AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development, Characterization and Evaluation of Nebivolol Hydrochloride Transdermal Drug Delivery Systems

Mettu Srikanth Reddy[1*], N. G. Raghavendra Rao[1], Shailaja[2]

1. Department of Pharmaceutics, Jyothishmathi Institute of Pharmaceutical Science, Thimmapur, Karimnagar - 505481, AP. India.

2. Department of Pharmaceutics, St. Peter's Institute of Pharmaceutical Sciences, Warangal - 506001, AP, India.

ABSTRACT

Nebivolol Hydrochloride is a third generation beta blocker used for the treatment of hypertention and heart failure. Nebivolol is rapidly absorbed following oral administration, reaching peak plasma concentrations in 0.5 – 4.0 hrs. The present study was designed to develop a suitable matrix type transdermal drug delivery system (TDDS) for Nebivolol Hydrochloride employing various ratios of hydrophilic and hydrophobic polymers by solvent casting technique. The developed patches were then evaluated for physicochemical characterization, ex-vivo permeation and skin irritation studies. The compatibility of drug with other ingredients was checked by FTIR studies. FTIR results revealed that there was no interaction between dug and other excipients. The transdermal patches obtained were transparent, smooth, uniform and flexible. The results of physicochemical properties were within the pharmacopoeial limits. All the formulations were subjected to ex-vivo skin permeation study by means of Franz's diffusion cell in order to optimize the suitable formulation. Two formulations with the polymeric blend 3:2 (HPMC E50: ERL 100 and HPMC E15: ERL100 respectively) showed an increase in permeation of drug via skin when compared with the formulations having less proportion of hydrophilic polymer (HPMC), however the formulation with HPMC E50 : ERL 100 showed overall improvement in flux and permeation, hence it was optimized as suitable matrix system. The drug release follows zero order kinetics with diffusion mechanism. The average steady state flux obtained with HPMC E50: ERL 100 (3:2) was 43.3 µg/cm^2/hr and the same was increased to 59.2 µg/cm^2/hr with the incorporation of 5% v/w of DMSO as permeation enhancer. In conclusion, the present data confirm the feasibility of developing Nebivolol Hydrochloride transdermal system. The release rate of drug through patches increased when the concentration of hydrophilic polymer was increased.

Keywords: Nebivolol Hydrochloride, HPMC E 50, HPMC E15, transdermal patches.

*Corresponding Author Email: ngraghu@rediffmail.com

Received 20 October 2012, Accepted 02 November 2012

Please cite this article in press as Reddy MS *et al.*, Development, Characterization and Evaluation of Nebivolol Hydrochloride Transdermal Drug Delivery Systems. American Journal of PharmTech Research 2012.

INTRODUCTION

Over the past few years, the field of transdermal drug delivery has experienced a rapid growth. One of the driving forces behind this growth is the increasing number of drugs that can be delivered in clinically effective concentrations, via the skin portal to the systemic circulation. This is in spite of the inherent protective function of the stratum corneum, which is primary one in excluding foreign substances from entering the body. Success has been achieved in the administration of several drugs transdermally via transdermal therapeutic systems (TTS) with a view of maintaining a constant plasma concentration of the respective drug over a pre-determined time period[1].

Hypertension is one of the main causes of heart disease, and in recent years, the age-adjusted hypertension and hypertensive disease death rates are increasing[2] (Hoyert et al., 2005). Consequently, the prevention and treatment of hypertension are of social significance[3].

Transdermal drug delivery is the non-invasive delivery of medications from the surface of skin- the largest and most accessible organ of human body- through its layers, to the circulatory system. Transdermal drug delivery system (TDDS) provides a means to sustain drug release as well as reduce the intensity of action and thus reduce the side effects associated with its oral therapy. Since 1980 this system has been available commercially. A number of therapeutic agents including antihypertensive, antianginal, antihistaminic, anti-inflammatory, analgesic, and anti-arthritic drugs are being investigated and developed for transdermal therapeutic systems either for academic research or for commercial purpose[4, 5].

TDDS has gained a lot of interest during the last decade as it offers many advantages over the conventional dosage forms and oral controlled release delivery systems notably avoidance of hepatic first pass metabolism, less frequency of administration, reduction in gastrointestinal side effects and improves patient compliance[6]. For example, it provides controlled release of the drug into the patient, and enables a steady blood-level profile, leading to reduced systemic side effects and, sometimes, improved efficacy over other dosage forms[7,8]. In addition, the transdermal patch dosage form is user-friendly, convenient, painless, and offers multiday dosing, it generally leads to improved patient compliance[9]. The transdermal therapeutic system is of particular clinical significance for prevention and long-term treatment of the chronic diseases like hypertension.

Nebivolol Hydrochloride is a third generation beta blocker used for the treatment of hypertention and heart failure. Nebivolol is a racemate (DL-nebivolol) made up of equal parts of the D- and the L-isomer of the drug. It is a highly β1-adrenergic selective β-blocker with distinct

vasodilatory capacities. The D-isomer primarily exerts β-blocking effects; and both the D-isomer and the L-isomer seem to have vasodilatory capacities. Nebivolol is rapidly absorbed following oral administration, reaching peak plasma concentrations in 0.5 – 4.0 h. Absorption is not affected by food and age. Nebivolol is subject to extensive first-pass effect by hepatic metabolism primarily through CYP450 2D6 enzymes[10].

In recent years, owing to advantages offered by transdermal administration and the extensive use of β-blockers in the treatment of various cardiovascular disorders, several authors have studied percutaneous permeation and TDD with patches of β-blockers across artificial animal[11-13] or human skin membrane[14] models.

The present study was designed to develop a suitable matrix type transdermal drug delivery system (TDDS) for Nebivolol Hydrochloride employing various ratios of hydrophilic (HPMC E5, HPMC E15 and HPMC E50) and hydrophobic polymers (Eudragit RL 100) by solvent casting technique. The developed patches were then evaluated for physicochemical characterization, ex-vivo permeation and skin irritation studies.

MATERIALS AND METHODOLOGY:

Materials:

Nebivolol hydrochloride was received as a gift sample from Cadila pharmaceuticals Ltd (Gujarat, India). Eudragit RL 100, HPMC E15 and HPMC E50 were received as a gift sample from Reddy's Laboratories Hyderabad. All other chemicals such as dichloromethane, methanol, propylene glycol and acetonitrile were of laboratory grade.

Preparation of transdermal patches:

Matrix type transdermal patches containing Nebivolol HCl were prepared using different ratios **(Table 1)** of Eudragit RL 100 (ERL 100) and hydroxy propyl methylcellulose (HPMC E 5, E15 and E50) by solvent casting technique. The polymers were weighed in requisite ratios by keeping the total polymer weight 1250 mg dissolved in 10ml of solvent mixture (1:1 ratio of dichloromethane, methanol) and allowed to swell for about 6 hrs, after 6 hrs 15% v/w (of dry polymer weight) propylene glycol was incorporated as plasticizer and drug dissolved in methanol was added slowly to the polymer solution and mixed thoroughly to obtain a uniform solution. This polymer drug solution was set aside for some time to exclude entrapped air. The polymer solution of drug was poured on to the anumbra petriplate (38 cm^2) and dried at room temperature in a dust free environment; the rate of evaporation of solvent was controlled by inverting cup funnel. After 24 hrs the film obtained was cut into 4cm^2 area patches and aluminium foil as a

backing membrane glued on, patches were stored in a desiccators for further use[15].

Table 1: Composition of Nebivolol HCl transdermal patches [The dose of the drug is 10mg]

Polymer blend	Formulation code								
	A1	**A2**	**A3**	**B1**	**B2**	**B3**	**C1**	**C2**	**C3**
E RL 100 : HPMC E5	**1:4**	**2:3**	**3:2**	-	-	-	-	-	-
E RL 100 : HPMC E15	-	-	-	**1:4**	**2:3**	**3:2**	-	-	-
E RL 100 : HPMC E50	-	-	-	-	-	-	**1:4**	**2:3**	**3:2**

HPMC E5-hydroxypropyl methylcellulose E 5, HPMC E15-hydroxypropyl methyl cellulose E 15, HPMC E50 hydroxy propyl methylcellulose, ERL 100- Eudragit RL 100.

Preparation of Rat Abdominal Skin:

Male wistar albino rats (150-200 g) were used for the study. The rats were sacrificed by using excess amount of anesthetic ether. Before surgical removal of the skin, hair on dorsal side was removed with hair clipper taking extreme precautions not to damage the skin. The epidermis was prepared by a heat separation technique[16], which involved soaking of the entire abdominal skin in water at 60°C for 45 sec, followed by careful removal of the fatty layer. The epidermis was washed with water, wrapped in aluminium foil and stored at -20°C till further use (should be used within 2weeks of preparation).

Drug- Polymer Interaction Study:

To study the possible interaction between Nebivolol HCl and polymeric materials of the patches, infrared (IR) spectroscopy was carried out on pure substances and their physical mixtures. The IR spectra are recorded using IR Spectrophotometer (Perkin Elmer FT-IR, Perkin Elmer Inst. USA) by KBr pellet method.

Evaluation of Transdermal Patches:

The developed patches were then evaluated for physicochemical characterization, ex-vivo permeation and skin irritation studies. The weight variation was determined by individually weighing three randomly selected patches for each formulation and average weight was calculated. The thicknesses of patches were measured at four points using digital vernier calipers. For each formulation three randomly selected patches were used. Folding endurance of the patch was determined manually by repeatedly folding a small strip of the medicated patch at the same place until broke. The number of times the strip could be folded at the same place without breaking gave the folding endurance number. Flatness was determined by randomly selected five longitudinal strips were cut out from medicated patch of each formulation; the length of each strip was measured before and after kept at room temperature for 30 min.

Variation in length due to non uniformity of flatness was measured by determining percent constriction, with 0% constriction considered as 100% flatness[17].

A patch of size 4cm^2 was cut into small pieces and put into a 100ml methanol ,shaken for 24 hrs (to extract the drug) to get a homogeneous solution and filtered. The drug content was estimated by suitable dilutions and absorbance was measured by UV-spectrophotometer at 281nm. The patches were weighed individually and kept in a desiccator containing calcium chloride at room temperature for 24 hrs. The final weights of individual patches were weighed repeatedly until they showed a constant weight. The percentage of moisture content was determined by the difference between initial and final weight with respect to initial weight[17]. The patches were weighed individually and kept in a desiccator containing 100 ml of saturated solution of potassium chloride to maintain 84% relative humidity at room temperature for 24 hrs. The final weights of individual patches were weighed repeatedly until they showed a constant weight. The percentage of moisture absorption was determined by the difference between final and initial weight with respect to initial weight.

***In vitro* skin permeation study:**

The study was conducted with the prior approval of Institutional Animal Ethical Committee, St. Peter's Institute of Pharmaceutical Sciences. *In vitro* permeation studies were carried out using unjacketed vertical Franz diffusion cells with a diffusional surface area of 4.153 cm^2 and 19 mL of receptor cell volume. The skin was brought to the room temperature and mounted between the donor and receiver compartment of the Franz diffusion cell, with the stratum corneum side facing the donor compartment, the stratum corneum side of the skin was kept in intimate contact with the release surface of the patch under test. The receptor compartment consisted of 10% acetonitrile, which was maintained at 37 ± 2° C under constant stirring upto 24 hrs. The donor chamber and the sampling port were covered by parafilm to prevent evaporation during the study. Aliquots of 1 mL samples were withdrawn periodically at different time intervals (0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hrs) and replaced with equal volume of buffer to maintain constant receptor phase volume. At the end of the study, the samples were suitably diluted and the amount of drug was determined spectrophotometrically at 281 nm[18].

Cumulative percentage drug released were calculated and plotted against time. The data was fitted to different kinetic models to explain the release mechanism and pattern using the following equations[19].

Skin Irritation Study:

The study was conducted with the prior approval of Institutional Animal Ethical Committee, St. Peter's Institute of Pharmaceutical Sciences. Skin irritation studies were carried out to investigate the potential for Nebivolol HCl to cause irritant or allergic reactions. Allergic reactions are usually to a specific base component. Irritant reactions are most frequent and more important. Many test procedures are described to test for irritancy levels, both in animals and humans. The skin irritation study was carried out in the healthy rabbits weighing 1.917 ± 0.098 kg. The dorsal surface of the rabbit was cleared and hair was removed by shaving. The patches were placed over the skin with the help of surgical adhesive tape. They were removed after 24 hr and the skin was examined for any untoward reaction.

Statistical Analysis:

The statistical analysis was analyzed by Students t test. The results were expressed as arithmetic mean ± SD. P value of 0.05 or less was considered to be significant.

RESULTS AND DISCUSSION:

The FT-IR spectral analysis of Nebivolol HCl, HPMC E15, HPMC E50 and ERL100 alone showed that the principal peaks were observed **(Figure 1)** that confirming the compatibility of the drug and polymer respectively.

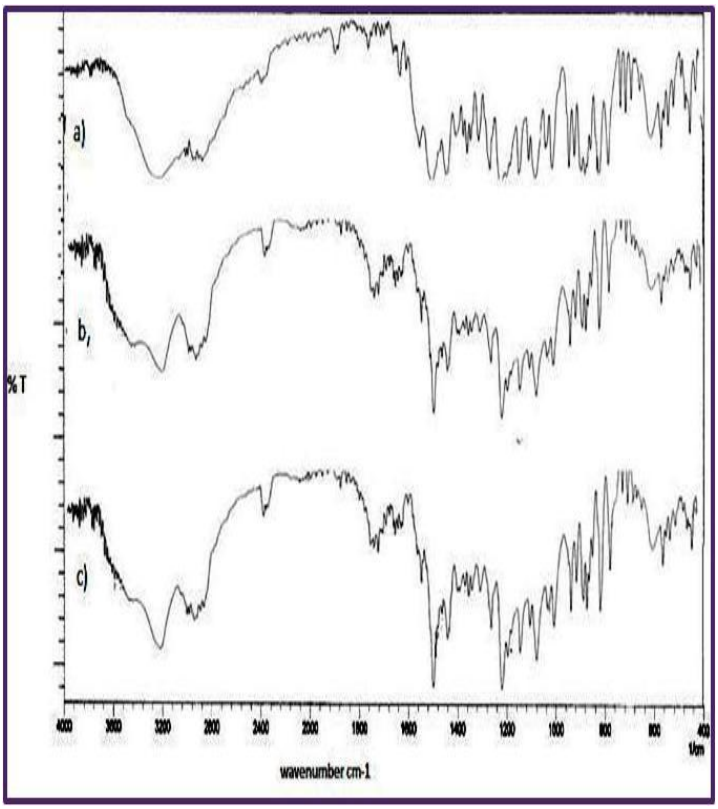

Figure 1: FT-IR spectra of a) Drug b) Physical mixture of drug, HPMC E15 and ERL 100 and c) Physical mixture of drug, HPMC E50 and ERL 100.

The FT-IR spectral analysis of Nebivolol HCl alone showed that the principal peaks were observed at wave numbers 3190.26 (O-H stretching), 2966.52 (C-H stretching), 1747.51 (C=O stretching), 1303.88 (C-N stretching) and 1101.35(C-O stretching Cyclic ether). The IR spectra of the physical mixture of drug, HPMC E15 and ERL 100 and that of drug, HPMC E50 and ERL100 the intensity of the peaks at 3190.26, 2966.52, 1303.88 and 1101.35 were reduced and at 1747.51 fused. However, some additional peaks were observed with the physical mixture, possibly because of the presence of polymers. These results suggest that there is no interaction between the drug and polymers used. It is well known that the common polymers such as HPMC and Eudragit RL are popular in Controlled/Sustained release matrix type patches because of their compatibility with a number of drugs[20].

The formulation A, that is combination of HPMC E5 and ERL 100 (A1-A3), does not gave the patch. The reason might be lack of sufficient viscosity. Hence the formulation is not considered for further studies.

Each formulated film was prepared in triplicate. The weights of all patches were in the range of 97.3 mg to 107.3 mg. Results of weight variation test indicated uniformity in weight of the patches as evidenced by standard deviation value, between 1-5. In thickness variation test, the thickness was found to be uniform. The thickness increased with increase in HPMC concentration. The thickness of all patches was found to be in the range of 220µm to 300 µm, thickness was changed from one formulation to other formulation, but individual strips of medicated patch carry uniform thickness, which indicates that total medicated patch carry uniform thickness. The folding endurance numbers of HPMC E15 containing patches has in the range of 144 to 175 and HPMC E50 containing patches has in the range of 205 to 220. The folding endurance number gives the mechanical property of the patches, high folding endurance number indicate that has high mechanical property. The folding endurance number was increased with increasing the HPMC content. These results indicated that the patches would not break and would maintain their integrity with general skin folding when applied.

Table 2: Physicochemical parameters of NEB HCl transdermal patches.

Code	Weight variation (mg)	Thickness (mm)	% Flatness	Folding endurance
B1	97.3 ± 6.8	0.22 ± 0.02	100 ± 0.0	175 ± 6.5
B2	97.7 ± 5.9	0.30 ± 0.0	100 ± 0.0	173 ± 9.2
B3	100 ± 4.2	0.50 ± 0.0	100 ± 0.0	144 ± 11.3
C1	99.6 ± 5.7	0.36 ± 0.05	100 ± 0.0	205 ± 8.0
C2	103.3 ± 6	0.46 ± 0.05	100 ± 0.0	212 ± 7.0
C3	107.3 ± 3.5	0.50 ± 0.0	100 ± 0.0	220 ± 15.0

All the formulations had the same strip length before and after cutted from medicated patch indicating 100% flatness, thus, no amount of constriction was observed. The percent constriction of all patches was found to be 0%, so all patches were carry 100% flatness. The results of weight variation, thickness, folding endurance and flatness were given in **Table 2.**

The drug content of all batches was in the range of 9.2 mg to 10.07 mg per $4cm^2$. Good uniformity in drug content was observed in all transdermal patches. The results revealed that the moisture content was found to increase with increasing concentration of hydrophilic polymer (HPMC). This may be due to hydrophilic nature of HPMC E15 and E50 which absorb moisture more compared to hydrophobic polymers (ERL 100). The small moisture loss in the formulations helps them to remain stable and from being a completely dried and brittle film (Muthalik and Udupa, 2004). The results revealed that the moisture uptake was found to increase with increasing concentration of hydrophilic polymer (HPMC). This may be due to hydrophilic nature of HPMC E15, which absorbs moisture more compared to hydrophobic polymers (ERL 100). The moisture uptake of the formulations was also low, which could protect the formulation from microbial contamination and reduce bulkiness[21] (Muthalik and Udupa, 2004). The results were mentioned in **Table 3 and Figure 2.**

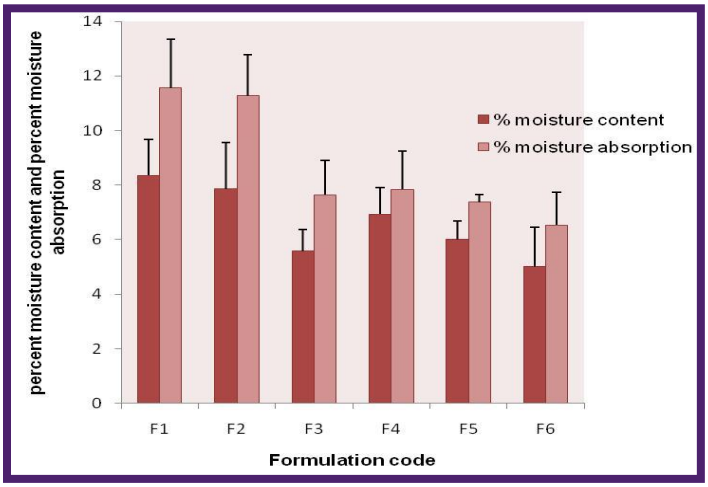

Figure 2: Moisture absorption and moisture content of nebivolol HCl transdermal patches.

Table 3: Physicochemical parameters of NEB HCl transdermal patches

Code	Drug content (mg)	% Moisture Content	% Moisture absorption
B1	09.40 ± 0.2	8.36 ± 1.30	11.56 ± 1.80
B2	09.30 ± 0.45	7.86 ± 1.70	11.28 ± 1.50
B3	10.07 ±.28	5.58 ± 0.80	07.64 ± 1.26
C1	09.30 ± 0.5	6.93 ± 0.99	07.85 ± 1.40
C2	09.20 ± 0.42	6.00 ± 0.67	07.37 ± 0.27
C3	10.00 ± 0.3	5.01 ± 1.44	06.53 ± 1.2

Table 4: Ex-vivo skin permeation study of B1-B3 (Cumulative amount permeated per cm^2(µg/cm^2) in 24 hrs

Time (hrs)	B1	B2	B3
0	0	0	0
0.5	17.23 ± 0.735	25.8 ± 0.20	26.00 ± 0.907
1	22.34 ± 0.355	28.3 ± 0.70	28.70 ± 0.702
2	23.94 ± 0.380	29.6 ± 1.02	32.00 ± 0.682
3	24.58 ± 0.219	30.56 ± 0.48	35.30 ± 1.350
4	25.28 ± 0.500	31.5 ± 0.75	38.20 ± 1.260
6	25.96 ± 0.850	35.5 ± 0.60	42.50 ± 1.010
8	28.55 ± 0.368	36.75 ± 0.54	45.34 ± 1.270
10	29.33 ± 0.237	44.85 ± 0.39	48.20 ± 1.210
12	30.24 ± 0.506	47.06 ± 0.62	51.00 ± 0.700
24	38.15 ± 0.752	58.00 ± 1.09	60.70 ± 1.66

Table 5: Ex-vivo skin permeation study of C1-C3 (Cumulative amount permeated percm^2 (µg/cm^2) in 24 hrs

Time (hrs)	C1	C2	C3
0	0	0	0
0.5	18.80 ± 1.27	19.60 ± 1.57	30.00 ± 0.66
1	19.50 ± 1.02	23.70 ± 1.80	33.70 ± 1.20
2	21.40 ± 0.55	26.80 ± 1.08	37.73 ± 0.91
3	24.05 ± 0.66	27.50 ± 0.68	40.90 ± 1.20
4	24.49 ± 0.57	31.50 ± 1.10	43.60 ± 0.50
6	24.92 ± 0.63	33.45 ± 0.76	45.80 ± 1.35
8	26.88 ± 1.19	37.65 ± 0.97	47.00 ± 0.60
10	28.52 ± 1.13	42.38 ± 1.02	49.70 ± 0.57
12	29.20 ± 1.08	45.70 ± 0.48	51.20 ± 0.58
24	39.34 ± 1.16	59.10 ± 1.22	63.20 ± 0.75

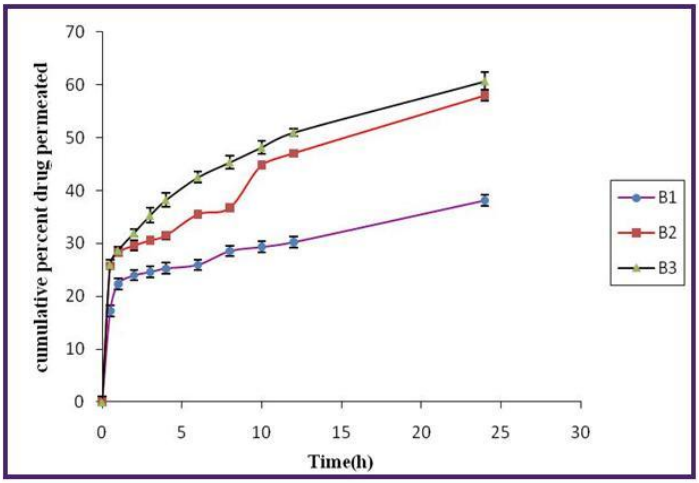

Figure3: Ex-vivo skin permeation profile of Nebivolol HCl from transdermal patches (B1-B3).

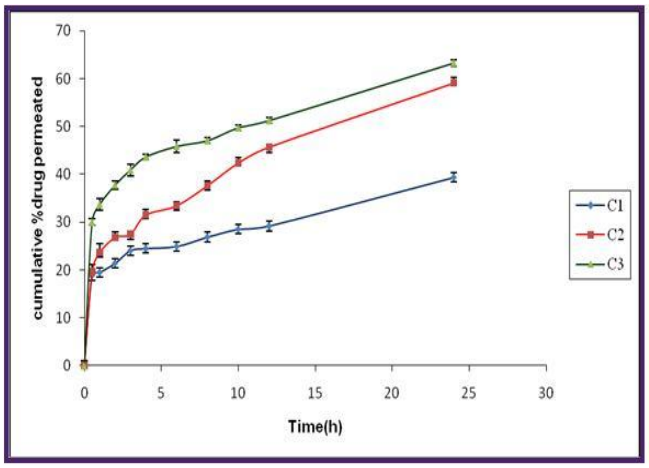

Figure 4: Ex-vivo skin permeation profile of Nebivolol HCl from transdermal patches (C1-C3).

The in-vitro skin permeability of Nebivolol HCl transdermal patch across excised rat skin was investigated. The results of in-vitro skin permeation of Nebivolol HCl from patches were shown in **Figure 3 & 4 Table 4 & 5**. The cumulative percent drug permeated and percent drug retained by the individual patch in the *in vitro* skin permeation studies were based on the mean amount of drug present in the respective patch. Formulations B3 and C3 (area 4cm^2) exhibited greatest amount of drug permeation than other formulations. The cumulative % drug permeated for formulation B3 is 60.7 and C3 is 63.2.

To further increase the permeability of Nebivolol HCl through rat skin the DMSO at concentrations of 2.5 and 5 % v/w was used as permeation enhancer in C3 formulation. However the percent drug release was increased from (63.2% to 81.5%) with DMSO at the concentration of 5% and flux was increased from (43.3 µg/cm^{2}h to 59.2 µg/cm^{2}h).DMSO enhanced the skin permeation by either reversibly changing the configuration of the protein structure of stratum corneum or by increasing the thermodynamic activity of the drug. DMSO also enhanced permeation by causing a swelling in the stratum corneum to induce formation of channels which decreased diffusional resistance.

The cumulative amounts of drug permeated per square centimeter of patches through the rat abdominal skin when plotted against time, the permeation profiles of drug seem to follow zero order kinetics as it is evidenced by correlation coefficients (0.6335 - 0.9576) better than first order (0.2137 - 0.9156) and Higuchi's equation (Higuchi square-root model) (0.7868 - 0.9478) **(Table 6)**. The kinetic data revealed that correlation coefficient values for zero order was high corresponding to first order indicating that the drug permeation from patches followed zero order followed diffusion mechanism.

Table 6: Correlation coefficients (r^2) and release exponent of formulations

Formulation Codes	r^2		
	Zero order	First	Higuchi
B1	0.905	0.8167	0.7868
B2	0.9576	0.9156	0.8774
B3	0.8998	0.8156	0.8857
C1	0.6335	0.2137	0.8255
C2	0.7876	0.8669	0.9478
C3	0.8739	0.8158	0.8103

The skin irritation studies were attempted to observe any visual skin irritation after the application of the patch to the Albino rabbits. The results indicated that upon topical application of matrix diffusion controlled TDDS the drug did not cause any noticeable irritation on the rabbit pinna skin throughout the study period.

CONCLUSION:

In conclusion, the present data confirm the feasibility of developing NEB HCL transdermal system. The release rate of drug through patches increased when the concentration of hydrophilic polymer was increased. Release of Nebivolol from the patches followed zero-order kinetics. Addition of enhancer in the patch increased the permeation of Nebivolol through hairless rat skin. The system was free from any apparent skin irritation.

REFERENCES:

1. Jain AK, Thomas NS and Panchagnula R. Transdermal drug delivery of imipramine hydrochloride. I. Effect of terpenes. J. Cont. Rel 2012’79: 93- 101
2. Hoyert DL, Kung HC, Smith BL. Deaths: preliminary data for 2003. Natl. Vital Stat. Rep. 2005; 53:1–48.
3. Kim J, Kitakaze M. Prevention and treatment of the hypersensitive organopathy - the necessity of cardiovascular protection. Nippon Rinsho; 2004; 62, 120–127.
4. Brown MB, Martin GP, Jones SA, Akomeah FK. Dermal and transdermal drug delivery systems: current and future prospects. Drug Del. 2006; 13:175–187.
5. Kurihara-BT, Good WR, Feusullin S and Signor C. Skin compatibility of transdermal drug delivery system. J Control Release. 1991; 15:271–278.
6. Patel RP, Patel G, Baria A. Formulation and evaluation of transdermal patches of aceclofenac, Int J Drug. Del 2009: 1; 41-51.
7. Ranade, VV. Drug delivery systems. 6. Transdermal drug delivery. J. Clin. Pharmacol. 1991; 31, 401–418.

8. Ke GM, Wang L, Xue HY, Lu WL, Zhang X, Zhang Q, Guo HY. In-vitro and in-vivo characterization of a newly developed clonidine transdermal patch for treatment of attention deficit hyperactivity disorder in children. Biol. Pharm.Bull. 2005; 28, 305–310.
9. Audet MC, Moreau M, Koltun WD, Waldbaum AS, Shangold G, Fisher AC, Creasy GW. Evaluation of contraceptive efficacy and cycle control of a transdermal contraceptive patch vs. an oral contraceptive: a randomized controlled trial. 2001; JAMA 5, 2347–2354.
10. Rudolf A de Boer, Adriaan A Voors and Dirk J van Veldhuisen; Nebivolol: third-generation β-blockade; Expert Opin. Pharmacother. 2007; 8(10).
11. Ghosh TK, Chiao CS, Gokhale RD. In-vitro permeation of some b-blockers across the hairless mouse skin. J. Pharm. Pharmacol. 1993; 45:218–219.
12. Demou JS, Sidhom MB, Plakogiannis FM. Comparative in vitro diffusion studies for atenolol transdermal delivery systems. Pharm. Acta Helv 1994; 68:215–219.
13. Ghosh TK, Adir J, Xiang SL, Onyilofur S. Transdermal delivery of metoprolol II: In-vitro skin permeation and bioavailability in hairless rats. J. Pharm.Sci.1995; 84:158–160.
14. Krishna R, Pandit JK. Carboxy methylcellulose sodium based transdermal drug delivery system for propranolol. J. Pharm. Pharmacol.1996; 48, 367–370.
15. Gupta JRD, Tripathi P, Irchhiaya R, Garud N, Dubey P, Patel JR, Formulation and evaluation of matrix tipe transdermal drug patches of Glibenclamide. Int J Pharm Sci Drug. Res. 2009: 1; 46-50.
16. Zhao K, Singh J. In vitro percutaneous absorption enhancement of propranolol hydrochloride through porcine epidermis by terpenes/ethaol. J Control Rel 1999; 62:359–66.
17. Mutalik S, Udupa N. Glibenclamide transdermal patches: physicochemical, pharmacodynamic, and pharmacokinetic evaluations. J Pharm Sci 2004; 93:1577-1594.
18. Ramesh G, Vamshi YV, Kishan V, Rao YM. Development of Nitrendipine Transdermal Patches: In vitro and Ex vivo Characterization. Current Drug. Del. 2007; 69-76: 4
19. Mamatha T, Venkateswara RJ, Mukkanti K and Ramesh G. Transdermal Drug Delivery System for Atomoxetine Hydrochloride – In vitro and Ex vivo Evaluation. Current Trends in Biotechnology and Pharmacy. 2009;3 (2) .
20. Wade A and Weller PJ. Hand book of pharmaceutical excipients. Washington, DC; American Pharmaceutical Publishing Assoc2000; 252-255 & 401-406
21. Mutalik S, Udupa N. Formulation development, in vitro and in vivo evaluation of membrane controlled transdermal systems of glibenclamide. J. Pharm. Pharma Sci 2005;8: 26-38.