Research Article

ELECTRICALLY ASSISTED TRANSDERMAL DELIVERY OF ESCIN

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ABSTRACT

Escin is a neutral glycosidic drug with anti-inflammatory, anti-edematous, vasoprotective and venotonic activity used in treatment of chronic venous insufficiency (CVI), hemorrhoids and post-operative oedema. The present study was carried out to enhance the transdermal delivery of escin which is a neutral high molecular weight compound. The effect of different variables on iontophoretic delivery of escin was assessed; also, the effect of the combined physical (iontophoresis) and chemical (penetration enhancers) modulation of skin barrier on the transdermal delivery of escin was investigated. Finally, the *in-vivo* activity of selected escin gel formulae was assessed in comparison to a marketed product. Laser Doppler flowmetry (LDF) was used to measure the change in skin perfusion following the passive and iontophoretic application of the commercial and selected formulae respectively. The results of the present study revealed that, iontophoresis can be a useful tool for enhancing escin transport across the skin although being neutral high molecular weight compound; also, iontophoresis and penetration enhancers synergistically increased escin transdermal delivery compared to any of them alone.

Keywords: Escin, Transdermal Iontophoresis, Electroosmosis, Chronic Venous Insufficiency (CVI), Vasoprotective Drug.

INTRODUCTION

Escin is a combination of approximately 30 saponines with the main aglucon protoaescigenin, which is extracted from horse chestnut seeds [Aesculus hippocastanum L.]. It has been proved to be effective in the localized treatments of edema, phlebitis, post-thrombotic syndrome and in the remission of the ulcerous symptoms in patients who are subjected to current thrombophlebitic attacks. Furthermore, it has also been used in cerebral edemas and intracranial aneurisms [1, 2], in the resolution of the edematous state in gynecology [3], and in odontotherapy [4] to improve gingival haemorrhages. The vasoprotective action of escin also constituted the basis for its application in dermatology, particularly in the treatment of chronic venous stasis and for the prevention/treatment of panniculopatia edematofibroschlerotica, so-called cellulitis [5].

Iontophoresis is a process that enhances the delivery of drugs through a biological membrane via the application of low-intensity electrical current [6-8].

Mechanisms of drug transport during iontophoresis are electrorepulsion and electroosmosis, a convective solvent flow, caused by the application of electric current on a charged membrane [9].

The contribution of each is determined mainly by the molecule of interest characters and the formulation parameters that affect both that molecule and the skin. That is to say electromigration is the main mechanism for the small ionized molecules, while electroosmosis is more important for the transport of neutral or large molecules [10].

In many cases, iontophoresis itself has been able to show an increased permeation of the drug molecule but, in many others, iontophoresis alone has not given the desired results. Therefore, iontophoresis, has been used along with chemicals like permeation enhancers to produce a synergistic effect on the permeation of many drugs e.g. insulin [11] buspirone hydrochloride [12] thiocolchicoside [13] LHRH (full name) [14] timolol Maleate (TM) [15], and R- Apomorphine [16].

To our knowledge, few articles on iontophoresis of neutral drugs are present in the literatures on this subject and little of them concerns high molecular weight compounds [11, 17-23].

The aim of this work was to study the effect of iontophoresis and the effect of combined chemical and physical modulation of skin barrier on the *in vitro* transdermal transport of escin and to assess the *in-vivo* iontophoretic delivery of escin from selected gel formulae using laser Doppler flowmeter (LDF) device that measure the extent of change in the skin microcirculation following application of the selected escin containing gel formulae in comparison to the marketed escin gel.

MATERIALS AND METHODS

Materials

Escin (Madaus GmbH, Germany) obtained as a gift from CID (Chemical Industrial Development Company, Egypt), Potassium dihydrogen ortho-phosphate and Disodium hydrogen phosphate (Winlab, U.K.), Deionized water of resistivity ≈ 18 MΩ cm, Normal Saline (El-Nasr company, Egypt), Chitosan (MW ≈ 60000) (Fluka, Germany), HydroxypropylMethylcellulose (Colorcon, Kent, U.K.), Carbopol 940 and Transcutol (Sigma, USA), Propylene glycol, Triethanolamine, Dimetheylformamide (DMF) and Urea all were analar grade and obtained from Adwic Company (Cairo, Egypt)., Sulfuric acid Glacial acetic acid was supplied from United Company for Chemical and Medical Preparations (Egypt).

Preparation of skin membranes

Full-thickness hairless albino rat skin was used as a model skin membrane [24-26]. Newly born albino rats (2-3 days age) were subjected to cervical dislocation and full-thickness skin was excised [27]. The dermal surface was carefully cleaned to remove subcutaneous tissue without damaging the epidermal surface. The skin was soaked for 24 hours in phosphate buffer pH 7.4 before storing in the frozen state in phosphate buffer pH 7.4 and left to thaw at room temperature before running the experiment.

Measurement of conductivity and pH of escin solutions (in deionized water and in normal saline) and the prepared Escin gels

The conductivity (Ecoscan CON 6, Eutech Instruments, Malaysia) and pH of escin solutions of different concentrations in deionized water and in normal saline and escin gels with different penetration enhancers were determined to measure their effect on iontophoretic transport of escin through hairless albino rat skin.

Analysis of escin in phosphate buffer pH 7.4 by colorimetric method [28]

1 ml of test sample was transferred to a test tube to which 4 ml ferric chloride was added; the tubes were capped with a stopper

then placed in a water bath at 60 °C for 25 minutes with occasional shaking. Absorbance of the solution was measured at 540 nm. Ferric chloride reagent was prepared immediately before use by dissolving 75 mg of ferric chloride in 100 phosphate buffer containing 10% sulfuric acid while shaking and cooling.

Preparation of the electric circuit used in the *in vitro* permeation studies

An electric circuit was custom designed [26] using 220 volts input source to deliver direct current. A rheostat was used to adjust the current intensity at the desired predetermined values. A potentiometer and transistor were used to adjust the current intensity at the desired predetermined values. Silver wires (50 mm x 1 mm; 99% purity) were used for making the electrodes (Ag/AgCl electrode) of effective length 1 cm each. The electrodes were connected to the power source using crocodile copper wires. A digital multimeter was used to monitor and assure that the current intensity is constant at the desired value.

Experimental Design

Optimization of the iontophoretic conditions for transdermal delivery of escin through hairless albino rat skin.

The effects of five variables which were expected to have pronounced effects on the iontophoretic permeation of escin were determined separately.

Effect of type of iontophoresis

Effect of type of iontophoresis on the delivery was studied by performing both cathodal and anodal iontophoresis for 3 hours at current density 0.25 mA/cm², drug concentration 2 mg/ml, continuous current and using deionized water as drug solvent.

Effect of current density

The effect of current density on delivery was studied to see the relation between the different current densities applied (0.25 mA/cm² and 0.5 mA/cm²) and the amount of drug delivered using anodal iontophoresis at drug concentration 2 mg/ml, continuous current and using deionized water as drug solvent.

Effect of drug concentration

The effect of escin concentration on the delivery was studied at 2 mg/ml and 4 mg/ml using anodal iontophoresis at current density 0.25 mA/cm², continuous current and using deionized water as drug solvent.

Effect of mode of iontophoresis

The effect of iontophoresis on drug delivery was studied by performing continuous current and pulsed current (30 min ON and 30 min OFF) anodal iontophoresis at current density 0.25 mA/cm², drug concentration 2 mg/ml and using deionized water as solvent.

Effect of type of drug solvent (Ionic Strength)

Effect of type of escin solvent used on drug delivery was studied by using deionized water and normal saline as drug solvents in donor compartment using anodal iontophoresis, current density 0.25 mA/cm², drug concentration 2 mg/ml and continuous current method.

Passive diffusion of escin across hairless albino rat skin

A preliminary passive diffusion experiment was done to test for the potential of escin passage passively through full-thickness hairless albino rat skin. 5 ml of 2 mg/ml escin solution was tested for permeation using the same conditions of permeation experiments but with no current.

Compatibility Study of Escin and Solid Additives

Differential Scanning Calorimetry (DSC)

Samples were placed in aluminum pan and heated from room temperature to 400°C at a rate of 10°C / min with indium in the reference pan, in an atmosphere of nitrogen. The DSC studies were carried out for escin, each of the solid additives and the drug-additive physical mixture (1:1). The 1:1 weight ratio will maximize the likelihood of observing any interaction.

Fourier transform infrared spectroscopy (FT-IR)

Infrared spectra of escin, each of the solid additives and the drug-additive physical mixture (1:1) were determined by the KBr disc technique.

Preparation of escin gel formulae

Twenty seven escin gel formulae were prepared using 3 different polymers (1.5% w/v); hydroxypropylmethyl cellulose (HPMC, neutral), Carbopol 940 (anionic) and chitosan (cationic) and 4 different penetration enhancers; Urea, Propylene glycol (PG), Transcutol and Dimethylformamide (DMF) at either 5% (w/v) or 10% (w/v). The composition of different formulae is shown in table (1). The drug concentration was kept at 2 mg/g.

Table 1: Composition of different escin gel formulae

Formula	Drug (2 mg/ml)	Polymer (Type/Concentration)			Enhancer (Type/Concentration)							
		HPMC (1.5%)	Chitosan (1.5%)	Carbopol (1.5%)	Urea (5%)	Urea (10%)	PG (5%)	PG (10%)	Transcutol (5%)	Transcutol (10%)	DMF (5%)	DMF (10%)
F1	+	+	-	-	-	-	-	-	-	-	-	-
F2	+	+	-	-	+	-	-	-	-	-	-	-
F3	+	+	-	-	-	+	-	-	-	-	-	-
F4	+	+	-	-	-	-	+	-	-	-	-	-
F5	+	+	-	-	-	-	-	+	-	-	-	-
F6	+	+	-	-	-	-	-	-	+	-	-	-
F7	+	+	-	-	-	-	-	-	-	+	-	-
F8	+	+	-	-	-	-	-	-	-	-	+	-
F9	+	+	-	-	-	-	-	-	-	-	-	+
F10	+	-	+	-	-	-	-	-	-	-	-	-
F11	+	-	+	-	+	-	-	-	-	-	-	-
F12	+	-	+	-	-	+	-	-	-	-	-	-
F13	+	-	+	-	-	-	+	-	-	-	-	-
F14	+	-	+	-	-	-	-	+	-	-	-	-
F15	+	-	+	-	-	-	-	-	+	-	-	-
F16	+	-	+	-	-	-	-	-	-	+	-	-
F17	+	-	+	-	-	-	-	-	-	-	+	-
F18	+	-	+	-	-	-	-	-	-	-	-	+
F19	+	-	-	+	-	-	-	-	-	-	-	-
F20	+	-	-	+	+	-	-	-	-	-	-	-
F21	+	-	-	+	-	+	-	-	-	-	-	-
F22	+	-	-	+	-	-	+	-	-	-	-	-
F23	+	-	-	+	-	-	-	+	-	-	-	-
F24	+	-	-	+	-	-	-	-	+	-	-	-
F25	+	-	-	+	-	-	-	-	-	+	-	-
F26	+	-	-	+	-	-	-	-	-	-	+	-
F27	+	-	-	+	-	-	-	-	-	-	-	+

Ex-vivo permeation studies of escin from different prepared solutions and gel.

Ex-vivo iontophoretic transport of escin from different solutions and gels across full-thickness hairless albino rat skin was studied for 3 hours (for gels permeation was continued for another 1 hour but without electric current). A vertical diffusion cells representing the finite dose technique as small amounts of drug can be placed on the skin was used [29]. The study was carried out using double open sided tube [30]. Deionized water was used for the preparation of all solutions and buffers [17, 31-33]. The donor vehicle was 5 ml of the escin solution (or 5 g of escin gel) under study placed on the dorsal side of the skin. The available skin diffusion area was 3.14 cm². The receptor medium was 75 ml of phosphate buffer pH 7.4 [25, 34, 35] agitated by magnetic stirrer at 500 rpm (by the aid of 3 cm teflon coated magnetic bar) to maintain sink condition. Experiments were carried out at 37±2 ℃. For anodal iontophoresis, an Ag wire was used as the anode in the donor compartment and an Ag/AgCl wire was used as the cathode in the receptor compartment and they were reversed with cathodal iontophoresis. Samples (5 ml) were withdrawn and compensated immediately with fresh receptor medium at predetermined intervals of 15, 30, 45, 60, 90, 120, 150 and 180 minutes (210 and 240 in case of gel) and analyzed colorimetrically. Care was taken to avoid any contact between the anode and the skin to avoid any high local voltage [27].

The drug concentration values were corrected for progressive dilution to obtain cumulative amount permeated using the following equation [12]:

$$Q't(n) = Vr \cdot Cn + Vs \cdot \Sigma Cm \quad (1)$$

Where:

- Q't(n) is the cumulative mass of drug transported across the membrane at time t,

- Cn represents the current concentration in the receptor medium

- Σ Cm denotes the summed total of the previous measured concentrations [m = 1 to n – 1]

- Vr is the volume of the receptor medium

- Vs corresponds to the volume of the sample removed for analysis.

The cumulative amounts of escin permeated per unit area of rat skin were plotted as a function of time. [36, 37]. The responses measured were Q180 (µg/cm²), which is the cumulative amount permeated per cm² immediately after 3 hours from the start of iontophoresis [37, 38], transdermal flux (J) (µg/cm²/hr) was calculated from the slope of linear portion of the cumulative amount permeated per cm² versus time plot [37, 39], the permeability coefficient (Kp) (cm/hr) was calculated according to the following equation Kp = Js/Cv, where Cv is the drug concentration in the donor solution [26, 40], and the enhancement factor (EF) which is the ratio between flux of iontophoretic delivery to flux of passive delivery EF = Jiont / Jpassive [26, 40].

All data were statistically analyzed using data analysis tool in Microsoft excel® software for personal computer version 2007. Means for each response individually were compared by one way ANOVA, Significance level was set at P < 0.05.

The mathematical models, zero order, first-order exponential function, Higuchi square root of time equation[41] and Korsemeyer-peppas[42] model equations were fitted to individual permeation data with the linear regression by means of personal computer; The best fitted mathematical model that describes the permeation profiles was determined.

Correlation between viscosity and permeation parameters

A correlation analysis between the viscosities of different formulae at 0.5 rpm and each of Flux (J), Q_{180}, Enhancement Factor (EF) or Permeability coefficient (Kp) was performed individually. The correlation coefficients (r) were tested for significance against zero according to equation (8).

$$t_{calc} = r \sqrt{n-2} / \sqrt{1-r^2} \text{ at (df = n – 2) (2)}[43]$$

Where:

- r is the correlation coefficient between the selected response and viscosity

- n is the number of x, y pairs

- df is the degrees of freedom of the tabulated t.

In-vivo transdermal delivery of escin from selected escin gel formulae

The protocol of this study was approved by the human ethical committee of the faculty of pharmacy, Cairo University. The experiments were done on three healthy human volunteers (3 volunteers provided us with 2 different readings and all volunteers gave us 6 different readings); the baseline skin perfusion (before each treatment) in the forearm area of both the right and left hands of each volunteer was measured using laser Doppler flowmeter (LDF) device (ML191 Blood Flowmeter, ADInstruments, Australia) with standard surface probe (MSP100XP, ADInstruments, Australia), then about 3 ml of the commercial gel was applied (using a syringe) on the skin of the forearm area (marked with a circle of diameter of 4 cm) of both hands daily for 30 minutes for 1 weak after which skin perfusion in the forearm area of both hands of each volunteer was measured again. After a wash-out period of 3 days, the selected escin gel formulae were applied for 30 min for 1 week using Phoresor® Unit II iontophoresis device (Chattanooga company, USA) with TransQe iontophoresis electrodes of active area 13.4 cm2 (IOMED Company, USA), current density was adjusted at 0.25mA/cm2.

The % reduction in skin perfusion before and after treatment was calculated. One Way ANOVA was used for the statistical analysis of data at level of significance p < 0.05 using data analysis tool in Microsoft excel® 2007.

RESULTS AND DISCUSSION

Measurement of Conductivity and pH of Escin solutions and gel formulae

Conductivity results of different escin solutions revealed that escin is a neutral molecule with the increase of conductivity is due to changing type of solvent only; the conductivity of 2 mg/ml and 4 mg/ml of escin in deionized water (DW) was 762.33±3.05 µS and 1123±21.37 µS respectively, the conductivity of the corresponding escin solutions in normal saline (NS) was 18860±10 µS and 19123.33±23.09 µS respectively. Conductivity results of gel formulae containing either cationic (chitosan, F16: 3909.67±56.8 µS) or anionic (Carbopol, F27: 2773±89.01 µS) polymer gave a higher conductivity values compared to those prepared using a neutral polymer (HPMC, F7: 2485±22.91 µS).

All pH values measured for escin solutions and for the prepared escin gel formulae were greater than 4 causing a net negative charge on the hairless albino rat skin membrane which will cause a convective flow from anode to cathode during anodal iontophoresis [44].

Compatibility study of escin and solid additives

Differential scanning calorimetry (DSC)

Figure (1) shows DSC thermograms of drug, polymers, solid additives, and (1:1) physical mixtures of them. Escin shows an endothermic peak which reaches its maximum at 280.13 ℃.

The DSC thermograms for the physical mixtures of escin with HPMC, chitosan, Carbopol and urea showed this same peak with a slight shift in case of HPMC, chitosan and Carbopol. In case of HPMC and chitosan, this endothermic peak was attenuated due to its presence in the same range with the exothermic peak of HPMC and Chitosan. No attenuation in this peak happened with urea. This result needs to be verified and will be further investigated in the FT-IR analysis.
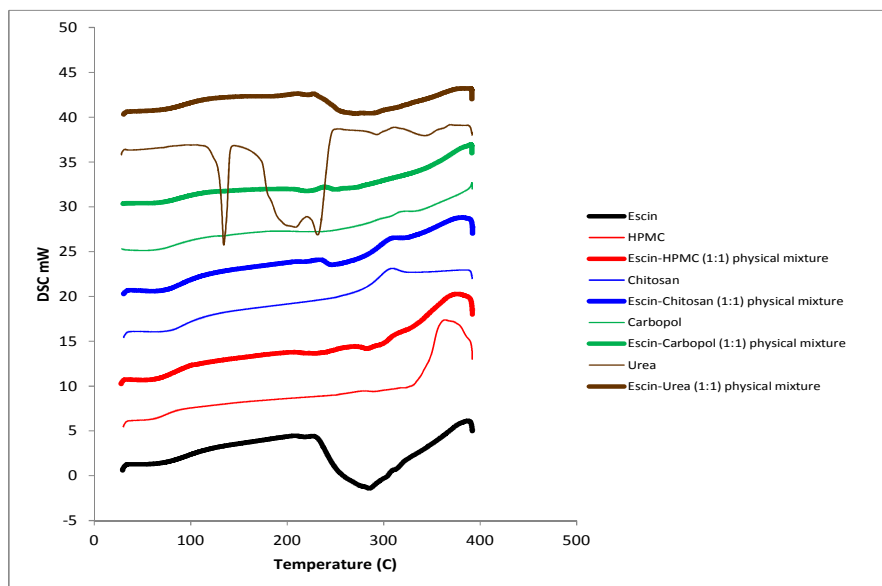
Fig. 1: DSC thermograms of escin, polymers/solid additive and escin-polymers/solid additive (1:1) physical mixtures.

FT-IR

Figure (2) shows the IR spectrum of escin, polymers, solid additives and (1:1) physical mixtures of them, it shows the characteristic peaks of escin which are O-H stretching at 3421.72 cm^{-1}, C-H aliphatic stretching at 2927.94 cm^{-1}, carbonyl C=O stretching at 1720.5 cm^{-1} and C=C stretching at 1647.21 cm^{-1}. The spectrum of escin physical mixture with HPMC, Chitosan and Carbopol shows the same characteristic peaks for escin.

The spectrum of escin physical mixture with urea shows the same characteristic peaks for escin which appears beside a forked peak due to the double N-H stretching of the urea NH2 groups at 3448.72 cm^{-1} and 3348.42 cm^{-1}.

The presence of the same characteristic peaks of escin ensures that there is no incompatibility exists between escin and any of the chosen solid additives in the four physical mixtures.
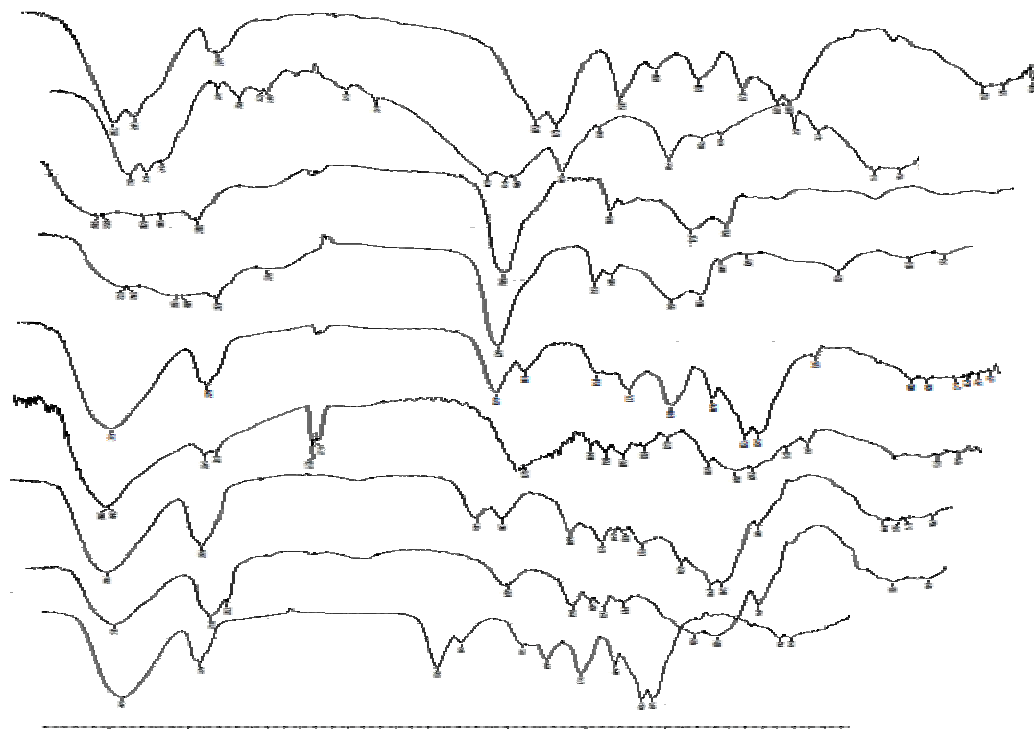

Fig. 2: FTIR spectrum of a) Escin, b) HPMC, c) Escin-HPMC (1:1) physical mixture, d) Chitosan, e) Escin-Chitosan (1:1) physical mixture, f) Carbopol, g) Escin-Carbopol (1:1) physical mixture, h) Urea, i) Escin-Urea (1:1) physical mixture.

Ex-vivo Permeation Study

Permeation profiles of different escin solutions are presented in figure (3). Different permeation parameters of both passive and iontophoretic transdermal delivery of different escin solutions are shown in table (2), it can be concluded that only anodal iontophoresis significantly increase escin transport through skin (p = 0.007) while cathodal one reduced transport, this increase can be solely attributed to the electroosmotic flow which is in the anode-to-cathode direction at pH 7.4, due to the negative charge of the skin. The presence of a convective solvent flow during transdermal iontophoresis was also reported [22]. Increasing the current density to

0.5 mA/cm^2 did not significantly increase (P = 0.621) Flux or Q180, being applied for a neutral drug molecule.

Using a discontinuous current mode did not significantly modify (P = 0.082) Flux or Q180 of escin through skin as the main contribution to transport which is electroosmosis together with passive diffusion is not changed. Increasing drug concentration from 2 mg/ml to 4 mg/ml significantly increased (p = 0.046) escin permeation through the skin however further experiments were continued using 2 mg/ml which is the drug concentration in the commercial marketed gel. Changing drug solvent from deionized water (DW) to normal saline (NS) did not significantly (p = 0.242) modify permeation parameters.
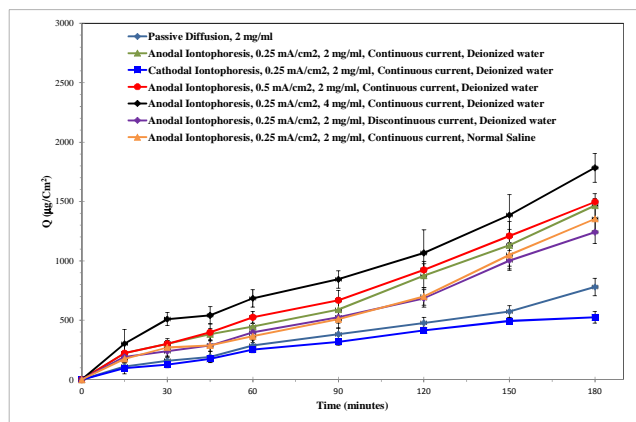

Fig. 3: Permeation profiles of different escin solutions for optimization of iontophoretic transdermal delivery of escin.

Table 2: Effect of different parameters on Flux (J, μg/cm^2/hr), Q$_{180}$ (μg/cm^2), Enhancement factor (EF) and Permeability coefficient (Kp, cm/hr) of escin from solution.

Experimental Design	J (μg/cm^2/hr) ±SD	Q$_{180}$ (μg/cm^2) ±SD	EF±SD	Kp (cm/hr) ±SD
- Passive Diffusion, 2 mg/ml	236.79±15.72	780.25±72.97	-	-
- Anodal Iontophoresis, 0.25 mA/cm^2, 2 mg/ml, Continuous current, Deionized water.	446.77±39.43	1464.96±99.44	1.88±0.05	0.22±0.02
- Cathodal Iontophoresis, 0.25mA/cm^2, 2 mg/ml, Continuous current, Deionized water	174.82±3.03	525.47±47.77	0.73±0.03	0.08±0.01
- Anodal Iontophoresis, 0.5 mA/cm^2, 2 mg/ml, Continuous current, Deionized water	466.80±10.04	1496.81±27.58	1.99±0.10	0.23±0.01
- Anodal Iontophoresis, 0.25mA/cm^2, 4 mg/ml, Continuous current, Deionized water	522.95±24.31	1783.43±120.20	1.50±0.06	0.13±0.01
- Anodal Iontophoresis, 0.25 mA/cm^2, 2 mg/ml, Discontinuous current, Deionized water	383.89±26.20	1242.03±95.541	1.62±0.19	0.19±0.01
- Anodal Iontophoresis, 0.25 mA/cm^2, 2 mg/ml, Continuous current, Normal saline	413.70±10.86	1353.50±99.443	1.75±0.15	0.20±0.01

It can be observed that the enhancement factor values obtained here are not very high, because the tested compound is neutral and then the only transport mechanism involved is electroosmosis, which is known to be poorly efficient. Therefore investigating the effect of combining chemical penetration enhancers along with anodal iontophoresis on drug transdermal delivery was necessary.

Twenty seven gel formulae were subjected to permeation studies using the same conditions as mentioned before. The experimental protocol consisted of two stages; stage I: iontophoresis (for 3 hours) and stage II: post-iontophoretic diffusion (for 1 hour). Representative permeation profile is shown in figure (4).

Permeation parameters of different escin gels are presented in table (3)

The results revealed that the cumulative amounts of escin (Q) permeated per cm^2 and flux (J) from gels containing different penetration enhancers combined with iontophoresis were greater than that of the same gel formula without iontophoresis or without iontophoresis and enhancer (table 3). In all the gel formulae tested, the rate of increase of the amount of escin permeating the skin decreased just after stopping the current in the post-iontophoretic diffusion stage, This indicates that the rat skin was regaining its barrier properties after the treatment with continuous current of 0.25 mA/cm^2 for 3 hours and any of the gel formulae used [12, 24].
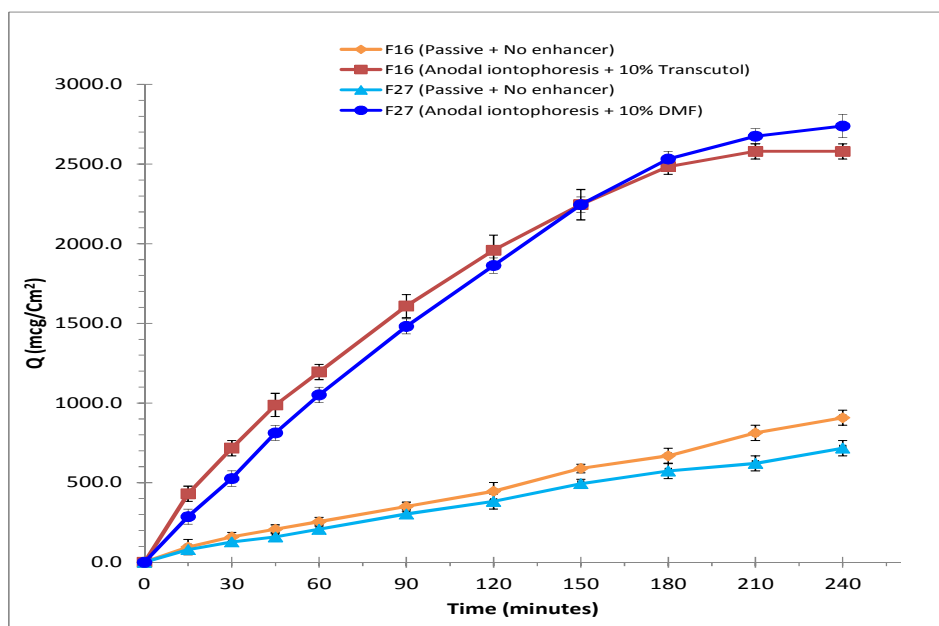
Fig. 4: Representative permeation profiles of F16 and F27 gel formulae in comparison to their passive permeation profiles.

Table 3: Permeation data [Flux; J (µg/cm²/hr), Q_{180} (µg/cm²), Enhancement factor; EF and Partition coefficient; Kp (ml/cm²/hr)] for iontophoretic delivery of escin through hairless albino rat skin from different escin gel formulae.

Formula	Flux, J (µg.hr/cm²)±SD	Q_{180} (µg/cm²) ±SD	Enhancement Factor (EF) ±SD	Permeability Coefficient, Kp (ml.hr/cm²) ±SD
1	467.77±8.98	1512.33±110.27	1.68±0.26	0.23±0.01
2	506.85±15.65	1704±198.87	1.82±0.30	0.25±0.01
3	583.19±39.95	1974.66±120.26	2.10±0.40	0.29±0.02
4	533.75±21.90	1815.28±47.77	1.91±0.21	0.26±0.01
5	609.90±51.07	2070.06±120.22	2.20±0.45	0.30±0.02
6	615.59±26.87	2117.83±55.16	2.21±0.36	0.30±0.01
7	710.62±19.32	2579.61±82.74	2.56±0.42	0.35±0.01
8	537.78±42.78	1815.28±95.54	1.94±0.42	0.26±0.02
9	697.98±36.57	2340.76±47.77	2.49±0.21	0.34±0.01
10	434.59±23.59	1353.50±72.97	2.02±0.18	0.21±0.01
11	566.67±20.13	1783.43±72.97	2.64±0.22	0.28±0.01
12	710.36±17.37	2261.14±99.44	3.32±0.36	0.35±0.01
13	593.61±7.93	1878.98±72.97	2.77±0.26	0.29±0.01
14	687.42±18.22	2181.52±55.16	3.21±0.35	0.34±0.01
15	622.12±4.96	1958.59±47.77	2.90±0.26	0.31±0.01
16	799.76±26.61	2484.07±47.77	3.73±0.29	0.39±0.01
17	600.20±5.52	1863.05±47.07	2.80±0.27	0.30±0.01
18	734.16±38.74	2261.14±72.91	3.43±0.44	0.36±0.01
19	368.96±14.34	1162.42±27.58	1.99±0.06	0.18±0.01
20	529.22±19.88	1671.97±95.54	2.85±0.19	0.26±0.01
21	660.58±5.859	2054.14±47.77	3.56±0.21	0.33±0.01
22	511.08±23.34	1624.20±47.77	2.75±0.06	0.25±0.01
23	694.08±6.03	2101.91±47.77	3.75±0.27	0.34±0.01
24	579.71±1.146	1767.51±47.77	3.13±0.22	0.28±0.01
25	754.60±16.67	2292.99±47.77	4.07±0.21	0.37±0.01
26	633.88±15.08	1942.67±27.58	3.42±0.31	0.31±0.01
27	847.34±13.14	2531.84±47.77	4.57±0.31	0.42±0.01

- The flux values for passively diffused enhancer free HPMC, Chitosan and Carbopol gels were 281.6± 37.98, 215.39± 22.60 and 185.63± 12.74 respectively.

In order to study effect of polymer type on flux and Q_{180}, the one way ANOVA test was performed on different formulae having the same enhancer type at its higher concentration but with different polymer type; and the results revealed that: At 10% Urea, there was a non significant difference between HPMC (F3) and each of Chitosan (F12) and Carbopol (F21) and a significant difference between Chitosan (F12) and Carbopol (F21). Thus the three polymers can be arranged in the following order with respect to their effect on flux and Q_{180}: Chitosan > HPMC > Carbopol. At 10% PG, there was a non significant difference between HPMC (F5) and Chitosan (F14), significant difference between HPMC (F5) and Carbopol (F23), and a non significant difference between Chitosan (F14) and Carbopol (F23). Thus the three polymers can be arranged in the following order with respect to their effect on flux and Q_{180}: Chitosan >

Carbopol > HPMC. At 10% Transcutol, there was a non significant difference between HPMC (F7) and each of Chitosan (F16) and Carbopol (F25), and a significant difference between Chitosan (F16) and Carbopol (F25). Thus the three polymers can be arranged in the following order with respect to their effect on flux and Q_{180}: HPMC > Chitosan > Carbopol. At 10% Dimethylformamide (DMF), there was a non significant difference between HPMC (F9) and Chitosan (F18), significant difference between HPMC (F9) and Carbopol (F27) and a significant difference between Chitosan (F18) and Carbopol (F27). Thus the three polymers can be arranged in the following order with respect to their effect on flux and Q_{180}: Carbopol > Chitosan > HPMC. As the results revealed, the effect of polymer type on flux and Q_{180} varied depending on the type of enhancer used which may be due to certain interaction between the polymer and the enhancer used.

In all formulae it was found that increasing enhancer concentration resulted in increase of all the determined parameters. The synergistic effect between chemicals and iontophoresis may be attributed to several mechanisms, if the enhancer is charged; iontophoresis should increase the rate of enhancer delivery into the skin. This should further increase transdermal drug transport. Chemical enhancers could dilate intercellular spaces and reduce skin impedance, thus increasing the effectiveness of iontophoresis [45]. The major advantages of iontophoresis together with chemicals over each of them alone include its effectiveness and ease of application.

The activity of propylene glycol is thought to result from solvation of keratin within the stratum corneum; the occupation of proteinaceous hydrogen bonding sites reducing drug-tissue binding and thus promoting permeation [46].

The activity of dimethylformamide at low concentrations is a result of partitioning into the keratin regions. At higher concentrations they increase lipid fluidity by disruption of lipid packing as a result of solvation shell formation around the polar head groups of the lipids [47].

F16 (Chitosan) and F27 (Carbopol) gel formulae were chosen for subsequent *in-vivo* testing based on that they gave the highest enhancement factors among all of the tested formulae.

Linear regression analysis of the release data of all formulae (table 4) reveals that R^2 values fitted to Higuchi diffusion mechanism with the exception of F7, F8, F9, F10, F15, F16, F17, F23, F24, F25, F26 and F27 that showed an anomalous non-fickian release kinetics applying Korsmeyer-Peppas equation {where *n:* indicates the general operating release mechanism. An *n* value of 0.43 indicates Fickian (case I) diffusion-mediated release, 0.43 <*n*<0.85 indicates non-Fickian (anomalous) release that is coupled diffusion and erosion, *n* = 0.85 indicates erosion mediated release (zero-order kinetics) and *n* > 0.85 indicates super case II type of release. The release exponent, *n*, is the slope of log fraction of drug release (*Mt/M∞*) versus log time plot}.

Table 4: Kinetic analysis for the estimation of the order followed by escin during permeation from different prepared escin gel formulae.

Formula	R^2			Korsmeyer-Peppas model data			
	Zero order	First order	Diffusion	K	n	R^2	Transport mechanism
1	0.9815	0.9740	0.9977	0.0112	0.6961	0.9675	Anomalous
2	0.9767	0.9817	0.9922	0.029	0.5414	0.9579	Anomalous
3	0.9826	0.9595	0.9920	0.0438	0.5999	0.9715	Anomalous
4	0.9671	0.9693	0.9989	0.0299	0.5482	0.9732	Anomalous
5	0.9804	0.9559	0.9939	0.0499	0.5858	0.9752	Anomalous
6	0.9869	0.9524	0.9930	0.0411	0.5219	0.9868	Anomalous
7	0.9615	0.8802	0.9881	0.0763	0.5489	0.9915	Anomalous
8	0.9665	0.8961	0.9784	0.0323	0.5475	0.9776	Anomalous
9	0.9769	0.8938	0.9977	0.0482	0.5255	0.9983	Anomalous
10	0.989	0.9422	0.9909	0.0064	0.7957	0.9916	Anomalous
11	0.9772	0.9357	0.9955	0.0149	0.6913	0.992	Anomalous
12	0.9829	0.925	0.9992	0.0257	0.6359	0.994	Anomalous
13	0.9798	0.9413	0.9932	0.017	0.6749	0.9909	Anomalous
14	0.9858	0.9427	0.9945	0.0244	0.6355	0.9884	Anomalous
15	0.9888	0.9326	0.9922	0.0144	0.7154	0.9953	Anomalous
16	0.9833	0.8750	0.9985	0.0202	0.7111	0.9986	Anomalous
17	0.9878	0.9171	0.9866	0.0113	0.7569	0.9981	Anomalous
18	0.9803	0.8708	0.9976	0.0176	0.7208	0.9976	Anomalous
19	0.9870	0.9408	0.9935	0.0043	0.8376	0.9922	Anomalous
20	0.9888	0.9419	0.9998	0.0082	0.7865	0.9928	Anomalous
21	0.9773	0.9344	0.9982	0.0132	0.7407	0.9927	Anomalous
22	0.9647	0.9456	0.9994	0.0073	0.7988	0.9897	Anomalous
23	0.9879	0.9231	0.9912	0.0093	0.8186	0.9964	Anomalous
24	0.9793	0.9153	0.9925	0.0069	0.8415	0.9988	Anomalous
25	0.9874	0.8975	0.9986	0.0093	0.8382	0.9997	Anomalous
26	0.9891	0.9049	0.9935	0.0063	0.8767	0.9992	Anomalous
27	0.9725	0.8709	0.9940	0.0084	0.8861	0.9978	Anomalous

Correlation between viscosity and permeation parameters

A correlation analysis figures which was performed between the viscosities of different formulae at 0.5 rpm and each response individually. The correlation coefficients (r) were 0.077459, 0.128, 0.3681 and 0.02236 in case of average J, Q_{180}, EF and Kp respectively. Testing these coefficients for significance against zero proved that all the correlation coefficients were insignificantly different from zero [43]. This reflected that the viscosity of the polymer matrices showed no relationship with the drug permeation rates, that is to say the diffusion of the drug through the polymer matrix is not the rate determining step. This is in accordance with the study by Fang et al [24], in which the authors reported that the viscosity of the polymer matrices showed no relationship with the diclofenac sodium permeation

rates. Another study conducted by Huang et al [35], however, found that the permeation through hydrogels and skin were both important rate determining factors for nalbuphine and its prodrugs investigated.

In-vivo transdermal delivery of escin from selected escin gel formulae

Transdermal iontophoresis of vasoactive substances has been an established as d noninvasive in vivo model for studying microvascular function [48]. The microvascular responses to the drugs applied using iontophoresis have most often been measured using laser Doppler flowmetry [49-51]. Laser Doppler flowmetry (LDF) and iontophoresis were first combined by Ekenvall and Lindblad[52] who administered the vasoconstrictor norepinephrine.

The LDF is a noninvasive method that continuously follows the flow of red blood cells [53]. It operates on the Doppler principle; employing low power helium–neon laser emitting red light at 632.8 nm and near infra-red light at 780 nm, output power is usually about 1 mW. The radiation is transmitted via an optical fiber to the skin. The radiation is diffusely scattered and its optical frequency is shifted by the moving red blood cells. The reflected light, being coherently mixed with another portion of the light scattered from static tissues, generates a Doppler beat signal in the photodetector output current. A quantitative estimation of the cutaneous blood flow derives from spectral analysis of the beat signal.

Table 5: % reduction in skin perfusion obtained after application of the selected escin gel formula 16 and 27 (iontophoresis) in comparison to the commercial escin gel formula (passive diffusion) during *in-vivo* study.

	Commercial	F16	F27
	7.73	17.59	21.98
	8.04	15.06	22.27
	6.05	20.02	19.45
	5.52	19.55	19.07
	9.03	18.03	20.79
	9.33	18.13	20.60
Average±SD	7.62±1.55	18.06±1.75	20.69±1.29

Figure (5) shows the effect of escin on microcirculation and skin perfusion; being a vasoprotective, anti-inflammatory and antiedematous agent, it reduces microcirculation and skin perfusion [54, 55].
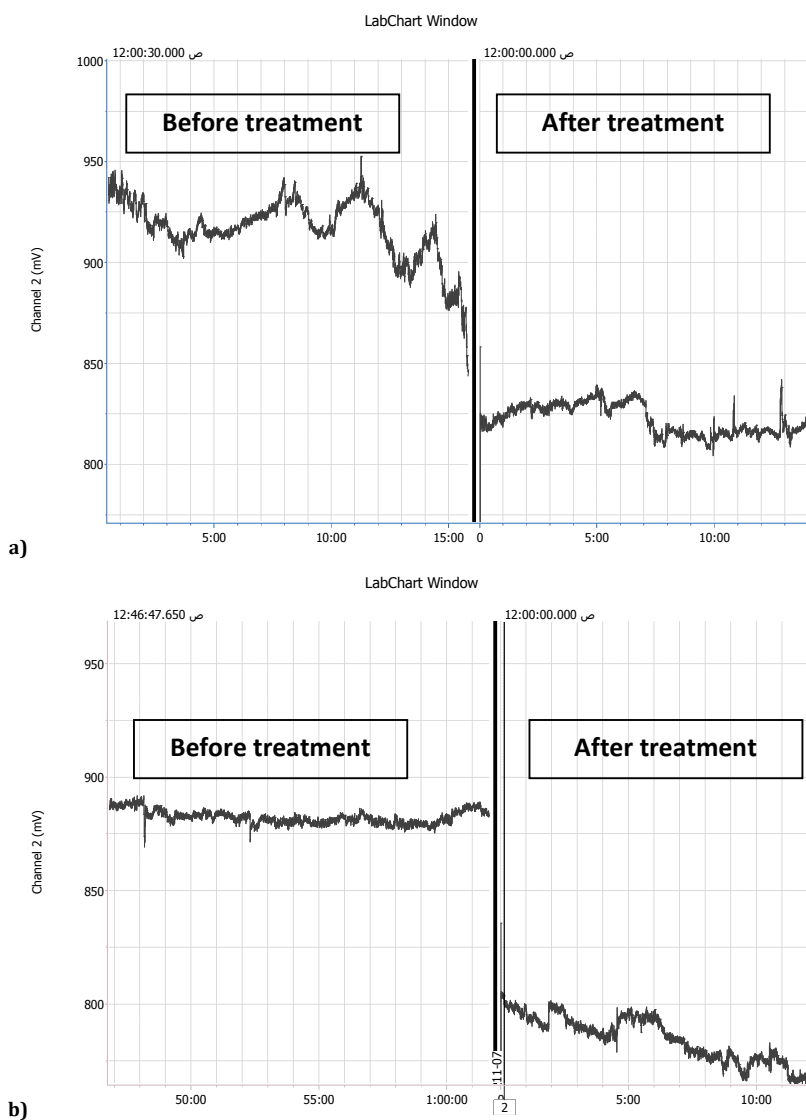
Fig. 5: Laser Doppler flowmetry chart for Skin perfusion before and after treatment for the *in-vivo* tested (a) F16 and (b) F27 escin gel formulae.

In all volunteers following the use of escin gels either the commercial escin gel by passive diffusion or the selected escin gel formulae by anodal iontophoresis, microcirculation and skin perfusion were reduced ($p < 0.05$) as proved by the laser Doppler flowmetry measurements on the skin of different human volunteers. The % reduction in skin perfusion was calculated following use of the different escin gels either the commercial escin gel by passive diffusion or the selected escin gel formulae by anodal iontophoresis (table 5). It was found that the % reduction in skin perfusion following anodal iontophoresis of the selected escin gel formulae (18.09%±1.75 and 20.67%±1.29 for F 16 and F 27 respectively) was significantly greater ($p < 0.05$) than the % reduction in skin perfusion obtained following simple rubbing (passive diffusion) of the commercial escin gel formula (7.59%±1.54). Moreover, the % reduction in skin perfusion calculated following iontophoretic application of the selected escin gel formulae did not differ significantly ($p > 0.05$). The increased efficiency is due to the combined effect of iontophoresis and penetration enhancers used in different prepared escin gel formulae which have been previously reported in others' work [40, 56-59].

CONCLUSION

The data obtained in this work suggests that it is possible to enhance the transdermal permeability of large molecules through hairless albino rat skin by the application of anodal iontophoresis combined with chemical penetration enhancers. In-vitro permeability enhancement factor obtained were about 4.57. This enhancement can be attributed to a convective solvent flow in the anode-to-cathode direction, caused by the presence of a net negative charge on the skin. In-vivo % reduction in skin perfusion following anodal iontophoresis of the selected gel formulae reached 20%. We think that our finding can have important clinical applications and pave the way to novel less invasive drug delivery methods.

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