Formulation and Evaluation of Matrix Membrane Moderated Transdermal Patches of Bosentan Monohydrate

Revathi Mannam, Indira Muzib Yallamalli

Abstract— The main aim of the study was to develop matrix membrane moderated transdermal drug delivery systems of Bosentan Monohydrate and to study the effect of different rate controlling membranes on the drug release pattern. The drug reservoir membranes were prepared by using HPMC and rate controlling membranes by HPMC K4M, HPMC K15M, HPMC K100M and E RL PO. The effect of rate controlling membrane on drug release pattern was studied by *in-vitro* and *ex-vivo* methods. All the formulated transdermal patches were tested for physical appearance and pharmacotechnical properties. F3 (HPMC K4M: ERL PO 1:0.4) has shown a drug release of 93.93±1.23% in 24 h with a flux of 2.101±0.021 (µg/cm^2/h). In case of HPMC K15M and HPMC K100M the drug release prolonged for more than 24h. Drug release kinetics was interpreted by using different kinetic models and the drug release followed mixed order kinetics, non-fickian diffusion model. The drug release mainly depended on the swelling behavior and matrix erosion of the polymer in rate controlling membrane. Stability studies were conducted according to ICH guidelines and the formulations were found to be stable. *Ex vivo* studies has shown a significant decrease in drug release compared to in vitro and F3 with highest drug release was selected for further studies. Based on the above studies it was revealed that by using rate controlling membrane desirable release patterns can be obtained and the films were found to be physically acceptable and are more durable.

Index Terms —Bosentan Monohydrate, E RL PO, *ex-vivo* studies , HPMC K4M, *in-vitro* studies, Reservoir membrane, Rate controlling membrane.

1 INTRODUCTION

Transdermal Drug Delivery System (TDDS) is an innovative drug delivery system, where drug can be released directly in to the systemic circulation to provide systemic effect or to provide local action through skin at site of delivery [1]. TDDS provides controlled release of drug by following zero order kinetics and reduces the drug toxicity by minimizing the plasma concentration of drug. The ideal transdermal patch is one where skin is not a rate controlling factor for drug release. For the effective passage of drug through the skin flux is very important criterion, i.e., by diffusion drug passes through the skin and enters in to systemic circulation where the concentration gradient is the driving force [2]. Selection of drug for TDDS is a very important step; the drug is selected based on the physicochemical properties like melting point, molecular weight, partition coefficient, skin permeability, dose, kinetic properties like half-life and bioavailability [3]. In the present work, transdermal drug delivery system was formulated with an approach where the drug is present in reservoir membrane and release is controlled by matrix membrane. Bosentan monohydrate is a new generation endothelin receptor antagonist used to treat pulmonary arterial hypertension, which is a very progressive disease. Bosentan monohydrate is used as a dual endothelin receptor antagonist which acts by blocking endothelin receptors there by reduces the constriction of pulmonary artery [4]. Bosentan monohydrate has hepatic first pass metabolism, terminal half-life of 5 hours, bioavailability of 50%, partition coefficient (3.4) and pKa (5.8), have rationalized the selection of drug for formulation of transdermal drug delivery system [5].

Polymer like Hydroxy Propyl Methyl Cellulose (HPMC) was selected as different grades of HPMC are available which acts as both film forming and release retarding agent. As they absorb high amount of moisture the polymer swells and increases the path length for the diffusion of drug, which results in retarding release of the drug. HPMC also helps in providing the adhesive action to the transdermal patch which synergists the performance of transdermal patch [6]. Eudragit RL PO (E RL PO)was selected as it forms matrix and further sustains the drug release through the patch [7, 8]. The polymers selected are non-toxic and are known for many years.

Membrane moderated transdermal systems were formulated by using E RL PO and different grades of hydrophilic polymers like HPMC, HPMC K4M, HPMC K15M, HPMC K100M. In this the transdermal patch consist of two layers where the primary layer is drug reservoir layer prepared by using HPMC and the secondary layer is rate controlling membrane prepared by using HPMC K4M, HPMC K15M, HPMC K100M and E RL PO. The drug release through rate controlling membrane was controlled by the matrix diffusion, swelling of the polymer and polymer relaxation rate.
In the present work an attempt was made to develop a passive type transdermal system, where the drug release was prolonged up to 24 h to provide relief from hypertension to

- *Revathi Mannam is currently pursuing doctoral degree program in pharmaceutical sciences in Sri Padmavathi Mahila University, India. E-mail: revathi.mannam@gmail.com*
- *Indira Muzib Yallamalli is currently professor in pharmaceutics in Sri Padmavathi Mahila University, India. E-mail: yindira1414@gmail.com*

improve the quality of life. The study comprises of physico-chemical characterization, *in-vitro* and *ex-vivo* studies.

2 Materials and Methods

2.1 Materials

Bosentan Monohydrate was a gift sample received from MSN Pharmaceuticals, Hyderabad. HPMC, HPMC K4M, HPMC K15M, HPMC K100M were gift samples provided by Colorcon Asia Pvt. Ltd., Mumbai. E RL PO was gift sample received from Zhaveri Pharma Chemicals, Mumbai. All reagents used were of analytical grade.

2.2 Partition coefficient

Partition coefficient of pure drug was performed by using n-octanol and water in a separating funnel. 50 mL of n-octanol and 50 mL water were taken and mixed well. To this drug was added and agitated at regular time intervals. After 24 h both octanol and water were separated and the amount of drug present was determined spectrophotometrically at 272nm. The ratio of both the values (partition coefficient) was calculated.

2.3 Preparation of drug reservoir patch

Drug reservoir patch of bosentan monohydrate was prepared by using HPMC as film forming agent and propylene glycol as plasticizer. Required amount of HPMC was dispersed in to solvent system (dichloromethane & methanol in 1:1 ratio) and kept for stirring until a homogenous mixture was obtained, drug and plasticizer were added simultaneously. The resultant matrix dispersion was sonicated for 2 min to remove the entrapped air bubbles and casted on a backing membrane (PVA-6%) in a glass mould 4x4 sq.cm. For uniform evaporation mould was covered with inverted funnel and dried at 40°C. Later the dried film was separated from mould and stored in a desiccator.

2.4 Preparation of rate controlling membrane

Rate controlling membrane was prepared by using different concentrations of HPMC K4M (Table 1), HPMC K15M (Table 2), HPMC K 100M (Table 3) and ERL PO. Required amount of polymer was dispersed in solvent system of (dichloromethane & methanol in 1:1 ratio). Plasticizer and penetration enhancer were added simultaneously. The resultant matrix dispersion was coated on a glass mould 4x4 sq.cm. For uniform evaporation glass mould was covered with inverted funnel and dried at 40°C. Later the dried film was separated from the mould and stored in a desiccator. Rate controlling membrane was glued to drug reservoir patch by applying moisture.

3 Evaluation of Transdermal Patch

3.1 Physical appearance

All prepared patches were inspected for clarity, color, flexibility, transparency and smoothness.

TABLE 1: RATE CONTROLLING MEMBRANE BY HPMC K4M

Ingredients	F1	F2	F3	F4
HPMC K4M	1%	1.5 %	1%	1%
E RL PO	-	-	0.4%	0.5%
*Propylene glycol	20%	20%	20%	20%
*DMSO	5%	5%	5%	5%
DCM: Methanol	1:1	1:1	1:1	1:1

**Quantities were taken in percentage weight of polymer*

TABLE 2: RATE CONTROLLING MEMBRANE BY HPMC K15M

Ingredients	F5	F6	F7	F8
HPMC K15M	1%	1.5 %	1%	1%
E RL PO	-	-	0.4%	0.5%
*Propylene glycol	20%	20%	20%	20%
*DMSO	5%	5%	5%	5%
DCM: Methanol	1:1	1:1	1:1	1:1

**Quantities were taken in percentage weight of polymer*

TABLE 3: RATE CONTROLLING MEMBRANE BY HPMC K100M

Ingredients	F9	F10	F11	F12
HPMC K100M	1%	1.5 %	1%	1%
E RL PO	-	-	0.4%	0.5%
*Propylene glycol	20%	20%	20%	20%
*DMSO	5%	5%	5%	5%
DCM: Methanol	1:1	1:1	1:1	1:1

**Quantities were taken in percentage weight of polymer*

3.2 Weight uniformity

Three films from each batch were selected randomly and weighed. Weight of three films was noted and the average weight was calculated.

3.3 Folding endurance

Folding endurance was measured manually for the prepared patches. A strip of the film was cut and repeatedly folded at the same place till it broke. The number of times a film could be folded with-out any cracking gave the value of folding endurance. The procedure was repeated for three samples.

3.4 Flatness

Longitudinal strips were cut out from the prepared films and the length of the strip was measured at two different places. Flatness was calculated to find out the constriction of the film after drying.

Constriction (%) = $(L_1\text{-}L_2/L_2) \times 100$

3.5 Thickness

Film thickness was measured by using screw gauge at three different sites and the mean average value was calculated.

3.6 Drug Content

The patch of area 1cm² was added to 100ml phosphate buffer saline (PBS). The medium was stirred in a magnetic stirrer for a period of 24 h. After 24 h the solution was filtered and required dilutions were made and absorbance was noted at 272 nm using UV-Visible spectrophotometer [9].

3.7 Percentage moisture content

Prepared films were weighed (Wi) and transferred in to a desiccator containing silica gel and weighed after a constant weight was achieved (Wc). Moisture content study was done by using the formula [10].

Moisture content (%) = (Wi-Wc) / Wc x100. (1)

3.8 Percentage moisture uptake

The films were kept in a desiccator with silica gel for 24h and weighed (Wi). The films were then transferred in to another desiccator containing NaCl solution until constant weight was obtained (Wm). Moisture uptake study was calculated according to the formula [11].

Moisture uptake capacity (%) = (Wm - Wi / Wi) x 100. (2)

3.9 pH measurement

pH of the swollen film was measured by using pH meter which was calibrated before use.

3.10 FT-IR Study

FT – IR study was done on the pure drug and physical mixture with IR grade KBr. Prepared KBr pellets were scanned between wave number regions of 4000-400 cm^{-1}.

3.11 Differential scanning calorimetric studies

Thermal analysis was carried out by using a Differential scanning calorimeter with a liquid nitrogen cooling accessory. Sample was placed in aluminum crucible cell and was firmly crimped with the lid. The sample was heated from ambient temperature to the required temperature at pre-programmed heating rate of 10 °C per min.

3.12 *In-vitro* Diffusion studies

in-vitro diffusion studies for the selected transdermal patch were carried out by using modified Franz-diffusion cell. The receptor compartment contains PBS and the donor compartment contains transdermal film of area 3.14cm² placed on dialysis membrane-150. The dialysis membrane was soaked previously in PBS over-night. The whole assembly was kept on a magnetic stirrer and stirred at 500 rpm and temperature maintained at 37±0.4°C. The amount of drug released was determined by collecting the sample of 3mL per interval. The drug content in the sample was analyzed spectrophotometrically at 272nm [12].

3.13. *Ex-vivo* release studies

Preparation of skin: Full thickness of dorsal skin was obtained from male Wistar rats (CPCSEA Registration No.:1677/PO/a/12/CPCSEA). The rat was anaesthetized by using chloroform and hair was shaved. The dorsal skin was surgically removed from the animal and the adhering fat was removed with the help of scalpels by keeping the skin in boiled water at 60°C. The prepared skin was cleaned with the help of PBS covered with aluminum foil and stored in -20°C. Generally, the skin can be used for 2 weeks [13].

Ex-vivo permeation studies for the selected transdermal patch were carried out using modified Franz-diffusion cell. The receptor compartment contains PBS and the donor compartment contains transdermal film of area 3.14cm² placed on skin with dermal side in contact with receptor phase, equilibrated for 1h. The whole assembly was kept on a magnetic stirrer stirred at 500 rpm and temperature was maintained at 37±0.4 °C. Amount of the drug released was determined by collecting the sample of 3mL per interval, analyzed spectrophotometrically at 272nm.

3.14 Scanning electron microscopy

Scanning electron microscope (Model JSM 6360, Jeol Make, United Kingdom) was used to characterize the surface of the transdermal patches.

3.15 Drug release kinetics

To study the release kinetics data obtained from in-vitro studies was fitted to various kinetic models such as zero order, first order, higuchi model and korsmeyer-peppas model and the one with highest correlation coefficient was considered to be the best suitable model.

3.16 Stability studies

Stability studies were conducted according to ICH guidelines. Optimized transdermal patches were stored under 40±2°C/75%RH in stability chamber (Thermo lab, Mumbai) for a period of 6 months. After 6 months, transdermal patches were tested for *in-vitro* drug release studies and drug content.

4 Statistical Analysis:

All the results were done in triplicate and represented as mean ± SD. Statistical analysis was done using Graph Pad Prism 5.0.

5 Results and Discussion:

Partition coefficient of drug was found to be 3.4 shows the drug is having desired lipophilicity and was found suitable for transdermal drug delivery system.

Film thickness values were in between 1.130±0.04 mm to 1.42±0.015 mm. The increase in thickness was due to increase in polymer concentration [14]. Folding endurance values were found to be more than 200 for all formulations which shows that transdermal films were more flexible and durable.

Uniformity in drug content is an important parameter which ensures a uniform controlled release of drug from patch [15]

TABLE 4: PHARMACOTECHNICAL PROPERTIES OF TRANSDERMAL PATCHES

Formulation	Thickness (mm)	Weight uniformity (mg)	Flatness (%)	Folding endurance	%Drug content	Surface pH	%Moisture content	%Moisture uptake
F1	1.130±0.04	35.6±1.17	100	320±0.57	99.73±0.28	7.13±0.12	1.42±0.05	1.57±0.21
F2	1.243±0.03	38.4±1.23	100	322±0.21	98.82±0.41	6.86±0.15	1.46±0.01	2.65±0.33
F3	1.132±0.03	39.6±0.98	100	321±0.57	99.23±0.51	7.17±0.11	1.42±0.01	1.57±0.11
F4	1.146±0.02	39.4±1.06	100	312±0.52	99.14±0.46	6.76±0.21	1.41±0.05	1.77±0.15
F5	1.263±0.02	37.8±1.77	100	317±0.51	100.05±0.4	6.76±0.31	1.45±0.05	2.95±0.28
F6	1.306±0.15	38.8±2.01	100	321±3.05	99.09±0.68	6.93±0.11	1.48±0.05	3.01±0.66
F7	1.236±0.02	37.5±1.56	100	319±0.52	98.56±0.26	6.96±0.21	1.55±0.02	2.84±1.73
F8	1.216±0.05	38.5±1.42	100	318±0.57	99.78±0.05	7.06±0.15	1.57±0.05	1.76±0.98
F9	1.333±0.05	38.9±1.67	100	321±0.15	98.56±0.43	7.23±0.05	1.75±0.02	3.08±1.45
F10	1.42±0.015	37.9±1.55	100	318±0.57	99.14±0.51	6.96±0.21	1.70±0.05	3.99±1.34
F11	1.323±0.21	38.5±2.08	100	323±0.08	99.25±0.23	6.86±0.05	1.67±0.05	2.09±1.67
F12	1.303±0.05	39.4±1.38	100	325±0.15	99.57±0.37	7.23±0.05	1.68±0.05	2.80±1.83

Note: All the values are mentioned as mean ± SD, n=3

and the percentage drug content values for all the transdermal films were found to be in the range of 100.05±0.4%-98.82±0.41%.

Flatness of the transdermal patch is important parameter which ensures that there is no constriction of film after drying, flatness of the film was evaluated by taking length of the film at different places and the results were found around 100%, which ensures that there is no constriction of film after drying and the patch can be applied evenly to the skin.

Surface pH was mainly done to know whether the film is acidic or basic. Irritation will persist if the film is more acidic or basic. Surface pH of the transdermal films was in between 6 and 7 which match to the pH of the skin, infers that the film is not having irritating property

Moisture content and moisture absorption are the main parameters to evaluate a film to know whether it can withstand with the atmospheric conditions or not. Stability of the transdermal film can be evaluated with this study. The percent moisture content of the transdermal film increased with increase in grade of HPMC, the increase in moisture content was mainly due to increase in hydrophilic nature of the polymer [16]. The moisture content values were low with less standard deviation values, indicate that the film was more durable and can withstand for long time. Moisture absorption values were less indicating the stability of the film for long term duration and protects the films from degradation and microbial contamination [17] (Table 4).

The FT-IR spectrum shows that there is no interaction between drug and polymers and the drug is compatible with the polymers (Table 5) (Figure 1, 2, 3). Drug characteristic peaks were observed at 3629 cm^{-1} (O-H), 3440 cm^{-1} (N-H Stretch), 2959 cm^{-1} (C-H), 1585 cm^{-1} (N-H bond), 1343 cm^{-1} (S=O).

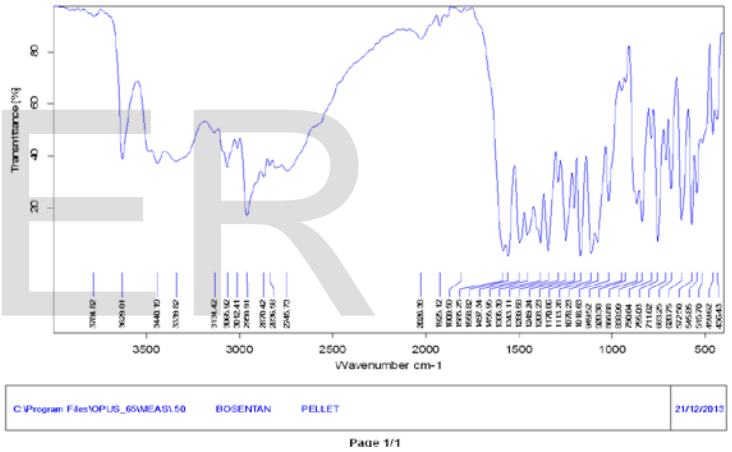

Fig 1: FT-IR spectra of pure drug

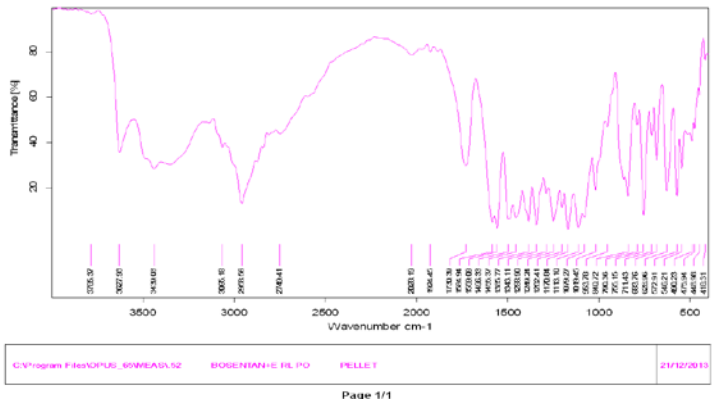

Fig 2: FT-IR spectra of drug and E RL PO

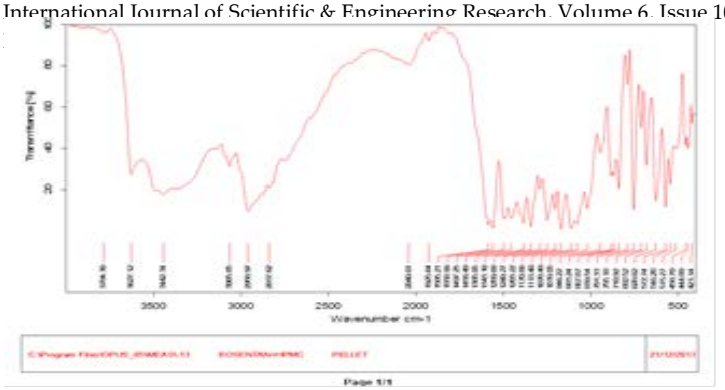

Fig 3: FT-IR spectra of drug and HPMC, HPMC K4M, HPMC K15M, HPMC K100M

DSC spectra of the pure drug and formulated patch reveal that there was no interaction of the drug with the polymers and no change in the physicochemical properties of the drug (Figure 4, 5).

TABLE 5: CHARACTERISTIC PEAKS OF BOSENTAN MONOHYDRATE OBSERVED IN PURE DRUG AND PHYSICAL MIXTURES

Bosentan Monohydrate		Bosentan Monohydrate + ERL PO		Bosentan Monohydrate + HPMC	
Wave number (CM^{-1})	Functional Group	Wave number (CM^{-1})	Functional Group	Wave number (CM^{-1})	Functional Group
3629	O-H	3627	O-H	3627	O-H
3440	N-H stretch	3439	N-H Stretch	3439	N-H Stretch
2959	C-H	2958	C-H	2957	C-H
1585	N-H Bend	1584	N-H Bend	1584	N-H Bend
1343	S=O	1343	S=O	1343	S=O

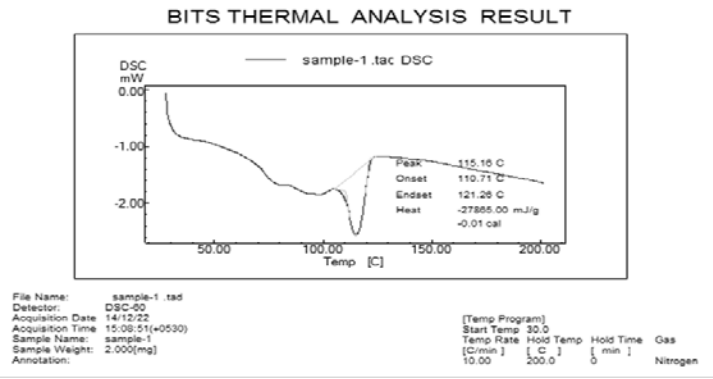

Fig 4: DSC spectra of pure drug

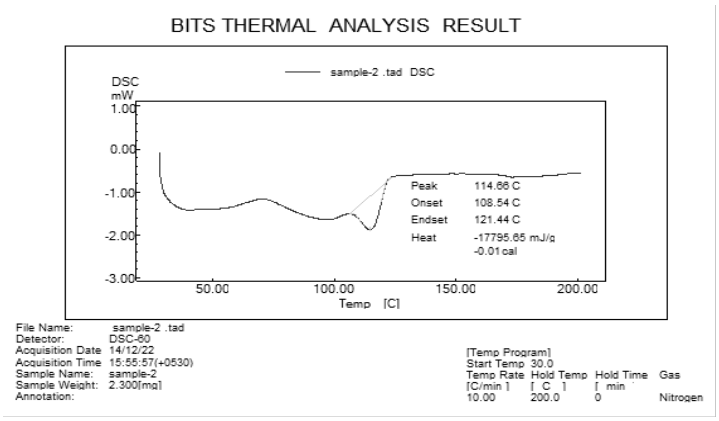

Fig 5: DSC spectra of formulated transdermal patch F3

SEM image of the F3 formulation shows that the patch has uniform surface and uniform distribution of drug throughout the patch (Figure 6).

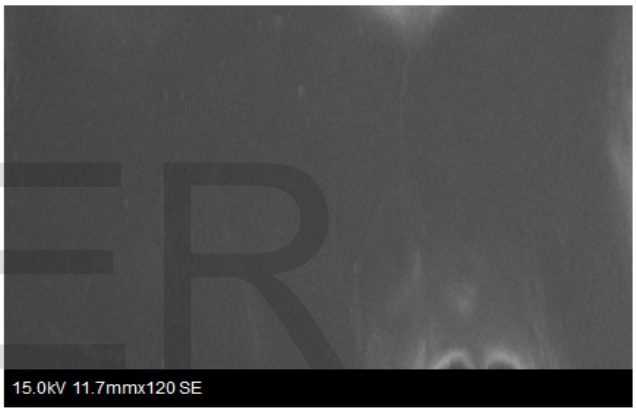

Fig 6: SEM image of HPMC K4M transdermal patch (F3)

5.1 *In-vitro* release studies

In-vitro release studies are important to assess the drug release behavior of transdermal dosage forms and these studies mimic the drug release behavior of transdermal film in *in-vivo*. *In vitro* studies were conducted according to the FDA guidelines [18]. In *in-vitro* studies along with the drug release studies different parameter like flux, permeability coefficient, lag time and diffusion coefficient were estimated.

Flux is defined as the rate at which drug was released in to the diffusion media and this mainly depends on the concentration gradient and thickness of the drug release barrier [19]. Flux was calculated from slope of the Cumulative drug release vs Time. Permeability coefficient was defined as the ratio of flux vs initial drug load [20]. Lag time was defined as the time taken for the release of drug in to the diffusion media at the initial stage, calculated by back extrapolation of the cumulative drug release Vs Time curve on X-axis (Table 6).

TABLE 6: *IN-VITRO* RELEASE STUDIES OF TRANSDERMAL PATCHES

Formulation	Flux (µg/hr/cm²)	Permeability coefficient (k)	Lag time(hr)	Diffusion coefficient (cm²/hr)
F1	8.320±0.013	1.664±0.21	0.12±0.17	0.020±0.01
F2	5.528±0.015	1.228±0.17	0.19±0.25	0.031±0.09
F3	2.101±0.021	0.420±0.14	0.23±0.09	0.038±0.11
F4	1.869±0.023	0.424±0.45	0.24±0.21	0.040±0.05
F5	5.460±0.021	1.092±0.09	0.15±0.33	0.025±0.21
F6	5.141±0.011	1.028±0.62	0.15±0.26	0.025±0.04
F7	1.971±0.028	0.438±0.76	0.24±0.08	0.040±0.19
F8	1.911±0.011	0.406±0.59	0.37±0.18	0.061±0.02
F9	5.120±0.016	0.948±0.32	0.30±0.23	0.050±0.16
F10	4.962±0.014	1.078±0.41	0.34±0.20	0.056±0.21
F11	1.810±0.002	0.362±0.21	0.38±0.26	0.063±0.22
F12	1.593±0.016	0.318±0.27	0.40±0.22	0.066±0.05

Note: All the values are mentioned as mean ± SD, n=3

In the present study *in-vitro* diffusion studies were carried out using modified Franz-diffusion cell with dialysis membrane as barrier for drug release. 3 mL of sample was collected at regular intervals of time and sink conditions were maintained. Drug release was estimated spectrophotometrically at 272 nm. A graph was plotted by taking cumulative percent drug release on y-axis and time on x-axis (Figure 7, 8, 9).

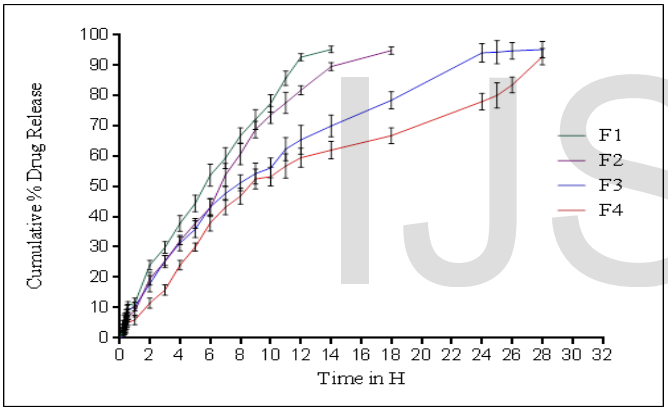

Fig 7: Cumulative *in-vitro* release profile of HPMC K4M transdermal patches

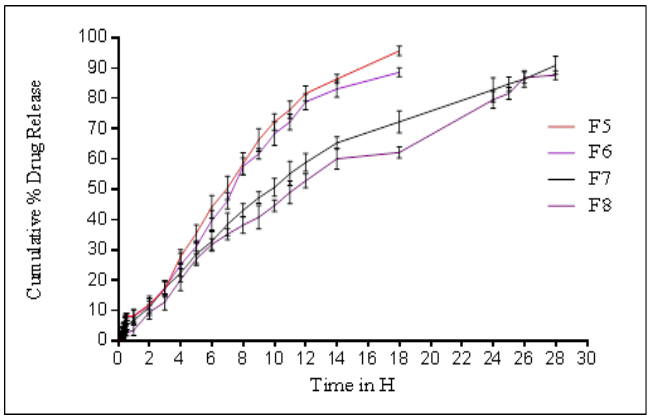

Fig 8: Cumulative *in-vitro* release profile of HPMC K15M transdermal patches

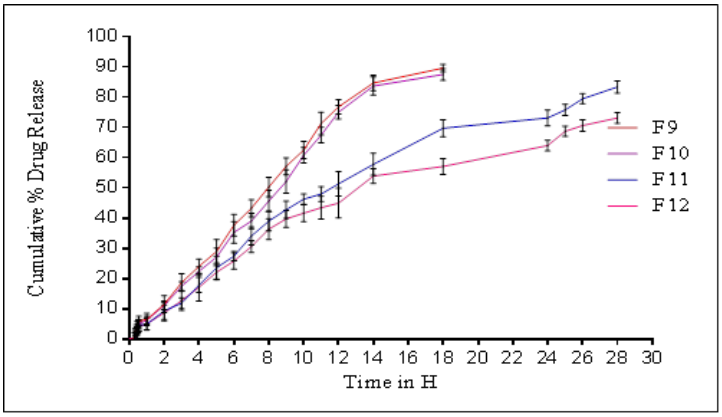

Fig 9: Cumulative *in-vitro* release profile of HPMC K100M transdermal patches

In case of transdermal patches formulated by using HPMC K4M 90% of drug was released at 14h (F1and F2) and by adding matrix forming polymer E RLPO drug release was extended up to 24h (F3 and F4). HPMC K15M was more hydrophilic compared to HPMC K4M and retarded the drug release more than HPMCK4M because the main mechanism in drug release is by swelling and erosion of polymer, more the hydrophilic more will be the swelling ratio of the polymer [21]. By using simple HPMC K15M release retarding membrane the drug release was extended up to18h. To extend the release of drug up to 24h, matrix forming polymer E RL PO [22] was used in F7 and F8 and the drug release was extended up to 24h. The addition of E RL PO in F7 and F8 had shown effect on release behavior.

Transdermal patches formulated by using HPMC K100M a more hydrophilic polymer had shown a huge impact on drug release where drug release rate was very less compared to HPMC K4M and HPMC K15M. When matrix forming agent E RL PO was used in the rate controlling membrane the drug release was extended for more than 24 h and at the end of 24h only 70 % of drug was released.

The drug release mechanism through rate controlling membranes using HPMC K4M, HPMC K15M and HPMC K100M was mainly due to swelling of the polymer and polymer chain relaxation [21]. More hydrophilic the polymer more will be the swelling rate and more will be the path length of the polymer for the release of drug. The initial increase in drug release was mainly due to diffusion of drug which is present on the surface of the drug reservoir film through the rate controlling membrane during storage. Releasing minimum amount of drug in initial stage will help to maintain therapeutic concentration and the extended release will provide constant drug release for a longer period. Formulations F3 and F7 were selected as optimized formulations based on the amount of drug released at the end of 24h.

The drug release kinetics of the formulations was studied by plotting zero order, first order, higuchi and korsemeyer-peppas (k-p) plots [23]. R^2 values in higuchi were more linear indicating the drug release is by diffusion mechanism [24].

TABLE 7: *IN VITRO* PHARMACOKINETICS OF TRANSDERMAL PATCHES

Formulation	Zero order	First order	Higuchi	k-p	
	R^2	R^2	R^2	R^2	n
F1	0.9864±0.001	0.9384±0.020	0.9833±0.014	0.9943±0.004	0.8004±0.01
F2	0.9684±0.002	0.9725±0.021	0.9779±0.020	0.9872±0.002	0.8470±0.03
F3	0.9290±0.001	0.9810±0.001	0.9952±0.001	0.9852±0.021	0.6560±0.04
F4	0.9158±0.004	0.9906±0.002	0.9860±0.017	0.9190±0.037	0.7804±0.01
F5	0.9744±0.001	0.9577±0.001	0.9656±0.041	0.9785±0.007	0.9565±0.03
F6	0.9714±0.001	0.9793±0.012	0.9615±0.028	0.9805±0.002	0.9413±0.02
F7	0.9482±0.003	0.9935±0.001	0.9889±0.020	0.9816±0.028	0.7863±0.16
F8	0.9638±0.001	0.9844±0.002	0.9835±0.031	0.9644±0.039	0.9035±0.01
F9	0.9824±0.001	0.9649±0.021	0.9589±0.037	0.9929±0.051	0.9747±0.03
F10	0.9843±0.001	0.9564±0.001	0.9504±0.026	0.9930±0.027	0.9775±0.04
F11	0.9513±0.011	0.9933±0.001	0.9826±0.018	0.9765±0.058	0.8506±0.02
F12	0.9444±0.001	0.9907±0.001	0.9860±0.027	0.9772±0.031	0.8043±0.01

Note: All the values are mentioned as mean ± SD, n=3

In order to characterize the drug release behavior of the polymeric transdermal film k-p plot was plotted; release exponent values (n) were in between 0.5 and 1 resulting that drug release followed non-fickian diffusion mechanism [23]. When liquid diffusion and polymeric diffusion rate are of same order in diffusion swellable systems drug release is by non-fickian diffusion mechanism [25]. The R^2 values of zero order and first order are almost similar indicating the drug release followed mixed order kinetics [26, 27] (Table 7).

5.2 Ex-vivo permeation studies

Ex-vivo studies through rat skin were conducted for F3 and F7 at the end of the study i.e., at 28h 86.03% of drug was released in case of F3 and 75.38% in F7 (Table 8) where there is a significant difference compared to *in-vitro* studies. The drug release in *ex-vivo* studies was less when compared to *in-vitro* studies which may be due to lipophilic nature and Log P of the drug. It was observed that the lipophilic drugs and drugs with Log P value greater than 3 shows high diffusion in to the stratum corneum and little transport in to systemic circulation [28] (Figure 10).

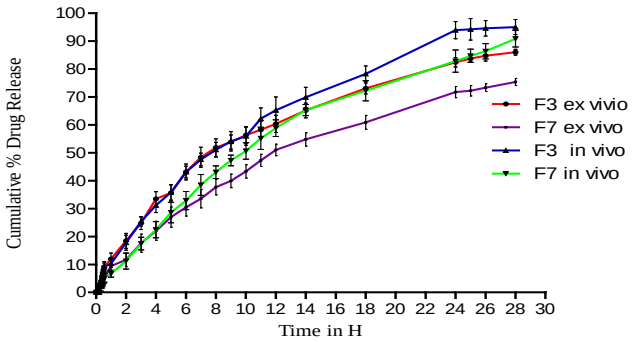

Fig 10: *ex-vivo* and *in-vitro* drug release studies of F3 and F7

TABLE 8: IN VITRO AND EX VIVO RELEASE OF TRANSDERMAL PATCHES

Formulation	Percentage Drug release at the end of 28h	
	in-vitro	ex-vivo
F3	95.04±2.68	86.031±1.025*
F7	90.89±2.99	75.38±1.28***

Note: All the values are mentioned as mean ± SD, n=3
** implies p<0.01 and *** implies p<0.0001 (p<0.05 was found statistically significant difference)*

5.3 Stability studies

Stability studies were conducted for the transdermal formulations F3 and F7 at 40±2°C/75%RH for 6 months in stability chamber, formulations were tested for drug content and *in-vitro* release. Physical appearance of the formulations did not show any crystal growth and all the transdermal patches were found to be transparent. Drug content and drug release studies at the end of stability study did not show any significant variation ($p<0.05$ statistically significant difference). This indicates that all the transdermal patches were stable after stability studies at accelerated condition (Table 9).

TABLE 9: STABILITY STUDIES OF TRANSDERMAL PATCHES

Formulation	Drug content (%)		in-vitro release at 28h (%)	
	Initial	After 6 months	Initial	After 6 months
F3	99.2±0.51	99.03±0.21	95.04±2.68	93.03±2.16
F7	98.56±0.26	96.79±0.78	90.89±2.99	88.05±1.77

Note: All the values are mentioned as mean ± SD, n=3

6 CONCLUSION

Bosentan monohydrate is an antihypertensive agent used to treat pulmonary arterial hypertension. A new approach of transdermal system i.e. matrix membrane moderated system was tried. All the formulations had good physical appearance and they comply with all the ideal physical characteristics of transdermal patch. Formulations using various grades of

HPMC produced extended drug release up to 12h, addition of matrix forming agent in rate controlling membrane further extended the drug release up to 24h and different drug release patterns were obtained. The swelling of the polymer and polymer erosion, polymer chain breaking is the main mechanism involved in the release of drug through the rate controlling membrane. Decrease in drug release in *ex-vivo* studies compared to *in-vitro* studies was observed and this is due to lipophilic nature of the drug. All the transdermal films in the study follow mixed order kinetics. Stability studies conducted indicated that all the formulations were stable.

ACKNOWLEDGEMENT

The authors would like to thank UGC-BSR-RFSMS for providing their financial assistance, DST-CURIE SPMVV for their technical support in FT-IR studies and BITS-HYDERABAD for their technical support in DSC studies.

REFERENCES

[1] T. Pongjangyakul , S. Prakongpan and A. Priprem, "Acrylic matrix type nicotine transdermal patches: In vitro evaluations and batch-to-batch uniformity," Drug Dev. Ind. Pharm, vol.29, no.8, pp.843-853, Sep.2003.

[2] M.R. Prausnitz, S. Mitragotri and R. Langer, "Current status and future potential of transdermal drug delivery," Nature Reviews Drug Discovery, vol.3, no.2, pp.115-124, Feb.2004.

[3] J.-R. Robinson and V.-H. Lee, Controlled Drug Delivery: Fundamentals and Applications. Marcel Dekker, New York, pp. 523-552, 1987.

[4] L. Stephen Archer, E. Kenneth Weir and Martin R Wilkins, "Basic Science of Pulmonary Arterial Hypertension for Clinicians New Concepts and Experimental Therapies Basic science for clinicians," Circulation, vol.121, no.18, pp.2045-2066,May.2010.

[5] Data sheet, Tracleer.www.medsafe.govt.nz/profs/datasheet/t/Tracleertab.pdf

[6] C. Valenta and B.G. Auner, "The use of polymers for dermal and transdermal delivery," Eur. J.Pharm. Biopharm., vol.58, no.2, pp.279-89, Sep.2004.

[7] V. Kusum Devi, S. Saisivam, G.R. Maria and P.U. Deepti, "Design and evaluation of matrix controlled transdermal patches of verapamil hydrochloride," Drug Dev. Ind. Pharm, vol.29, no.5, pp.495-503, May.2003.

[8] S.P. Gupta and S.K. Jain, "Development of matrix-membrane transdermal drug delivery system for atenolol," Drug Delivery, vol.11, no.5, pp281-6, Sep.2004.

[9] R.P. Patel, G. Patel and A. Baria, "Formulation and evaluation of transdermal patch of aceclofenac," Int. J. Drug Delivery, vol.1, no.1, pp.41-51, Ja .2009.

[10] C. Amnuaikit, I. Ikeuchi, K. Ogawara, K. Higaki and T. Kimura, "Skin permeation of propranolol from polymeric film containing terpene enhancers for transdermal use," Int. J. Pharm., vol.289, no.1-2, pp.167-178, Jan.2005.

[11] B. Mukherjee, S. Mahapatra , R. Gupta, B.A. Patra, A. Tiwari and P. Arora, "A comparison between povidone–ethylcellulose and povidone–eudragit transdermal dexamethasone matrix patches based on in vitro skin permeation,"Eur. J. Pharm. Biopharm., vol.59, no.3, pp.475-83,Apr.2005.

[12] H. Bundgaard, T. Norgaard and N.M. Nielsen, "Photodegradation and hydrolysis of furosemide and furosemide esters in aqueous solutions,"Int. J. Pharm., vol. 42, no.1-3, pp.217-224, March.1988.

[13] K. Zhao and J. Singh, "In vitro percutaneous absorption enhancement of propranolol hydrochloride through porcine epidermis by terpenes/ethanol," J. Controll. Rel, vol.62, no.3, pp.359-366, Dec.1999.

[14] T. Mamatha, J.V. Rao, K.Mukkanti and G. Ramesh G, "Development of matrix type transdermal patches of lercanidipine hydrochloride: physicochemical and in vitro characterization," DARU J Faculty Phar, vol.18, no.1, pp.9-16, Jan.2010.

[15] P. Rama Rao and P.V. Diwan, "Formulation and in vitro evaluation of polymeric films of diltiazem hydrochloride and indomethacin for transdermal administration," Drug Dev. Ind. Pharm, vol.24, no.4, pp.327-336, April.1998.

[16] S. Mutalik and N. Udupa, "Glibenclamide transdermal patches: physicochemical, pharmacodynamic, and pharmacokinetic evaluations," J. Pharm Sci., vol.93, no.6, pp.1577-94, June.2004.

[17] P. Arora and B. Mukherjee, "Design, development, physicochemical, and in vitro and in vivo evaluation of transdermal patches containing diclofenac diethyl ammonium salt," J. Pharm. Sci., vol.91, no.9, pp.2076-2089, Sep.2002.

[18] X. Liu, H. Liu, J. Liu , Z. He, C. Ding, G. Huang, W. Zhou and L. Zhou, "Preparation of a ligustrazine ethosome patch and its evaluation in vitro and in vivo," Int. J. Nanomedicine, vol.6, pp.241-247, Jan.2011.

[19] S. Peltola, P. Saarinen-Savolainen, J. Kiesvaara, T.M. Suhonen and A. Urtti, "Microemulsions for topical delivery of estradiol,"Int. J. Pharm., vol.254, no.2, pp.99-107, Mar.2003.

[20] S. Nuntakan, T. Phensri and U. Kaisri, "Transdermal Delivery of Zidovudine (AZT): The effects of vehicles, enhancers, and polymer membranes on permeation across cadaver pig skin," AAPS Pharm. Sci. Tech, vol.5, no.3, pp.82-89, Sep.2004.

[21] P.R.P. Verma and Ashok R. Chandak, "Development of matrix controlled transdermal delivery systems of pentazocine: In vitro/in vivo performance," Acta Pharma, vol.59, pp.171-186, March.2009.

[22] U. Ubaidulla, M.V.S. Reddy, K. Ruckmani, F.J. Ahmad and R.K. Khar, "Transdermal therapeutic system of carvedilol: Effect of hydrophilic and hydrophobic matrix on in vitro and in vivo characteristics," AAPS Pharm. Sci. Tech, vol.8, no.1, pp E1-E8, Jan.2007.

[23] R.W. Korsmeyer, R. Gurny, E. Doelker, P. Buri and N.A. Peppas, "Mechanism of solute release from porous hydrophilic polymers," Int. J. Pharm, vol.15, no.1, pp.25-35,May.1983.

[24] T. Higuchi , "Mechanism of sustained action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices," J. Pharm. Sci., vol.52, no.12, pp.1145-1149, Dec.1963.

[25] L. Perioli, V. Ambrogi, D. Rubini, S. Giovagnoli, M. Ricci, P. Blasi and C. Rossi, "Novel mucoadhesive buccal formulation containing metronidazole for the treatment of periodontal disease," J. Control. Rel., vol.95, no.3, pp.521-33, Mar .2004.

[26] M. Donbrow and Y. Samuelov, "Zero order drug delivery from double layered porous films: release rate profiles of ethyl cellulose, hydroxypropyl cellulose and polyethylene glycol mixtures," J. Pharm. Pharmacol., vol.32, no.7, pp.463-70, Jul.1980.

[27] J.G. Wagner, "Interpretation of percent dissolved-time plots derived from in vitro testing of conventional tablets and capsules," J. Pharm. Sci., vol.58, no.10, pp.1253-7, Oct.1969.

[28] P. Lewi, E. Arnold, K. Andries, H. Bohets, H. Borghys, A. Clark, F. Daeyaert, K. Das, M.P. de Béthune , M. de Jonge , J. Heeres , L.Koymans, J. Leempoels , J. Peeters, P. Timmerman, W.Van den Broeck, F. Vanhoutte , G. Van't Klooster , M. Vinkers , Y. Volovik and P.A Janssen, "Correlations between factors determining the pharmacokinetics and antiviral activity of HIV-1 non-nucleoside reverse transcriptase inhibitors of the diaryltriazine and diarylpyrimidine classes of compounds," Drugs R D, vol.5, no.5, pp.245-57, Sep .2004.

International Journal of Scientific and Engineering Research

COPYRIGHT AGREEMENT

Title of paper: Formulation and Evaluation of Matrix Membrane Moderated Transdermal Patches of Bosentan Monohydrate

Author(s): Revathi Mannam

The undersigned hereby transfer any and all rights in and to the paper including without limitation all copyrights to the IJSER. The undersigned hereby represents and warrants that the paper is original and that he/she is the author of the paper, except for material that is clearly identified as to its original source, with permission notices from the copyright owners where required. The undersigned represents that he/she has the power and authority to make and execute this assignment.

This agreement is to be signed by at least one of the authors who have obtained the assent of the co-author(s) where applicable.

Revathi 31/08/2015

Author's Signature & Date

Revathi Mannam
Institute of Pharmaceutical Technology,
Sri Padmavathi Mahila Visvavidyalayam,
Tirupati-517502

Send to: mailto:ijser.editor@ijser.org