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Transdermal Iontophoretic Delivery of Atenolol in Combination with Penetration Enhancers: Optimization and Evaluation on Solution and Gels

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ABSTRACT

In the present investigation, we prepared Atenolol (1.5 % w/w) solution and various polymer formulations by incorporating the tween-20 or L-menthol, as a penetration enhancer and its effect on permeation of the drug through the excised abdominal rat skin were used to examined by using the vertical Franz-type diffusion cell. The physicochemical interactions between Atenolol and various polymers were investigated by performing the assay, ultra violet absorption maxima, Fourier transform infrared spectroscopy and it was further confirmed by thin layer chromatography studies, from which drug did not show any evidence of interaction with the polymers. We found that, L-menthol was superior than tween-20 to iontophoresis [current density applied 0.5 mA/cm^2 and 90:10 (on: off) ratio], in enhancing the transdermal permeation of Atenolol; it enhanced the flux of Atenolol by more than 2-folds, comparison to the preparations without penetration enhancer via passive diffusion, and 3 folds increased using iontophoresis alone with a shorter lag time. Atenolol also showed good stability in gel formulations. The basic parameters like % loading dose released at the end of the study, permeation coefficient and steady state flux (J_{ss}) were calculated and showed statistically significant difference ($p<0.05$). The results indicated that suitable iontophoretic delivery with desired permeability could be appeared and the cumulative amount-time curves were suitable to fit by a zero order equations which indicated a steady state permeation rate or sustained effect could be achieved from hydrogel; when it is combined with penetration enhancer, L-menthol. The results demonstrate that the semisolid gel formulations are more applicable than solution as a transdermal iontophoretic delivery system to administer clinically. Electrically assisted transdermal delivery of Atenolol significantly increased transport compared to passive delivery. Also, rapid and modulated delivery was shown to be feasible by programming the electrical parameters.

Keywords: transdermal delivery; iontophoresis; Atenolol; solution; gel formulations; penetration enhancers; Franz cell; rat skin.

INTRODUCTION

A number of approaches have been developed to enhance and control transport across the skin, and expand the range of drugs delivered. These involve chemical and physical methods, based on two strategies: increasing skin permeability and/or providing driving force acting on the drug. [1]A recent review by Barry showed that the transdermal route now vies with oral treatment as the most successful innovative research area in drug delivery, with around 40 % of drug delivery candidate products under clinical evaluation related to transdermal or dermal systems. [2]

The iontophoretic delivery is based on the fact that solute ions will be repelled by an electrode of like charge and migrates to an electrode of opposite charge under the influence of an electromotive force. [3] Iontophoresis is the administration of drug through the skin by application of an electric current (<0.5 mA/cm^2 for min or h). [4]

The commercial successes of iontophoretic systems have raised the interest in such promising delivery of the drug in

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the form of gel or solution based formulations. Wearable iontophoretic patch (E-TRANS ®) for delivery of fentanyl is currently under commercial development. Vyteris Lidosite™ (Lidocaine) iontophoretic system, marketed by B. Braun-Healthcare Products and Services Company. Wearable Electronic Disposable Drug (WEDD®), is a self-contained, portable and disposable patch and manufactured by Brich Point Medical. Iontopatch (IOMED Phoresor ® PM850 & PM 900) which are used by physical therapist and sports medicines professional containing Lidocaine HCl: epinephrine (1:100, 00). [5-7] In spite of the advantages of the transdermal delivery, only a small percentage of drugs can be delivered transdermally due to the barrier properties of the skin; only small potent lipophilic drugs can be delivered at therapeutic rates by passive diffusion. [1]

Atenolol is a β-adrenergic blocking agent used in the treatment of various cardiovascular disorders; with an oral bioavailability of 50%, the transdermal delivery of these drugs could be a potential alternative to oral delivery to increase therapeutic efficacy, bypassing hepatic first-pass metabolism and low oral absorption. [8] The physicochemical properties of atenolol made it a suitable iontophoretic model compound. The hydrophilic atenolol compound (pKa =9.6) has one positive charge at physiological pH (octanol /Phosphate buffer saline pH 7.4), resulting in a limited passive permeability. [9] The gel base often provides a fast release of drug substance and a high degree of clarity in the appearance. Moreover, there is always a great volume of water employed in gel formulation which exhibits a high electrical conductivity. [10] Penetration enhancers may be applied to the skin prior to application of the drug, co-applied with the drug or used in the vehicle matrix. More recently naturally occurring compounds, including terpenes from the chemical classes of hydrocarbons, alcohols, ketones and oxides and long-chain fatty acids when applied to the skin in a suitable co-solvent such as propylene glycol have been used. [11] Though studies of iontophoretic drug delivery utilize the *in vitro* animal models such as rat, mouse, rabbit or guinea pig, much less is known about their suitability as a model for *in vivo* human skin. [12]

The purpose of this study was to evaluate the potential transdermal delivery of Atenolol using iontophoresis and to evaluate the parameters affecting the efficacy of the enhancement technique. Moreover, the study involved the possibility of using penetration enhancers to potentiate the effect of iontophoresis. Atenolol was chosen as the model candidate for this study, since it possesses near ideal characteristics that a drug should have in formulating an iontophoretic transdermal drug delivery system: Short biological half life (6-8 hours), low molecular mass (266.34), pKa=9.6; low lipid solubility, effective in low plasma concentration as well as a high degree of first pass metabolism. It also means multiple daily administrations with subsequent lack of patient compliance.

Aim of the present study was to develop a suitable formulation and to investigate the efficacy of transdermal administration of drug from the prepared solution and gels. The investigation consisted essentially of the following steps: (a) preparation of medicated solution, and gels using various polymers with different penetration enhancers, (b) testing of the effects of various formulations and penetration enhancers on the *in vitro* permeation of drug through rat abdominal skin for the initial screening of the suitable iontophoretic drug delivery carrier.(c) lastly to choose proper formulation, as to increase the bioavailability and achieve a controlled release delivery system. An extensive application area may be needed for a therapeutic effect, and this may prevent good patient acceptance and compliance.

MATERIALS AND METHODS

Atenolol was donated as a gift sample by Stadmed Private Ltd. (Kolkata, India). Medium grade sodium carboxy methylcellulose (Na-CMC) and Methyl Cellulose (viscosity of 2 % solution 300-500cps) were obtained from HPC (New Delhi, India). Silver chloride (99 % pure) and silver wire metal (1 mm diameter, 99.9 % pure) were purchased from Loba Chemicals (Mumbai, India). De-mineralized water was used throughout the study. All other reagents were of analytical grade.

Preparation of formulations

Preparation of Atenolol solution

Atenolol 1.5 % w/w was dissolved in phosphate buffer as required volume and adjusted the pH to 7.4 with 1M NaOH and/or 1M HCl solutions.

Preparation of gel formulations

Gel formulations were prepared by modifying some reported procedures, which is given as follows: Sodium carboxy methyl cellulose (medium grade) gel was prepared by dissolving polymer (3 % w/w), with sufficient amount of phosphate buffer (pH 7.4) by heating on a water bath for 30 min to effect complete solution and kept it to cool at the room temperature and stirred that preparation using mixer until a cleared gel were obtained.

Methyl cellulose (viscosity of 2 % solution 300-500cps) gel was prepared by dissolving polymer (3 % w/w), as required amount of phosphate buffer (pH 7.4) without heating and stirred that preparation using mixer until a clear gel were obtained.

Methyl paraben (0.2 % w/w) and propyl paraben (0.02 % w/w) solution were prepared by heating with the some portion of propylene glycol until a clear solution was obtained. This preservative solution was mixed individually with the gel preparation, prepared by polymer.

Atenolol 1.5 % w/w and L-menthol (2 % w/w) dissolved into the propylene glycol and incorporated to the polymer solutions and mixed it by continuous stirring at about 500 rpm for 30 min. pH of the resulting final preparations were then adjusted to pH 7.4 by the addition of the 1M NaOH and/ or 1 M HCl solution. The solution was stored overnight at room temperature to ensure complete polymer dissolution in an air tight glass container. [12-13]

Interaction studies

Interaction studies were conducted on the medicated formulations by comparing them with the pure drug and placebo formulations on the basis of assay, UV, FTIR and TLC analysis. [14- 16]

Assay

The TDDS was dissolved in Phosphate buffer (pH 7.4) and phosphate buffer with 5% methanol and the drug content was determined by UV analysis method (Shimadzu UV-pharmaspec 1700, Japan).

UV analysis

The phosphate buffer (pH 7.4) with 5 % methanolic solutions of the pure drug, medicated and placebo formulations were filtered through Whatmann filter paper and scanned spectrophotometrically between 210-300 nm.

Table 1: Specification for Iontophoretic device used throughout the experiments

Output current range	Compliance Voltage	Mode of operation	Pulse duration	Duty cycle used	Power source	Electrodes	Adjustable peak voltage
0±4mA or 0±20mA (Interval 0.05 &0.25 mA per unit)	24 V maximum	Timed / pulsed current	5 sec	90:10 (on: off)	20V DC external power supply (rechargeable)	Ag/Agcl	0-20 V

Table 2: Compositions of various Atenolol formulations
Compositions of various Atenolol formulations

Formulation	Atenolol (%w/w)	Sodium carboxy methyl cellulose (%w/w)	Methyl cellulose (%w/w)	Methyl paraben (%w/w)	Propyl paraben (%w/w)	Propylene glycol (%w/w)	Permeation Enhancer (%w/w)	
							L- Menthol	Tween 20
F-1	1.5	-	-	-	-	-	-	-
F-2	1.5	3.0	-	0.2	0.02	30	-	-
F-3	1.5	3.0	-	0.2	0.02	30	-	5
F-4	1.5	3.0	-	0.2	0.02	30	2	-
F-5	1.5	-	3.0	0.2	0.02	30	-	-
F-6	1.5	-	3.0	0.2	0.02	30	-	5
F-7	1.5	-	3.0	0.2	0.02	30	2	-

FTIR analysis

The IR absorption spectra of the pure, medicated and placebo formulations were taken in the range of 4000-400cm^{-1} using the KBr disc method (Shimadzu IR -Prestige-21, Japan).

Thin Layer Chromatography (TLC) studies

TLC plates of 0.25 to 0.50 mm thickness were prepared with Silica gel GF_{254} using dioxane: acetonitrile: methanol: 25 % v/v ammonia (60: 36: 5: 4) as solvent. Sample solution of pure drug was prepared by dissolving 50 mg pure Atenolol in 5 ml purified water and solutions of various gel formulations were prepared by using equivalent to 50 mg of Atenolol gel formulations in 5 ml purified water. The sample solutions were spotted on the TLC plate and subjected to ascending chromatographic analysis. After the migration of the solvent system, the plate was air dried and visualized in CAMAG UV TLC cabinet at 254 nm, whereas; iodine vapor were used as visualizing agent.

Specification of Iontophoresis device

The specifications for iontophoresis device (Table 1) were modified from several previously reported papers. [6, 17-18]

Preparation of electrodes

Iontophoresis experiments were conducted using silver/ silver chloride electrodes. The silver chloride electrodes were prepared as follows: Prior to chloridation of silver electrodes, they were dipped in deionized water, ethanol, fuming nitric acid and rinsed thoroughly with deionized water. Silver wires (1 mm diameters, 9 cm longs) were immersed in 0.1 N HCl solution and connected to the anode of an electric current source of 1mA connected with 12 V DC. Silver chloride powder was melted in a basin and picked up by another silver wire which was connected to the negative pole of the current source. A gray silver chloride layer was gradually coated on the anodal silver wires, and after 24 h. This wire was ready for use as iontophoresis cathodal electrodes. [9]

Preparation of skin membranes

Male hairless Albino rat were sacrificed and the skin from the abdominal surface was excised, and the adherent fat and subcutaneous tissue were removed. The skins were kept at 4°C until used. Immediately before the experiment, the skins were taken out and left to thaw at room temperature. The skins were cuts into small pieces as per required for the in vitro testing. The pieces were carefully mounted on top of the diffusion cells and left to hydrate for 1 h before the application of the formulations. The thicknesses of the skin were used approximately one mm. [10, 19]

Evaluation of formulations

Homogeneity

The formulations were tested for the homogeneity up to 3 months by visual appearance and by touch after the gels have been set in the container.

Adjustments of pH of the gel formulations

The Atenolol solution and gel formulations were adjusted to pH 7.4 by the addition of the 1M NaOH and/ or 1 M HCl solution. The pH of different gel formulations and simple Atenolol solution were measured by diluting it with the purified water, using µ pH meter 361 (Systronics, Model-361). During the *in vitro* studies, the final pH of the donor and receptor solutions was measured after 12 hrs of the experiments.

Table 3: TLC results of the pure drug sample, methyl paraben, propyl paraben and gel formulations of Atenolol

Sample	R_f value (Mean ±SD), n=3	% Coefficient of variation
Pure drug (Atenolol)	0.425±0.0158	3.717
Methyl paraben	0.876±0.016	1.826
Propyl paraben	0.885±0.041	4.632
PF-2	0.841±0.031	3.686
F-2	0.415±0.013	3.132
	0.853±0.044	5.158
PF-4	0.850±0.010	1.1764
F-4	0.409±0.0025	0.611
	0.852±0.044	5.164
PF-3	0.830±0.026	3.132
F-3	0.410±0.004	0.976
	0.852±0.044	5.164
PF-5	0.880±0.020	2.272
F-5	0.408±0.0057	1.397
	0.868±0.0076	0.8755
PF-7	0.904±0.055	6.084
F-7	0.407±0.0015	0.368
	0.867±0.0213	2.456
PF-6	0.872±0.040	4.575
F-6	0.408±0.0015	0.367
	0.867±0.030	3.46

PF-2, PF-3, PF-4-Placebo gel formulations of SCMC, PF-5, PF-6, PF-7-Placebo gel formulations of MC
(All formulations prepared without drug and kept other compositions same as mentioned in table 2)

Drug Content Uniformity Studies

After accurately weighing the different gel preparations**,** containing equivalent to 15 mg of Atenolol, were transferred to 100 ml volumetric flasks and 50 ml of solvent was added. Phosphate buffer (pH 7.4) and phosphate buffer with 5 % methanol was used as extractive solvents. Flasks were shaken for about 15 min to solubilize the drug, and volumes were

made up to the mark with respective solvent. Finally, solutions were filtered through Whatmann filter paper. The 1ml and 2 ml filtrates of these each solutions were taken and volume made up to 10 ml with the respective solvent. Blank preparations were also carried out by using placebo formulations by the same method, and the absorbance were noted at 224 against corresponding blank preparation. [9, 20-22]

Stability studies

The gel formulations samples were stored in well sealed glass containers for a period of 90 days at 25ºC, 40 ºC. At predetermined time intervals; 0, 15, 30, 60, and 90 days, samples were collected and physical appearance was evaluated. Atenolol content of samples was determined by UV analysis (Shimadzu UV-pharmaspec 1700, Japan). Prior to analysis of gel samples, a weighed amount was reconstituted with phosphate buffer solution (pH 7.4) with 5 % methanol in order to allow the extract of atenolol from the formulations and filtered it out with the Whatmann filter paper. [18, 23]

***In vitro* Permeation studies**

This study was conducted after modification of previously reported methods. [10, 12, 15]

Rat skin was mounted on top of vertical diffusion cells, with a surface area available for diffusion of 0.64 cm^2 and 45 ml receptor compartment volume. The receptor compartments of diffusion cells were filled with isotonic phosphate buffer saline (pH 7.4) and were stirred at 500 rpm. The receptor fluids were thermo stated at 37±1°C under this condition the temperature at the skin surface was 35±1°C.Hairless rat skins were left for 1 h to hydrate before the start of the experiment. After this hydration period 1 g of drug solutions or gel were applied on top of each skin. The donor compartments of diffusion cells were covered with a triple layer of Para film and single layer of aluminum foil. A silver wire representing the anode was placed in the donor compartment, and a silver chloride cathode was placed in the receptor compartment. Both electrodes were attached to 12 V DC supply 5 sec (90:10) cut off duty cycle and current regulated at 0.5 mA/cm^2 area of the skin.

At predetermined time intervals 1, 2, 4, 5, 6, 8, 10, 11, 12 hrs 5 ml samples were withdrawn from the receptor compartment and were immediately replaced by the same volume of the buffer solution (37°C). The samples after diluting suitably were then analyzed spectrophotometrically, at a wavelength of 224 nm for Atenolol content against blank.

These samples were accounted for in the calculation of the corrected Atenolol concentration in the samples for the subsequent calculation of cumulative amount of Atenolol permeated through the skin. All samples were kept at freeze prior to analysis. All experiments were conducted 12 h and also the pH changes of the final donor and receptor solutions were measured after 12 h of the experiments.

Leakiness studies

After completing the permeation studies the leakiness of the mounted skin was investigated applying 2 ml of 0.5 % methylene blue solution to the donor chamber. After 20min, the receptor solution was visually inspected for blue staining. No staining of receptor solutions was only accepted. [9]

RESULTS AND DISCUSSION

Formulations were tested for homogeneity, changes of the pH during storage, drug content uniformity studies, drug stability and *in vitro* permeation studies.

To determine stability of the formulations, those were tested for homogeneity and changes of the pH with respect to time. It has been found that pH of the formulations was not significantly change and all formulations were homogeneous up to 3 months (Table 4).

Table 4: Homogeneity and changes of the pH of the different gel formulations with function of time after adjusting the initial pH at 7.4.

Gel formulation	Time interval			
	15 days[a]	1 month[a]	2 months[a]	3 months[a]
F-2**	7.487±0.0124	7.497±0.0124	7.523±0.0124	7.543±0.0124
F-4**	7.490±0.008	7.517±0.0124	7.550±0.0124	7.560±0.008
F-3**	7.457±0.0286	7.460±0.0163	7.50±0.008	7.510±0.008
F-5**	7.520±0.0163	7.563±0.0124	7.593±0.0124	7.597±0.0124
F-7**	7.497±0.0169	7.530±0.0294	7.553±0.038	7.550±0.0294
F-6*	7.493±0.0205	7.50±0.0216	7.543±0.0294	7.558±0.0249

a- Mean ±SD (n=4), Homogeneity up to 3 months-**Good, *Satisfactory.

In our previous study, gels were tested for the drug content uniformity from each formulations and drug content were found uniform. [20]

Interaction studies were carried out to ascertain any interaction of the drug with the excipients used in the preparation of transdermal drug delivery system (TDDSs), which may be interfere the permeation of the drug actively as well as stability of the product. Therefore, medicated and placebo formulations along with the pure drug sample were subjected to assay, UV, FTIR (fig 1 and 2) and TLC analyses (Table 3). [14-16]

On performing the assay, as much as 100 % of the drug were recovered from the optimized formulations, when phosphate buffer solution (pH 7.4) with 5 % methanol solution used as extracting solvent, but it were lesser in case while only phosphate buffer solution (pH 7.4) used. The results of recovery studies, which indicated that the % recovery by using Phosphate buffer (pH 7.4) alone, gave the estimated values 98.843±0.5086, 99.655±0.291, 99.3945±0.3783 in case of F-2, F-3, F-4 and for F-5, F-6, F-7 the values were 98.395±0.757, 98.455±0.585, 97.816±0.889 with the respective formulations, but when Phosphate buffer (pH 7.4) was combined with 5 % (v/v) methanol which gave the values 99.86±0.547, 100.42±0.299, 100.128±0.238 for F-2, F-3, F-4 and for F-5, F-6, F-7 values were 99.727±0.653, 100.789±0.436, 100.6±0.742 respectively.

The UV absorption maxima (Shimadzu UV-pharmaspec 1700, Japan) for the pure drug and the medicated formulations were found at about nearest to 224 nm.

In analyses of the FTIR spectra (Shimadzu-Prestige-21, Japan) of the pure drug and the medicated formulations, mentioned in Fig. 1 and 2, and no difference were observed in the absorption peak pattern. The UV and FTIR spectra of the placebo formulations gave entirely different absorbance profiles from those of the pure drug and medicated formulations.

Small variations of R_f values of Atenolol as a pure drug and in medicated formulations are evident from table 3.

Therefore the results indicate that the drug remained intact in gel formulations and that there were negligible chemical interaction between the drug and the excipients therein.

Gel formulations at different temperatures showed good physical stability, as there were no discolorations, precipitation, or any other physical changes after storage. The chemical stability of Atenolol in gel formulations was stored at different temperatures for period of 90 days. [15, 20] Atenolol showed good chemical stability in gel formulations.

Table 5: Drug released kinetic model report.

Formulations		Correlation coefficient factor for drug released profile (r)						
		F-1	F-2	F-3	F-4	F-5	F-6	F-7
Passive	Zero order	0.7546 P<0.1	0.9964 P<0.05	0.9963 P<0.05	0.997 P<0.02	0.9928 P<0.05	0.9831 P<0.1	0.9966 P<0.05
	First order	0.7993 P<0.1	0.9909 P<0.1	0.9968 P<0.05	0.9844 P<0.1	0.9959 P<0.05	0.9938 P<0.05	0.9861 P<0.1
	Higuchi model	0.9247 P<0.1	0.9674 P<0.1	0.983 P<0.1	0.9622 P<0.1	0.9889 P<0.05	0.9949 P<0.05	0.9599 P<0.1
Iontophoresis	Zero order	0.959 P<0.1	0.9985 P<0.02	0.9945 P<0.05	0.9939 P<0.05	0.999 P<0.02	0.996 P<0.05	0.9977 P<0.02
	First order	0.9816 P<0.1	0.9934 P<0.05	0.9975 P<0.02	0.9685 P<0.1	0.9893 P<0.05	0.9801 P<0.1	0.9574 P<0.1
	Higuchi model	0.9848 P<0.1	0.9733 P<0.1	0.9895 P<0.05	0.9756 P<0.1	0.9723 P<0.1	0.9737 P<0.1	0.9605 P<0.05

Table 6: Effect of penetration enhancers on permeation characteristics and changes of pH to the both compartment at the end of the in-vitro studies, of various formulations across Wister rat skin via passive diffusion only

Formulation	Permeation parameters							
	$Flux_{sst}$[a] (µg.cm^{-2} .min^{-1})	Coefficient of Correlation[a,b] (r)	L_t[a] (min)	P[a] (cm. min^{-1}) x10^3	EF %	Amts. Permeated[a*] (mg/cm^2)	pH changes**	
							pH (D)	pH (A)
F-1	41.833±1.619	0.975±0.002	22.06±3.19	2.789±0.107	-	8.860±0.062	7.3±0.02	7.67±0.01
F-2	8.70±0.1732	0.9944±0.0026	18.173±5.9	0.5707±0.011	-	5.84±0.132	7.3±0.01	7.41±0.01
F-3	13.166±0.057	0.9987±0.0006	7.627±2.27	0.83±0.0036	145.4	8.748±0.032	7.2±0.02	7.31±0.02
F-4	18.033±0.057	0.9915±0.0013	-	1.165±0.0037	201.8	12.885±0.159	7.31±0.2	7.37±0.01
F-5	9.833±0.153	0.9944±0.0031	7.105±5.15	0.624±0.0097	-	6.32±0.10	7.0±0.02	7.3±0.015
F-6	15.933±0.321	0.998±0.001	6.558±3.18	1.01±0.0204	161.8	9.343±0.132	7.0±0.05	7.20±0.05
F-7	17.766±0.152	0.9917±0.0022	-	1.089±0.0092	172.9	12.977±0.081	7.2±0.01	7.3±0.015

a, Mean ± S.D.,(n=4), b, p<0.01, Fluxsst- Steady state Flux (µg.cm-2.min-1),Lt-Lag time (min),P-Permeability coefficient (cm.min1), EF%-Enhancement factor, *Amounts permeated through the rat skin after 12 hrs of the experiment (mg/cm2), ** Changes of the pH of the various gel formulations at the end of the study; after adjusting the initial pH at 7.4, pH (D)-pH of Donor compartment, pH(A)- pH of Acceptor compartment.

Table 7: Effect of different penetration enhancers on permeation characteristics and changes of pH to the both compartment at the end of the in-vitro studies from various formulations across Wister rat skin via iontophoretic delivery system.

Formulation	Permeation parameters						
	$Flux_{sst}$[a] (µg.cm^{-2}.min^{-1})	Coefficient of Correlation[a,b] (r)	P[a] (cm. min^{-1}) x10^3	EF %	Amts. Permeated[a*] (mg/cm^2)	pH changes**	
						pH (D)	pH (A)
F-1	44.10± 0.2	0.9822±0.0019	2.94± 0.013	105.4	20.96± 0.032	6.87±0.026	7.52±0.106
F-2	14.766± 0.115	0.9947±0.0010	0.94± 0.007	165.2	10.51± 0.175	6.71±0.072	7.73±0.286
F-3	19.233±0.057	0.9831±0.0017	1.21± 0.003	211.67	13.26± 0.113	7.15±0.1	7.883±0.02
F-4	25.80± 0.173	0.9735±0.0025	1.667± 0.01	288.2	18.13± 0.155	6.286±0.67	7.78±0.025
F-5	16.76± 0.057	0.9722±0.0019	1.06±0.003	170.5	12.16± 0.085	6.48±0.1	7.87±0.015
F-6	19.9± 0.173	0.981± 0.0011	1.268± 0.01	203.2	14.14± 0.213	6.76±0.045	7.89±0.01
F-7	25.43± 0.115	0.994±0.002	1.54±0.007	244.7	18.29± 0.102	6.283±0.015	7.7±0.0153

a, Mean ± S.D.,(n=4), b, p<0.01,Fluxsst- Steady state Flux (µg.cm-2.min-1),Lt-Lag time (min),P-Permeability coefficient (cm.min-1), EF%-Enhancement factor, *Amounts permeated through the rat skin after 12 hrs of the experiment (mg/cm2), ** Changes of the pH of the various gel formulations at the end of the study; after adjusting the initial pH at 7.4, pH (D)-pH of Donor compartment, pH(A)- pH of Acceptor compartment.

On performing stability studies shelf life of various formulations F-2, F-3, F-4, F-5, F-6 and F-7 were observed 912±0.898, 570±0.757, 760±0.989, 114±0.676, 651±0.742 and 912± 0.876 days respectively.

The permeation of drug from the different formulations via passive and iontophoretic system through the Wister rat skin are evident the drug released kinetic model (Table-5) and various permeation characteristics (Table-6 and 7).

***In vitro* Permeation data analysis**

In vitro Permeation data analysis method was adopted from the reported formulas. [9, 12, 14] The cumulative amount of the permeated Atenolol in receptor chamber per unit surface area after correction of sample removal was plotted versus time and the cumulative flux (*J*ss, µg.cm^{-2}.min^{-1}) was calculated from the steady-state slope of the linear portion of the plot using the following equation:

$$Flux_{sst} = Q_t/A\ (t-L_t) \quad (1)$$

Where, Q_t was the total cumulated amount of atenolol in receptor chamber at time *t*, *A* is the cross-sectional area of donor–receptor opening and L_t represented the lag time obtained by extrapolation to the *x*-axis of the linear part of the plot. The presented value of steady state flux was the mean of $Flux_{sst}$.

The apparent permeability coefficients (P_{app}, cm.min^{-1}) were calculated using equation:

$$P_{app} = dQ/dt * 1/AC_0 \quad (2)$$

where, d*Q*/d*t* signified the steady state appearance rate of atenolol in the receptor chamber after correction for sample removal, *A* was the cross-sectional area of donor–receptor opening, and C_0 represented the initial concentration of atenolol in donor chamber.

The percentage enhancement factor (EF) was calculated using equation 3 and 4 for chemical enhancer and chemical enhancer with iontophoresis, respectively.

$$EF\ (\%) = (P_{enh}/P_{control}) \times 100 \quad (3)$$

Where, P_{enh} was the permeability coefficient obtained for the gel containing enhancer and $P_{control}$ is the permeability coefficient for the gel without enhancer.

$$EF\ (\%) = (\text{Iontophoretic with enhancer } P_{ionto+enh} / \text{Passive Control } P_{app}) \times 100 \quad (4)$$

where, the iontophoretic *P* represented the $P_{ionto+enh}$ value obtained in the presence of iontophoresis and passive P_{app} value of control was obtained without using any penetration

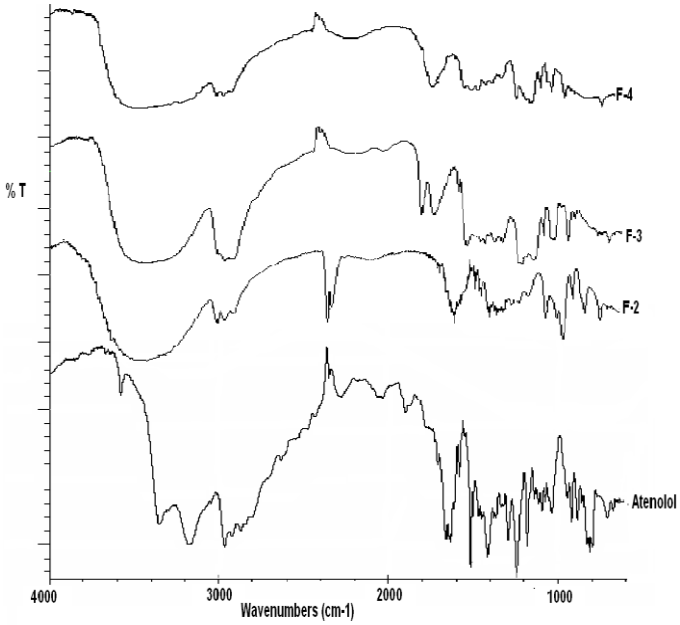

Fig. (1) FTIR spectra of pure atenolol and gel formulations prepared by SCMC (F-2, F-3 and F-4).

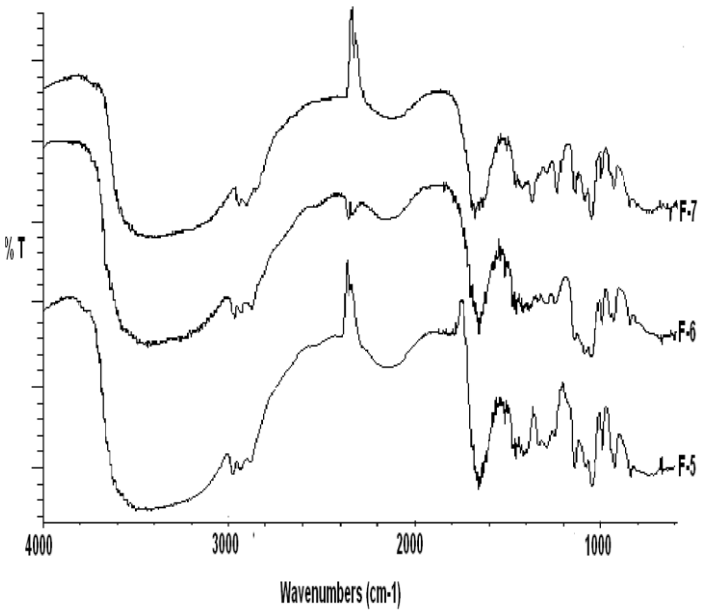

Fig. (2) FTIR spectra of pure atenolol and gel formulations prepared by MC (F-5, F-6 and F-7).

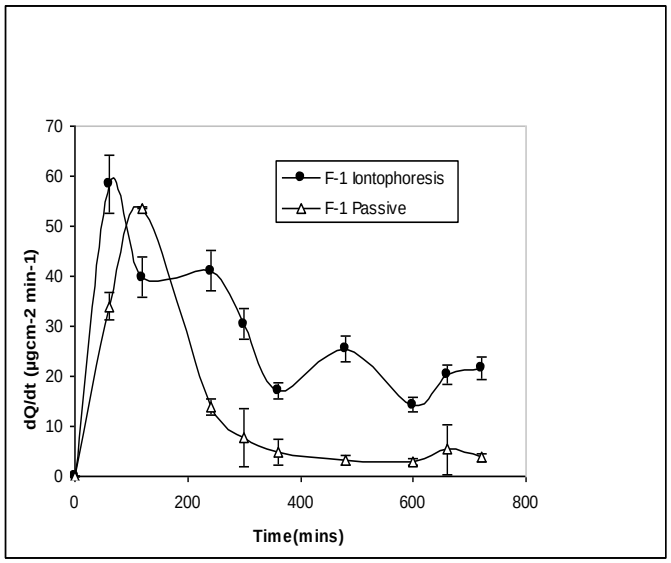

Fig. (3) Plot of flux versus time of Atenolol solution (F-1) via passive diffusion and iontophoretic system only.

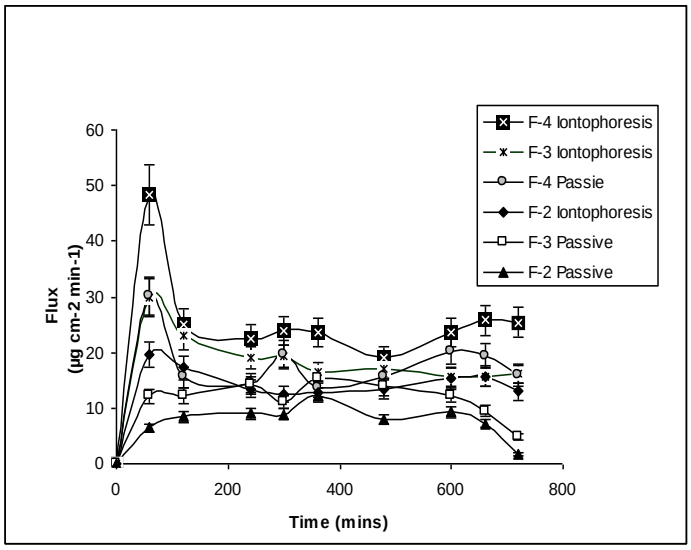

Fig. (4) Plot of flux versus time of Atenolol gel formulations prepared with polymer SCMC, by the passive diffusion and iontophoretic delivery system.

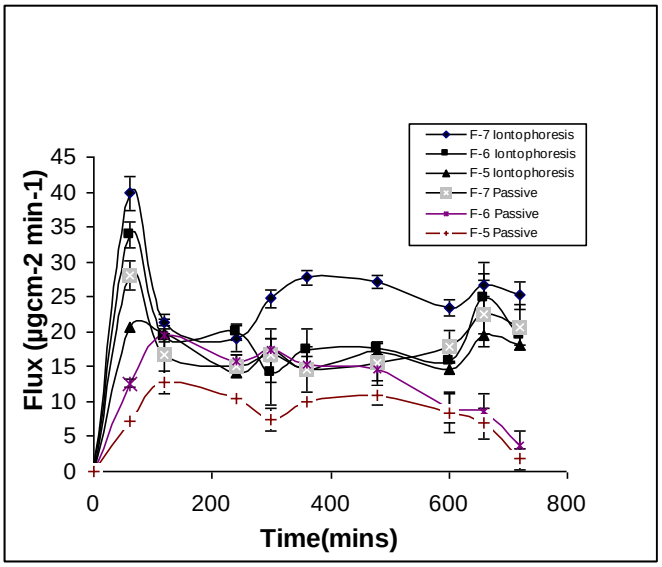

Fig. (5) Plot of flux versus time of Atenolol gel formulations prepared with polymer MC, by the passive diffusion and iontophoretic delivery system.

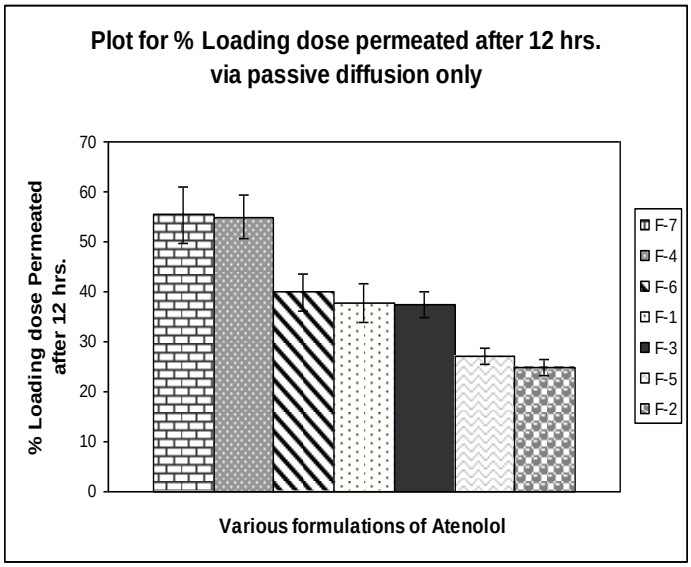

Fig. (6) Plot for % loading dose permeated at the end of the study (12hrs.), from various formulations via passive diffusion .

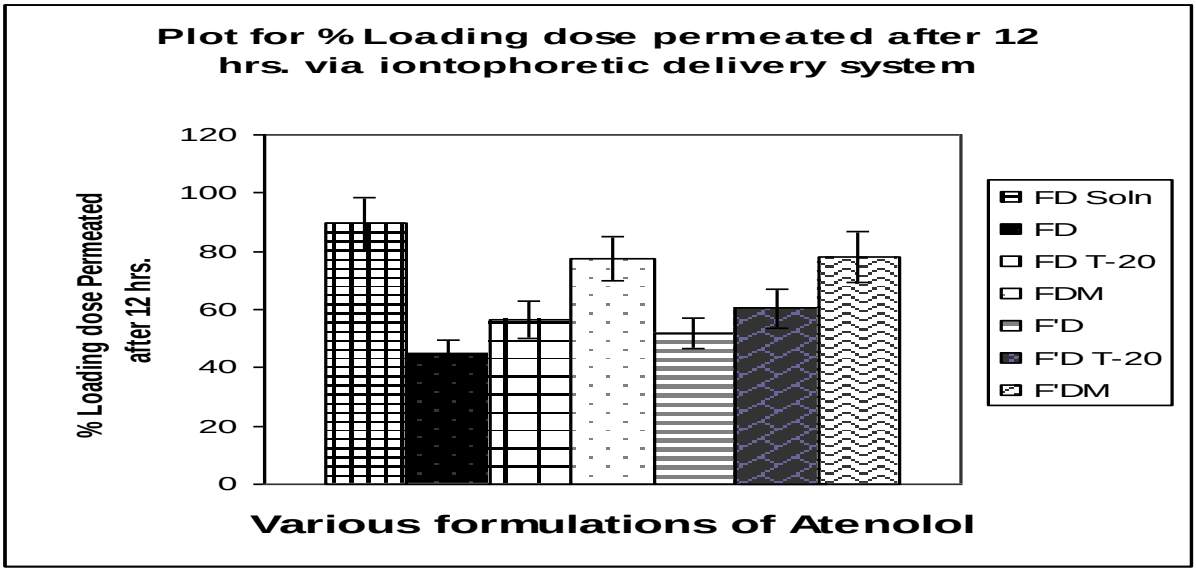

Fig. (7) Plot for % loading dose permeated at the end of the study (12hrs.), from various formulations via iontophoretic delivery system.

enhancers and current application (via Passive permeation only).

The permeation rate of drug from various formulations were studied by the passive diffusion and iontophoretic system, it is concluded that the permeation of the drug through the Wister rat skin were relatively superior via iontophoretic system than passive diffusion.

F-ratio test (one way ANOVA) was employed considering the % loading dose released at the end of the study in order to find out whether the fourteen sets of data obtained after permeation study are the same or they differ from each other. The magnitude of % loading dose released at the end of the study, from fourteen sets of Atenolol formulations yielded a statistically significant F value at the 5 % level (F=2.40). Hence, it may be inferred that the test preparations are not the same, but are different in their formulation /composition and also the method of permeation applying were different.

Further, the t-test was then applied to find out whether the difference is significant or not, using the following three parameters, % loading dose released at the end of the study (Fig. 6 and 7), Permeation coefficient (table 6 and 7) and Steady state flux (Jss) (table 6 and 7).It is concluded from t-test that the level of significance are almost $p < 0.05$.

The passive diffusion of Atenolol using deionized water solutions resulted in steady state flux and Q_{12} values were 41.833 ± 1.619 μg/(min.cm^2) and 8.860 ± 0.062 mg/cm^2, respectively. This passive delivery of Atenolol was inadequate in providing the clinically required amounts of drug in control manner. Since human skin is less permeable than hairless rat skin, so it is apparent to significantly enhance Atenolol transdermal delivery.

Iontophoresis is potentially effective in enhancing the permeation of most ionic compounds. In this study, it was taken up with iontophoretic delivery using deionized water solutions containing 1.5 % of Atenolol and current density applied 0.5 mA/cm^2. The current represents the driving force for the movement of ionic species across the skin.

Iontophoresis enhances drug delivery across the skin by electro repulsion and electro osmosis. At pH values higher than 4, the skin is negatively charged and cation perm selective. Thus, current passage causes a net convective solvent flow from the anode to the cathode, facilitating cation transport, inhibiting anion transport, and enabling the enhanced transport of neutral polar solutes. The relative importance of electro repulsion and electro osmosis depends on the physicochemical and electrical characteristics of the membrane and of the permeant. The skin's negative charge can be reduced, neutralized, or even reversed by the iontophoresis of certain cationic, lipophilic species. The negatively charged skin at pH 7.4 is permselective to the highly mobile K+ ions over the less mobile negative phosphate ion. This permselectivity creates a net movement of the solvent from the cathode to the anode, facilitating the movement of both the ionized and the unionized neutral forms of Atenolol. [11, 19, 23] In terms of clinical use, the iontophoretic patch or semisolid dosage form will most likely incorporate a polymeric delivery system. These systems can control the drug release rate over a long period of time, and the manufacturing process is straightforward. Moreover, because of their high water content, polymeric gels exhibit a high electrical conductivity, making them the vehicles of choice for electrically assisted trans-dermal drug delivery. Many researchers have reported successful iontophoretic delivery of various drug molecules from gel bases. Many researchers have reported successful iontophoretic delivery of various drug molecules from gel bases. [14, 19, 24]

In this study, two types of polymer gels (Table 2) were prepared using SCMC and MC at proximate viscosity in order to eliminate the viscosity effect on drug delivery. At higher viscosities there is decreased drug release from gel bases, decreased vehicular conductivity, and reduced iontophoretic drug transport. Higher polymer concentrations are thought to decrease the proportionality of water in the vehicle, resulting in a decrease in the conductivity of the formulation. [11, 25]

The permeation profile of Atenolol from SCMC and MC gels was not significantly different from each other, through passive diffusion as well as iontophoretic delivery system.

Transdermal systems may require a large application area to be therapeutically effective, and this may decrease patient compliance. Moreover, the combination of enhancers and iontophoresis would moderate the iontophoretic regimen required to achieve the target flux and thus improve the tolerability of the skin. Terpenes are naturally occurring

volatile oils that exhibit high percutaneous enhancement abilities and low cutaneous irritancy at low concentrations (1-5 % w/w). [26]

As seen in figure 3, 4, 5 and table 7, 8 terpene enhancers (L-menthol) were superior to iontophoresis in enhancing the transdermal permeation of Atenolol. In general, they enhanced the flux of Atenolol by more than 2-fold relative to control preparation via passive diffusion and a 3-fold increase using iontophoresis alone. L-menthol showed the higher activity compared to tween-20.

Polar terpenes were shown to be more effective in enhancing the permeation of polar drugs, while nonpolar terpenes were more effective in enhancing the permeation of lipophilic drugs. It has been also showed, in case of Indomethacin (lipophilic) drug absorption, which was shown to be markedly enhanced by the addition of hydrophobic cyclic monoterpenes, while hydrophilic terpenes showed minor effects. In contrast, under similar conditions, alcohol terpene (L-menthol) was found to be the most effective in enhancing the permeation of diclofenac sodium (hydrophilic). [19]

Additions of chemical enhancers is one way to potentiate the effectiveness of iontophoresis. Chemical enhancers that disrupt intercellular lipid organization can have a synergistic effect on iontophoresis, and terpenes belong to this category. Fig. 3, 4 and 5 showed the effect of the combination of L-menthol and iontophoresis on the permeation of Atenolol. Under most conditions, combination treatment synergistically increased the flux and Q_{12} of Atenolol (Table 6 and 7) relative to iontophoresis or enhancers alone.

The combination of L-menthol and iontophoresis showed the highest activity relative to tween-20 as an enhancer-iontophoretic combination (Table 6 and 7). In case of SCMC gel, Atenolol flux by passive diffusion were 8.7 ± 0.1732 (without L-menthol), and 18.033 ± .057 (with L-menthol) and the flux for iontophoretic was increased to 25.8 ± 0.173 µg/ (min.cm^2) when they were combined with penetration enhancer (L-menthol). But in MC gel, Atenolol flux increased by passive diffusion were 9.833 ± 0.153 (without L-menthol), and 17.766 ± .152 (with L-menthol) and the flux for iontophoretic was increased to 25.43 ± 0.1154 µg/(min.cm^2) when they were combined with the penetration enhancer (L-menthol). L-menthol was the most active enhancer, and when combined with iontophoresis it was possible to deliver 36-37 mg/cm^2/day of Atenolol.

Amongst the seven formulations made with varying compositions, six gel formulations were by using two types of polymers sodium carboxy methyl cellulose and methyl cellulose with and without penetration enhancers (L-menthol and tween-20), and another one was simple Atenolol solution. On conducting the permeation studies of those formulations by passive and iontophoretic system, three formulations(F-1, F-4 and F-7) was found best based on physical properties and in-vitro release profile via iontophoretic delivery system only. Although the release rate of the Atenolol solution (F-1) is higher than the other two (F-4 and F-7) formulations prepared by the menthol with the SCMC and MC polymer. So it is also proved that the L-menthol is good penetration enhancers than the tween-20, and also the permeation rate of the drug can be controlled in better way by using the iontophoretic system with the penetration enhancers together from the gel formulations than the simple solution form.

From the 'r' values of drug permeation profile of various gel formulations (Table-5), it can be concluded that the formulation F-1 dose not follows zero order and first order kinetics rather it follows Higuchi model kinetics via both passive diffusion and iontophoretic delivery system, and formulations F-2, F-3, F-4, F-5, F-6 and F-7 are follows first order release kinetics via passive diffusion only, but through iontophoretic system they mostly follows the zero order pattern. It indicates that the steady state permeation rate may be achieved and sustained effect of drug may be obtained.

The delivery of Atenolol from the release L-menthol containing gel formulations was substantially enhanced by iontophoresis and the rates were shown to be approximately proportional to the time. The data clearly indicates the iontophoretic process to be significantly less efficient without presence of penetration enhancers.

The study has determined separately the effects on these release and transport processes of the inclusion of L-menthol capable of penetration enhancements into the systems and of the application of iontophoretic assistance. Finally, synergistic effects of simultaneous chemical and electrical enhancement were observed. This may be due to increased membrane permeability and altered tissue extensibility.

Moreover, L-menthol is the most effective enhancer relative to tween-20 and the combination of iontophoresis with L-menthol is the best drug delivery system to achieve desire level of drug. Although the electrical resistance is several times higher in human skin than the rat skin. This present study is holds promise for the further clinical study of iontophoretic regimens of Atenolol from polymer hydro gels.

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