Original Article

FORMULATION DEVELOPMENT AND STATISTICAL OPTIMIZATION OF A BILAYER TABLET OF BOSENTAN MONOHYDRATE AND SILDENAFIL CITRATE IN MANAGEMENT OF PULMONARY ARTERIAL HYPERTENSION

PEARL PIRES DIGHE[1,2]*, H. M. TANK[3]

[1]Department of Pharmaceutics, PES's Rajaram and Tarabai Bandekar College of Pharmacy, Ponda, Goa, [2]Faculty of Doctoral Studies and Research, School of Pharmacy, RK University, Rajkot, Gujarat, India, [3]Department of Pharmaceutics, Atmiya Institute of Pharmacy, Rajkot, Gujarat, India
Email: pirespearl@gmail.com

ABSTRACT

Objective: The current study aims at fabrication of an oral bilayer matrix tablet of bosentan monohydrate and sildenafil citrate; the optimisation of their *in vitro* release and characterization, thereby reducing the side effects associated with bosentan, reducing dosing frequency and increasing patient compliance in the management of pulmonary arterial hypertension.

Methods: Methocel K4M Premium DC2, a directly compressible HPMC grade was used as the sustained release polymer. Pregelatinised starch is used as a diluent and release modifier and sodium lauryl sulphate (SLS) as a solubiliser. The blends of both layers were prepared, evaluated for precompression characteristics and compressed by direct compression. The compressed bilayer tablets were evaluated for their hardness, weight variation, friability, content uniformity and swelling index. The principle objective was to assess the influence of the above variables on *in vitro* drug release of Bosentan using a 2^3 factorial design. Responses are measured as drug release at 2h (Q2), 6h (Q6) and 10h (Q10).

Results: HPMC and pregelatinized starch form a synergistic gel thereby controlling drug release of bosentan for a 12 hour period. Batch BS09 consisting of 40 mg HPMC, 30 mg Pregeletinized starch and 5 mg SLS showed adequate controlled release for a 12 h period. Immediate release layer of sildenafil citrate showed optimum drug release of 102.96% within 30 min.

Conclusion: Bilayer tablet of bosentan and sildenafil is an ideal combination for patients failing monotherapy in pulmonary arterial hypertension.

Keywords: Bilayer tablet, Bosentan monohydrate, Sildenafil citrate, HPMCK4M DC2Premium, Preletinized starch, SLS, Design expert

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare disease in which remodelling of the pulmonary vasculature progressively leads to increased pulmonary vascular resistance, right ventricular failure. PAH is a specific type of pulmonary hypertension that is caused by the development of scar tissue in the tiny blood vessels of the lung. This scar tissue blocks the blood flow through the lungs and causes the pressure in those blood vessels to increase [1].

The dual endothelin receptor antagonist, bosentan, is an orally active therapy, which is effective in the treatment of pulmonary arterial hypertension the most significant adverse event in patients on bosentan treatment is the potential development of abnormal hepatic function and specifically a rise in hepatic amino transaminases [2]. It has an oral bioavailability of 50% and plasma elimination half-life of 5 h [3]. Administration of bosentan as a controlled release formulation due to the short half-life would be desirable in maintaining the plasma therapeutic levels and thereby reducing adverse effects.

Sildenafil is an orally active, potent and selective inhibitor of phosphodiesterase type 5 (PDE5) present throughout the body and is found in high concentrations in the lungs [4]. Inhibition of PDE5 enhances the vasodilator effects of nitric oxide in pulmonary hypertension by preventing the degradation of cyclic guanosine monophosphate (cGMP), which promotes relaxation of vascular smooth muscle and increases blood flow. Sildenafil has been found to produce a relatively selective reduction in pulmonary artery pressure without adverse systemic hemodynamic effects [5].

Pulmonary arterial hypertension is a disease with complex pathogenesis, for which combination therapy is an attractive option. Studies have assessed the impact of sequential combination therapy of bosentan and sildenafil on both short-term responses and long-term outcomes in patients failing on monotherapy [6].

The multi-layered tablet concept has been utilized to develop sustained release formulations and co-administers two drugs as a fixed dose combination [7]. The present study aims to combine the two drugs bosentan and sildenafil for the treatment of pulmonary arterial hypertension as a bilayer tablet. The combination therapy would help to lower pulmonary hypertension by different mechanisms of action in patients failing monotherapy, thereby reducing frequency of dose, side effects and thus improving patient compliance. The objective was to formulate a bilayer tablet wherein bosentan is sustained release and sildenafil is the immediate release layer. Methocel K4M Premium DC2, a directly compressible hydroxypropyl methylcellulose (HPMC) grade has been used as the sustained release polymer. Pregelatinized starch is used as a diluent and release modifier and sodium lauryl sulphate (SLS) as a solubiliser. The further objective was to assess the influence of the above variables on drug release of bosentan. This was accomplished using the statistical design of experiments using the Design expert software, trial version 11.

MATERIALS AND METHODS

Bosentan monohydrate was gifted by Cipla Ltd. Goa, sildenafil citrate was gifted by Unichem Laboratories Pvt. Ltd. Methocel K4M Premium DC was gifted by Colorcon Asia Pvt. Ltd. Goa; pregelatinized starch byIndocco Remeides Pvt. Ltd. Goa; MCC pH 102 and other excipients from Arihant chemicals, Mumbai.

Formulation of sildenafil citrate immediate release blend

The dose of sildenafil which is the immediate release layer is 20 mg. 22.47 mg of sildenafil citrate equivalent to sildenafil is weighed. The blend for immediate release layer as given in table 1was prepared by mixing the drug with super disintegrant in the mortar and pestle, following which other ingredients were blended into, mixed for 15 min and sieved through sieve no# 80. The tablets of sildenafil were compressed using Rimek Mini Press II MT (Karnavati Engineering Private Ltd.). Final weight of the tablet was 130 mg [8].

Table 1: Formulation of sildenafil citrate blends

Ingredients (mg/tab)	Formulations			
	SC01	SC02	SC03	SC04
Sildenafil citrate	22.47	22.47	22.47	22.47
Crosscarmellose sodium	5	10	---	----
Crosspovidone	---	----	5	10
Calcium Hydrogen Phosphate anhydrous	40	40	40	40
MCC Ph102	61	61	61	61
Magnesium stearate	1	1	1	1
Aerosil	0.53	0.53	0.53	0.53
Total tablet weight (mg)	130	135	130	135

MCC: Microcrystalline Cellulose

Formulation of bosentan monohydrate sustained release component using 2^3 factorial design

Various preformulation trials were carried out for selection of matrix polymer to sustain the release of bosentan. Methocel K4M PemiumDC2 was found to show promising sustained release effect for desired period of time. The sustained release layer was formulated with the drug, Methocel K4M PemiumDC2, pregelatinized starch as a diluent and release modifier and sodium lauryl sulfate (SLS) as a solubilizer. The effect of each key ingredient was studied using a 2^3 factorial design containing 3 factors at 2 levels and center point batches repeated four times to assess the experimental error of the model. The dependent response variables measured were the percentage of bosentan release at 2, 6 and 10h. Table 2 shows the variables and their concentrations according to the design expert software trial version 11.

Table 2: Experimental design for bosentan monohydrate component as per design expert

Coded values	Actual value		
	X_1	X_2	X_3
	Concentration of methocel K4M premium DC2 per tablet (mg)	Concentration of pregelatinized starch per tablet (mg)	Concentration of SLS per tablet (mg)
-1	30	20	0
+1	50	40	10

A total of twelve batches were compressed (table 3), of which eight batches-BS01-BS08, comprised of a varying concentration of independent variables and BS09-BS12 were the four repeated center point batches. The blend of sustained release was prepared by mixing drug with Methocel K4M premium DC2 and SLS in a laboratory blender for 15 min. The rest of the ingredients were blended into for the next 10 min. Finally, magnesium stearate was

added and blended. The tablets were compressed using Rimek Mini Press II MT of Karnavati Engineering Private Ltd. The final weight of the sustained release component was 170 mg.

Both the immediate and sustained release blends were evaluated for precompression parameters such as bulk density, tapped density, angle of repose and carr's compressibility index [9, 10].

Table 3: Formulation blends of bosentan monohydrate layer

Composition	BS01	BS02	BS03	BS04	BS05	BS06	BS07	BS08	BS09	BS010	BS11	BS12
Bosentan monohydrate	64.54	64.54	64.54	64.54	64.54	64.54	64.54	64.54	64.54	64.54	64.54	64.54
HPMC K4M DC	30	50	30	50	30	50	30	50	40	40	40	40
Pregelatinized starch	20	20	40	40	20	20	40	40	30	30	30	30
SLS	0	0	0	0	10	10	10	10	5	5	5	5
sunset yellow FCF	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
magnesium stearate	2	2	2	2	2	2	2	2	2	2	2	2
MCC ph102 (qs)	53	33	33	13	43	23	23	3	28	28	28	28
Total weight (mg)	170	170	170	170	170	170	170	170	170	170	170	170

Formulation of bilayered tablet of bosentan monohydrate and sildenafil citrate

The twelve blends of bosentan monohydrate were compressed with the optimised sildenafil blend SC01 using tablet compression machine (Rimek Mini Press II MT (Karnavati Engineering Private Ltd) and were further evaluated. The bilayer tablet was prepared by adding the sustained release blend of bosentan into the die cavity and compressing lightly followed by compressing the immediate release blend of sildenafil over it thereby obtaining the bilayer tablet [11].

Evaluation

The bilayered tablet was evaluated for physical tests like hardness (Monsanto hardness tester-Pathak Electrical Works), friability (Roche friabilator-Pathak Electricals), thickness (Vernier calliper-Bliss Classic), drug content, uniformity of weight and swelling index.

Incompatibilities between drugs and excipients were ruled out by DSC and FTIR studies. *In vitro* disintegration time for sildenafil immediate release component was evaluated using USP tablet disintegration apparatus with 0.1 N Hcl as medium. The volume of medium used was 900 ml and the temperature was 37±0.5 °C. The time taken for complete disintegration of the IR layer was measured [9-11].

***In vitro* drug release**

The *in vitro* drug release of bilayered tablet was studied using 900 ml 0.1 N HCl for the first one hour followed by 11 h in 900 ml phosphate buffer pH 7.2 using USP type II paddle apparatus using at 50 rpm at 37±0.5 °C. The samples were withdrawn every one hour for a period of 12 h and replaced with the fresh buffer. The samples were analysed using double beam UV-Visible spectrophotometer at 271 nm and 290 nm for bosentan and sildenafil respectively [12].

Swelling studies

The tablets were weighed (W0) and placed in a petridish containing buffer and incubated at 37±1 °C. At regular time intervals until 12 h, the tablet was removed carefully and the excess buffer was removed with blotting paper. The swollen tablet was then reweighed (Wt) and the percent swelling was calculated as follows:

$$\% \text{ swelling} = \{(Wt - W0)/W0\} \times 100$$

Where Wt is the weight of tablet at time t and W0 is the initial weight of tablet. The swelling index was calculated [13].

Statistical analysis

Statistical analysis was done using Design Expert® 11 software. The responses analysed were the drug release Q2 (at 2 h), Q6 (at 6 h) and Q10 (10 h). The effect of independent variables on response was represented through contour plots and response surface plots. Significance of results was studied through two-way analysis of variance (ANOVA) wherein P<0.05 was considered significant [13-14].

Drug release kinetics

Kinetics of drug release was studied by analysing the dissolution data through zero order, first order, Higuchi's and Korsmeyer Peppas equations [15].

Accelerated stability study

The optimized batch was subjected to stability study as per ICH guidelines [16].

RESULTS AND DISCUSSION

The results of precompression characteristic have been tabulated in table 4 and 5 for immediate release and sustained release blend respectively. Bulk and tapped densities indicate good packing properties. The carr's index for all formulations was below 17.3% indicating good flow and compressibility. The angle of repose for all blends lies between the ranges of 12.64 °-22.75 ° further ascertaining good flow.

The compressed tablets were evaluated for their hardness, weight variation, friability, content uniformity, and diameter. As shown in table 6, all the parameters were found to be within range.

Table 4: Precompression characteristics of sildenafil citrate blends

Formulation code	Angle of repose[a]°	Bulk density (g/ml)[a]	Tapped density [a](g/ml)	Carrs Index [a](%)
SC01	21.84±0.57	0.43±0.03	0.51±0.03	15.68
SC02	21.37±0.19	0.43±0.04	0.52±0.08	17.30
SC03	22.49±0.42	0.42±0.05	0.50±0.07	16
SC04	22.75±0.38	0.40±0.03	0.48±0.05	16.66

[a]mean±SD, n = 3

Table 5: Precompression characteristics of bosentan monohydrate blends

Formulation code	Angle of repose[a]	Bulk density[a] (g/ml)	Tapped density [a] (g/ml)	Carrs index (%)
BS01	21.57±0.62	0.41±0.08	0.46±0.04	10.86
BS02	20.14±0.22	0.42±0.05	0.48±0.11	12.5
BS03	18.54±0.87	0.43±0.07	0.49±0.09	12.22
BS04	19.31±1.0	0.38±0.1	0.42±0.08	9.52
BS05	18.54±0.32	0.36±0.08	0.41±0.06	12.19
BS06	16.66±0.54	0.35±0.04	0.42±0.05	16.66
BS07	15.41±0.87	0.36±0.09	0.40±0.08	10.0
BS08	12.64±0.55	0.34±0.08	0.39±0.09	12.8
BS09-BS12	14.65±0.21	0.33±0.04	0.38±0.24	13.15

[a]mean±SD, n = 3

Table 6: Post compression characteristics of bilayered tablet

Formulation code	Hardness (Kg/cm²)[a]	Thickness (mm)	Weight Variation[a] (mg)	Friability [a] (%)	DT for IR layer (s)	Drug content[a] (SR layer)(%)	Drug content[a] (IR layer)(%)
BS01	8.5±0.23	4	300±0.56	0.29±0.0021	79	99.12±0.67	99.21±0.57
BS02	10.3±0.35	4	300±0.34	0.41±0.0074	85	99.87±1.07	99.57±0.64
BS03	9.8±0.87	4	300±0.57	0.51±0.0041	84	100.35±0.44	98.64±1.28
BS04	10.5±0.47	4	300±0.69	0.38±0.005	90	99.74±0.56	99.24±0.55
BS05	8.7.0±0.17	4	300±0.46	0.63±0.0087	75	100.67±0.32	98.54±0.97
BS06	10.1±0.61	4	300±0.49	0.49±0.0097	89	98.62±1.06	99.69±1.64
BS07	9.5±0.31	4	300±0.78	0.55±0.0035	88	99.79±1.13	98.82±1.47
BS08	11.0±0.45	4	300±0.81	0.17±0.0047	100	100.54±0.64	99.16±0.57
BS09-BS12	10.3±0.36	4	300±0.74	0.26±0.0046	89	99.34±0.61	98.54±1.22

[a]mean±SD, n = 3

Drug-excipient compatibility

DSC studies

The thermograph of pure drug bosentan monohydrate showed the melting point to be 197.94 °C and of sildenafil citrate 187.30 °C, which is as reported in the literature. The thermographs of the mixture of both drugs, as well as drug and excipient mixtures, does not reveal a significant change in the melting points of bosentan and sildenafil, thus ruling out in-compatibilities as depicted in fig. 1.

DSC of pure drug Bosentan Monohydrate

DSC of pure drug Sildenafil Citrate

DSC of mixture of Bosentan and sildenafil citrate

DSC of mixture of Bosentan Monohydrate with excipients

DSC of mixture of Sildenafil Citrate with excipients
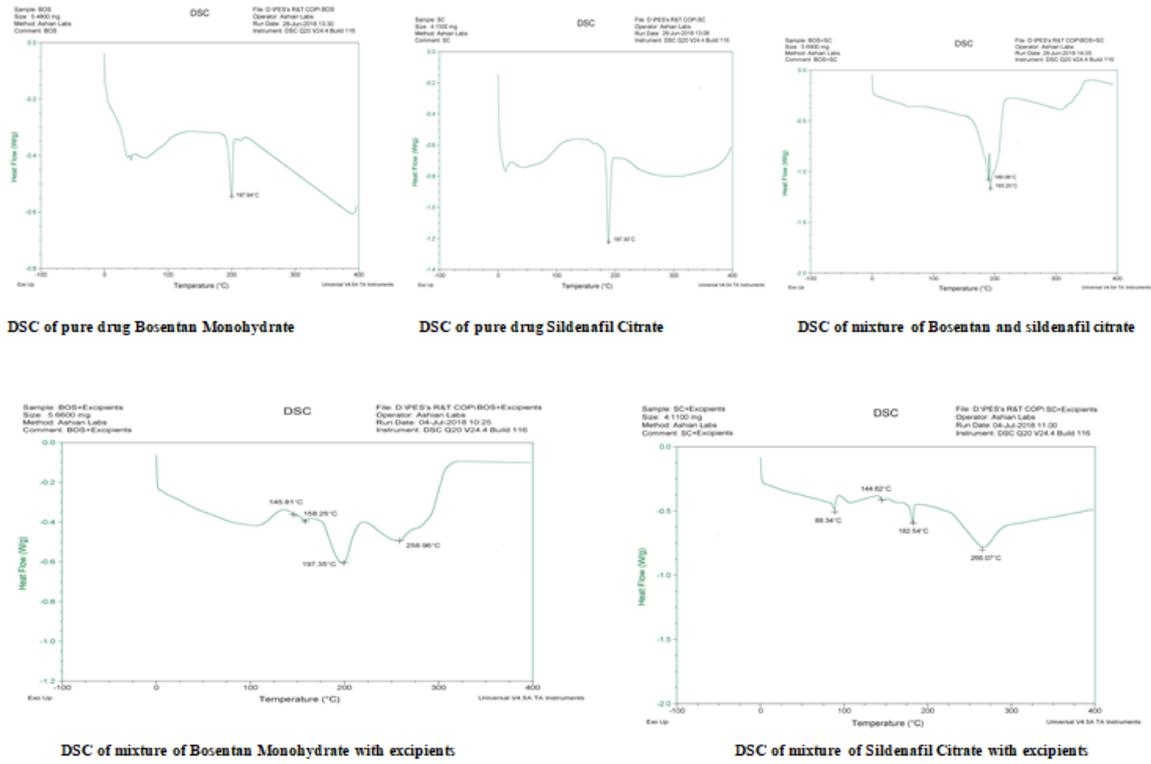
Fig. 1: Compatibility studies of drugs and excipients using DSC

In vitro drug release

From the four batches of sildenafil citrate, SC01 containing croscarmellose sodium as disintegrant showed optimum drug release of 102.96% within 30 min. Hence this blend was further selected to be compressed against the 12 batches of bosentan monohydrate sustained release blends.
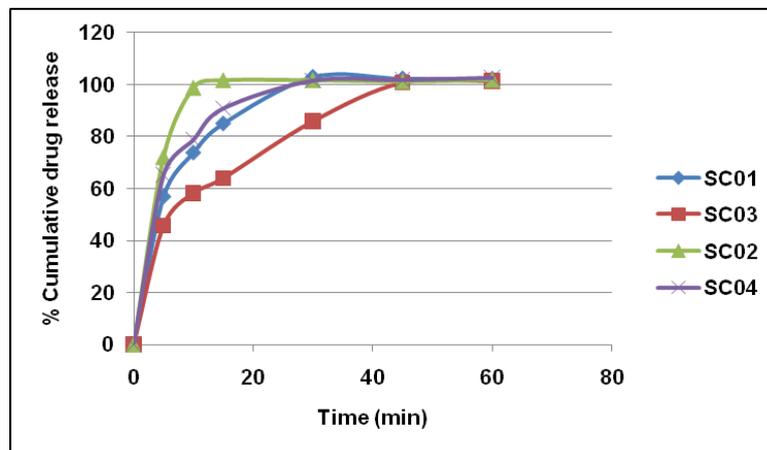
Fig. 2: Cumulative drug release profiles of sildenafil citrate immediate release component (mean±SD, n = 3)

The release of sustained release bosentan monohydrate from the 12 batches of bilayer tablets is as given in fig. 3. All batches showed drug release varying from 8-12 h depending on the concentration of independent variables. Batches BS01-BS04 that do not contain solubiliser SLS do not show complete drug release at the end of 12 h. This may be attributed to the saturation of the drug released in the dissolution vessel after 5-6 h. Formulations BS05-BS09 containing SLS as solubilizer showed adequate drug release. Formulations BS05 and BS07 containing 30 mg of HPMC K4M DC2 Premium per tablet could control the release for 8-9 h, whereas formulations BS06 and BS08 containing 50 mg HPMC showed adequate control for a 12 h period as desired. Pregelatinized starch was observed to have a synergistic effect along with HPMC on controlling the drug release. Formulations BS09 which was the center point batch containing 40 mg HPMC, 30 mg pregelatinized starch and 5 mg SLS per tablet was observed to be the best-optimised formulation. The dissolution profile of the optimised bilayer tablet is depicted in fig. 4.
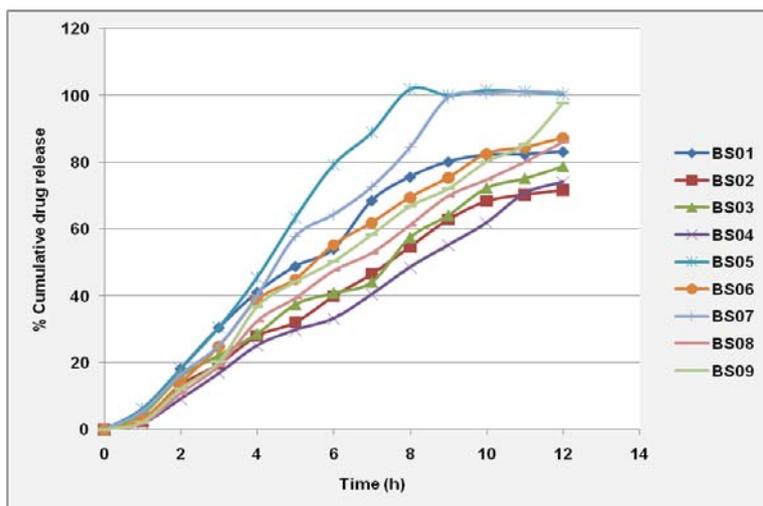
Fig. 3: Cumulative drug release profiles of bosentan monohydrate sustained release component (mean±SD, n = 3)
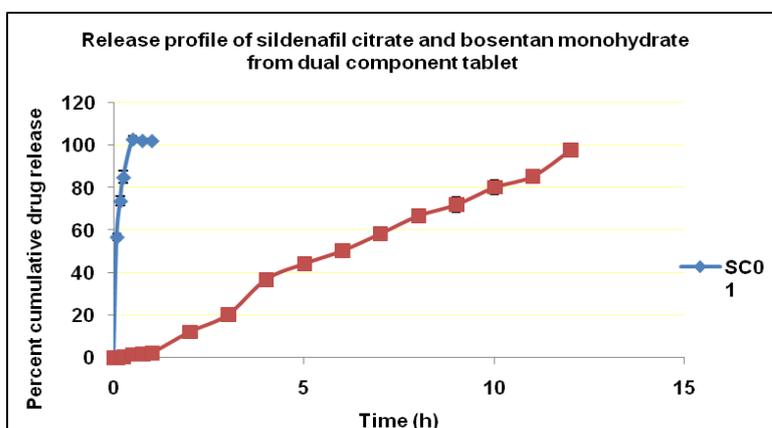
Fig. 4: Dissolution profile of optimised batch of bilayer tablet (mean±SD, n = 3)

To describe the entire dissolution profile, three-time points were considered in the design expert software. Fig. 5 shows the response surface plot for drug release at 2 h (Q2). As depicted, as the concentration of HPMC increases from 30 mg to 50 mg per tablet, the percent drug released is controlled. The same phenomenon is observed with pregelatinized starch as well, but to a lesser extent than HPMC. SLS does not have a significant effect at 2 h.
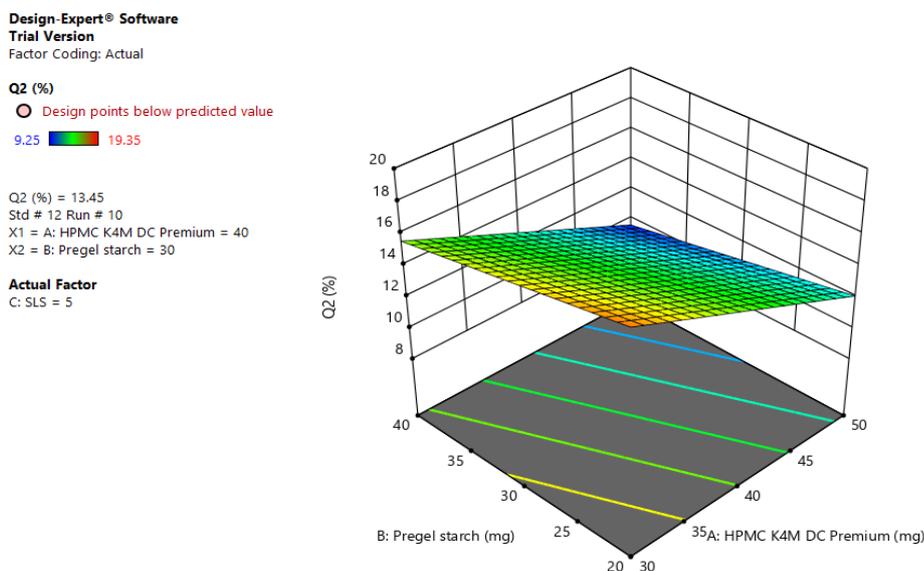
Fig. 5: Response surface plot for drug release at 2 h (Q2) (n=3)

At 6 h and 10 h, HPMC continues to show a dominant effect on controlling the drug release along with pregelatinized starch. This is attributed to the formation of swollen gel of HPMC. HPMC along with pregelatinized starch forms a synergistic matrix gel thereby influentially controlling the drug release for 12 h period. The influence of pregelatinized starch is most dominant at 6 h. A very significant influence of SLS was observed at 6 h and 10 h. As depicted in fig. 6 and

7 it was observed that as SLS concentration increases from 0 to 10 mg per tablet the release of drug increases at 6 h and 10 h. This may be attributed to the fact that bosentan being a BCS class II drug, having a solubility of around 40 mg/100 ml in phosphate buffer pH 7.2, may pose a problem in the drug getting solubilised in the dissolution vessel after 6 h. Therefore the addition of SLS as a solubiliser in the tablet helps to enhance the solubility of the drug as it is released.

(a)

(b)

(c)
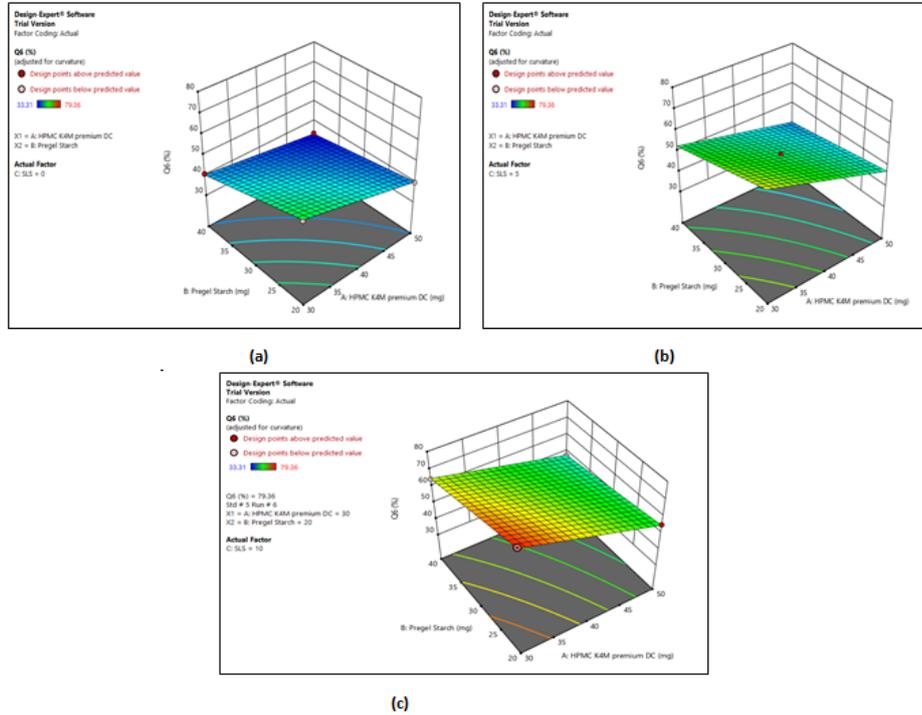
Fig. 6: Response surface plot for drug release at 6 h (Q6) (n=3), fig. 6(a) Drug release at 6 h when concentration of SLS is 0, fig. 6(b) Drug release at 6 h when concentration of SLS is 5, fig. 6(c) Drug release at 6 h when concentration of SLS is 10

(a)

(b)

(c)
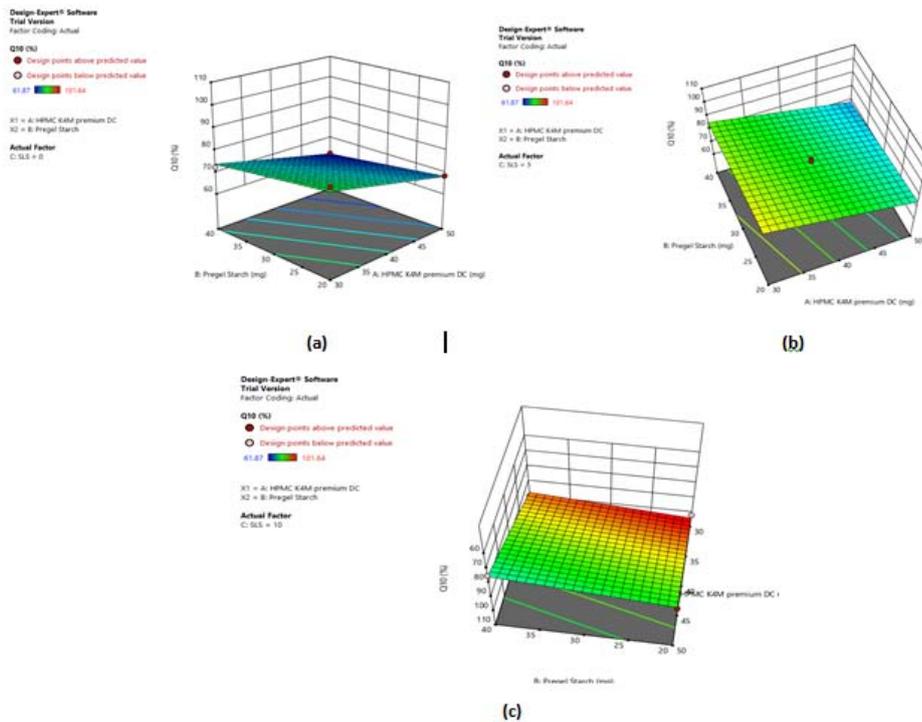
Fig. 7 Response surface plot for drug release at 10 h (Q10) (n=3), Fig. 7(a) Drug release at 10 h when concentration of SLS is 0, Fig.7(b) Drug release at 10 h when concentration of SLS is 5, Fig. 7(c) Drug release at 10 h when concentration of SLS is 10

The statistical data obtained from ANOVA for the models is represented in table VII. From the ANOVA analysis, it can be observed that HPMC K4M DC2 Premium(X1) polymer has effect on all responses Q2, Q6 and Q10reinforcing the fact that it is the main release retarding polymer that controls the drug release upto12 h. At two hours the coefficient, as observed from the equation in table 7 has a lesser value as this is the period of establishment of a fully swollen gel matrix. This is also reinforced by the swelling index of the tablet as depicted in fig. 8 for optimised batch BS09. It was also observed that as the HPMC content per tablet increases the swelling index also increases.

Pregelatinized starch has significant influence at 6 h as observed from the equation in table. It forms a synergistic gel matrix with HPMC. The influence of SLS as solubilser for drug is most predominant from 6 h onwards till 12 h. Since P<0.05 for all three responses, indicates that the model is significant. R^2values indicate a high level of correlation between experimental and predicted responses. The closeness of the adjusted and predicted R^2 values explains the reliability of the model. The adequate precision value of greater than 4 indicates that the model is discriminating. Since coefficient of variation CV is less than 10 the model can be considered reproducible.

Table 7: ANOVA influence of formulation variables on response factors

Regression model	R^2	Adjusted R^2	Predicted R^2	Model P value	% CV	Adequate precision
Q2= 13.90-3.05 X1–1.31 X2+0.540 X3	0.9134	0.8810	0.8384	0.0001	7.45	16.3950
Q6= 51.86–7.83 X1–5.31 X2+9.80 X3+1.71 X1*X2–2.39 X1*X3	0.9937	0.9884	0.9730	<0.0001	2.50	50.4568
Q10 = 80.22–8.52 X1–3.28 X2+9.23 X3–2.45 X1*X3	0.9686	0.9506	0.9096	<0.0001	3.17	25.6130
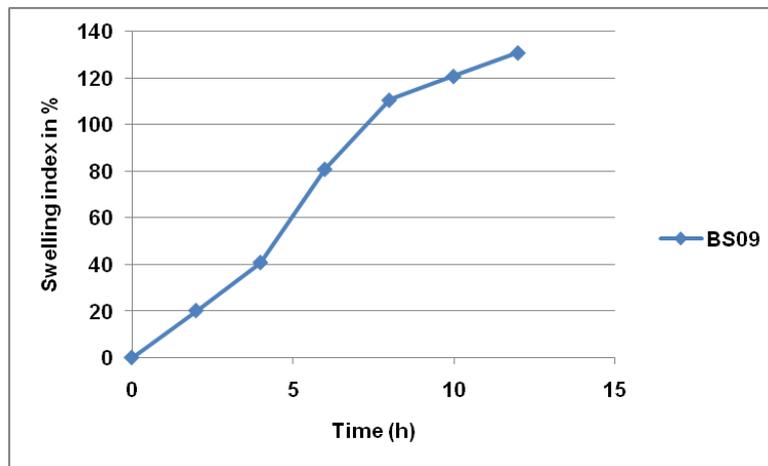
Fig. 8: Swelling index of optimised batch BS09 (mean±SD, n = 3)

The release kinetics of all the formulations were checked by fitting the release data to various kinetic models, and the release was best fitted to zero order release mechanism. Further by fitting the data to the Korsmeyer‑Peppas equation and the n value for all the formulations obtained was>1 which suggests that the release followed the super case II transport mechanism. This suggests more than one mechanism involved in drug release from matrix. The r^2 values for all the models are shown in table 8.

Stability results of the optimised batch BS09 showed optimum drug release of sildenafil citrate within 30 min and controlled release of bosentan over a 12 h period.

Table 8: Correlation coefficients of different pharmacokinetic models for release

Formulation	zero order	first order	Higuchi	Hixon crowel	Korsmeyer-peppas r^2	n
BS 01	0.9479	0.9703	0.949	0.972	0.8756	1.6175
BS 02	0.9872	0.981	0.9299	0.9889	0.8924	1.5739
BS 03	0.9936	0.969	0.9253	0.9859	0.8634	1.5766
BS 04	0.9963	0.9542	0.8917	0.9762	0.9289	1.6068
BS 05	0.8972	0.8722	0.9086	0.8444	0.9148	1.7362
BS 06	0.9727	0.9809	0.9408	0.9933	0.918	1.6662
BS 07	0.9669	0.864	0.9251	0.867	0.9093	1.7243
BS 08	0.9927	0.9556	0.9201	0.9851	0.9383	1.6739
BS 09	0.9947	0.7802	0.9149	0.9119	0.9267	1.6989
BS 10	0.9881	0.7819	0.9179	0.9005	0.9129	1.6613
BS 11	0.997	0.6618	0.9073	0.8632	0.9265	1.7089
BS 12	0.9925	0.8644	0.9266	0.9498	0.9169	1.6773

CONCLUSION

The above study demonstrates the use of a factorial design approach in an optimisation of bilayer tablet dosage form of bosentan monohydrate and sildenafil citrate in the management of pulmonary arterial hypertension. The amount of HPMC and amount of pregelatinized starch has the significant influence on *in vitro* drug release. SLS acts as a solubilizer exhibiting significant influence after 6 h. The statistical tool design expert trial version 11 was used to predict the interaction between the variables and the extent of their influence on drug release.

The direct compression technique for manufacture of this bilayer tablet is a safe and effective tool for patients failing monotherapy in pulmonary arterial hypertension. Bilayer tablets are an ideal way of administering drugs as fixed dose combinations for lifestyle diseases.

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AUTHORS CONTRIBUTIONS

Pearl Pires Dighe, corresponding author for this publication, working as Assistant Professor has carried out all the above research work in the premises of PES's Rajaram and Tarabai Bandekar College of Pharmacy under the guidance of her Ph. D guide Dr. H. M. Tank, Principal, Atmiya Institute of Pharmacy, Dist. Rajkot.

CONFLICT OF INTERESTS

All authors have none to declare

REFERENCES

1. Corris P, Degano B. Severe pulmonary arterial hypertension: treatment options and the bridge to transplantation. Eur Respir Rev 2014;23:488-97.
2. Gabbay E, Fraser J, McNeil K. Review of bosentan in the management of pulmonary arterial hypertension. Vasc Health Risk Manage 2007;3:887–900.
3. Weber C, Schmitt R, Birnboeck H, Hopfgartner G, Van Marle SP, Peeters PA, *et al.* Pharmacokinetics and pharmacodynamics of the endothelin-receptor antagonist bosentan in healthy human subjects. Clin Pharmacol Ther 1996;60:124-37.
4. Ghazwan B. The role of phosphodiesterase inhibitors in the management of pulmonary vascular diseases. Glob Cardiol Sci Pract 2014;3:257–90.
5. Klodell CT Jr, Morey TE, Lobato EB, Aranda JM Jr, Staples ED, Schofield RS, *et al.* Effect of sildenafil on pulmonary artery pressure, systemic pressure, and nitric oxide utilization in patients with left ventricular assist devices. Ann Thorac Surg 2007;83:68–71.
6. Dardi F, Manes A, Palazzini M, Bachetti C, Mazzanti G, Rinaldi A, *et al.* Combining bosentan and sildenafil in pulmonary arterial hypertension patients failing monotherapy: real-world insights. Eur Respir J 2015;46:414-21.
7. Vishwakarma AG, Mogal RT, Pawar AY. Bi-Layer tablet-a new ways in oral drug delivery system. Int J Pharm Tech Res 2014;6:1416-28.
8. Arunprashad B, Teja GK. Design and evaluation of bilayer tablets to treat respiratory tract infection. Int J Pharm Pharm Sci 2013;5:250-5.
9. Roy S, Naskar S, Kundu S, Kpoutsu K. Formulation and evaluation of sustained release bilayer tablets of propranolol hydrochloride. Int J Pharm Pharm Sci 2015;7:264-9.
10. Kumar U, Md Samiull, Halder S, Rouf A. Assessment of once daily sustained release hydrophilic matrix tablet of carvedilol. Dhaka Univ J Pharm Sci 2017;16:43-53.
11. Brito SR, Sravani G, Bhanupriya N, Veerupakshi M, Anil Kumar B, Wasim Raja S. Design and evaluation of sustained release bilayer tablet of metformin hydrochloride with metoprolol tartrate. Int J Novel Trends Pharm Sci 2011;1:10-7.
12. Prabhakar S. Formulation and evaluation of bilayer tablets of diclofenac sodium with ranitidine HCL for sustained and immediate release. J Appl Pharma Sci 2012;2:136-41.
13. Jivani RR, Patel CN, Jivani NP. Statistical design of experiments on the fabrication of bilayer tablet of narrow absorption window drug: development and *in vitro* characterisation. Indian J Pharm Sci 2012;74:302–11.
14. Bobde SS, Tank HM. Design and statistical optimization of mouth dissolving sublingual film of fixed dose combination of doxylamine succinate and pyridoxine hydrochloride using the design of experiment in the treatment if nausea and vomiting. Asian J Pharm Clin Res 2018;11:202-8.
15. Chime SA, Onunkwo GC, Onyishi II. Kinetics and mechanisms of drug release from swellable and non swellable matrices: a review. Res J Pharm Biol Chem Sci 2013;4:97-103.
16. International Conference of Harmonization (ICH). Harmonized Tripartide Guidelines for Stability Testing of New Drug Substances and Products Q1A (R2). Rockville, MD: United States Pharmacopoeial Convention, Inc.; 2003. p. 6.