# DESIGN DEVELOPMENT AND OPTIMIZATION OF A SUSTAINED RELEASE TABLET OF BOSENTAN MONOHYDRATE FOR PULMONARY ARTERIAL HYPERTENSION

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(Received 25 January 2019) (Accepted 11 February 2019)

**ABSTRACT**

Pulmonary arterial hypertension (PAH) means high blood pressure in the lungs caused by obstruction in the small arteries of the lungs.The current study involves the fabrication of oral matrix sustained release tablet of bosentan monohydrate, a dual endothelin receptor antagonist, the optimisation of its *in vitro* release and characterisation. Methocel K4M PremiumDC2, a directly compressible HPMC grade has been used as the sustained release polymer. Pregelatinised starch is used as a diluent and release modifier and sodium lauryl sulphate as a solubiliser. The influence of the above variables on drug release is measured using a $2^3$ factorial design using design expert software. Surface response plots show significant interaction among the formulation variables thus aiding in optimization of bilayer tablet.

**Keywords:** Bosentan monohydrate, sustained release, HPMC K4M PremiumDC2, 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 (PAH).Bosentan is used in idiopathic and familial PAH, in PAH associated with connective tissue disease and in PAH which may develop in association with other conditions. 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 to the short half-life would be desirable in maintaining the plasma therapeutic levels and thereby reducing adverse effects.

The goal of the present study is to design a sustained release matrix tablet of bosentan monohydrate for treatment of pulmonary arterial hypertension, thereby reducing the side effects associated and increasing patient compliance. MethocelK4M Premium DC2, a directly compressible HydroxyPropyl Methyl Cellulose (HPMC) grade has been 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 further objective is to assess the influence of the above variables on drug release of bosentan. This was accomplished using statistical design of experiments using the Design expert software, trial version 11.

## MATERIALS AND METHODS

Bosentan Monohydratewas gifted byCipla Ltd. Goa.Methocel K4M Premium DC was gifted by ColorconAsia Pvt. Ltd. Goa;pregelatinized starch byIndoccoRemeidesPvt. Ltd. Goa;MCC pH 102 and other excipients from Arihant chemicals, Mumbai.

### Formulation of bosentan monohydrate sustained release tablet using $2^3$ factorial design

Various preformulation trials were carried out for selection of matrix polymer to sustain the release of bosentan. Methocel K4M Pemium DC2 was found to show promising sustained release effect for desired period of

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**Table I: 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 | 40 | 20 | 0 |
| +1 | 60 | 40 | 10 |

**Table II: Formulation blends of bosentan monohydrate layer**

| COMPOSITION | BS01 | BS02 | BS03 | BS04 | BS05 | BS06 | BS07 | BS08 |
|---|---|---|---|---|---|---|---|---|
| Bosentan monohydrate | 64.54 | 64.54 | 64.54 | 64.54 | 64.54 | 64.54 | 64.54 | 64.54 |
| HPMC K4M DC | 40 | 60 | 40 | 60 | 40 | 60 | 40 | 60 |
| Pregelatinized starch | 20 | 20 | 40 | 40 | 20 | 20 | 40 | 40 |
| SLS | 0 | 0 | 0 | 0 | 10 | 10 | 10 | 10 |
| sunset yellow FCF | 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 |
| MCC ph102 (qs) | 63 | 43 | 43 | 23 | 53 | 33 | 33 | 13 |
| Total weight (mg) | 190 | 190 | 190 | 190 | 190 | 190 | 190 | 190 |

time. The sustained release blend was formulated with drug, Methocel K4MPemium DC 2, 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. The dependent response variables measured were percentage of bosentan release at 2, 8 and 20 h. Table Ishows the variables and their concentrations according to the design expert software trial version 11.

A total of eight batches were compressed (table II) comprising of varying concentration of independent variables. 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 mins. 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 tabletwas 190mg.

Theblends were evaluated for precompression parameters such as bulk density, tapped density, angle of repose and Carr's compressibility index[4].

## Evaluation

The sustained releasetablet 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 and uniformity of weight. Incompatibilities between drugs and excipients were ruled out by DSC and FTIR studies[5].

## *In vitro* drug release

The *in vitro* drug release of bilayered tablet was studied using 900 mL phosphate buffer pH 7.2 using USP type II paddle apparatus using at 50 rpm at 37±0.5°C for a period of 24 h. The samples were withdrawn every two hours for a period of 24 h and replaced with the fresh buffer. The samples were analysed using double beam UV-Visible spectrophotometer at 271 nm which is the $\lambda_{max}$ of bosentan[6].

## Swelling studies

The swelling behavior of tablet described as the water absorbing capacity. The tablets were weighed individually (W0) and placed separately in petridish containing cellophane membrane and incubated at 37 ± 1°C. At regular time intervals until 12 hours, the tablet was removed carefully. The swollen tablet was then reweighed (Wt) and the % swelling were calculated using the following formula:

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

**Table III: Precompression characteristics ofbosentan monohydrate blends**

| Formulation Code | Angle of repose[a] | Bulk density[a] (g/mL) | Tapped density [a] (g/mL) | Carrs Index (%) |
|---|---|---|---|---|
| BS01 | 22.47±0.87 | 0.42±0.03 | 0.47±0.06 | 10.63 |
| BS02 | 19.14±0.95 | 0.43±0.08 | 0.49±0.07 | 12.24 |
| BS03 | 20.54±0.76 | 0.44±0.06 | 0.5±0.1 | 12 |
| BS04 | 18.31±0.56 | 0.39±0.06 | 0.43±0.09 | 9.3 |
| BS05 | 18.64±0.7 | 0.37±0.02 | 0.42±0.08 | 11.90 |
| BS06 | 16.49±0.88 | 0.36±0.09 | 0.43±0.05 | 16.27 |
| BS07 | 16.90±0.92 | 0.37±0.04 | 0.41±0.06 | 9.75 |
| BS08 | 15.04±0.69 | 0.35±0.07 | 0.40±0.09 | 12.5 |

*[a]mean±SD, n = 3*

**Table IV:Post compression characteristics of sustained release tablet**

| Formulation Code | Hardness (Kg/cm$^2$) [a] | Thickness (mm) | Weight Variation [a] (mg) | Friability[a] (%) | Drug content [a] (%) |
|---|---|---|---|---|---|
| **BS01** | 8.5±0.23 | 4 | 190±0.56 | 0.29±0.0021 | 99.12±0.67 |
| **BS02** | 10.3±0.35 | 4 | 190±0.34 | 0.41±0.0074 | 99.87±1.07 |
| **BS03** | 9.8±0.87 | 4 | 190±0.57 | 0.51±0.0041 | 100.35±0.44 |
| **BS04** | 10.5±0.47 | 4 | 190±0.69 | 0.38±0.005 | 99.74±0.56 |
| **BS05** | 8.7±0.17 | 4 | 190±0.46 | 0.63±0.0087 | 100.67±0.32 |
| **BS06** | 10.1±0.61 | 4 | 190±0.49 | 0.49±0.0097 | 98.62±1.06 |
| **BS07** | 9.5±0.31 | 4 | 190±0.78 | 0.55±0.0035 | 99.79±1.13 |
| **BS08** | 11.0±0.45 | 4 | 190±0.81 | 0.17±0.0047 | 100.54±0.64 |

*[a]mean±SD, n = 3*

**Table V: 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= 21.99-1.41 X1 – 0.2925 X2 + 0.63 X3-0.7825X1*X2 | 0.9810 | 0.9557 | 0.8650 | 0.0065 | 1.81 | 17.9015 |
| Q8= 52.47 – 7.74 X1 – 4.13 X2 | 0.9856 | 0.9799 | 0.9632 | < 0.0001 | 2.55 | 28.9290 |
| Q20 = 90.27 – 1.94 X1 – 1.53 X2 + 8.88 X3 – 1.62 X1*X2 | 0.9961 | 0.9908 | 0.9721 | 0.0006 | 1.07 | 32.7917 |

**Table VI: Correlation coefficients of different pharmacokinetic models for release**

| Formulation | zero order | first order | higuchi | hixoncrowel | korsmeyerpeppas | |
|---|---|---|---|---|---|---|
| | | | | | $r^2$ | n |
| BS 01 | 0.9745 | 0.9926 | 0.9675 | 0.9955 | 0.8223 | 1.5322 |
| BS 02 | 0.9849 | 0.9741 | 0.95 | 0.9858 | 0.8169 | 1.4567 |
| BS 03 | 0.971 | 0.9603 | 0.959 | 0.9746 | 0.8101 | 1.5137 |
| BS 04 | 0.9788 | 0.9746 | 0.9569 | 0.9819 | 0.8139 | 1.4033 |
| BS 05 | 0.9906 | 0.9243 | 0.9567 | 0.9732 | 0.8237 | 1.5583 |
| BS 06 | 0.9783 | 0.9789 | 0.9526 | 0.9854 | 0.8055 | 1.4374 |
| BS 07 | 0.9854 | 0.8692 | 0.9263 | 0.9325 | 0.8168 | 1.5252 |
| BS 08 | 0.9594 | 0.9562 | 0.9528 | 0.9631 | 0.7855 | 1.3542 |

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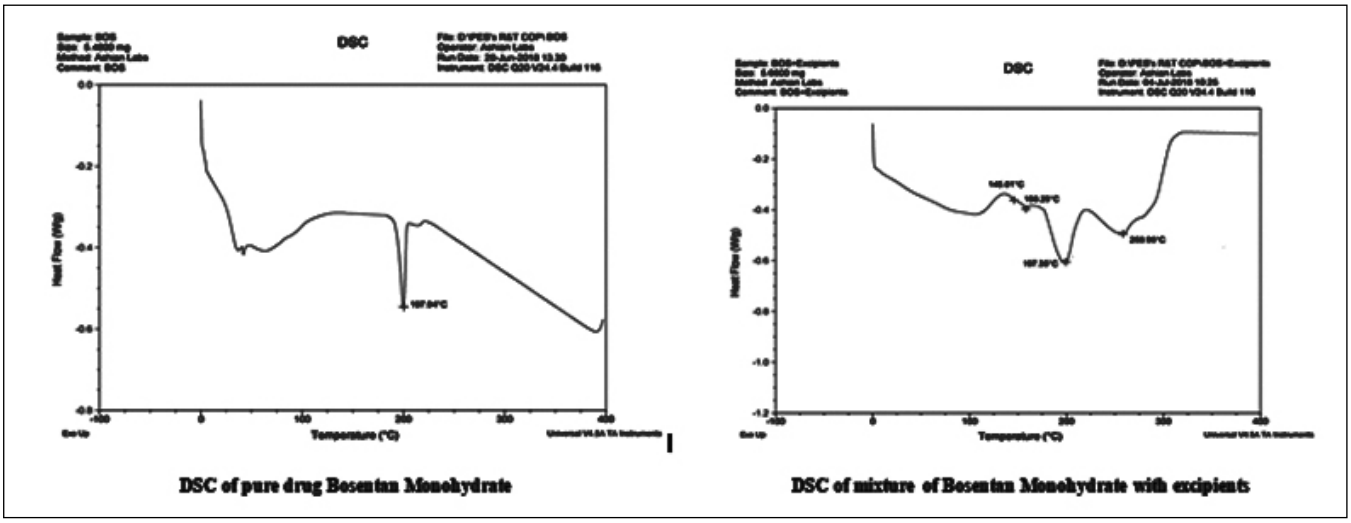


**Fig. 1: Compatibility studies of drugs and excipients using DSC**

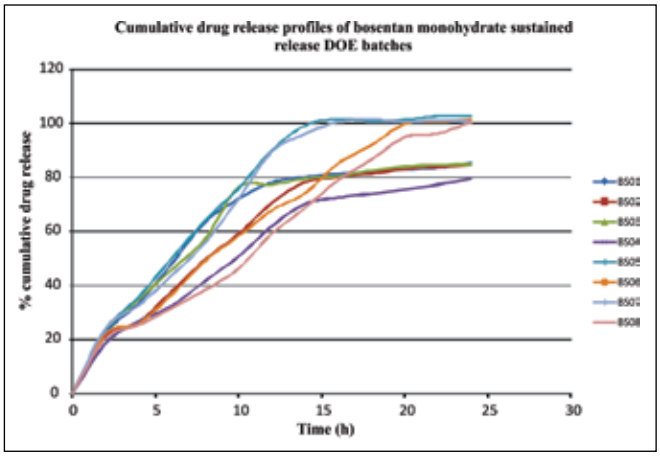


**Fig. 2: Cumulative drug release profiles of bosentan monohydrate sustained release batches**

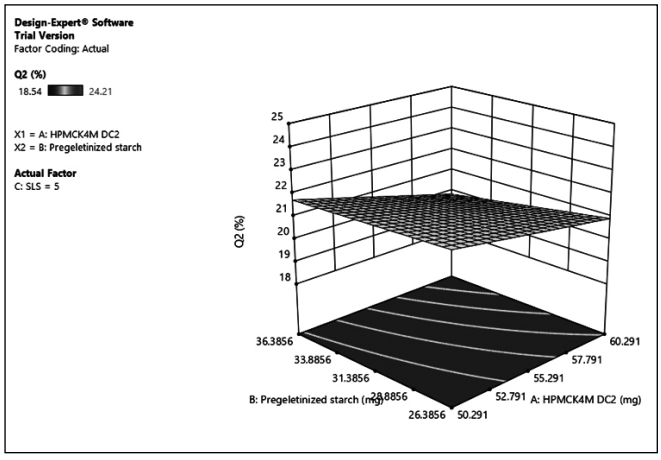


**Fig. 3: Response surface plot for drug release at 2 h (Q2)**

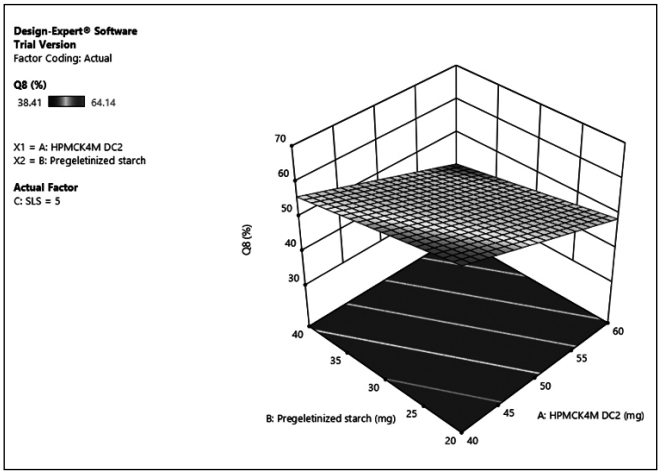


**Fig. 4: Response surface plot for drug release at 8 h (Q8)**

Where Wt is the weight of tablet at time t and W0 is the initial weight of tablet. The swelling index was calculated[7].

## Statistical analysis

Statistical analysis was done using Design Expert® 11 software. The responses analysed were the drug release Q2 (at 2 h), Q8 (at 8 h) and Q20 (20 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) where in $P<0.05$ was considered significant[7,8].

## Drug release kinetics

Kinetics of drug release was studied by analysing the

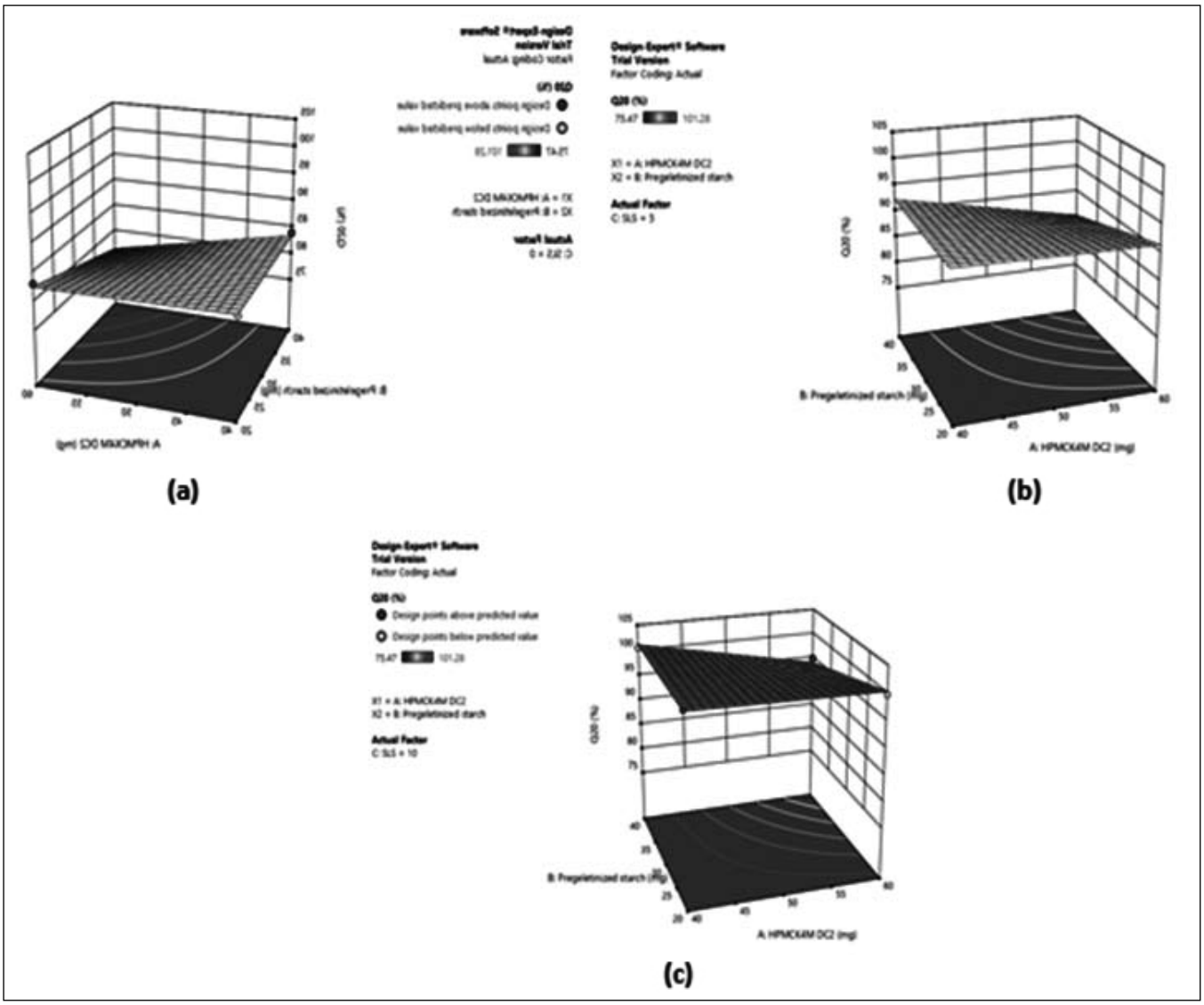


**Fig. 5: Response surface plot for drug release at 20 h (Q20)**
**(a) Drug release at 20 h when concentration of SLS is 0**
**(b) Drug release at 20 h when concentration of SLS is 5**
**(c) Drug release at 20 h when concentration of SLS is 10**

dissolution data through zero order, first order, Higuchi's and Korsmeyer Peppas equations[9].

### Accelerated stability study

The optimized batch was subjected to stability study as per ICH guidelines[10].

## RESULTS AND DISCUSSION

The results of precompression characteristic have been tabulated in table III. Bulk and tapped densities indicate good packing properties.The Carr's index for all formulations was below 16.27% indicating good flow and compressibility. The angle of repose for all blends lies between a range of $16.49^0$ - $22.47^0$ further ascertaining good flow.

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

## DRUG EXCIPIENT COMPATIBILITY

### DSC studies

The thermograph of pure drug bosentan monohydrate showed the melting point to be 197.94 $^0$C, which is as reported in literature. The thermograph of drug and

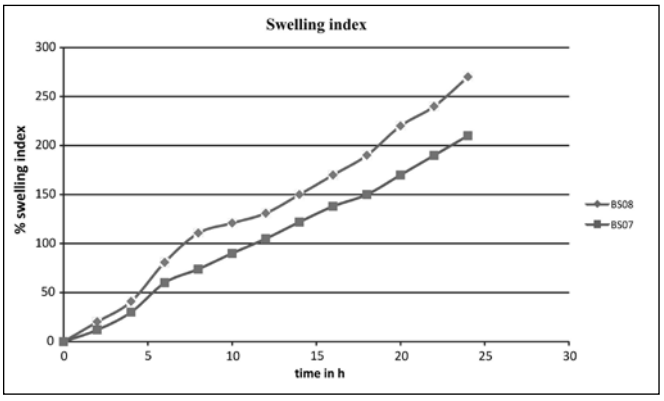


**Fig.6. Swelling index of formulation BS07 and BS08**

excipient mixtures does not reveal significant change in the melting points thus ruling out incompatibilities as depicted in Fig. 1.

### *In vitro* drug release

The release of sustained release bosentan monohydratefrom the 8batches is as given in figure 2.All batches showed drug release varying from 14-24 hours depending on the concentration of independent variables. Batches BS01-BS04that do not contain solubilser SLS do not show complete drug release at the end of 24 h. This may be attributed to the saturation of the drug released in the dissolution vessel after 14 hours. Formulations BS05-BS08 containing SLS as solubilizer showed adequate drug release. Formulations BS05 and BS07 containing 40 mg of HPMC K4M DC2 Premium per tablet could control the release for 14-16 hours, whereas formulations BS06 and BS08 containing 60mg HPMC showed adequate control for a 24 h period as desired. Pregelatinized starch was observed to have a synergistic effect along with HPMC on controlling the drug release.

To describe the entire dissolution profile, three time points were considered in the design expert software. Fig.3 shows the response surface plot for drug release at 2 h (Q2). As depicted, as the concentration of HPMC increases from 40mg to 60 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 significant effect at 2 h.

At 8 hours, 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 matrix of HPMC. HPMC along with pregelatinized starch forms a synergistic matrix gel thereby influentially controlling the drug release for 24 h period as depicted in Fig. 4.The effect of SLS is not significant at 8 h.

A very significant influence of SLS was observed at 20 h. As depicted in Fig. 5,it was observed that as SLS concentration increases from 0 to 10 mg per tablet the release of drug increases at 20 h. This may be attributed to the fact that bosentan being a BCS class II drug, having a solubility of around 40mg/100 mL in phosphate buffer pH 7.2, may pose a problem in the drug getting solubilised in the dissolution vessel after 16 hours. Therefore addition of SLS as a solubiliser in the tablet helps enhance the solubility of the drug as it is released.

The statistical data obtained from ANOVA for the models is represented in Table V. From the ANOVA analysis it can be observed that HPMC K4M DC2 Premium(X1) polymer has effect on all responses Q2, Q8 and Q20reinforcing the fact that it is the main release retarding polymer that controls the drug release upto24 h. At two hours the coefficient, as observed from the equation in table V 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. 6.It was also observed that as the HPMC content per tablet increases the swelling index also increases. Pregelatinized starch has significant influence at 8 hours 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 16 h onwards till 24 h. Since $P<0.05$ for all three responses, indicates that the model is significant.$R^2$values 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 significant. Since coefficient of variation CV is less than 10 the model can be considered reproducible.

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 thesuper 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 VI.

## CONCLUSION

In the present research, a sustained release tablet of bosentan monohydrate using combination of HPMC K4M Premium DC2 and pregelationized starch was developed. SLS was used as a solubilizer in the formulation which showed significant influence on the drug release. The formulation was optimised by using the design expert software which predicted the influence of the above formulation variables on drug release at various time points. The optimised formulation BS08 showed a controlled drug release upto a 24 h period.

Thus, the developed sustained release tablet of bosentan monohydrate can be useful in reducing the side effects associated with bosentan, reducing dosing frequency and increasing patient compliance. Statistical tools like design expert are an ideal way of predicting the influence of various variables in the optimisation of a formulation.


## ACKNOWLEDGEMENTS

My sincere gratitude to my guide Dr. H. M. Tank, for his guidanceand support throughout the work.The authors are thankful to Mr.AnantNaik of Unichem Laboratories and Cipla Ltd. for providing gift sample of drugs.


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