Iranian Journal of Pharmaceutical Sciences
2016: 12 (2): 9-20
www.ijps.ir

Original Article

Synergistic Effect of Chemical Enhancer and Iontophoresis for Transdermal Delivery of Nebivolol Hydrochloride

Sriram Pavani[a, b], Yamsani Madusudhan Rao[b*], Yamsani Shravan Kumar[b]

[a]*Department of Pharmacy, JNTU, Kukatpally, Hyderabad, India,* [b]*Department of Pharmaceutics, Vaagdevi College of Pharmacy, India*

Abstract

The aim of the present study was to develop and investigate the feasibility of delivering Nebivolol as a transdermal patch by iontophoresis. The passive and electrically assisted transdermal delivery of Nebivolol hydrochloride by iontophoresis will improve the therapeutic efficacy and overcome the difficulties raised in oral drug delivery. Because of its extensive hepatic metabolism and low dose, Nebivolol hydrochloride become a suitable candidate for transdermal administration. The matrix transdermal patches of Nebivolol hydrochloride were prepared and tested for *in-vitro* drug release and *ex-vivo* permeation. The study was conducted with the help of silver-silver chloride electrodes by iontophoresis across hairless rat skin. Drug release was evaluated in the presence of iontophoresis field using a current density of 0.5 mA/cm^2 or without electric field i.e passive diffusion by the process of electro-migration and electro-osmosis. Drug was measured spectrophotometrically and flux was determined. The flux of Nebivolol significantly increased ($P<0.05$) with increase in current strength from 0.5 – 1.0 mA/cm^2. The findings show that Nebivolol hydrochloride matrix transdermal therapeutic systems could be prepared with the required flux having suitable mechanical properties**.** It can be concluded from the results that the appliance of iontophoresis with penetration enhancer enhances the flux compared to the passive diffusion.

Keywords: Nebivolol hydrochloride, iontophoresis, permeation enhancer, transdermal, hypertension, flux

Corresponding Author: Yamsani Madusudhan Rao, Department of Pharmacy, JNTU, Kukatpally, Hyderabad, India. bDepartment of Pharmaceutics, Vaagdevi College of Pharmacy, India
Tel: 08099762637
E-Mail: pavanisrm@gmail.com
Cite this article as: Pavani S, Madusudhan Rao Y, Shravan Kumar Y, Synergistic Effect of Chemical Enhancer and Iontophoresis for Transdermal Delivery of Nebivolol Hydrochloride.Iranian Journal of Pharmaceutical Sciences, 2016, 12 (2): 9-20.

1. Introduction

There is a necessity for new drug delivery system that improves the therapeutic efficacy of drugs so that, the drug directly enters the blood stream through the skin, keep diffusing for long period maintaining drug concentration at constant level. Enhancement of drug penetration across the skin is facilitated by using chemical

enhancers and/or physical techniques. Among physical approaches, phonophoresis, electroporation and iontophoresis, *i.e*. the applying of a low voltage electric current (<0.5 mA/cm), are progressively used to enhance drug transport into and through the skin with one or combination of the following mechanisms: electro-migration or electro-osmosis, the charged drugs and other ions are carried across the skin [1].

Iontophoretic method of drug therapy is gaining a wide popularity especially in pain relief, diabetes, hypertension and rheumatoid arthritis. It involves the application of a low-level electric current either directly (DC) to the skin or indirectly (AC) through the dosage form to enhance permeation of a topically applied therapeutic agent [2, 3]. It increases the permeation of ionic drugs and flux into surface tissues by repulsion of ions at the active electrode [4, 5]. In iontophoretic delivery, onset of action is rapid in contrast to passive treatment [6]. This method of administration has drawn interests of various formulation technologists and practitioners due to its certain advantages over oral administration such as bypassing the hepatic metabolism, avoiding GI side effects and drug deposition. [7].

Nebivolol hydrochloride is a β1- receptor selective antagonist with vasodilatory property used to treat hypertension. Long-term oral administration of drug side effects might be overcome by using a transdermal formulation with a high degree of drug permeation through skin. But, low aqueous solubility and intact barrier function of stratum corneum are two major limiting factors in formulation of a desired topical preparation [8].

Several approaches including formulation of various solvent mixtures and application of natural or chemical penetration enhancers [9] have been tried to increase the penetration of drug. No study has been reported on the application of iontophoresis for permeation of Nebivolol across the skin. So, the aim of the present study was to develop a transdermal patch of Nebivolol suited for iontophoretic delivery and to investigate the effects like concentration of drug and polymer, current density, pulsatile duration and synergistic effect of enhancer with iontophoresis on permeation of drug through excised rat skin. Because of its low bioavailability (12%), low dose, lipophilic nature and due to extensive hepatic metabolism, Nebivolol, is a suitable candidate for transdermal administration [10, 11].

2. Materials and Methods

2.1. *Materials*

The active pharmaceutical ingredient Nebivolol was obtained from Aurobindo Pharmaceuticals, India as a kind gift sample and other excipients used were from Origin Pharma Company, Hyderabad and Hi-media laboratories. Silver wire was from SD fine chemicals. All chemicals and reagents used are of analytical grade.

2.2. *Drug-Excipient Compatibility Study*

The IR spectra were being recorded using an IR-spectrophotometer (FT-IR; Bruker) using

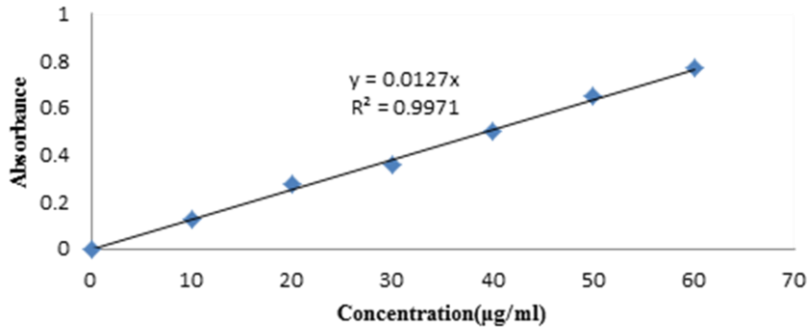

Figure 1. Standard graph of Nebivolol Hydrochloride in pH 7.4 phosphate buffer.

Table 1. Composition of Nebivolol transdermal patches.

Formulation code	Drug (mg)	HPMC E15 (mg)	Eudragit L100 (mg)	D-Limonene (ml)	Current mA/cm^2
F1	50	600	-	0.03	-
F2	50	400	200	0.03	-
F3	50	450	150	0.03	-
F4	50	500	100	0.03	-
F5	50	550	50	0.03	-
F6	50	350	250	0.03	-
F7	50	600	-	0.03	1
F8	50	400	200	0.03	1
F9	50	450	150	0.03	1
F10	50	500	100	0.03	1
F11	50	550	50	0.03	1
F12	50	350	250	0.03	1

KBr pellet method to study the possible interaction between drug and polymers.

2.3. Construction of Standard Graph of Nebivolol

The calibration curve was obtained by preparing stock solution (1000 mcg/ml) and dilutions from where absorbance was obtained using UV-Visible spectrophotometer (Shimadzu, Japan) at 283 nm against phosphate buffer pH 7.4 as blank figure 1.

2.4. Preparation of Nebivolol Transdermal Patches

Matrix type transdermal patches containing Nebivolol hydrochloride was prepared by solvent evaporation technique using different ratios of HPMC E15, ER L 100 in methanol and

chloroform solvent mixture (1:1). The polymers were weighed as shown in Table 1 and allowed for swelling for 6 h in solvent mixture. To the above polymer solution 15% v/w polyethylene glycol was being incorporated as plasticizer with continuous stirring, followed by addition of drug solution. The solution was checked for their turbidity, colour and presence of air bubbles and casted on anumbra plate and allowed for air drying. After drying the entire sheet was cut into small patches with an area of 4.9 cm^2 containing 3.67 mg of Nebivolol Hydrochloride.

2.5. Preparation of Ag/Agcl Electrodes and Skin

The silver-silver chloride electrodes used for the study were prepared by dipping the 0.5 mm diameter silver wire into molten silver chloride to form a thin uniform coat. Before using, the electrodes were being immersed in 0.1 M HCl.

2.6. Preparation of Skin

The male albino rats were sacrificed and the full skin was being removed from abdominal region, the epidermis was prepared surgically by heating in water for 45 min at 60^{o}C, washed, dried in desiccator and stored at 4^{o}C until used. At the time of use the epidermis was re-hydrated by immersing in water at room temperature for one hour [12].

2.7. Evaluation of Nebivolol Transdermal Patches [13, 14]

The films prepared by general procedure were tested for physical parameters like thickness, weight variation, folding endurance.

2.7.1. Weight Variation, Thickness

Six patches were randomly taken; weighed and average weight was recorded. The thickness of the films was measured using screw gauge and average was determined.

2.7.2. Drug Content

The drug content was measured for each formulation by taking a patch and soaked with solvent mixture overnight. Drug concentration was determined at 283 nm using a UV-Visible spectrophotometer against blank.

2.7.3. Folding Endurance

It was done by folding the patch repeatedly at the same place until it breaks. The number of times the patch folded at the same place without breaking gives the folding endurance.

2.7.4. Moisture Absorption Studies

Moisture absorption studies and moisture content was measured for each formulation. The pre-weighed patches are placed in a desiccator along with saturated solution of aluminum chloride. After three days the final weight was noted and percentage of moisture absorption was calculated.

2.7.5. Moisture Content Studies

Initially weighed patches were placed with calcium chloride in a desiccator at 40^{o}C, after 24 h final weight was recorded and the percentage of moisture loss was calculated.

2.7.6. Measurement of Mechanical Properties

Mechanical properties of the films were tested using microprocessor-based advanced force gauze equipped with a 25 kg load cell for a selected patch. A film of 6 cm, free from imperfections held between two clamps with a distance of 3 cm, the force and elongation break was measured [14].

2.7.7. In Vitro Drug Release Studies

In vitro drug release studies was performed using Franz diffusion cells made of two components, donor and receptor, separated by a membrane barrier. The cellulose acetate membrane which was mounted between two chambers was held with a clamp. The receiver chamber was filled with phosphate buffer and patch was positioned on membrane facing the donor chamber. The receiver contents were stirred continuously using a magnetic stirrer. Samples were withdrawn at regular intervals and replaced with freshly prepared buffer to maintain the sink condition. The samples were analyzed by UV Visible spectrophotometer (Shimadzu) at 283 nm for drug content and mean percentage of drug released was plotted. All experiments are carried out in triplicate.

2.7.8. Ex-Vivo Permeation Studies

Transport of Nebivolol across the skin from patch was carried out by *ex vivo* permeation studies. The epidermis prepared by heat separation method was re-hydrated and used for *ex-vivo* permeation studies. Rat skin was held between the donor and reservoir compartment of the diffusion cell, the transdermal patch was placed over the skin, samples were withdrawn periodically from recipient compartment and amount of drug permeated was measured by reading the absorbance at 283 nm spectrophotometrically. Cumulative amount of drug permeated in $\mu g/cm^2$ was calculated and plotted against time [15].

The flux was calculated by using the following equation.

$J = C_{ss}Cl_TBW/A$ where, J is the flux, A is surface area of the patch, BW the body weight of standard human body, $C_{ss,}$ the steady state concentration at therapeutic level and Cl_T total clearance.

2.7.9. Permeation Studies with Iontophoresis

Both *in vitro* drug release and *ex vivo* drug transport experiments were carried out without application of any electrical current (passive diffusion). In the iontophoretic experiments, a portable iontophoresis system applied to generate a weak current of 0.5 mA/cm^2 using silver/silver chloride electrodes, shown to have both reversibility and stability [15]. Pure silver wire as anodal electrode, and silver chloride (AgCl) electrode as cathodal electrode was connected to a power source. The receptor compartment filled with phosphate buffer was used. For skin integrity test for 3 h, methyl red solution was added to donor compartment. Thereafter, the skin was washed and mounted between the compartments and the patch was positioned above the skin and a small current was supplied for about 2 h, thereafter, the power

Table 2. Drug Excipient Compatibility study –FTIR Analysis.

IR Spectra	**Peak of functional groups [wavelength(cm-1)]**							
	Alkane Stretching	C-C	C-H	C-O	O-H	C-N	N-H	C-F
Nebivolol hydrochloride	2901.5	1491.1	870.4	1212	3715	1258.3	3184	1073
Nebivolol+ HPMC E15	2876.6	1491.8	870.4	1213	3653	1258.5	3374	1071
Nebivolol+ HPMC E15 +EL100	2955.6	1491.7	870.9	1213	3737	1258.6	3253	1073

was discontinued and followed by passive diffusion. The effect of various iontophoresis limits including applied current density, pulsatile condition was studied. Similar experiments were accomplished without application of any electrical current (passive flux) as control [16].

2.8. Stability Studies

The stability studies were conducted for optimized formulations according to ICH guidelines for 3 months, and the samples were collected after 1, 2 and 3 months and analyzed for various parameters.

3. Results and Discussion

3.1. FTIR Studies

The Fourier transforms infrared spectroscopy studies were carried out for pure drug, excipients and physical mixture. From the Table 2, it could be seen that the major functional groups of Nebivolol hydrochloride and excipients were present in the spectrums of pure and physical mixture. It shows that there is no interaction as the major peaks remained same and they were compatible with each other.

3.2. Physicochemical Parameters

The results of Physico-chemical properties of patches prepared were shown in Table 3**,** the weight of the patches increased with increase in HPMC E15 concentration. The results showed uniformity in weight of patches. Thickness also increased with increase in HPMC E15 and found to be uniform. The thickness ranged from 0.19±1.54 mm for F6 to 0.26±0.67 mm for F7. The folding endurance numbers of HPMC E15 containing patches were between 562 to 566 whereas HPMC E15 with Eudragit L100 were between 435 to 563. The folding endurance number gives the mechanical property of the patches, high folding endurance have high mechanical property. The results (Table 3) showed that the patches would not break and would maintain their integrity with general skin folding when applied. Good uniformity in drug content was observed in all transdermal patches as evidenced by low SD values. The drug content ranged from 2.73±0.55 mg in formulation F6 (HPMC E15 & Eudragit L100) to 3.42±1.37 mg in formulation F7 (HPMC

Table 3. Evaluation for physical and physico-chemical properties of transdermal patches.

Formulation	Weight variation (mg)	Thickness (mm)	Folding endurance	Drug content (mg)	%Moisture absorbed	%Moisture Content
F1	46.9±1.53	0.25±0.79	562.45±0.53	3.35±0.96	10.87±1.58	9.34±0.96
F2	33.76±0.97	0.2±1.27	435.12±1.38	2.83±1.29	7.92±1.82	4.62±0.85
F3	38.26±0.59	0.22±0.95	489.57±0.75	3.05±0.84	9.67±0.95	5.97±1.17
F4	42.41±1.26	0.23±0.83	550.77±0.93	3.26±1.18	8.39±1.46	8.35 ±1.32
F5	45.75±0.78	0.24±0.56	558.98±0.88	3.29±1.04	10.45±0.93	8.45±1.95
F6	32.37±0.49	0.19±1.54	432.48±0.64	2.73±0.55	6.42±1.25	4.58±0.77
F7	47.55±0.55	0.26±0.67	566.92±1.29	3.42±1.37	11.44±1.03	9.35±0.94
F8	35.45±1.12	0.205±0.98	454.1±1.02	2.99±0.92	8.35±0.89	5.21±0.55
F9	39.62±1.43	0.21±1.38	490.7±0.74	3.16±0.75	8.86±0.64	6.32±0.79
F10	40.78±0.89	0.24±1.26	558.57±0.62	3.32±1.55	9.34±0.59	7.56±0.82
F11	43.51±0.95	0.25±0.58	563.46±1.14	3.38±1.27	10.48±1.19	9.12±0.93
F12	33.25±0.67	0.215±0.63	470.79±1.09	2.76±0.86	6.54±1.53	5.89±1.87

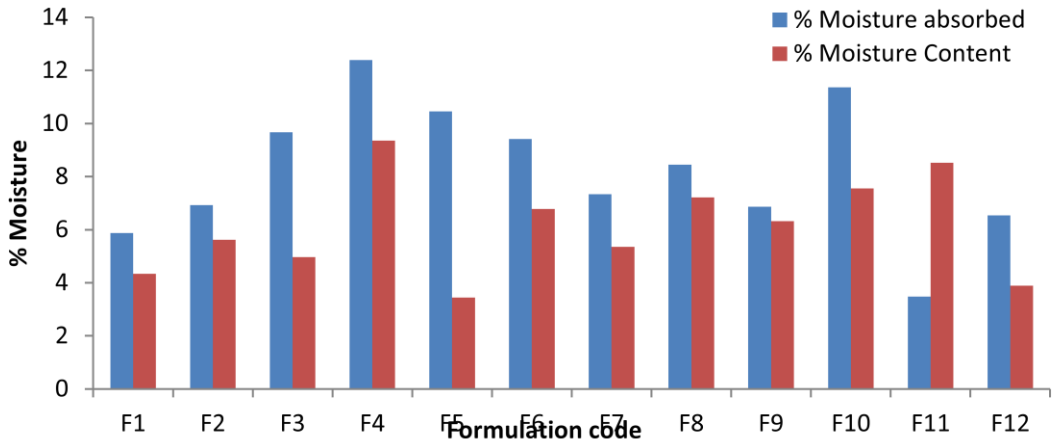

Figure 2. % Moisture absorbed and %Moisture content of Nebivolol transdermal patches.

E15). The drug content was greatest in the formulation containing more amount of hydrophilic polymer.

The moisture content of the patches ranged from 4.58±0.77% for F6 (HPMC E15 & Eudragit L100) to 9.35±0.94% for formulation F7 with HPMC E15). The moisture absorption in the formulations is ranged from 6.42±1.25% for F6 (HPMC E15 & Eudragit L100) to 11.44±1.0 % for F7 (HPMC E15). The results showed that the moisture absorption and moisture content increases with increasing the amount of hydrophilic polymer (HPMC E15). The smaller moisture content in the formulations helps them to remain stable from being dried and brittle. (Figure 2)

3.3. Ex Vivo Permeation Studies, Synergistic Effect of Penetration Enhancer and Iontophoresis and Effect of Pulsatile Current

with iontophoresis showed largest (3569.78 µg) cumulative permeation and a flux of 44.46 µg/cm^2/h compared with formulation F4 and

Table 4. Cumulative amount of drug permeated and flux.

Formulation code	Cumulative amount of drug permeated (µg/cm^2)	Flux, J_{ss} (µg/cm^2/h)
F1	2357.3±19.46	26.54± 1.05
F2	2530.67±9.3	29.4± 0.93
F3	2754.54±9.1	29.74 ±0.72
F4	**3062.63±14.2**	**33.24 ±1.36**
F5	2269.35±6.7	25.47± 0.85
F6	2437.82±8.32	28.32± 0.64
F7	2597.64±10.5	29.15± 1.54
F8	2695.45±7.7	31.04± 1.13
F9	2963.54±3.24	31.6± 0.56
F10	**3569.84±9.82**	**44.46±0.73**
F11	2411.93±9.25	28.24±1.28
F12	2600±10.95	30.2±1.18

Table 5. Comparative study of Nebivolol Hydrochloride permeation.

Time (h)	**Cumulative amount of drug permeated (µg/cm^2)**	
	F4	**F10**
0	0	0
1	269.01±10.5	602.98±6.45
2	412.14±7.5	961.54±10.55
3	583.53±9.3	1228.34±14.52
4	751.24±8.9	1529.28±11.12
5	940.62±7.5	1844.9±7.29
6	1143.93±9.92	2014.83±5.49
7	1351.66±9.35	2160.52±9.58
8	1545.07±13.56	2403.85±8.54
9	1773.71±14.5	2617.81±8.22
10	2001.25±9.58	2771.95±5.89
12	2252.27±12.12	2982.6±12.38
24	3062.63±14.2	3569.84±9.82
Flux J_{ss}	32.82±1.36	44.46±0.73

The results are shown in Table 4 and 5 of Nebivolol Hydrochloride permeation through the rat skin from patches. The formulation F4 exhibited the maximum (3062.63 µg) cumulative amount of drug permeation in 24 h, F1 2357.3µg, and F10 i.e., permeation enhancer

others.

On ionization, Nebivolol attains a positive charge and pushes ions into and transport drug across the skin compared to passive diffusion. But on continuous use of direct current the skin results in polarization which reduces the

Table 6. Effect of Pulsatile and continuous current on various permeation parameters.

Current	Q_{24}	$J_{ss, Flux}$	K_p	Er
Continuous current	2695.23	32.04	0.0087	1
Pulsatile current	3569.78	44.46	0.0121	1.38

Table 7. Mechanical properties of optimized formulations.

Formulation code	Tensile strength (kg/m^2)	Elongation at break (%mm^{-2})
F4	1.38±0.58	24±1.42
F10	1.46±0.78	22.53±0.98

iontophoretic delivery efficiency which is proportional to length of current density. The polarization effect can be overcome by using pulsed current that is delivered intermittently. So, further enhancement of permeation and flux across the skin can be occurred with Pulsed iontophoresis.

However, the flux increases with pulsatile current (Table 6). The pulsatile current allows the skin to depolarize and return to initial state when current is off for a fraction of time. So, the *ex vivo* studies for F6 to F12 were performed using pulsatile current. The Formulations F1 and F7 composed of HPMC E15 showed less drug permeation as rigid films were obtained. The formulations F1 to F6 except F4 could not get the required flux (33.24 µg/cm^2/h). F4 containing only penetration enhancer showed flux of 33.4 µg/cm^2/h (flux greater than F1 to F6). Formulations F4, F8, F9 have shown required flux. The transdermal patches planned with only penetration enhancer could obtain the required flux whereas the formulation F10, permeation enhancer was combined with iontophoresis exhibited maximum drug permeation and the required flux increased to 44.46 µg/cm^2/h. The results (Table 4-6) of drug permeation from transdermal patches of Nebivolol Hydrochloride through rat abdominal skin confirmed that Nebivolol permeated through the rat skin as planned and hence could permeate through human skin [10, 11]. Iontophoresis markedly enhances the permeation of Nebivolol Hydrochloride.

3.4. Mechanical Properties

The results of mechanical properties are shown in Table 7 (tensile strength, elongation at break, elastic modulus, and strain) show that the optimized formulations were strong and flexible but not brittle. As the concentration of HPMC increases, tensile strength and elastic modulus also increases but elongation break would decrease.

3.5. Release Kinetics

The increasing order of drug release with permeation enhancers was F5<F1<F6<F2<F3<F4. The release data were fit into different kinetics to determine the release mechanism and n values. The Higuchi model was the appropriate model describing the release kinetics from all patches having the correlation coefficient between 0.962 and 0.994. The n

value (0.22–0.41) shows that the amount of drug released was due to Fickian diffusion [17]. So, the optimized formula F4 along with current density (F10) has shown maximum flux.

4. Conclusion

Iontophoresis is a hopeful physical technique used to enhance transdermal drug delivery with safety and high efficiency. The observations and findings of the present study showed that Nebivolol transdermal flux through rat skin increases with current density and pulsatile condition. Because of its ionization iontophoretic drug transport was almost 1.5 times more than passive flux. The results suggest that Nebivolol may be considered as a suitable candidate for transdermal transport through iontophoresis to treat hypertension. However, to support *in vitro* and *ex vivo* results, *in vivo* conclusions and correlations are required.

Acknowledgements

The authors are thankful to Origin Pharma for providing gift sample also thankful to management of college for providing facilities to carry out this work.

References

[1] Eiichiro M, Sachihiko N, Kenji S, Yasunori M. Analysis of skin permeation enhancing mechanism of iontophoresis using hydrodynamic pore therapy. J Control Release (2000) 66:149–158.

[2] Jorge LL, Feres CC, Teles V. Topical preparations for pain relief: efficacy and patient adherence. J Pain Res (2011)4:11–24.

[3] Dixit N, Bali V, Baboota S, Ahuja A, Ali J. Iontophoresisan approach for controlled drug elivery: a review. Curr Drug Deliv (2007)4:1–10.

[4] Ashok Kumar J. Transdermal drug delivery system: an overview. Int J Pharm Sci Rev Res (2010)3:49–54.

[5] Yan G, K Li, WI Higuchi. Evaluation of constant current alternating current iontophoresis for transdermal drug delivery. J Control Release (2005)110:141–150.

[6] Banga AK, Chien YW. Iontophoretic delivery of drugs: fundamentals, developments and biomedical applications. J. Control release (1988)7:1-24.

[7] Tavakoli N, Minaiyan M, Saghaei E. Preparation of diltiazem topical gel for the treatment of anal fissure and *invitro*, exvivo drug release evaluations. J Kerman Univ Med Sci. (2007)14:167–175

[8] Farmer, ER., Hood., Anoinette, H., 2000. The Pathology of the Skin, 2nd edition.

[9] Heather, AE., Benson. Transdermal drug delivery: Penetration enhancement techniques. (2005)2:23-33.

[10] Packer M, Coats AJ, Fowler MB, Katus HA, DeMets DL Effect of Carvedilol o survival in severe chronic heart failure. N Engl J Med (2001) 344:1651–1658.

[11] Vinay V. Various penetration enhancement techniques in transdermal drug delivery. Int J Pharma Technol (2011)3:2373–2401.

[12] Gupta R, Mukharjee B. Development and *in vitro* evaluation of diltiazem hydrochloride transdermal patches based on povidone ethyl cellulose matrices. Drug Dev Ind Pharm (2003)29:1–7.

[13] Yousuf M, Ahmad M. Ketotifenfumarate and salbutamol sulphate combined transdermal patch formulations: *in vitro* release and *ex vivo* permeation studies. In J Pharm Sci (2013)75:569–577.

[14] Jitendra B. Formulation, optimization and evaluation of matrix type transdermal system of Lisinopril dehydrate using penetration enhancers. Drug Invention Today (2010)2:134–137.

[15] Sebastiani P, Nicoli S, Santi P. Effect of lactic acid and iontophoresis on drug permeation across rabbit ear skin. Int J Pharm. (2005)292:119–126.

[16] Kotwal V, Bhise K, Thube R. Enhancement of iontophoretic transport of diphenhydramine hydrochloride thermosensitive gel by optimization of pH, polymer concentration, electrode design, and pulse rate. AAPS Pharm SciTech. (2007)28:E120.

[17] Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers. Pharm ActaHelv (1985)60:110–111.

ONLINE SUBMISSION

www.ijps.ir