Original Article

Design, development and permeation studies of nebivolol hydrochloride from novel matrix type transdermal patches

Vijay Singh Jatav, Jitender Singh Saggu[1], Ashish Kumar Sharma, Anil Sharma, Rakesh Kumar Jat

Department of Pharmaceutics, Gyan Vihar School of Pharmacy, SGVU, Jaipur, [1]Department of Pharmaceutical Chemistry, Lordshiva College of Pharmacy, Sirsa, Haryana, India

Abstract

Background: Nebivolol hydrochloride is a third generation β-blocker with highly selective β_1-receptor antagonist with antihypertensive properties having plasma half life of 10 h and 12% oral bioavailability. The aim of the present investigation was to form matrix type transdermal patches containing Nebivolol hydrochloride to avoid its extensive hepatic first pass metabolism, lesser side effect and increase bioavailability of drug.

Materials and Methods: Matrix type transdermal patches containing Nebivolol hydrochloride were prepared using EudragitRS100, HPMC K100M (2:8) polymers by solvent evaporation technique. Aluminum foil was used as a backing membrane. Polyethylene glycol (PEG) 400 was used as plasticizer and Dimethyl sulfoxide (DMSO) was used as a penetration enhancer. Drug polymer interactions determined by FTIR and standard calibration curve of Nebivolol hydrochloride were determined by using UV estimation.

Result: The systems were evaluated physicochemical parameters and drug present in the patches was determined by scanning electron microscopy. All prepared formulations indicated good physical stability. *In vitro* drug permeation studies of formulations were performed by using Franz diffusion cells using abdomen skin of *Wistar* albino rat. Result showed best *in vitro* skin permeation through rat skin as compared to all other formulations prepared with hydrophilic polymer containing permeation enhancer.

Conclusions: It was observed that the formulation containing HPMC: EudragitRS100 (8:2) showed ideal higuchi release kinetics. On the basis of *in vitro* drug release through skin permeation performance, Formulation F1 was found to be better than other formulations and it was selected as the optimized formulation.

Key Words: Nebivolol hydrochloride, transdermal patch, EudragitRS100, HPMC K100M, solvent evaporation technique, *In vitro* Skin permeation

Address for correspondence:
Mr. Vijay Singh Jatav, Gyan Vihar School of Pharmacy, SGVU, Jaipur, India. E-mail: jatavvijay@rediffmail.com
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INTRODUCTION

Transdermal delivery of drugs through the skin to the systemic circulation provides a convenient route of administration for a variety of clinical indications. Pharmaceutical scientists have accepted the challenge of transdermal drug delivery over the last 25 years. Most recently, there is an increasing

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recognition that the skin can also serve as the port of administration for systemically active drugs. In this case, the drug applied topically will be absorbed first into blood circulation and then be transported to target tissues. This could be rather remote from the site of drug application, to achieve its therapeutic purpose.[1] Recently, it is becoming evident that the benefits of i.v. drug infusion can be closely duplicated, without its hazards, by using the skin as the port of drug administration to provide continuous transdermal drug infusion into the systemic circulation. The oral route of administration has certain disadvantages such as destruction of drugs by hepatic first pass metabolism and enzymatic degradation within the gastrointestinal tract. Continuous intravenous administration at a programmed rate has been recognized as a superior mode of drug delivery not only to bypass hepatic first pass effect, but also to maintain a constant, prolonged, and therapeutically effective drug level in the body.[2]

The simply designed transdermal patch has undergone a dramatic transformation over the past decade. All transdermal systems attempt to create a balance between a number of key factors including size of patch or coverage area, concentration of the drug, duration of therapeutic drug level, and use of a skin penetration enhancer.[3]

The transdermal drug delivery systems are devoid of these disadvantages, in addition, their potential benefits include easy terminal drug input in case of adverse effects, permits use of drugs with a short biological half life, avoidance of absorption variability, and differential metabolism associated with oral therapy.[4] The statical data showed a market of $12.7 billion in the year 2005 which is assumed to increase by $21.5 billion in the year 2010 and $31.5 billion in the year 2015. Almost all the pharmaceutical companies are developing transdermal drug delivery systems.[5]

It is marketed in Europe for the treatment of hypertension and heart failure and is currently being reviewed for use in the US by the Food and Drug Administration. Nebivolol appears to be well tolerated with an adverse event profile that is at least similar, if not better, than that of other beta-adrenergic blockers. Studies suggest that long-term therapy with nebivolol improves left ventricular function, exercise capacity, and clinical endpoints of death and cardiovascular hospital admissions in patients with stable heart failure.[6]

Nebivolol is a third generation beta-blocker, with highly selective for the β_1-adrenoceptors (AR) and endowed with the ability to release nitric oxide from the cardiovascular endothelium.[7] In animal models Nebivolol has been shown to induce endothelium-dependent arterial relaxation in a dose dependent manner, by stimulation of the release of endothelial nitric oxide.[8] Nebivolol hydrochloride (M.W. 441.9) showed the favorable logarithmic value of partition coefficient (Log *P* (octanol/water): 3.23; 4.03 (pH 11.8, 23°C). and negligible skin degradation. The plasma half life is about 8-10 hours which make frequently dosing necessary to maintain the therapeutic blood levels of drug for a long term treatment.[9]

MATERIALS AND METHODS

Materials

Nebivolol hydrochloride was a gift sample from Zydus cadila, Health care ltd., Ahmedabad (Gujarat), and Hydroxy Propyl Methyl Cellulose (HPMC) and Eudragit RS 100 were gift sample from Akums Drugs and Pharmaceutical LTD, Haridwar, Polyethylene glycol 400 (PEG 400) was purchased from Central Drug House Ltd., New Delhi and Dimethyl sulfoxide (DMSO) was purchased from Merck Specialities Pvt., Worli, Mumbai, India.

Analytical method for nebivolol hydrochloride

A total of 10 mg of accurately weighed quantity of Nebivolol Hydrochloride was dissolved in 100 ml of methanol (concentration 100 mcg/ml). From the above stock solution, 60 ml was taken and diluted with methanol to made it 100 ml to get the concentration of 60 mcg/ml. In order to generate a calibration curve, 5 to 60 µg/mL of primary standard were prepared and the calibration curve was obtained by measuring their absorbance at predetermined UV–VIS spectrophotometer at 282 nm shown in Figure 1. The volumetric flask was first rapped with black paper and then it was covered with aluminum foil to avoid the problem of drug photosensitivity. The concentration of Nebivolol hydrochloride was calculated using the linear regression equation of the calibration curve (Absorbance = 0.015 × concentration –0.009,

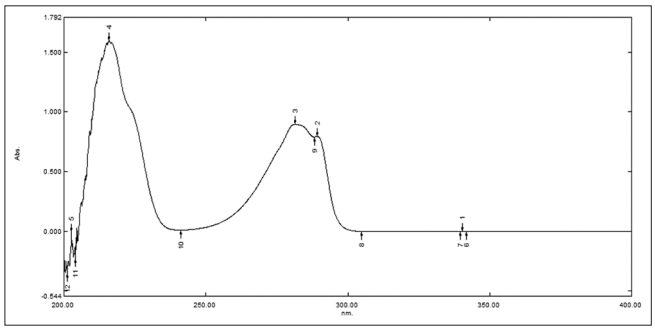

Figure 1: The UV scan of Nebivolol Hydrochloride in methanol

r^2 = 0.9989). When a standard drug solution was assayed repeatedly (n = 6), mean standard error (accuracy) and RSD (precision) were found to be ±0.35 and ±0.65, respectively.

Physico-chemical compatibility of drug and polymer

The physicochemical compatibility between Nebivolol hydrochloride and polymers used in the films was studied by using fourier transform infrared (FTIR 8300, Shimadzu Co., Kyoto, Japan) spectroscopy. The infrared spectra were recorded using an FTIR by the KBr pellet method and spectra were recorded in the wavelength region between 4000 cm^{-1} and 400 cm^{-1}. The spectra obtained for Nebivolol hydrochloride, polymers, and physical mixtures of Nebivolol hydrochloride with polymers were compared.

Preparation of transdermal films

In the present study, drug-loaded matrix-type transdermal films of Nebivolol hydrochloride were prepared by solvent evaporation method[10-13] using different ratios of ERS-100 and HPMC K100M polymers [Table 1]. The polymers were weighed in requisite ratios by keeping the total polymer weight at 1.0 g added in solvent mixture (3:2 ratio of methanol: Chloroform). Propylene glycol was incorporated as plasticizer and DSMO as penetration enhancer were used. A known quantity of drug was added slowly to the solution and dissolved by continuous stirring for 30 min. The aluminums foil was spread uniformly on a glass petri dish and solution poured in it for the formulation of transdermal patch. The disk was kept on a horizontal surface for uniformity. The solution was poured on the foil into a petri dish of about 70 cm^2. The solvent was allowed to evaporate for 24 hours by inverting a funnel over a disk. The polymer was found to be self-adhesive due to the presence of Eudragit polymer along with plasticizer. The patches were cut out to give required area for evaluation.

Evaluation of transdermal patch of nebivolol hydrochloride

Physicochemical properties such as physical appearance, thickness,[14] weight variation,[15] folding endurance,[16,17] content uniformity,[18] were determined on developed patches.

Scanning electron microscopic studies

SEM photography was taken for the Nebivolol hydrochloride and various samples to assess the surface topography of the drug and formulations. The samples were deposited on the aluminum hold and sputtered with gold. This was done for all the samples as they were coated uniformly with gold after fixing them individually. The SEM photography was taken on the Zeiss EVO 50 scanning electron microscope (textile department, IIT, Delhi) at the required magnification at room temperature.

In vitro permeation study

The *in vitro* permeation study of fabricated transdermal patches of Nebivolol hydrochloride was carried out by using excised rat abdominal skin and franz diffusion cell.[14] The skin was sandwiched between donor and receptor compartments of the diffusion cell. The patch of per cm^2 was placed in intimate contact with the stratum corneum side of the skin; the top side was covered with aluminum foil as a backing membrane. Teflon bead was placed in the receptor compartment filled with 12 ml of phosphate buffer pH 7.4. The cell contents were stirred with a magnetic stirrer and a temperature of 37 ± 0.5°C was maintained throughout the experiment. Samples of 2 ml were withdrawn through the sampling port at different time intervals for a period of 48 h, simultaneously replacing equal volume by phosphate buffer pH 7.4 after each withdrawal. The samples were Analyzed in spectrophotometer at 282 nm. Based on the results of *in vitro* permeation profiles of preliminary batches of Nebivolol hydrochloride transdermal patches the optimum composition of checkpoint batches of Nebivolol hydrochloride transdermal patch was optimized.

Stability studies

Optimized medicated films were subjected to short-term stability testing. Films were placed in a petri disk lined with aluminum foil and kept in a humidity chamber (desiccators) maintained at 40 ± 2°C and 75 ± 5% RH for 6 month as per

Table 1: Composition of transdermal patches

Formulation code	Drug (mg)	Polymers ratio ERS 100:HPMC K100M	DMSO (%)	PEG 400 (%)	Solvents ratio (Methanol: Chloroform)
F1	100	2:8	20	30	3:2
F2	100	4:6	20	30	3:2
F3	100	6:4	20	30	3:2
F4	100	8:2	20	30	3:2

Table 2: Physiochemical evaluation of transdermal patches

Formulation code	F1	F2	F3	F4
Appearance	Thin, transparent, and flexible	Thin, opaque, and flexible	Thin, opaque, and flexible	Thick, non flexible, and opaque
Thickness	0.263±0.67	0.289±0.55	0.301±0.61	0.219±0.75
Weight (mg)	51.01±0.80	52.15±0.68	50.5±0.75	52.02±2.15
Drug content (2.64 mg/cm^2)	3.75±1.08	3.87±0.98	3.61±0.13	3.67±0.28
Folding endurance	>100	>100	>100	>100

ICH guidelines.[19] Changes in the appearance and drug content of the stored films were investigated after storage at the end of every week. The data presented were the mean of three determinations.

RESULTS

Evaluation of transdermal patch

The prepared transdermal patches were evaluated for their physicochemical characteristics such as appearance, weight variation, thickness, folding endurance, drug content, [Table 2], and *in-vitro* drug permeation through albino rat skin [Table 3]. The physical appearance of the various formulations in terms of their transparency, smoothness, flexibility, stickiness, homogenicity, and opaque properties were recorded. The formulation F-1 was found to be thin, transparent, and flexible, formulation F-2 was found to be thin, opaque, and flexible, formulation F-3 was found to be thin, opaque; and flexible and formulation F-4 was found to be thick, non flexible, and opaque. The SEM analysis of the Nebivolol hydrochloride patch was done to study the surface characteristics of the film. Here it was observed that the drugs were presented in the patch. The formulation F-1 gave the most suitable transdermal film with all desirable physico-chemical properties. The thickness of the patches was varied from 0.219 ± 0.75 mm to 0.301 ± 0.61 mm. From the result, uniformity of the patches was showed prepared by solvent evaporation while low standard deviation values ensued by thickness measurements of film. The weights ranged between 50.5 ± 0.75 mg and 52.15 ± 2.15 mg, which indicates that different batches patch weights, were relatively similar. Folding endurance was found to be >100 that is satisfactory weight of the patches, drug content was found to be 3.61 ± 0.13 mg to 3.87 ± 0.98 mg. Here it was observed that the drugs were uniformity distributed in the patch. The cumulative percentage drug permeated and percentage drug retained by the individual path in the *in vitro* skin permeation studies were based on the mean amount of drug present in the respective patch. The cumulative percentage drug release for F1 was found to be 91.21 ± 2.14% at 48 h and for F4 it was found to be 68.16 ± 5.57% at 48 h. The formulation, F1 [HPMC K100M, ERS-100 (8:2)] is considered as a best formulation, since it shows maximum *in vitro* drug release as 91.21 ± 2.14% at 48 h showed in Figure 2.

DISCUSSION

Trasdermal drug delivery system increases the bioavailability of drug by avoiding the first pass metabolism and increases the therapeutic efficacy of drug by reaching into the systemic circulation and also most suitable system for a long-term treatment or for a multi-dose treatment because transdermal patches are prepared for a long period of time in a single dose providing treatment from a day to even up to 7 days. Polymers HPMC K100M and ERS-100 were selected on the basis of their adhering property and non toxicity. The result of the study showed excellent adhering property and controlled release. Result from present study concluded that Nebivolol hydrochloride in combination with HPMC K100M, ERS-100, and with incorporation of PEG 400 (30%) and DMSO (20%) produced smooth, flexible, and transparent film. FT-IR studies showed characteristic peaks of Nebivolol hydrochloride, confirming the purity of the drug. FT-IR spectral studies indicated there was no interaction between Nebivolol hydrochloride and polymers used [Figure 3]. Nebivolol hydrochloride patches were prepared with combination of these polymers and evaluated it for physical parameters such as thickness, drug content, weight variation, % moisture loss, and % moisture absorption. It was observed from these results, that thickness, weight variation, drug content, low moisture loss, low moisture absorption, tensile strength were suitable for maximum stability of the prepared formulations. The drug content of TDDS patches ranged from 3.61 ± 0.13 to 3.87 ± 0.98 mg. The film prepared from HPMC

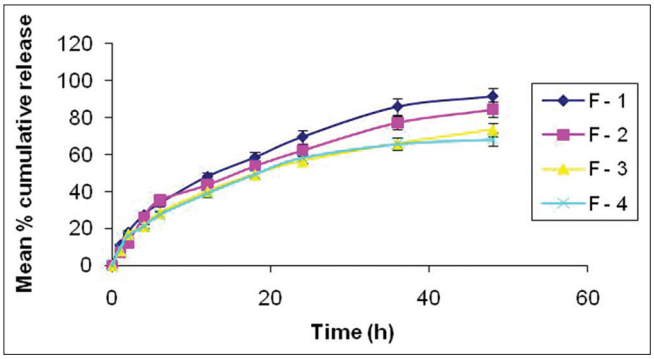

Figure 2: Comparative drug permeation profile. Data represented as mean ± SD ($n = 6$)

Table 3: *In vitro* drug permeation profile of Nebivolol hydrochloride transdermal patches

Formulation code	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Korsmeyer-peppas (R^2)	Diffusion Exponent (n)	Permeation rate (Flux) (mcg/cm²/h)
F1	0.9094	0.9956	0.9963	0.9948	0.54	0.35
F2	0.8929	0.9918	0.9878	0.9623	0.62	0.25
F3	0.8919	0.9749	0.9934	0.9790	0.50	0.28
F4	0.8655	0.9403	0.9831	0.9870	0.52	0.28

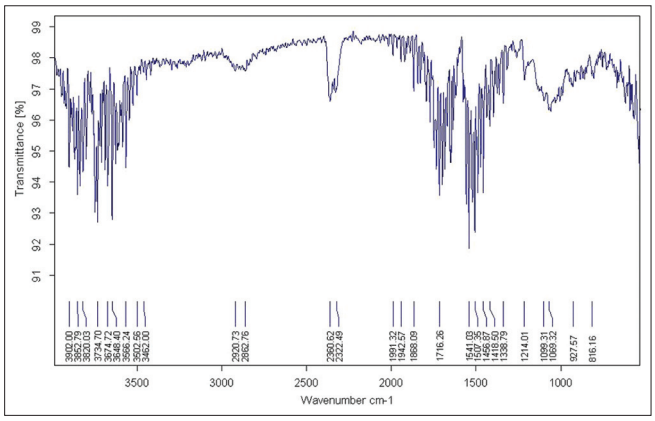

Figure 3: FTIR Spectra of nebivolol hydrochloride with polymers

K100M with increasing concentration of ERS-100 showed a significant difference between the results. The cumulative percentage drug release for F1 was found to be 91.21 ± 2.14% at 48 h and for F4 it was found to be 68.16 ± 5.57% at 48 h. The formulation, F1 [HPMC K100M, ERS-100 (8:2)] is considered as a best formulation, since it shows maximum *in vitro* drug release as 91.21 ± 2.14% at 48 h. The drug release kinetics studies showed that the majority of formulations were governed by Higuchi model and mechanism of release was non-Fickian mediated. Higuchi developed an equation for the release of a drug from a homogeneous-polymer matrix-type delivery system that indicates the amount of drug releases is proportional to the square root of time.[20] When plotted against square root of time, the release of drug from the transdermal film showed a straight line, it indicates that the release pattern is obeying Higuchi's kinetics. In our experiments, *in vitro* release profiles of all the different formulations of transdermal patches could be best expressed by Higuchi's equation, for release of drug from a homogeneous-polymer matrix-type delivery system that depends mostly on diffusion characteristics.

From the *in vitro* permeation profile data of all the formulations through rat skin, kinetics of drug release were found for zero-order, first-order, Higuchi-type release kinetics, and Korsmeyer-Peppas type release kinetics. The coefficient of correlation (R^2) of each of these release kinetics were calculated and compared [Table 3]. The data revealed that the release pattern of selected formulations was best fitted for Higuchi kinetics, as the formulation coefficient values predominate over zero-order, first-order, and Korsmeyer-Peppas type release kinetics, which again confirmed with Higuchi's equation for the drug release from matrix. Thus, a slow and controlled release as observed is indicating that the drug release mechanism is non Fickian model, as proposed by Higuchi.

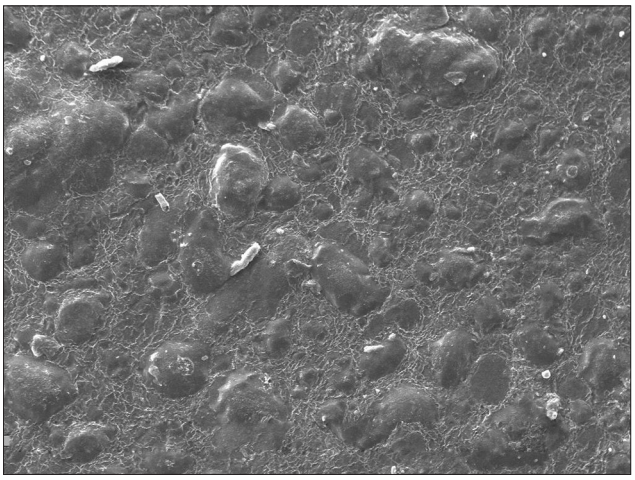

Figure 4: Scanning electron microscopy of nebivolol hydrochloride loaded patch

The regression analysis of the *in vitro* permeation curves were carried out for *in vitro* permeation studies in rat skin. The slope of the straight line obtained after plotting the mean cumulative amount released per Cm. Square patch vs. square root of time was taken as the experimental flux for Nebivolol hydrochloride. The flux obtained for all formulations were in the range of 0.25-0.35 mcg/cm^2/h. Among all these formulations, the formulation F-1 showed the maximum % drug cumulative release i.e., 91.21% up to 48 hours of the study. All the formulations showed Higuchi-type release kinetics. Regression analyses of the *in vitro* permeation curves were carried out. The slope of the straight line obtained after plotting the mean cumulative amount released per Cm. Square patch vs. square root of time was taken as the experimental flux for Nebivolol hydrochloride. In our studies the n values calculated from the slope of the Korsmeyer-Peppas Kinetic model, which were found to be 0.54, 0.62, 0.50, and 0.52 for F-1, F-2, F-3, and F-4 patches, respectively. These *n* values showed the release mechanism following non Fickian diffusion. Figure 4 represents the SEM photographs of nebivolol hydrochloride-loaded transdermal patch before the permeation study. The SEM photograph of the drug-loaded patch confirmed the drug present in the patch. The drug particles are uniformly distributed and seen on the surface of the patch.

CONCLUSIONS

In conclusion, controlled release TDDS patches of Nebivolol hydrochloride can be prepared using the polymer combinations, HPMC K100M, ERS-100 (8:2) with PEG 400 and DMSO as plasticizer and enhancer, respectively. The release rate of drug through patched increased simultaneously as concentration of hydrophilic polymer was increased. However, the

mechanism of drug release of all formulations was non Fickian. The properties of film did not change during the period of study. Further, *in vivo* studies have to be performed to correlate with *in vitro* release data for the development of suitable controlled release patches for Nebivolol hydrochloride.

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