Original Article

Iontophoretic delivery of 5-fluorouracil through excised human stratum corneum

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ABSTRACT: The objective of this study was to determine the effects of ionization, current density and penetration enhancers on the iontophoretic delivery of 5-fluorouracil (5-FU) through excised human stratum corneum (HSC). The iontophoretic (cathodal) transport of 5-FU was assessed *in vitro* at three physiologically relevant pH values of 5.0, 7.4 and 8.0, at various levels of current density ranging between 0.15 to 0.98 mA/cm^2, and in the presence of suitable penetration enhancers, namely Azone® (AZ), lauryl alcohol (LA), and isopropyl myristate (IPM). The steady-state flux at constant current density (0.47 mA/cm^2) was increased by approximately 19, 10 and 27 fold at pH 5, 7.4 and 8.0, respectively. The effect of current density at pH 7.4 exhibited a linear correlation between current density and steady-state flux (r = 0.98, p = 0.002), which indicates the potential of iontophoresis for controlled transdermal delivery of 5-FU. The combination of cathodal iontophoresis with IPM produced an additive enhancement which may be attributed to aggravated skin perturbation effect and increased skin conductivity. Other enhancers such as AZ and LA produced negative or no further enhancement respectively, when used in conjunction with cathodal iontophoresis. It may be therefore concluded that pH and current density play critical role during iontophoretic delivery of 5-FU, and combination of a chemical penetration enhancer and iontophoresis can not be always viewed as a synergistic strategy which should be evaluated on a case-by-case basis for each drug candidate/enhancer combination.

Keywords: 5-FU, Cathodal iontophoresis, Penetration enhancers, Transdermal, Current density

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1. Introduction

5-fluorouracil (5-FU) is an antineoplastic antimetabolite, which is commercially available as topical solution and cream (Effudex®; Valeant Pharmaceuticals, CA, USA). Another topical cream formulation is marketed as Fluoroplex® by Allergan, Inc. (CA, USA). These formulations are recommended for the topical treatment of multiple actinic (solar) keratoses, and have also been recently approved by the US FDA for the treatment of superficial basal cell carcinomas when conventional (*i.e.*, surgical) methods are not feasible. Topical 5-FU has also been considered as an effective treatment for psoriasis and Bowen's disease. Although conventional topical formulations of 5-FU are effective, their efficacy may be limited because of inadequate penetration through the stratum corneum, leading to suboptimal concentrations of 5-FU into the target tissue. Other disadvantages may include poor patient compliance because of prolonged treatment period required (*1*), frequent remission (*2*), and unacceptable irritation (*3*). Consequently, there remains need for a convenient, safe and efficacious method of 5-FU delivery for treatment of skin disorders, particularly basal cell carcinomas and psoriasis.

The inadequate penetration of 5-FU through the stratum corneum is mainly attributed to poor transcellular transport due to its hydrophilic nature (*4*), and restricted pore transport due to permselective properties of the skin. The isoelectric point (pI) of the human stratum corneum (HSC) is 3.7 (*5*), which implies that membrane is essentially neutral at pH ≈ 4. The pores are positively charged at pH below 4 and above 4 they bear a negative charge. Consequently, under normal physiological conditions (pH 7.4), the transport of 5-FU, an anionic drug (pK_a = 8.0), through charged pores is less likely to be favored owing to electrostatic repulsion across the negatively charged skin membrane. Therefore, to maximize the delivery of 5-FU, the formulation pH should be acidic (pH ≤ 5) which can affect drug solubility and skin irritation. One potential approach to overcome these limitations is iontophoresis, which utilizes an additional driving

force, namely an electrical potential gradient across the skin and takes advantage of the fact that like charges repel (*6*).

The clinical benefit of iontophoresis has been well documented for the treatment of psoriasis, Bowen's disease and various types of skin cancer (*1,7-9*). More recently, it has been utilized for delivery of acyclovir for the treatment of cold sores (*10*). An alternate approach to effectively deliver anticancer drugs across skin is electroporation (*11*).

The key advantage of iontophoretic delivery is that flux of a therapeutic drug can be controlled externally (by adjusting the applied current), thus tailored to the specific needs of the patient. In addition, a drug can be delivered in a pulsatile manner, which is advantageous for effective topical therapy of psoriasis (*2*). Yet, another important benefit of iontophoresis, which is clinically relevant for the treatment of Bowen's disease and other localized malignancies of the skin is that high concentrations of ionized drugs can be efficiently introduced in a relatively limited area with minimal exposure to normal surrounding tissues (*12*). This localized delivery, besides reducing the toxic effects, also has potential to avoid the necessity of a surgical treatment which is most common for Bowen's disease (*1*). These potential advantages of iontophoresis and the limited efficacy of conventional approaches make 5-FU an ideal candidate for iontophoretic delivery. Since 5-FU exists in anionic forms in solution (at pH > 5), a cathodal iontophoresis was used in the present investigation.

The specific objectives of this study were to (a) determine the degree to which the transdermal flux of 5-FU can be enhanced by constant current iontophoresis; (b) examine the role of pH; (c) study the effects of current density; and (d) to explore whether iontophoresis of the drug in the presence of chemical penetration enhancers further potentiates percutaneous transport. To the best of our knowledge, this is first reported investigation on the *in-vitro* iontophoretic delivery of 5-FU through human skin, both alone as well as in combination with penetration enhancers.

2. Materials and Methods

2.1. *Materials*

5-FU and Azone® (1-dodecylazacycloheptan-2-one) were gifts from Biochem Pharmaceutical Industries Ltd. (Mumbai, India) and Nelson Research and Development Co. (Irvine, CA, USA), respectively. Lauryl alcohol (1-dodecanol) and isopropyl myristate were purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). All other chemicals were of analytical grade and used without any further purification.

2.2. *Fabrication of iontophoretic delivery device*

A constant current power source was designed and fabricated at the University Science and Instrumentation Center of Banaras Hindu University. The unit could supply a direct current (DC) ranging between 50 μA to 25 mA. Platinum wires (99.9% purity, 0.5 mm dia; Jai Scientific Corporation, Varanasi, India) were used as electrodes, which had an effective working length of 10 mm.

2.3. *Iontophoretic transport studies*

The experimental methods for preparation of HSC and *in-vitro* transport studies have been described previously (*4*). Briefly, the HSC was rehydrated in water for 1 h and subsequently mounted in side-by-side permeation chambers with the support of a wire mesh. The dermal side of HSC faced the receiver chamber filled with phosphate buffer pH 7.4, while the donor chamber contained 4 mL of solution formulations of 5-FU (1 mg/mL). Samples of receiver solution (0.5 mL) were taken at regular intervals, and were replenished with same volume of fresh receiver solution. To perform cathodal iontophoresis, a pair of platinum electrodes was immersed in the solutions with the cathode in the donor chamber and anode in the receiver chamber. These electrodes were connected to an adjustable constant current source with simple DC features as described in section 2.2. The electric current was applied continuously, without interruption (except during sampling the current was turned 'off) for 6 h. The effective surface area of HSC available for drug permeation was 2.54 cm^2. All experiments were performed in triplicates at 37 ± 0.5°C.

2.4. *Effect of ionization*

The effect of ionization was determined at three physiologically relevant pH (donor solution) values of 5.0, 7.4 and 8.0 at a constant current density of 0.47 mA/cm^2 while maintaining the pH of receiver solution at 7.4. The buffer solutions were composed of equal proportions (30 mM) each of trisodium citrate, sodium dihydrogen orthophosphate and glycine. Minor changes in pH during iontophoresis were monitored and corrected for by the addition of micoliter amounts of 1 M HCl or 1 M NaOH solutions. By this method, the pH was kept within ± 0.2 units of the desired pH as determined at the end of the experiment.

2.5. *Effect of current density*

The influence of current density was investigated at the experimental conditions as described in section 2.3. The current density was varied between 0.15 to 0.98

mA/cm^2. The pH of both donor and receiver solutions was maintained at 7.4.

2.6. *Effect of iontophoresis in combination with penetration enhancers*

Three penetration enhancers, namely Azone®, lauryl alcohol and isopropyl myristate were studied. The concentrations of these enhancers in the respective donor solutions were 3% (w/v), 5% (w/v), and 5% (w/w), respectively. The preparation of donor solution with Azone® prompted an emulsification with 0.11% (w/v) of polysorbate 20, as suggested previously (*13*). All of these studies were performed at the physiological pH (that is, the pH of the donor and receiver solutions were 7.4) and at a constant current density of 0.47 mA/cm^2.

2.7. *Analytical method and data analysis*

The steady-state flux (*J*ss) and enhancement factor were determined using equations as described in previous publication (*4*). The fraction change in steady-state flux was determined using the following equation (*14*):

$$\text{Fraction change in the flux} = \frac{(\text{Iontophoretic flux} - \text{Passive flux})}{\text{Iontophoretic flux}}$$

Other formulae used in data analysis are given in Tables 1-3. The method for drug analysis was same as described previously (*4*).

2.8. *Statistical analyses*

Statistical comparisons were made using Student's paired *t*-test, with a two-tailed distribution. For the evaluation of any correlation, Pearson's correlation test was performed, and the correlation coefficients and associated probability values (two-tailed) were calculated using a statistical software (Graph PAD Instat®, CA, USA). The level of significance was considered at $p < 0.05$.

3. Results and Discussion

3.1. *Effect of ionization*

Formulation pH is a critical variable in iontophoresis because it affects drug ionization, net charge on the skin and electroosmotic flow (*15*). Accordingly, in the present study, three pH values higher than pI of the HSC were selected to determine the potential of iontophoresis at various ionization levels while simultaneously taking the advantage of the permselective property of the skin. In addition, minor drifts in pH that occurred during iontophoresis were monitored and adjusted when the difference between observed and original values were higher than ± 0.2 units. Such shifts in pH are often associated with use of platinum electrodes due to hydrolysis of water, which generates hydrogen ions. This in turn can affect the ionization state of the drug molecules and hence rate of iontophoretic transport (*16*).

The cumulative amount of 5-FU permeated at various pH is shown in Figure 1. The calculated values of fraction change in the steady-state flux and enhancement factor for iontophoretic transport of 5-FU are shown in Table 1. The data clearly show that at pH 5, 7.4 and 8.0, the flux is increased during iontophoresis by approximately 19, 10 and 27 fold, respectively. Apparently, the values of fraction change in the steady-state flux for 5-FU during iontophoresis are not solely related to their corresponding degrees of ionization.

It is clear from Table 1 that as the pH is increased from 7.4 to 8.0, the iontophoretic flux increased

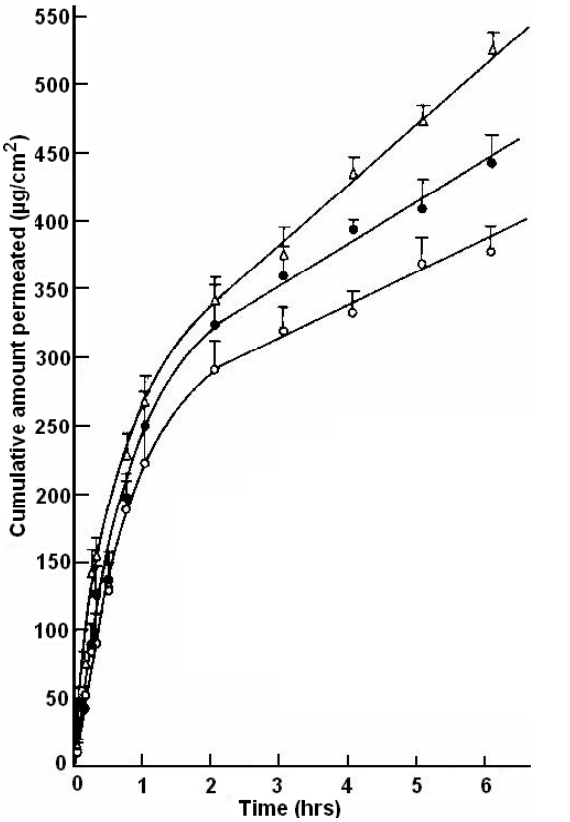


Figure 1. *In-vitro* permeation profiles of 5-FU through human stratum corneum at different pH values during cathodal iontophoresis (▵, pH 7.4; ●, pH 8; ○, pH 5) (Mean ± SD of three determinations; Only half bars have been shown for clarity).

Table 1. Effect of pH on the steady-state flux, fraction change in the flux and enhancement factor (Mean ± S.D. of three determinations) for iontophoretic transport of 5-FU through excised HSC at 0.47 mA/cm^2

pH	Fraction ionized[a]	Steady-state flux (µg/cm^2/h)	Fraction change in the flux[b]	Enhancement factor[c]
5.0	0.001	201.57 ± 8.81	0.948 ± 0.002	19.11 ± 0.78
7.4	0.201	170.06 ± 3.40	0.904 ± 0.006	10.40 ± 0.60
8.0	0.500	242.40 ± 6.58	0.962 ± 0.005	26.84 ± 3.50

[a] Calculated by considering its pK_{a1} value of 8.0
[b] Calculated using passive flux values from Singh *et al*. (*4*)
[c] Enhancement factor = $\frac{\text{Iontophoretic flux}}{\text{Passive flux}}$

which may be attributed to increase in the extent of ionization. However, the flux decreased unexpectedly when pH was increased from 5.0 to 7.4. Theoretically, a lower iontophoretic (cathodal) flux is expected at pH 5.0 compared to 7.4. The higher flux at pH 5 may be explained as follows.

At a pH of 5.0, the skin bears a net negative charge and only a small fraction of 5-FU (0.1%) is ionized in the donor solution. Under these conditions, the total flux of 5-FU during cathodal iontophoresis (J_t) may be represented by following equation which is modified from Merino *et al*. (*17*):

$$J_t = J_p + J_{er} - J_{eo} \qquad (1)$$

where J_p is the passive flux, J_{er} represents flux due to electrorepulsive migration (*i.e*., anions repelled into the skin from the cathode), and J_{eo} is electroosmotic flux which represents net transport of uncharged molecules of 5-FU due to electroosmosis. This transport occurs due to electrically induced convective solvent flow that carries uncharged molecules in the direction of positive ions flow *i.e*., anode-to-cathode direction (*18*). The negative sign signifies the fact that J_{eo} has a negative contribution to the total flux of 5-FU because it occurs in the direction opposite to electrorepulsive delivery (cathode-to-anode direction) (*17*).

Despite the fact that J_p at pH 7.4 (16.4 ± 1.28 μg/cm^2/h) was higher than at pH 5.0 (10.54 ± 0.04 μg/cm^2/h) (*4*), and that a higher electrorepulsive contribution is expected at pH 7.4, the total flux at pH 5 is higher than at pH 7.4. This clearly suggests that negative contribution of electroosmotic flow was relatively smaller (or negligible) at pH 5.0 than at pH 7.4.

Theoretical aspects of our interpretation may be further corroborated by acknowledging an apparent relationship between pH, electroosmotic flow and permselective properties of the skin. When the skin bears a net negative charge (at pH > pI), it preferentially allows the passage of counterions (*i.e*., cations). This cation permselectivity of the skin is an inherent property and is unlikely to be abolished due to imposition of an asymmetric pH gradient across the skin (in our study, 5.0 in cathode chamber, 7.4 in the anode chamber) (*19*). However, at lower donor pH of 5.0 it is possible that net negative charge at the skin surface may be decreased due to partial neutralization by hydronium ions. In fact, an excess concentration of hydronium ions at a very acidic donor pH (~3) has been observed to reverse the net charge of skin and therefore the direction of electroosmotic flow to cathode-to-anode direction (*17*). Based on this mechanism, the degree of preferential cation passage would be abated at pH 5.0. Accordingly, the magnitude of J_{eo} will be lower, and hence J_t will be higher at pH 5.0 compared to pH 7.4.

Finally, at pH 8.0, 5-FU is 50% ionized and the flux is 1.43 times higher than pH 7.4. This indicates that contribution of electrorepulsive migration is significant, which overwhelms the negative effect of electroosmosis (*6*). Collectively, these findings suggest that the relative contribution of electrorepulsive migration, electroosmotic flow and passive transport to total flux of 5-FU may be different than theoretically expected depending on the pH.

3.2. *Effect of current density*

In the absence of an electric current, 5-FU permeates through the skin at a low rate (J_{ss} = 16.4 ± 1.28 μg/cm^2/h). This permeation rate was significantly enhanced by cathodal iontophoresis at all current density values ($p < 0.05$). The corresponding values of enhancement factor are shown in Table 2. As predicted theoretically, the cumulative permeation of 5-FU was observed to increase with increasing applied current density (Figure 2).

The application of smaller iontophoretic current (0.16 and 0.31 mA/cm^2) produced a linear increase in the cumulative drug transport up to 6 h. Interestingly, doubling the current density from 0.16 to 0.31 mA/cm^2 increased the steady-state flux by almost two-fold. Further increases in current density also caused enhancement in the flux but did not commensurate in the same proportion. Overall, a positive linear relationship between current density and steady-state flux was observed (Figure 3), which was significant ($r = 0.98$, $p = 0.002$).

The linear dependence of the 5-FU flux on the applied current density may be described by Faraday's law which is represented by following equation (*20*):

$$J_i = \frac{t_i I}{F Z_i} \qquad (2)$$

where J_i, Z_i are the flux and charge (valency) of 5-FU anion, I is the applied current density and F is the Faraday's constant. In Eq (2), t_i is a proportionality constant which denotes the transport number of 5-FU anion. Remarkably, a linear relationship between 5-FU transport and current strength has also been demonstrated for *in-vivo* iontophoresis to rabbit eyes. In

Table 2. Effect of current density on the steady-state flux and enhancement factor (Mean ± S.D. of three determinations) for iontophoretic transport of 5-FU through excised HSC at pH 7.4

Current intensity (mA)	Current density (mA/cm^2)	Steady-state flux (μg/cm^2/h)	Enhancement factor[b]
0.0 (Passive)	-	16.40 ± 1.28[a]	1.0
0.4	0.16	26.53 ± 0.99*	1.62 ± 0.08
0.8	0.31	48.67 ± 0.73*	2.98 ± 0.19
1.2	0.47	63.37 ± 2.44*	3.87 ± 0.20
2.5	0.98	92.69 ± 2.34*	5.67 ± 0.41

* Significantly different from passive flux ($p < 0.05$)
[a] From Singh *et al*. (*4*)
[b] Enhancement factor = $\frac{\text{Iontophoretic flux}}{\text{Passive flux}}$

this particular study, the mean 5-FU concentration in the cornea increased linearly (R^2 = 0.97) when the current was increased from 0 - 0.75 mA (*12*).

In the present study, the ionic strength and pH of donor solution (*i.e.*, the % ionized fraction) was kept constant for all current density levels tested. It therefore follows that linear increase in flux occurred primarily due to proportional decreases in electrical resistance of the HSC, which is supported by previous findings (*21*).

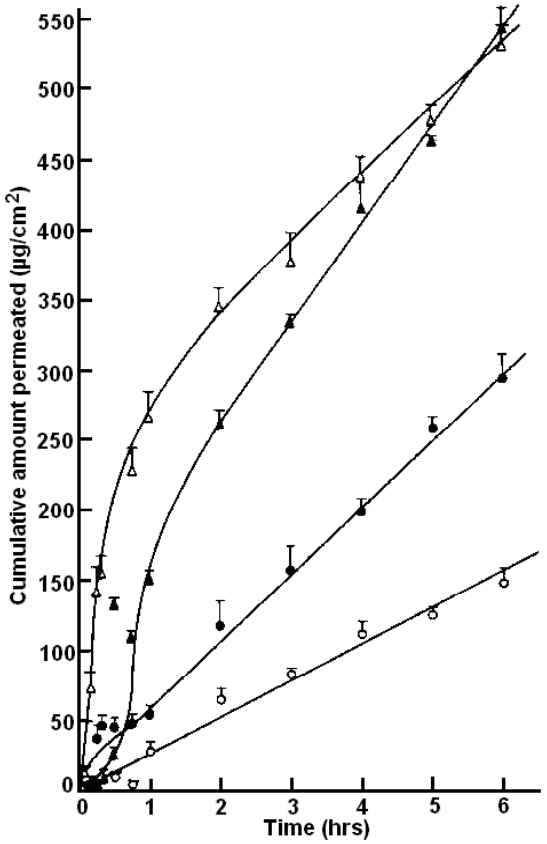


Figure 2. *In-vitro* permeation profiles of 5-FU through human stratum corneum at different current densities (▲, 0.98 mA/cm^2; ▵, 0.47 mA/cm^2; ●, 0.31 mA/cm^2; ○, 0.16 mA/cm^2) (Mean ± S.D. of three determinations; Only half bars have been shown for clarity).

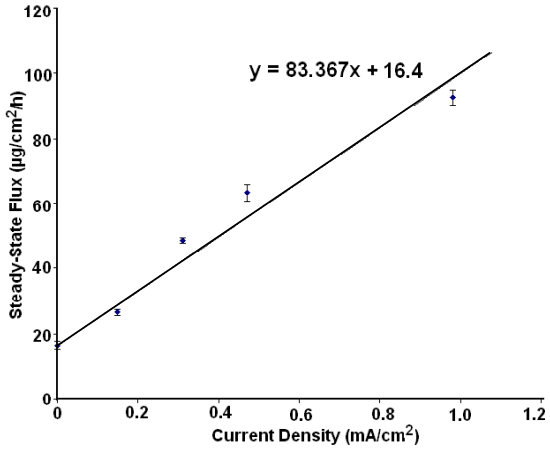


Figure 3. Relationship between steady-state flux of 5-FU and applied current density (Mean ± S.D. of three determinations).

Further, studies have also shown that changes in electrical properties of the skin are associated with proportional microscopic changes in the structure of stratum corneum. Craane-van Hinsberg *et al.* (*22*) studied the ultrastructure of the HSC after iontophoresis using current density in the range of 0.013 - 13 mA/cm^2. Their findings demonstrated that application of iontophoretic current causes disordering of the intercellular lipids of HSC and these perturbations are more pronounced with increasing current density. They attributed the lipid disordering effect to the polarization of the lipid head groups induced by the electric field, followed by mutual repulsion. These changes are microscopically noticed as loosening of epidermal cells and distention in intercellular space that commensurate with increasing current density (*23*).

The existence of a linear relationship between current density and flux has a considerable therapeutic relevance because it indicates the potential of 5-FU iontophoresis for controlled and individualized dose administration in patients.

3.3. *Effect of iontophoresis in combination with penetration enhancers*

The effect of various penetration enhancers on the iontophoretic transport of 5-FU across excised HSC is shown in Figure 4. All these experiments were performed

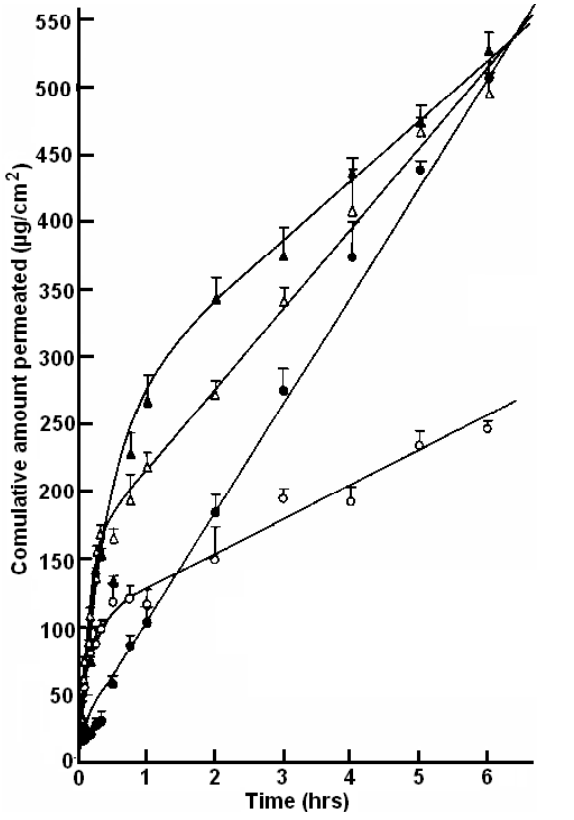
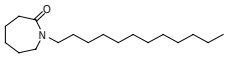
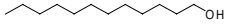
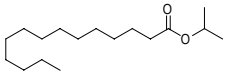


Figure 4. Effect of various penetration enhancers on the iontophoretic permeation of 5-FU through human stratum corneum (○, AZ; ●, IPM; ▵, LA; ▲, Control) (Mean ± S.D. of three determinations; Only half bars have been shown for clarity).

Table 3. Effect of penetration enhancers on the steady-state flux, enhancement factor, and synergy factor (Mean ± S.D. of three determinations) for iontophoretic transport of 5-FU through excised HSC at pH 7.4 and current density of 0.47 mA/cm^2

Penetration enhancers (PE)	Conc.	Steady-state flux (μg/cm^2/h)	K_p (cm/h × 10^4)	Enhancement factor[a]	Synergy factor[b]
None (Control)	N/A	63.37 ± 2.44	3.17 ± 0.12	1.0	-
 Azone (Mol. Wt = 281.49)	3% w/v	28.79 ± 3.17	1.44 ± 0.16*	0.45 ± 0.05	0.06 ± 0.01
 Lauryl alcohol (Mol. Wt. = 186.34)	5% w/v	63.74 ± 2.44	3.19 ± 0.12	1.01 ± 0.08	0.52 ± 0.07
 Isopropyl myristate (Mol. Wt. = 270.46)	5% w/w	85.36 ± 1.14	4.27 ± 0.06*	1.35 ± 0.04	0.79 ± 0.03

* Significantly different from control ($p < 0.05$)

[a] Enhancement factor = $\frac{\text{Iontophoretic } K_p \text{ with PE}}{\text{Iontophoretic } K_p \text{ w/o PE}}$

[b] Synergy factor = $\frac{\text{Iontophoretic } K_p \text{ with PE}}{\text{Iontophoretic } K_p \text{ w/o PE} + {}^{c}\text{Passive } K_p \text{ with PE}}$

c = Passive K_p from Singh *et al*. (*4*)

at constant current density of 0.47 mA/cm^2, which is close to the acceptable limit of current for tolerable iontophoretic delivery (0.5 mA/cm^2, Ref. *24*). The values of steady-state flux for iontophoretic and passive delivery in presence of various enhancers are compared in Figure 5. The corresponding data along with calculated values of permeability coefficient (K_p), enhancement and synergy factors are summarized in Table 3.

The permeability coefficient of 5-FU was significantly increased ($p < 0.05$) in the presence of IPM when compared to the control. However, the permeability coefficient remained unaltered ($p > 0.05$) or significantly decreased ($p < 0.05$) in presence of LA and AZ, respectively. In the presence of IPM, the cumulative drug transport increased linearly (Figure 4), and the enhancement in K_p of 5-FU was 1.35 times greater compared to control (Table 3). Furthermore, the combination of IPM and iontophoresis produced greater enhancement (5.2-fold) than iontophoresis alone (3.9-fold) and passive transport in presence of IPM (2.7-fold, Ref. *4*). Apparently, this was an additive effect, which is also evident by a lower than unity value of synergy factor (Table 3).

The additive enhancement may be attributed to direct and indirect effects of IPM. The direct effect of IPM involves interaction with the skin resulting in the perturbation of the lipid bilayers of the stratum corneum (*4*). The indirect effect has been attributed to ability of IPM to retard the rate of water loss from the skin, which results in an increase of skin conductivity and iontophoretic flux of the drug (*25*). Nevertheless, this finding is relevant because IPM is included in one of commercially available cream formulations of 5-FU (Fluoroplex®).

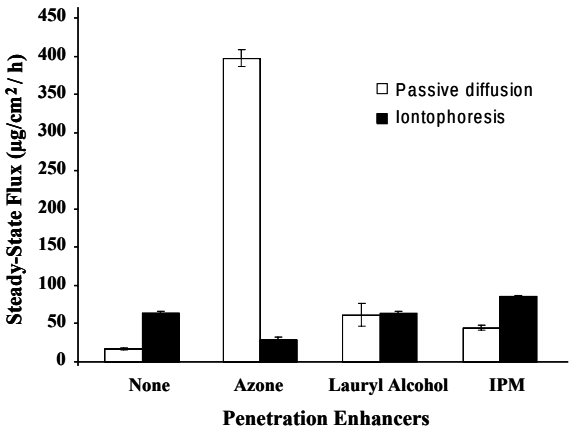


Figure 5. Comparison of steady-state flux during passive diffusion and iontophoresis of 5-FU in the presence of penetration enhancers (Mean ± S.D. of three determinations). Passive diffusion data replotted from Singh et al. (*4*).

As shown in Figure 5, the steady-state flux for combination (AZ + iontophoresis) is significantly lower compared to control (iontophoresis w/o AZ) as well as passive transport in presence of AZ. This further implies that combination of AZ and iontophoresis inhibits transport of 5-FU through both transcellular as well as hydrophilic (pores) pathways. The chemical structure of AZ is similar to that of a surfactant comprising of a polar head (lactam ring) and a lipophilic (dodecyl) side chain (Table 3). From mechanistic point of view, this structure provides an active configuration ("spoon-shaped form") in which polar head group is at an angle relative to the alkyl chain (*26*). Accordingly, it is possible that inhibitory effect of AZ in the present study might be related to the effect of electric current on the charge distribution of the polar region of AZ molecules

which in turn might affect their ability to interact with the components of the stratum corneum. Another possible reason might be due to an interaction between 5-FU and AZ which has been implicated for reduced enhancing efficiency (*27*). However, the role of skin-current interactions leading to development of a skin polarization potential can not be completed ignored. The latter has been known to reduce the efficiency of iontophoresis (*28*). Other investigators have also observed negative effect of AZ on the iontophoretic transport (*25,29*), but in other cases a synergistic enhancement has been reported (*28,30-31*). The lack of a synergistic effect of IPM and an effect of LA in the present study indicates that concentrations of these enhancers and current density level were probably not optimal which should be further explored both *in-vitro* as well as *in-vivo*.

It is obvious that three selected enhancers in this study produced distinctly different results when combined with iontophoresis. Some of the observed differences between findings of our study and other studies could be explained based on differences in the experimental protocol. For instance, in our study, the enhancers were added together with the drug. This is in sharp contrast to most of the reported studies in which skin specimens are pretreated with enhancers prior to iontophoretic transport studies, and other cosolvents like propylene glycol and ethanol are often used in combination with penetration enhancers. Skin pretreatment with a penetration enhancer has been reported to produce greater enhancement than the coapplication of the drug and an enhancer in the vehicle (*27*). Other variables that might explain differences are physicochemical properties of the drug (pK_a, solubility), formulation composition (pH, drug and enhancer concentrations, ionic strength), current density level, and differences in skin types. Consequently, the combination of iontophoresis with chemical enhancers can not be always viewed as a 'magic strategy' and should be evaluated on a case-by-case basis for each drug/enhancer combination.

4. Conclusions

The results generated in this series of investigation have demonstrated that, with the application of cathodal iontophoresis, the transdermal delivery of 5-FU could be substantially enhanced compared to passive diffusion. Moreover, this work explores the feasibility of a combination strategy involving a chemical penetration enhancer and iontophoresis as a technique for controlled transdermal delivery of 5-FU. Other enhancer systems more suited for practical applications need to be investigated. A successful combination of iontophoresis and a well-tolerated permeation enhancer such as IPM appears promising for controlled transdermal delivery of 5-FU. Thus by proper optimization of the electrical and physico-chemical factors, 5-FU can be delivered through the intact skin at a controlled rate and with a desirable dosing pattern. Eventually, such controlled delivery systems can offer convenience of outpatient therapy and assuage patients plagued by the side effects of chemotherapy with 5-FU.

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References

1. Welch ML, Grabski WJ, McCollough ML, Skelton HG, Smith KJ, Menon PA, Anderson LL. 5-fluorouracil iontophoretic therapy for Bowen's disease. J Am Acad Dermatol 1997; 36:956-958.
2. Pearlman DL. Method for treating psoriasis with cytotoxic agents. U.S. Patent 4853388, August 1, 1989.
3. Goette DK. Topical chemotherapy with 5-fluorouracil. A review. J Am Acad Dermatol 1981; 4:633-649.
4. Singh BN, Singh RB, Singh J. Effects of ionization and penetration enhancers on the transdermal delivery of 5-fluorouracil through excised human stratum corneum. Int J Pharm 2005; 298:98-107.
5. Wilkerson VA. The chemistry of human epidermis. II. The isoelectric points of the stratum corneum, hair, and nails as determined by electrophoresis. J Biol Chem 1935; 112:329-335.
6. Kalia YN, Naik A, Garrison J, Guy RH. Iontophoretic drug delivery. Adv Drug Del Rev 2004; 56:619-658.
7. Bacro TR, Holladay EB, Stith MJ, Maize JC, Smith CM. Iontophoresis treatment of basal cell carcinoma with cisplatin: a case report. Cancer Detect Prev 2000; 24:610-619.
8. Tiwari SB, Ravikumar BC, Udupa N, Balachandran C. Topical methotrexate delivered by iontophoresis in the treatment of recalcitrant psoriasis – a case report. Int J Dermatol 2003; 42:157-159.
9. Smith KJ, Konzelman JL, Lombardo FA, Skelton HG 3rd, Holland TT, Yeager J, Wagner KF, Oster CN, Chung R. Iontophoresis of vinblastine into normal skin and for treatment of Kaposi's sarcoma in human immunodeficiency virus-positive patients. The military medical consortium for applied retroviral research. Arch Dermatol 1992; 128:1365-1370.
10. Morrel EM, Spruance SL, Goldberg DI. Iontophoretic acyclovir cold sore study group. Topical iontophoretic administration of acyclovir for the episodic treatment of herpes labialis: a randomized, double-blind, placebo-

controlled, clinic-initiated trial. Clin Infect Dis 2006; 15:460-467.
11. Singh BN, Dwivedi C. Antitumor drug delivery by tissue electroporation. Anti-Cancer Drugs 1999; 10:139-146.
12. Kondo M, Araie M. Iontophoresis of 5-fluorouracil into conjunctiva and sclera. Invest Ophthalmol Vis Sci 1989; 30:583-585.
13. Morimoto Y, Sugibayashi K, Hosoya K, Higuchi WI. Penetration enhancing effect of Azone on the transport of 5-fluorouracil across the hairless rat skin. Int J Pharm 1986; 32:31-38.
14. Siddiqui O, Roberts MS, Polack AE. Iontophoretic transport of weak electrolytes through the excised human stratum corneum. J Pharm Pharmacol 1989; 41:430-432.
15. Costello CT, Jeske AH. Iontophoresis: applications in transdermal medication delivery. Phys Ther 1995; 75:554-563.
16. Hale RL, Lu A, Solas D, Selick HE, Oldenburg KR, Zaffaroni AC. Compositions and methods for enhanced drug delivery. U.S. Patent 5607691, March 4, 1997.
17. Merino V, López A, Kalia YN, Guy RH. Electrorepulsion versus electroosmosis: effect of pH on the iontophoretic flux of 5-fluorouracil. Pharm Res 1999; 16:758-761.
18. Pikal MJ. The role of electroosmotic flow in transdermal iontophoresis. Adv Drug Del Rev 2001; 46:281-305.
19. Kim A, Green PG, Rao G, Guy RH. Convective solvent flow across the skin during iontophoresis. Pharm Res 1993; 10:1315-1320.
20. Phipps JB, Gyory JR. Transdermal ion migration. Adv Drug Del Rev 1992; 9:137-176.
21. Inada H, Ghanem AH, Higuchi WI. Studies on the effects of applied voltage and duration on human epidermal membrane alteration/recovery and the resultant effects upon iontophoresis. Pharm Res 1994; 11:687-697.
22. Craane-van Hinsberg IW, Verhoef JC, Spies F, Bouwstra JA, Gooris GS, Junginger HE, Boddé HE. Electroperturbation of the human skin barrier *in vitro*: II. Effects on stratum corneum lipid ordering and ultrastructure. Microsc Res Tech 1997; 37:200-213.
23. Singh S, Bi M, Jayaswal SB, Singh J. Effect of current density on the iontophoretic permeability of benzyl alcohol and surface characteristics of human epidermis. Int J Pharm 1998; 166:157-166.
24. Prausnitz MR. The effects of electric current applied to skin: a review for transdermal drug delivery. Adv Drug Del Rev 1996; 18:395-425.
25. Fang JY, Huang YB, Lin HH, Tsai YH. Transdermal iontophoresis of sodium nonivamide acetate. IV. Effect of polymer formulations. Int J Pharm 1998; 173:127-140.
26. Kim N, El-Kattan AF, Asbill CS, Kennette RJ, Sowell JW Sr, Latour R, Michniak BB. Evaluation of derivatives of 3-(2-oxo-1-pyrrolidine) hexahydro-1H-azepine-2-one as dermal penetration enhancers: side chain length variation and molecular modeling. J Controlled Rel 2001; 73:183-196.
27. Chow DS, Kaka I, Wang TI. Concentration-dependent enhancement of 1-dodecylazacyclohetan-2-one on the percutaneous penetration kinetics of triamcinolone acetonide. J Pharm Sci 1984; 73:1794-1799.
28. Meidan VM, Al-Khalili M, Michniak BB. Enhanced iontophoretic delivery of buspirone hydrochloride across human skin using chemical enhancers. Int J Pharm 2003; 264:73-83.
29. Hirvonen J, Kontturi K, Murtomäki L, Paronen P, Urtti A. Transdermal iontophoresis of sotalol and salicylate; the effect of skin charge and penetration enhancers. J Controlled Rel 1993; 26:109-117.
30. Ganga S, Ramarao P, Singh J. Effect of Azone on the iontophoretic transdermal delivery of metoprolol tartrate through human epidermis *in vitro*. J Controlled Rel 1996; 42:57-64.
31. Femenía-Font A, Balaguer-Fernández C, Merino V, López-Castellano A. Combination strategies for enhancing transdermal absorption of sumatriptan through skin. Int J Pharm 2006; 323:125-130.

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