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International Journal of Pharmaceutics 254 (2003) 271–280
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Transdermal iontophoresis of insulin II. Physicochemical considerations

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Received 2 September 2002; received in revised form 10 January 2003; accepted 10 January 2003

Abstract

Transdermal iontophoresis is one of the potential enhancement strategies for the delivery of large and charged molecules. Insulin, a polypeptide of 6 kDa was used as a model for large peptides to understand the influence of peptide concentration, NaCl concentration, buffer type and its concentration on the transport efficiency of iontophoresis. Maximum enhancement was found at 3 mg/ml (75 IU/ml). The permeation of insulin was found to increase up to 0.05 M NaCl and decreased at higher concentrations of NaCl. The glucose permeation studies showed that permeation of insulin increased in the presence of NaCl due to ion induced convective flow. The flux enhancement of insulin in the presence of phthalate buffer was higher in comparison to citrate buffer, but the enhancement in these two buffers was the same in the presence of 0.05 M NaCl, which was also supported by a similar trend in conductivity values. However, the solution conductivity values did not reflect the influence of co-ions and counter ions on the transport of large peptides across the skin. Overall the findings revealed that the transport efficiency of large peptides like insulin may be improved by the optimisation of competing ions in solution.

Keywords: Transdermal iontophoresis; Transport efficiency; Co-ions; Counter ions; Ion induced convective flow; Electroosmosis; Conductivity

1. Introduction

Transdermal iontophoresis (TI) is a physical enhancement technique used primarily to facilitate the delivery of charged molecules across the skin. The recent interest in this 100-year-old technique is due to the emergence of a large number of peptide drugs (Panchagnula et al., 2000). The delivery of peptides by TI is influenced by a complex interplay of several factors, which has been reviewed elsewhere (Pillai et al., 1999; Cullander and Guy, 1992). However, one of the main issues in optimising the iontophoretic delivery of peptides is to improve the transport efficiency. The influence of co-ions and counter ions is given in the following equation (Nair et al., 1999), where flux (J_i) is expressed in terms of transport number (t_i) of a solute;

$$J_i = \frac{t_i I_T}{z_i F} \quad (1)$$

In this equation, I_T is the total current density, z_i the charge of the ion and F the Faraday's constant. The transport number of an ion in solution reflects a propor-

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doi:10.1016/S0378-5173(03)00034-6

tion of the current carried by a given solute. Co-ions and counter ions have a significant influence on the fraction of charge carried by the drug as these ions may compete with the drug for carrying the electric charge based on their size, charge and mobility (Phipps and Gyory, 1992). Although there are number of reports on the influence of extraneous ions on the transport of small drug molecules, there is an obvious lack of studies on large peptides like insulin. Unlike conventional small molecules, where transport efficiency may be improved by increasing the quantity of loaded drug, the high cost precludes such an approach for peptide drugs (Sage, 1993). On the other hand, another approach to improve the delivery efficiency is to optimise the number of competing ions in the drug formulation. These extraneous ions may have a pronounced and complicated influence on the fraction of peptide transported.

Insulin was chosen as a model peptide based on its commercial and therapeutic potential. Most of the earlier studies with insulin have been carried out in vivo using various animal models, where insulin was found to be permeable by iontophoresis and cause reduction in blood glucose levels (Kari, 1986; Siddiqui et al., 1987). The objective of this study was to examine the effect of various physicochemical parameters like peptide concentration, electrolyte concentration, buffer type and its concentration on the in vitro iontophoretic transport of insulin. All the studies were done at pH 3.6, as it was found to be the optimum pH in our earlier studies (unpublished data). At this pH there was maximum insulin flux and minimal pH shift.

2. Materials and methods

2.1. Materials

Bovine insulin was received as a *gratis sample* from Knoll Pharmaceuticals (Mumbai, India). ^{125}I-insulin (80–100 μCi/g) and ^{14}C-glucose (250 μCi/g) was provided by Board of Radiation and Isotope Technology (BRIT, Mumbai, India). All other chemicals and reagents used were of analytical grade. In all the experiments, ultrapure water prepared by reverse osmosis using Elgastat (ELGA Ltd., UK) was used and had a resistivity of 18 MΩ or greater.

2.2. Skin preparation

The animal experimentation protocols were approved by the Institutional Animal Ethics Committee (IAEC) of NIPER. Male Sprague-Dawley rats (200–250 g) obtained from central animal facility at NIPER were sacrificed by excessive ether anesthesia. The hair was removed from dorsal portion of the rat using an animal hair clipper (Aesculap, Germany) and subsequently, full thickness skin was harvested. Then the fat adhering to the dermis side was removed by using a surgical scalpel and isopropyl alcohol. Finally, the skin was rinsed in tap water and packed in aluminum foil. The skin samples were stored at −20 °C and was used within a week.

2.3. Ex vivo permeation studies

Phosphate buffered saline (0.1 M; pH 7.4) was sonicated for 30 min to remove dissolved gases and was placed in the receptor compartment of unjacketed Franz diffusion cells (area 0.79 cm^2) followed by equilibration for overnight at 37 °C and 900 rpm in a heating-stirring module (Pierce, USA). The skin piece was mounted with the *Stratum corneum* side facing the donor compartment and was equilibrated for 1 h. In the donor compartment, 500 μl of peptide solution (pH 3.6; insulin spiked with 80–100 μCi of radioactive insulin) was placed and a constant current of 0.5 mA/cm^2 was applied through platinum electrodes (2 cm × 0.5 mm diameter) for 6 h using a six-channel constant power supply unit (Ultrapure Scientifics, Mumbai, India). Both the donor and receptor compartments contained urea (2 mg/ml) to prevent adsorption of insulin to glass surfaces (Sato et al., 1983) and sodium azide (0.0025% w/v) to prevent any microbial growth. Anodal iontophoresis was carried out with the anode in the donor compartment and the cathode in the receptor compartment. The samples were periodically withdrawn from receptor compartment upto 48 h and were counted on an automatic gamma scintillation counter (1470, Wallac, Finland). At the end of the study, the pH of donor and receptor solutions were measured using a glass microelectrode (Eutech Instruments, USA) while, the skin area exposed to drug solution was washed with water, blotted, dried and weighed. The skin pieces were then digested overnight with tissue solubiliser (NCS-II, Amersham,

UK) in a shaker water bath (Julabo, Germany) at 37 °C and 100 rpm to obtain skin homogenates of which 200 μl was used for radioactive counting. Based on the ratio of labeled and non-labeled insulin, the amount of insulin in the samples was calculated. Radioactivity recovery from the donor and receptor solutions was analysed using a TLC method. The mobile phase consisted of *n*-butanol:glacial acetic acid:pyridine:water (38:25:7:30). After developing the pre-coated plate (Merck, Germany), it was cut into 1 cm pieces and counted for radioactivity. Percentage of the counts corresponding to R_f of insulin was calculated from the total count of all the pieces.

In order to characterize the electroosmotic flow, glucose was used as a marker. In separate experiments, skin pieces were soaked overnight in glucose solution (2.5% w/v) to saturate the glucose metabolizing enzymes in the skin (Rao et al., 1993). The donor solution was composed of glucose (50 mg/ml) and ^{14}C labeled glucose (250 μCi/g) with varying ionic strength either in absence or presence of insulin. The samples (200 μl) were withdrawn periodically upto 12 h and were analysed in a liquid scintillation counter (1409, Wallac, Finland) after addition of 3 ml of scintillation cocktail (BCS 104, Amersham, UK).

2.4. Effect of insulin concentration

The concentration of insulin in the donor solution was varied from 0.2 to 10 mg/ml (5–250 IU/ml) in citrate–phosphate buffer (CB, 0.24 M; pH 3.6). The electroosmotic experiments were conducted with 0.2, 3 and 10 mg/ml solutions.

2.5. Effect of buffer salt concentration

To study the influence of concentration of buffer ions, the total concentration of citric acid and sodium phosphate was varied from 60 to 480 mM, while keeping the ratio between the salts constant and insulin concentration was kept constant (3 mg/ml).

2.6. Effect of sodium chloride concentration

The effect of added ions on TI of insulin (3 mg/ml) was studied by varying sodium chloride concentration from 0.01 to 1 M, in CB (0.24 M). The electroosmotic experiments were conducted at 0.01, 0.05 and 1 M concentrations of sodium chloride.

2.7. Effect of type of buffer speices

In order to study the type of buffer species on iontophoretic transport, potassium biphthalate (PB; 0.1 M) was used both in the presence and absence of 0.05 M NaCl. The results were compared with that of CB (0.24 M). The concentration of buffer species used was based on the standard concentrations recommended for preparation of these buffers (Diem and Lenter, 1975).

2.8. Conductivity measurements

The conductivity of buffer solutions with and without sodium chloride was measured with a conductivity meter (Eutech Instruments, USA) after calibration with standard electrolyte solutions at room temperature using a cell constant of one. Insulin is reported to have negligible conductivity compared to the highly mobile buffer species (Banga and Chien, 1993). Hence, in a separate experiment, the specific conductivity of varying concentration of insulin was measured in deionized water to understand the role of solution conductivity on iontophoretic transport.

2.9. Data treatment

The cumulative amount of insulin permeated was plotted against time and the skin permeation parameters were calculated (Ritschel et al., 1989). The flux was calculated from the linear portion of the curve and the lag time was read from the intercept on *x*-axis. The permeability coefficient was calculated by dividing the flux by concentration of insulin in the donor compartment. For calculation of skin affinity values, the method described by Panchagnula and Patel (1997) was followed, wherein the drug concentration in skin (μg/mg) was divided by the concentration of insulin in the receptor compartment at the end of 48 h (μg/mg—taking density of receptor fluid as 1 g/ml). In glucose permeation experiments, the electroosmotic ratio was calculated by dividing the concentration of glucose in the receptor compartment by that in the donor compartment at the end of iontophoresis.

All experiments were done in triplicate and the values were expressed as mean±S.E.M., unless specified. Passive permeation experiments were used as control and the data was subjected to one-way ANOVA at a significance level of $P < 0.05$ using SIGMASTAT® (Jandel Scientifics, USA).

3. Results and discussion

3.1. Effect of insulin concentration

The change in permeability coefficient of insulin as a function of increasing insulin concentration is shown in Fig. 1a. There was a disproportionate increase in permeability with increase in insulin concentration and the permeability coefficient was minimum at 50 IU/ml of insulin. At concentrations >50 IU/ml, the permeability increased and then decreased linearly from 75 to 250 IU/ml. On the other hand, it was found that the iontophoretic flux enhancement increased with insulin concentration up to 75 IU/ml (Fig. 1b). Maximum enhancement was observed at 75 IU/ml, while higher concentrations of insulin resulted in very less enhancement. Fig. 1c shows the specific conductivity of insulin in water at different concentrations used in skin permeation studies. The specific conductivity did not change much in the concentration range of 50–100 IU/ml of insulin and there was no significant increase in specific conductivity with further increase in insulin concentration.

Insulin is known to form aggregates of varying degree from dimers to hexamers depending on the pH, concentration and ionic strength of the solution (Brange and Langkjaer, 1993). It is reported to exist as monomer below 6 μg/ml and as dimer at high concentrations relevant to pharmaceutical formulations in the pH range of 4–8. These aggregates are larger in size (dimer 12 kDa; tetramer 24 kDa; hexamer 36 kDa) than monomeric insulin and are hence expected to be less mobile and less permeable. Furthermore, in solution there is an equilibrium between the different aggregated states of insulin (Jeffrey and Coates, 1966), which may have an influence on the mobility and permeation through skin. On the other hand, there was no significant ($P > 0.05$) influence of insulin concentration (0.2, 3 and 10 mg/ml) on electroosmotic flow (data not shown), unlike that observed with some lipophilic cationic peptides (Hirvonen and Guy, 1997). Banga and Chien (1993) have reported that there was no significant effect of insulin concentration (0.2–2 mg/ml) on tritiated water flux. In another study, Kanikkannan et al. (1999) have found that there was no significant influence of insulin concentration (0.4–10 mg/ml) on plasma glucose reduction in diabetic rats.

3.2. Effect of buffer salt concentration

The buffering capacity of the system has to be high enough to accommodate hydrogen ions produced at the anode surface of platinum electrodes. A very low concentration of buffer ions would result in generation of hydrogen ions, which are highly mobile species due to their unique ‘transport mechanism’ in water (Lelawongs et al., 1990). At the same time, the use of very high buffer concentration results in greater competition of buffer ions with the peptide for carrying charge. Both the situations may compromise the transport efficiency of a large peptide like insulin. Therefore, the influence of varying concentration of buffer salts was studied to optimize the buffer concentration. The skin permeation parameters at varying buffer salt concentration are given in Table 1. Iontophoretic flux was significantly high with 120 mM of buffer salt, while there was no significant difference ($P > 0.05$) in the iontophoretic flux between 240 and 480 mM of CB. Similarly, there was no significant difference ($P > 0.05$) in the cumulative amount of insulin permeated in 48 h with various concentrations of CB. The solution conductivity of CB increased with increasing concentration of buffer salt and was not in agreement with the trend observed in iontophoretic flux of insulin (Table 1). On the other hand, flux enhancement (Fig. 2a) was significantly high ($P < 0.05$) with 240 mM of CB. The shift in pH as a function of buffer salt concentration is shown in Fig. 2b and the pH shift was high at ≤120 mM, while the buffering capacity was high at 480 mM of CB.

As the buffer salt concentration increases, the number of competing ions also increases, which influence the iontophoretic flux of insulin. The higher buffer salt concentration increases the buffering capacity, which reduces the pH shift caused by electrolysis of water at the surface of platinum electrodes. This pH shift can have an profound influence on the attainment of steady

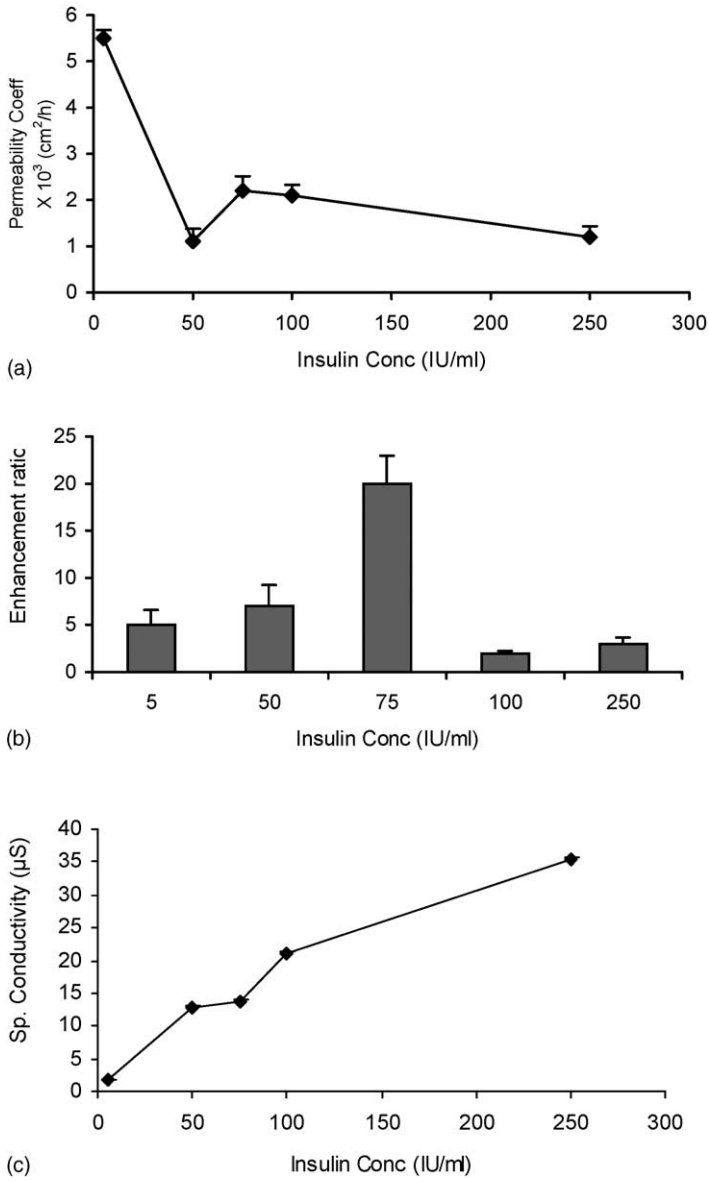

Fig. 1. Influence of varying concentration of insulin. (a) Iontophoretic permeability as a function of insulin concentration; (b) iontophoretic flux enhancement of insulin as a function of insulin concentration in the donor solution; (c) specific conductivity of insulin in deionized water at varying concentrations. Each data point is represented as mean ± S.EM. ($n = 3$). Conductivity measurements are represented as mean of two values.

state flux of peptides (Lelawongs et al., 1990). Similar to our findings, Kumar et al. (1992) found that there was no direct correlation between solution conductivity of varying concentration of buffer salts and flux of growth hormone releasing factor. However, among the buffer salt concentrations tested, 240 mM of CB was found to be optimum in terms of flux enhancement and pH shift.

Table 1
Solution conductivity of buffer salt and skin permeation parameters of insulin at varying buffer salt concentrations

Buffer concentration (mM)[a]	Specific conductivity (mS)[b]	Flux (μg/cm^2/h)	Cumulative amount permeated (μg)
60	2.22	2.74 (0.24)	101.20 (19.85)
120	4.43	4.17 (0.28)*	142.20 (15.64)
240	7.90	2.44 (0.10)	98.79 (1.89)
480	13.37	2.35 (0.19)	112.74 (8.08)
PB[c]	4.62	1.94 (0.28)	67.62 (7.65)

[a] The pH was 3.6. Citrate–phosphate buffer was used unless specified.
[b] Specific conductivity of buffer salts was measured in deionized water and the values are mean of two measurements. All other values are mean of three values with S.E.M. in parentheses ($n = 3$).
[c] PB is potassium biphthalate buffer (0.1 M; pH 3.6).
* Statistically significant difference ($P < 0.05$).

3.3. Effect of sodium chloride concentration

Insulin has a very low transport number (Banga and Chien, 1993) compared to other ions in the buffer system and its contribution to the total conductivity of solution is negligible (0.084%). Hence, the amount of electrolyte with smaller molecular size is important in allowing the effective transport of a large peptide like insulin, as each ion has a independent ionic mobility in an electric field. This implies that ions compete with

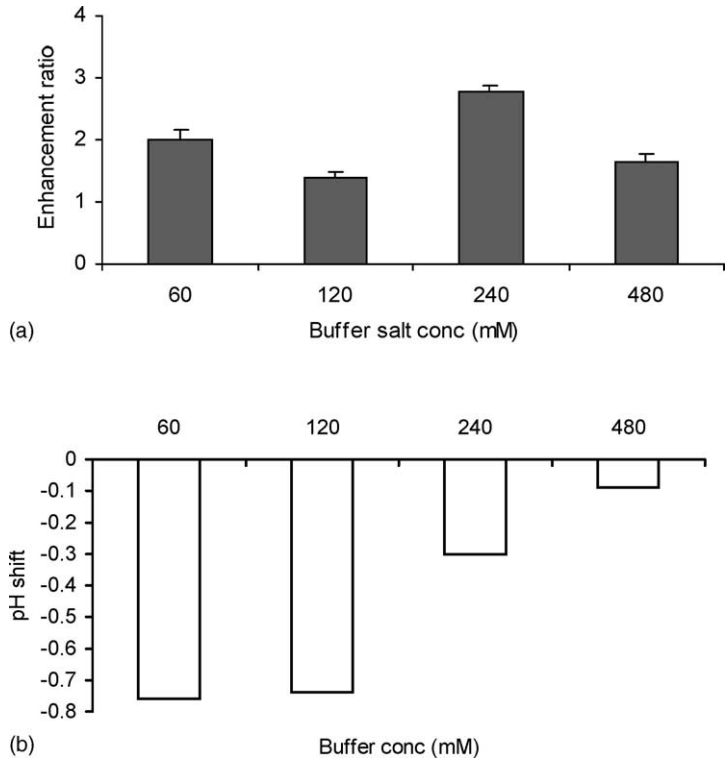

Fig. 2. Influence of varying citrate–phosphate buffer concentration on iontophoretic transport of insulin. (a) Iontophoretic flux enhancement of insulin as a function of varying buffer salt concentration in the donor solution; (b) shift in pH as a function of buffer salt concentration. pH shift was calculated as the difference in final and initial donor solution pH. Each data point is represented as mean ± S.E.M. ($n = 3$). Only the mean value of the pH shift is given.

Table 2
Solution conductivity of sodium chloride and skin permeation parameters of insulin at varying sodium chloride concentrations

NaCl concentration (mM)[a]	Specific conductivity (mS)[b]	Flux (μg/cm^2/h)	E_r[c]
10	7.18	6.12 (0.37)*	2.46 (0.15)
50	10.18	5.88 (1.03)*	2.84 (0.50)
100	19.85	4.92 (1.22)*	1.46 (0.36)
500	46.35	0.76 (0.06)	1.36 (0.10)
1000	77.35	1.50 (0.14)	1.30 (0.20)
PB + 50 mM[d]	9.23	3.22 (0.85)	2.97 (0.84)

[a] Citrate–phosphate buffer was used unless specified.
[b] Specific conductivity of varying sodium chloride concentrations was measured in citrate–phosphate buffer (0.24 M; pH 3.6) or in PB.
[c] Iontophoretic flux divided by flux during passive permeation.
[d] PB indicates potassium biphthalate buffer (0.1 M; pH 3.6) + 50 mM NaCl.
* Statistically no significant difference ($P > 0.05$). Conductivity values are mean of two measurements. All the other values are mean of three values with S.E.M. in parentheses.

a given solute in a differing magnitude in relation to its rate of transport (Yoshida and Roberts, 1995). In this context, sodium chloride concentration was varied from 0 to 1 M and its influence on iontophoretic transport of insulin was studied.

Fig. 3a and Table 2 depict the permeation parameters for insulin in presence of varying concentration of NaCl. The cumulative amount of insulin permeated in 48 h increased upto 0.05 M NaCl and subsequently decreased at >0.05 M NaCl. There was no significant difference in flux with 0.01 and 0.05 M of NaCl ($P > 0.05$). As shown in Fig. 3b, the skin affinity of insulin was high, when the NaCl concentration was >0.1 M. The electroosmotic flow was found to be high with 0.05 M NaCl, while it decreased at higher concentrations of NaCl (Fig. 3c). Though the solution conductivity of NaCl in buffer solution was directly proportional to its concentration ($R^2 = 0.99$), it was not in agreement with the skin permeation results of insulin.

The transport of ions in a charged membrane is coupled with the volume flow and in presence of an electric field, it is the summation of both convective flow as well as electroosmosis (Wearley and Chien, 1990). Therefore, the convective flow through the membrane increases with increase in the concentration of ions. Gangarosa et al. (1980) described this phenomenon as iontohydrokinesis where, both cations and anions carry water along with them by one or more mechanisms during iontophoresis through the different types of pore in the skin. These pores differ in their charge and concentration (Pikal, 1990). The increase in the amount of insulin permeated with 0.05 M NaCl is attributed to the associated increase in ion induced convective flow. Similar results have been observed earlier with small polar drugs (Wearley and Chien, 1990). However, unlike small molecules, this convective flow assumes greater importance in the transport of a large peptide like insulin (Guy et al., 2001).

Yoshida and Roberts (1994) have reported that at >50 mM NaCl, there is saturation of pores which retards further transport of sodium ions and the associated water flow into the pores. This decrease in convective flow is observed from the glucose permeation studies as shown in Fig. 3c. Presumably, the decrease in ion induced convective flow resulted in lesser enhancement and amount of insulin permeated above 50 mM of NaCl. This also resulted in higher fraction of insulin retained inside the skin (Fig. 3b). Further at high concentrations of NaCl (>0.1 M), insulin undergoes aggregation (Sundby, 1962; Jeffrey and Coates, 1966).

On the other hand, the solution conductivity may not precisely reflect the effect of solute concentration, as change in conductivity occurs when an ion passes through the membrane at higher concentrations (Yoshida and Roberts, 1994). Further, this is substantiated from the findings of Oh et al. (1993), where solution conductivity decreased by 80-fold on decreasing the NaCl concentration from 1 to 0.01 M, while the decrease in skin resistance was only 10-fold.

3.4. Influence of type of buffer species

As shown in Tables 1 and 2, the iontophoretic flux was significantly higher with CB. The amount of insulin permeated in 48 h was significantly higher

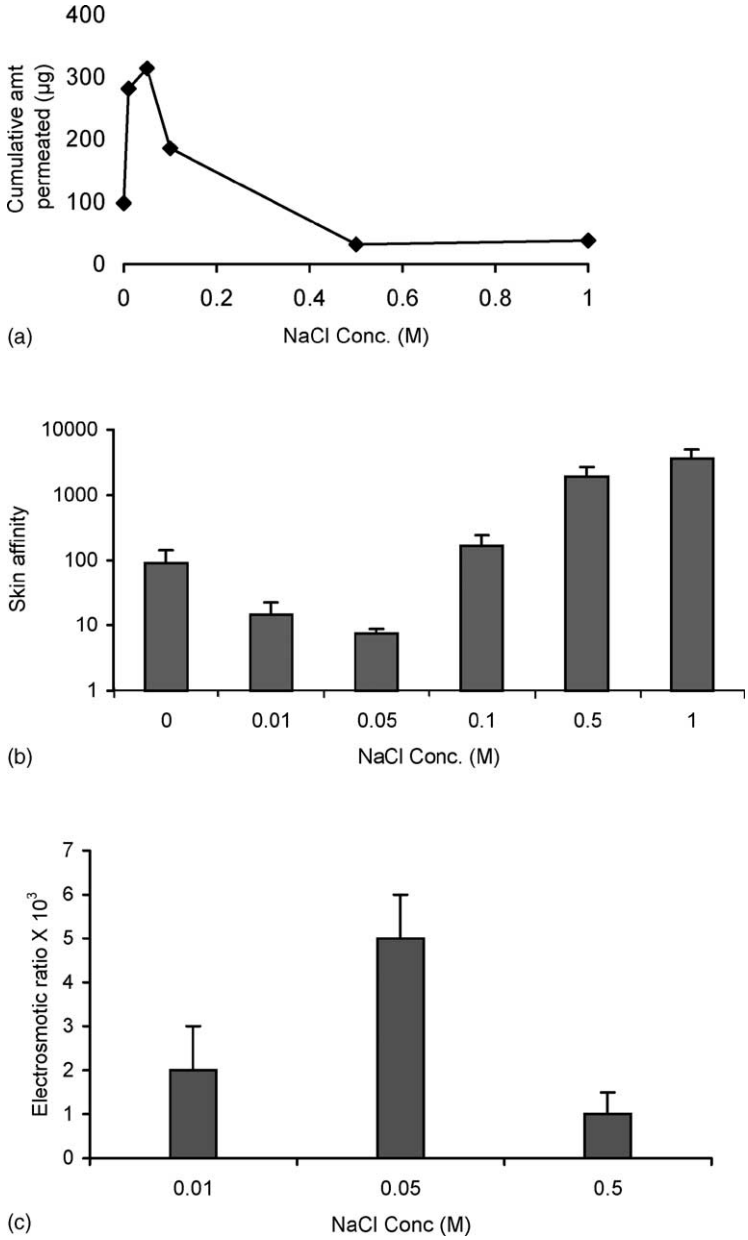

Fig. 3. Influence of varying sodium chloride concentration on iontophoretic transport of insulin. (a) Cumulative amount of insulin permeated in 48 h as a function of varying sodium chloride concentration in the donor solution. Only the mean values ($n = 3$) are represented here; (b) skin affinity values of insulin as a function of sodium chloride concentration in the donor solution. The *y*-axis is in logarithmic scale; (c) electroosmotic ratio as a function of sodium chloride concentration. The electroosmotic flow is represented as the ratio between the concentration of glucose in the receptor compartment to that in the donor compartment at the end of 6 h of iontophoresis. Values are represented as mean ± S.E.M. ($n = 3$).

($P < 0.05$) in CB with NaCl (314.37 ± 46.30 μg) in comparison to PB with and without NaCl (67.62 ± 7.65 and 122.73 ± 36.81 μg, respectively). However, in absence of NaCl, iontophoretic enhancement was significantly higher in PB compared to that with CB (5.89 versus 2.81), whereas in presence of NaCl, the enhancement was similar (2.84 times with CB and 2.97 times with PB). The shift in pH was relatively less in CB (0.31 pH units) compared to that in PB (1.34 units).

These differences and similarities between the two buffers was also evident from the conductivity values given in Tables 1 and 2, where conductivity was the least in PB without NaCl, but was similar for both the buffers in presence of NaCl. The lower conductivity of phthalate ion is due to the relatively larger size of the former compared to the latter. However, on addition of 0.05 M NaCl, the higher mobility of sodium and chloride ions seem to nullify the differences in conductivity of the two buffers. Since the PB components are less mobile, they compete relatively less compared to CB components, thereby resulting in greater enhancement. The addition of NaCl has a significant influence on increasing the total amount of insulin permeated by ion induced convective flow in CB compared to PB. This difference in cumulative amount of insulin permeated with the two buffers is due to the difference in the transport number in solution compared to that in the membrane and diffusivity through the membrane (Wearley et al., 1989; Phipps and Gyory, 1992). As the buffering capacity and the total amount of insulin permeated was higher with CB relative to that in PB, the former was found to be better.

4. Conclusions

For large peptides like insulin, it is difficult to improve the transport efficiency by increasing the concentration of the peptide due to the limitations in terms of cost and physical stability. On the other hand, the transport efficiency may be improved by optimizing co-ions and counter ions. An optimum concentration of sodium chloride would increase the overall permeation of large peptides like insulin by ion induced convective flow. The conductivity measurements of solutes may not truly reflect the skin transport behavior of large peptides such as insulin, unlike with smaller drug molecules. This necessitates the use of advanced techniques like capillary electrophoresis (VanOrman et al., 1995) to understand the complex behaviour of ions on iontophoretic transport of peptides.

Acknowledgements

This work is part of a grant from Department of Science and Technology (DST), New Delhi, India. The authors acknowledge the support of Board of Radiation and Isotope Technology (BRIT), Mumbai, India for supplying the radioiodinated insulin for the study. The useful discussions with Prof. Richard Guy, during the CRS symposium at Ooty, India in October, 2000 were helpful in preparation of this manuscript.

References

Banga, A.K., Chien, Y.W., 1993. Characterization of in vitro transdermal iontophoretic delivery of insulin. Drug Dev. Ind. Pharm. 19, 2069–2087.

Brange, J., Langkjaer, L., 1993. Insulin structure and stability. In: Wang, Y.J., Pearlman, R. (Eds.), Stability and Characterization of Protein and Peptide Drugs: Case Histories. Plenum Press, New York, pp. 315–350.

Cullander, C., Guy, R.H., 1992. Transdermal delivery of peptides and proteins. Adv. Drug Deliv. Rev. 8, 291–329.

Diem, K., Lenter, C., 1975. In: Geigy Scientific Tables. Geigy Pharmaceuticals, New York, pp. 280–281.

Gangarosa, L., Park, N., Wiggins, C., Hill, J., 1980. Increased penetration of non-electrolytes into mouse skin during iontophoretic water transport (iontohydrokinesis). J. Pharmacol. Exp. Ther. 212, 377–381.

Guy, R.H., Charro, M.B.D., Kalia, Y.N., 2001. Iontophoretic transport across the skin. Skin Pharmacol. Appl. Skin Physiol. 14, 35–40.

Hirvonen, J., Guy, R.H., 1997. Iontophoretic delivery across the skin: electroosmosis and its modulation by drug substances. Pharm. Res. 14, 1258–1263.

VanOrman, H.B., Liversidge, G.G., McIntire, G.L., 1995. The elctrophoretic mobility of tripeptides as a function of pH and ionic strength: comparison with iontophoretic flux data. Pharm. Res. 12, 751–755.

Jeffrey, P.D., Coates, J.H., 1966. An equilibrium ultracentrifuge study of the effect of ionic strength on the self-association of bovine insulin. Biochemistry 5, 3820–3823.

Kanikkannan, N., Singh, J., Ramarao, P., 1999. Transdermal iontophoretic delivery of bovine insulin and monomeric human insulin analogue. J. Control. Release 59, 99–105.

Kari, B., 1986. Control of blood glucose levels in alloxan-diabetic rats by iontophoresis of insulin. Diabetes 35, 217–221.

Kumar, S., Char, H., Patel, S., Piemontese, D., Iqbal, K., Malick, A.W., Neuroschel, E., Behl, C.R., 1992. Effect of iontophoresis on in vitro skin permeation of an analogue of growth hormone releasing factor in the hairless guinea pig model. J. Pharm. Sci. 88, 635–639.

Lelawongs, P., Liu, J.C., Chien, Y.W., 1990. Transdermal iontophoretic delivery of arginine-vasopressin (II): evaluation of electrical and operational factors. Int. J. Pharm. 61, 179–188.

Nair, V., Pillai, O., Poduri, R., Panchagnula, R., 1999. Transdermal iontophoresis. Part I. Basic principles and considerations. Methods Find. Exp. Clin. Pharmacol. 21, 139–151.

Oh, S.Y., Leung, L., Bommannan, D., Guy, R.H., Potts, R.O., 1993. Effect of current, ionic strength and temperature on the electrical properties of skin. J. Control Release 27, 115–125.

Panchagnula, R., Patel, J., 1997. Transdermal delivery of azidothymidine (AZT) through rat skin. Ex-vivo Pharm. Sci. 3, 83–87.

Panchagnula, R., Pillai, O., Nair, V.B., Ramarao, P., 2000. Transdermal iontophoresis revisited. Curr. Opin. Chem. Biol. 4, 468–473.

Phipps, J.B., Gyory, J.R., 1992. Transdermal ion migration. Adv. Drug Deliv. Rev. 9, 137–176.

Pikal, M.J., 1990. Transport mechanisms in iontophoresis. Part I. A theoretical model for the effect of electroosmotic flow on flux enhancement in transdermal iontophoresis. Pharm. Res. 7, 118–126.

Pillai, O., Nair, V., Pillai, O., Poduri, R., Panchagnula, R., 1999. Transdermal iontophoresis. Part II: Peptide and protein delivery. Methods Find. Exp. Clin. Pharmacol. 21, 229–240.

Rao, G., Gilkfeld, P., Guy, R.H., 1993. Reverse iontophoresis: development of a noninvasive approach for glucose monitoring. Pharm. Res. 10, 1751–1755.

Ritschel, W.A., Starzacher, A., Sabouni, A., Hussain, A.S., Kock, P., 1989. Percutaneous absorption of rosmarinic acid in the rat. Methods Find. Exp. Clin. Pharmacol. 11, 345–352.

Sage, B.H., 1993. Iontophoresis. In: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology, vol. 3. Marcel Dekker, New York, pp. 217–247.

Sato, S., Ebert, C.D., Kim, S.W., 1983. Prevention of insulin self-association and surface adsorption. J. Pharm. Sci. 72, 228–232.

Siddiqui, O., Sun, Y., Liu, J.C., Chien, Y.W., 1987. Facilitated transport of insulin. J. Pharm. Sci. 76, 341–345.

Sundby, F., 1962. Separation and characterization of acid induced insulin transformation products by paper electrophoresis in 7 M urea. J. Biol. Chem. 237, 3406–3411.

Wearley, L., Chien, Y.W., 1990. Enhancement of the in vitro skin permeability of azidothymidine (AZT) via iontophoresis and chemical enhancer. Pharm. Res. 7, 118–126.

Wearley, L., Liu, J.L., Chien, Y.W., 1989. Iontophoresis facilitated transdermal delivery of verapamil I. In vitro evaluation and mechanistic studies. J. Control. Release 8, 237–250.

Yoshida, N.H., Roberts, M.S., 1994. Role of conductivity in iontophoresis. Part 2. Anodal iontophoretic transport of phylethylamine and sodium across excised human skin. J. Pharm. Sci. 83, 344–350.

Yoshida, N.H., Roberts, M.S., 1995. Prediction of iontophoretic transport of various anions across excised skin from different vehicles using conductivity measurements. J. Pharm. Pharmacol. 47, 833–890.