartrate was obtained from Aurabindo Pharma Ltd., Hyderabad, as gift sample, HPMC K4M, Di butyl phthalate, Poly vinyl alcohol, Dichloromethane, ethanol were obtained from SD fine chemicals, Mumbai. Eudragit RSPO (Evonik) and purified water (ACG) were used as received.

4.2. Methods

4.2.1. Drug-excipient compatibility study:

Tolterodine tartrate and selected excipients were subjected for drug excipient compatibility studies. The drug and individual excipients were mixed in equal proportions by weight and filled in glass vials stoppered with Teflon plugs and sealed with aluminum seals. These samples were stored in incubators at 40°C/75% RH. Samples were analyzed for the solid-state property of the drug in the blended mixtures using differential scanning colorimeter (DSC) at initial and 1 month (40°C/75% RH) [8]. The results were shown in Figure:2

4.3. Experimental design

Design of experiment (DOE version 11.0.1)) was used to design the formulation compositions with 5 independent variables and 6 dependent responses by response surface methodology with central composite design. DOE generated 50 formulations with 8 replicates at center point which were used for the experimental work to find out intra and inter independent variables interaction and their effect on dependent responses and to find out optimized formulation with response surface method.

The selected list of independent and dependent variables based on literature survey and preliminary studies were mentioned in Table 1 with desirability limits. Each independent variable was studied at 3 levels. Formulation batch with experimental results were showed in Table 2. The experimental data was analyzed with second order polynomial multiple regression equations. [5, 9, 22].

Table 1. Variables of formulations in central composite design

Independent variables	category	levels used –actual, coded		
		Low (-1)	Medium(0)	High(+1)
A) Amount of Eudragit RSPO (mg) (X_1)	Polymer/rate retardant	100	175	250
B) Amount of HPMC K4 M (mg) (X_2)	Polymer/rate retardant	50	100	150
C) Dichloromethane: Ethanol(ml) (X_3)	Internal phase	5	7.5	10
D) Dibutyl phthalate (%w/v) (X_4)	plasticizer	1	1.25	1.5
E) RPM (X_5)	Rotations per minute	500	625	750

Dependent variables		Desirability limit
% product yield	(Y_1)	maximum
% Drug entrapment efficiency	(Y_2)	maximum
Particle size (µm)	(Y_3)	minimum
% drug release at 1st h	(Y_4)	Up to 30%
% drug release at 2nd h	(Y_5)	30-50%
% drug release at 4th h	(Y_6)	65-90%
% drug release at 8 th h	(Y_7)	80-100 %

4.4. Preparation of ER microsponges [10]

The microsponges containing Tolterodine tartrate were prepared by quasi emulsion solvent diffusion method by using different amounts of rate retardants/polymers. To prepare the internal phase, polymers were dissolved in mixture of Dichloromethane and ethanol 95% (1:1) in addition to dibutyl phthalate (%w/v). Then, Tolterodine tartrate was added and the final mixture was dissolved under ultra-sonication at 35°C at 70 - kHz frequency for 2 min. The internal phase then poured into the external phase containing Poly vinyl alcohol solution (1.5% w/v of Poly vinyl alcohol) with constant stirring at a rate of 500-750 RPM for the formulation runs as specified in Table-2 for 6 h. The O/W emulsion was produced. During this time, the dichloromethane and ethanol were completely removed by diffusion into PVA solution and evaporation through the air/liquid interface. The developed microsponges were filtered and washed with 50 mL of distilled water. The microsponges were dried in an air-heated oven at 40°C for 12 h and packed in air tight container for further study. For all the formulations the volume of external phase (1.5 % w/v of PVA solution) and amount of drug (100 mg) was kept constant.

4.5. Evaluation of Tolterodine tartrate ER microsponges [11-13]

Micrometrics of ER micro-sponges were determined by USP method using a Tapped density tester and the results were estimated by the following formule.

Bulk density: $\frac{Weight\ of\ sample\ in\ (g)}{untapped\ volume(ml)}$

Tapped density: $\frac{weight\ of\ sample(g)}{tapped\ volume\ (ml)}$

Hausner ratio: $\frac{Tapped\ density}{Bulk\ density}$

Percent yield: $\frac{weight\ of\ microsponges\ recovered}{weight\ of\ drug+polymer}\ x100$

Percent Drug Entrapment efficiency: $\frac{Actual\ drug\ content}{Theorotical\ drug\ content}x\ 100$

4.6. Assay of Tolterodine tartrate microsponges [14]

Tolterodine tartrate ER microsponges equivalent to 20 mg of Tolterodine Tartrate were transferred into 100 mL volumetric flask. Mobile phase was added and sonicated for 15 min to dissolve and made the volume up to the mark. 10 mL of this solution was transferred to 20 mL volumetric flask and made the volume up to the mark and the solution was filtered with 0.45 µ nylon membrane filter. The chromatographic conditions employed for analysis were as follows:

HPLC:

Detector: 220 nm, Column: Kromosil 60, C_{18} (150 x 4.6 mm), 5µm, Flow rate: 1.0 mL/min. Mobile phase: Methanol: Phosphate buffer pH 7(40:60) v/v, Limit: 97.0%–103.0% (as-is basis) and Runtime: 10 min.

$\frac{AT}{AS}X\frac{WS}{100}X\frac{10}{20}X\frac{100}{WT}\times\frac{20}{10}X\frac{P}{100}X$ A.W = mg /Tablet (Assay)

% Label amount = $\frac{Assay\ of\ Tolterodine\ Tartarate}{Label\ claim}$ X100

Where,
AT = Peak area of Tolterodine tartrate obtained from the sample solution,
AS = Average peak area of Tolterodine Tartrate obtained from the standard solution,
WS = Weight of Tolterodine tartrate working standard taken in mg,
WT = Weight of tolterodine tartrate microsponges sample taken in mg,
P = Potency of Tolterodine tartrate working standard used (on as is basis),
A = Average weight of the tablet in mg.

4.7. Determination of particle size and porosity:

The particle sizes of produced microsponges were analyzed by optical microscopy. The instrument was calibrated to find out the value of 1 unit of eye piece. Sizes of 100 microsponges were analyzed in x10.

Mercury Intrusion Porosimeter (Autoscan 60, Quantachrome, USA) was used to determine porous properties of optimized formulation in the pressure range 0–4,000 kg/cm^2 [15].

4.8. Scanning electron microscopy:

The morphology and surface characteristics of the microsponges were analyzed using Scanning Electron Microscope. Gold/palladium alloy under vacuum was used to coat the microsponges by sputter coater for a minute. Coated samples were examined under SEM; JEOL-JSM, 7900F, USA under vacuum at room temperature [16].

4.9. Formulation of tablets

The microsponges equivalents to 4 mg dose were prepared into tablets by direct compression technique. Micro crystalline cellulose: 33.7%, Mg. Stearate: 2% and Talc: 2% were used as excipients and Total weight of the tablet: 200 mg [17].

5. Evaluation of Tolterodine Tartrate microsponge tablets

Prepared tablets were evaluated for tablet thickness, friability, weight variation and content uniformity as per USP guidelines [18].

5.1. In vitro drug release studies

In vitro-drug release studies were performed for the prepared tolterodine tartrate microsponge tablets with dissolution test apparatus: USP Type II. The volume of the dissolution medium was 900 ml with a stirring speed of 50 rpm, and the temperature was maintained at 37°C ± 0.5°C. The study was carried out in pH 6.8 phosphate buffer at 0.5,1, 2, 3, 4, 5, 6,7,8, 10 and 12 h. 10 ml of sample was withdrawn periodically and replaced with equal volume of fresh dissolution medium. The collected samples were filtered with 0.45 µ nylon membrane filter and analyzed to assess the % drug dissolved by employing same chromatographic conditions as that of assay.

The % labeled amount of Tolterodine tartrate dissolved at respective time intervals was estimated from following formula:

$$= \frac{AT}{AS} \times \frac{WS}{100} \times \frac{900}{LC} \times \frac{P}{100} X100 = - - -\%$$

Where,
AT = Peak area of Tolterodine tartrate obtained from the sample solution,
AS = Average peak area of Tolterodine tartrate obtained from the standard solution,
WS = Weight of Tolterodine tartrate working standard taken in mg,
P = Potency of Tolterodine tartrate working standard used (on as is basis),
LC = Label claim.

5.2. Drug release kinetics [20]

Drug release kinetics of formulations were studied to find out dissolution patterns like order of drug release and mechanism of drug release by fitting into mathematical equations and shown in Table 6.

6. Design validation and optimization [21]

The Design Expert 11.0 (Stat-Ease Inc., Minneapolis, MN, USA) software., was used to design the experiment and to perform statistical analysis. The coefficients of determination (R^2) and analysis of variance (ANOVA) were used to assess the regression models and for goodness of fit. The optimal extraction conditions of the five independent variables and each dependent variable were estimated by applying the RSM technique with CCD. All experiments were performed in triplicate.

7. Stability studies [22]

Stability studies were conducted for optimized formulation as per ICH guidelines. Optimized formulation of Tolterodine tartrate micro sponge tablets were filled in clean, air tight aluminium containers and kept in humidity chamber at a temperature of 40±2 °C and 75±5 % relative humidity. Tablets were assessed for change in appearance, friability, drug content and *in vitro* release profile at an interval of 30, 90 and 180 days. The results were shown in table 7.

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Author contributions: Concept – S.N.; Design – S.N., P.A., R.G; Supervision – S.N.; P.A.; Materials – R.G.; Data Collection and/or Processing – R.G.; Analysis and/or Interpretation – S.N., P.A.; Literature Search – R.G., A.A., Writing – R.G.; Critical Reviews – S.N., P.A, A.A.

Conflict of interest statement: The authors declared no conflict of interest.

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