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Original Research Paper

EFFECT OF DIMETHYL SULPHOXIDES AS PERMEATION ENHANCER ON TRANSDERMAL PATCH OF NEBIVOLOL HYDROCHLORIDE

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ABSTRACT

A matrix type transdermal patch for delivery of Nebivolol hydrochloride (NEB), is unique as a beta-blocker. Studies were carried out to investigate the effect of permeation enhancers on the *in vitro* permeation of Nebivolol hydrochloride across rat skin. Films were prepared by using Eudragit RS100 (ERS100) and hydroxy propyl methyl cellulose (HPMC K100M) polymers by incorporating polypropylene glycol (PEG 400) as plasticizer using solvent evaporating method. A total of eight formulations were prepared by using same drug and different polymer ratio and some formulation prepared without (E1-E4)/with (F1-F4) Dimethyl sulphoxides (DMSO) as permeation enhancers in same concentrations. The maximum percent of drug permeation was observed with ERS100 and HPMC K100M (2:8) monolithic system containing 20% Dimethyl sulphoxides (F1). The *in vitro* release studies revealed that Dimethyl sulphoxides showed better permeation enhancement than without DMSO and the release was sustained up to 48 h and it follows Higuchi kinetics. All the films were found to be stable at temperature of 40±2°C and 75±5% relative humidity (RH) as per the international conference on harmonization (ICH) guidelines (Zone IV) for the period of six months with respect to their physical parameters.

Keywords: Permeation enhancer, Polymers, Transdermal patch, Nebivolol hydrochloride, Dimethyl sulphoxides.

INTRODUCTION

Recently, the most common form of delivery of drugs is the oral route. However this system has its own notable advantage of easy administration, it also has significant drawbacks; namely poor bioavailability due to first pass effect and the tendency to produce rapid both high and low blood level, leading to a need for high and/or frequent dosing, which can be both cost prohibitive and inconvenient.¹ Penetration enhancers are the substances that facilitate the absorption of penetrant through the skin by

temporarily diminishing the impermeability of the skin. Ideally, these materials should be pharmacologically inert, nontoxic, nonirritating, nonallergenic, and compatible with the drug and excipients, odorless, tasteless, colorless, and inexpensive and have good solvent properties.^{2,3} The site of action of the chemical skin penetration enhancers is located in the stratum corneum.⁴ Chemical enhancers can be divided into two broad categories: Those that change partitioning into the stratum corneum and those that influence diffusion across the stratum corneum⁵ such as dimethylsulfoxide or DMSO, alcohols. The

enhancer should not lead to the loss of body fluids, electrolytes, and other endogenous materials, and skin should immediately regain its barrier properties on its removal.⁶ Dimethyl sulphoxides (DMSO) is the most important compound belong to the category of sulfoxide and similar compound enhances the transdermal permeation of a variety of drugs like B- blockers and other antihypertensive drugs.^{7,8} Dimethyl sulfoxide, or DMSO, all-natural substance derived from wood pulp. Through the normal decomposition of plants DMSO is produced.⁹

Dimethyl sulphoxides (DMSO) is one of the earliest and most widely studied penetration enhancers. It is a powerful aprotic solvent which hydrogen bonds with itself rather than with water. It is colourless, odourless and is hydroscopic and is often used in many areas of pharmaceutical sciences as a “universal solvent”. DMSO alone has been applied topically to treat systemic inflammation. DMSO works rapidly as a penetration enhancer - spillage of the material onto the skin can be tasted in the mouth within a second. Although DMSO is an excellent accelerant, it does create problems. DMSO changes the stratum corneum keratin from α -helicals to β - sheet conformations. At concentration $> 60\%$ v/v DMSO are needed for optimum enhancement efficacy.¹⁰ However, at these relative high concentrations, DMSO can cause erythema and wheal of the stratum corneum. Denaturing of some skin proteins results in erythema, scaling, contact urticaria, stinging and burning sensation.^[11] Concentration greater than 60% DMSO enhances the flux, there was evidence of its interaction with stratum corneum lipids. It also produces alteration in protein structure, but may also be related to alterations in stratum corneum organization besides any increased drug-partitioning effect.¹²

In the present study, transdermal monothilic films of Nebivolol hydrochloride were prepared using various film forming agents and no report are available on the comparative evaluation between with and without permeation enhancer on transdermal patches of Nebivolol hydrochloride.

MATERIAL AND METHODS

Materials

Nebivolol hydrochloride was a gift sample from Zydus cadila, Health care ltd., Ahmedabad (Gujrat), and 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.

Preparation of Transdermal Films

In the present study, drug loaded matrix type transdermal films of Nebivolol hydrochloride were prepared by solvent evaporation method^{13,14,15} 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 gm added in solvent mixture (3:2 ratio of chloroform, methanol). Propylene glycol was incorporated as plasticizer and DMSO as penetration enhancer were used in F1 – F4 formulation. The drug was added slowly to the solution and dissolved by continuous stirring for 30 min. For the formulation of transdermal patch, the aluminum foil was spread uniformly on a glass petri dish. The mould was kept on a horizontal surface. The solution was poured on the foil into a petri dish of about 70 cm². The rate of evaporation was controlled by inverting a funnel over the mould. Aluminum foil was used as backing film. The solvent was allowed to evaporate for 24 hrs. The polymer was found to be self adhesive due to the presence of Eudragit polymer along with plasticizer. The patches were cut to give required area and used for evaluation.

Physicochemical Evaluation

Physicochemical properties such as physical appearance, thickness, content uniformity, weight variation, folding endurance, tensile strength and percentage moisture absorption were determined on developed patches.

Investigation of Physicochemical 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 Alpha, Bruker, Bangalore) spectroscopy. The infrared (IR) 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.

DSC Studies

The optimized formulations were subjected to differential scanning calorimeter at a heating rate of 10°C/min over a temperature range of 0-300°C. The sample of pure drug and optimized patches were hermetically sealed in an aluminum pen. Nitrogen gas was purged at the rate of 10 mL/min for maintained inert atmosphere.

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.¹⁶ The skin was sandwiched between donor and receptor compartments of the diffusion cell. The patch of 2.64 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 2ml 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 spectrophotometrically 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 of the optimized patch

Formulation F-1 was then subjected to the accelerated stability studies at temperature of 40±2°C and 75±5% relative humidity (RH) as per

the international conference on harmonization (ICH) guidelines (Zone IV) for the period of six months. The optimized patches (formulation F-1) were subjected to accelerated stability studies to evaluate any change in the performance when exposed to accelerated conditions of environment during storage, handling, transport and use. Patches were lined with aluminium foil and packed in plastic covers and kept in desiccator maintained at 75±5% RH at 40±2°C temperature for six months. The hot-air oven was set at temperature 40°C and the relative humidity (RH) of 75.3% was maintained by using saturated solution of sodium chloride (NaCl).¹⁷

RESULTS

Evaluation of Transdermal Patch

The prepared transdermal patches were evaluated for their physicochemical characteristics such as appearance, weight variation, thickness, % moisture loss, % moisture absorption, folding endurance, drug content, tensile strength and *in vitro* drug permeation through albino rat skin. The physical appearance of the various formulations in terms of their uniformity, transparency, smoothness, flexibility, stickiness, homogeneity and opaque properties were recorded. The formulation E-1 was found to be Sticky, thin, transparent and flexible, E-2 was found to be Thin, opaque and flexible, E-3 & E-4 was found to be Thick, not flexible and opaque, F-1 was found to be thin, transparent and flexible, formulation F-2 & F-3 was found to be thin, opaque and flexible and formulation F-4 was found to be thick, not flexible and opaque. The formulation F-1 gave the most suitable transdermal film with all desirable physicochemical properties. The thickness of the patches was varied from 0.211 ± 0.012 mm to 0.301 ± 0.61 mm.

Low standard deviation values in the film thickness measurements ensured uniformity of the patches prepared by solvent evaporation. The weights ranged between 49.16 ± 0.81 mg and 52.49 ± 0.65 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. The cumulative % drug permeated and % 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 E-1, E-2, E-3, E-4, F-1, F-2, F-3 and F-4 was found to be $85.97 \pm 3.04\%$, $63.21 \pm 5.70\%$, $54.06 \pm 5.68\%$, $57.97 \pm 6.75\%$, $91.21 \pm 2.14\%$, $83.16 \pm 7.16\%$, $73.20 \pm 7.39\%$ at 48 h and $68.16 \pm 5.57\%$ at 48 h respectively. The formulation, F1 [HPMC K100M, ERS-100 (8:2)] with DMSO as permeation enhancer is considered as a best formulation, since it shows maximum *in vitro* drug release as $91.21 \pm 2.14\%$ at 48 h.

The *in vitro* release profile is an important tool that predicts in advance how a drug will behave *in vivo*. The results of *in vitro* skin permeation studies of Nebivolol hydrochloride from transdermal patches are shown in Figures 1. In the present study hydrophobic Eudragit RS100 (ERS100) and hydrophilic hydroxy propyl methyl cellulose (HPMC K100M) polymers are used to prepared patches. Formulation E3 exhibit lowest $54.06 \pm 1.789\%$ of drug release value, while formulation F1 exhibited greatest $91.21 \pm 3.39\%$ of drug release value. The cumulative amount of drug released from formulations containing hydrophilic polymer release drug at faster rate than hydrophobic polymer. The drug release from the patch is ordered as $F1 > E1 > F2 > F3 > F4 > E2 > E4 > E3$. Unlike the formulations F2, F3, F4, E1, E2, E3 and E4, the formulations F1 achieved a high cumulative amount of drug permeation at the end of 48 hours. Based on physiochemical and *in vitro* release experiments, F1 was chosen for further studies.

DISCUSSION

A comparative evaluation of the permeability of without penetration enhancer films and the influence of DMSO as penetration enhancer on the film permeability in case was studied. Polymeric films can be prepared by solvent evaporation techniques produces uniform and reproducible films. In each case films were prepared using concentrations 20% of natural enhancer to evaluate the influence of the enhancer

on the permeability properties of the film. The percent penetration was reached to a maximum 92% by the addition of 20% enhancer to Nebivolol hydrochloride formulation with implying the ability of DMSO to increase the drug diffusion by modifying the barrier properties of stratum corneum.

Eudragit RS100 (ERS100) and hydrophilic hydroxy propyl methyl cellulose (HPMC K100M) polymers was used in patch as a rate controlling polymer which was observed to be effective by controlling the release rate of drug up to 48 hours of time period. In order to study the effect of DMSO as penetration enhancer on Nebivolol hydrochloride permeation through albino rat skin, the Nebivolol hydrochloride penetration from formulations with concentrations of penetration enhancer (20%) was determined by excised albino rat skin. From the *in vitro* permeation profile data of the formulations, the kinetics of drug release were found for the zero order, the first-order shown figure 3, Higuchi-type release kinetic shown figure 2 and Korsmeyer-Peppas type release kinetic shown in figure 4. It could be observed from the figure 1 that the penetration of Nebivolol hydrochloride across rat skin without enhancer was small but was by adding the amount of enhancer with increasing hydrophilic polymers range to the formulations and a maximum 92% drug was penetrated. Similar results were found in an investigative study (Gupta *et al.*, 2009).

The samples were analyzed at zero, 3 months and 6 months as per the ICH guidelines. Changes in the appearance and drug content of the stored films were investigated after storage at the end of every week. The data presented in figure 5 were the mean of three determinations.

CONCLUSION

Nebivolol hydrochloride transdermal patches was successfully prepared and evaluated with a high *in vitro* penetration rate. From the above results DMSO was found to be effective enhancer with increasing hydrophilic polymers range for the *in vitro* skin permeation of Nebivolol hydrochloride. It was also observed that 20% concentration of DMSO was more effective by enhancing penetration of drug through stratum corneum. The

properties of film did not change during the period of study. The transdermal Nebivolol

hydrochloride patches will be further investigated for in vivo studies in laboratory animals.

Table 1: Composition of transdermal patches

Formulation code	Drug (mg)	Polymers ratio ERS100:HPMC K100M	DMSO	PEG 400	Solvents ratio (Methanol : Chloroform)
E1	100	2:8	--	30%	3:2
E2	100	4:6	--	30%	3:2
E3	100	6:4	--	30%	3:2
E4	100	8:2	--	30%	3:2
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

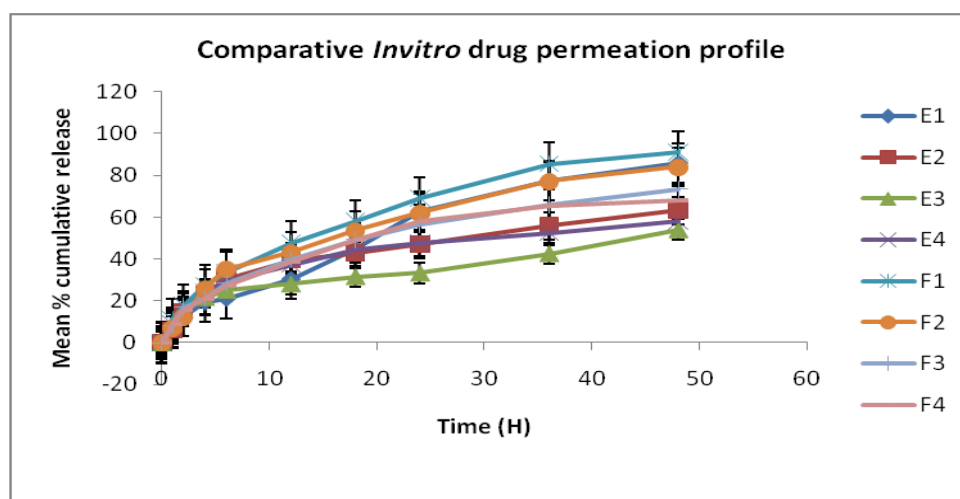


Figure 1: Comparative *in vitro* drug permeation profile. Data represented as means \pm SD (n=6)

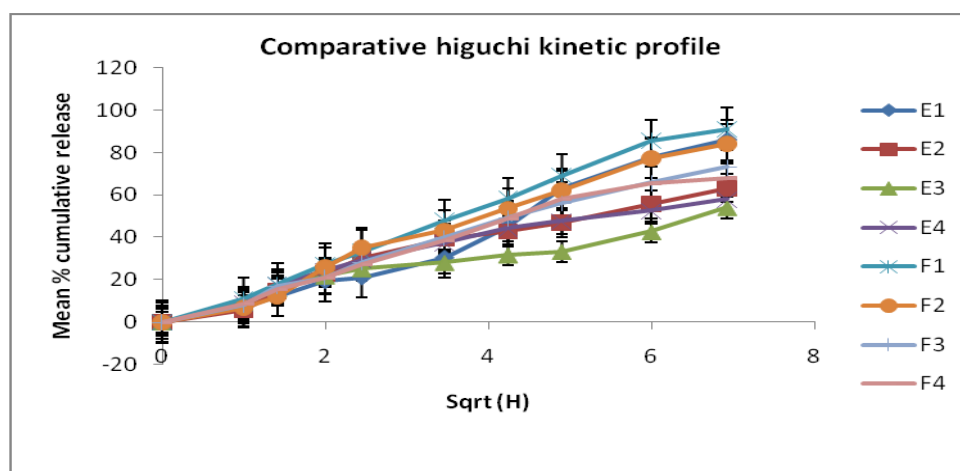


Figure 2: Comparative Higuchi drug permeation profile. Data represented as means \pm SD (n=6)

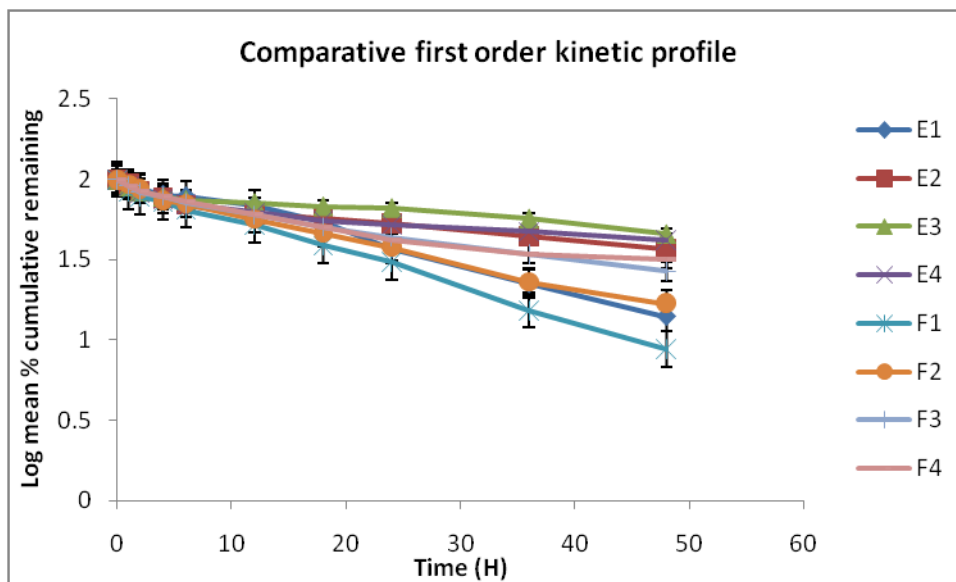


Figure 3: Comparative higuchi drug permeation profile. Data represented as means ±SD (n=6)

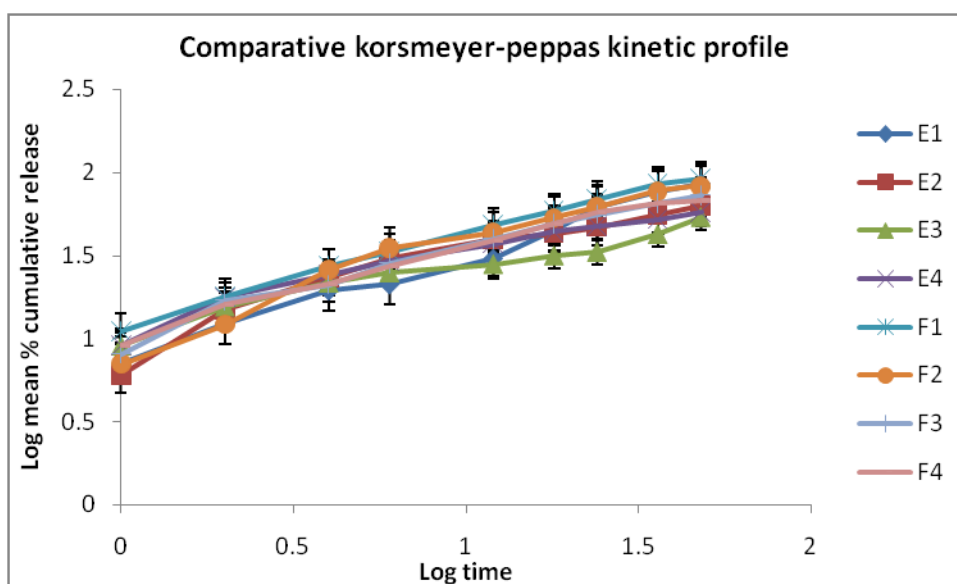


Figure 4: Comparative korsmeyer-peppas drug permeation profile. Data represented as means ±SD (n=6)

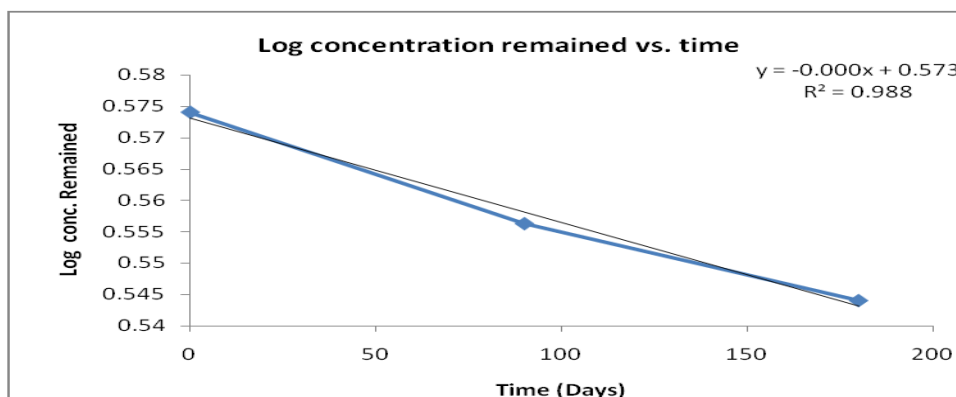


Figure 5: A plot of log concentration vs. time in days for formulation F-1 (Stability studies)

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