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Research Article

# Human Insulin Diffusion Profile from a Gel Formulation through Hair-less Mouse Skin: Influence of Permeation Enhancers

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## Abstract

We investigated the diffusion profile of human insulin (HINS) through an excised hair-less mouse skin using Franz diffusion cells. We pre-treated the surface of the skin with a permeation enhancer or a control vehicle prior to applying a simple gel formulation (50 IU/mL) to the skin surface. One milliliter of the gel was placed on the surface of the skin, and samples were collected from the receiver side over a 7-hour period. The samples were analyzed on HPLC for their content of HINS. The results showed that pretreatment with a limonene solution in alcohol (5%) allowed the diffusion of 1.23 IU of HINS/cm2/ hour, which mimics the pancreatic secretion rate of insulin (0.25 – 1.85 IU/hour). The calculated permeability coefficient (kp) for HINS following pretreatment with limonene in alcohol (5%), iodine tincture, and ethyl acetate in alcohol (1:1) was 9.47, 4.42, and 2.78 ($10^{-6}$) cm/sec, respectively, with limonene alcoholic solution producing a statistically significantly higher kp value than the other two enhancers ($p < 0.0001$). The lag time for the three enhancers was 142.0, 78.6, and 121.0 minutes, respectively. All other enhancers and control vehicles did not show any significant practical diffusion of HINS through the mouse skin.

**Key Words:** Human Insulin; Permeation Enhancers; Diffusion; Franz Diffusion Cell

## Introduction

The skin represents a formidable barrier for the diffusion of many substances from entering the body, in particular those that are highly hydrophilic. For those compounds that can permeate the skin (normally low molecular weight, lipophilic, and low-melting point), their diffusion through the skin may be facilitated through the use of permeation enhancers. Diffusion enhancers work by reducing the diffusional resistance (various mechanisms) of the skin allowing molecules to travel easier through the skin layers. Typical factors that affect the diffusion of substances through the skin include the applied concentration, partition coefficient, surface area of the application, the thickness of the stratum corneum, and the degree of skin hydration, among others. Human insulin (HINS) is currently used in the management of patients suffering from diabetic mellitus. The only route of administration for HINS currently available in the United States is the parenteral route which requires the patient to self-inject the drug several times daily. Although inhaled HINS was commercially available for a short time in the United States, this form of administration has been withdrawn from the market by the manufacturer.[1,2] HINS is also available in some countries in the form of an aerosol for buccal administration.[3] Research to find a more convenient route of administration for HINS has been the subject of many intense investigations since 1960s. The pulmonary, oral, nasal, and transdermal routes have been among the most investigated, and excellent review papers on this research are available in the literature.[4-8] Traditional skin applications to the skin surface for delivering drugs include ointments, creams, pastes, and gels. Drugs are applied on the surface of the skin for external and local effect or for achieving a systemic effect. The latter is limited to drugs that can easily penetrate the stratum corneum (e.g., nitroglycerin, scopolamine). Attempts have been made to facilitate the diffusion of substances that are impermeable to the stratum corneum. Iontophoresis, electroporation, sonophoresis, microneedles, and the use of chemical permeation enhancers are just a few well documented methods for enhancing drug's diffusion through the skin.[6,9-15] In this study, we investigated the use of various chemical permeation enhancers on the diffusion of HINS through a hair-less mouse skin. Our aim was to compare these enhancers as to their ability to promote the diffusion of HINS through the skin. HINS, being a large molecule (MW 6000 Dalton) and highly hydrophilic,[16] is incapable for a significant spontaneous passive diffusion through the skin.

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## Material and Methods

### Materials

Human Insulin (Humulin R®, Eli Lilly) was purchased from NC Mutual, NC, Lot #A380572 and A383239). Phosphate buffer solution (10x; Lot # 1174B27) and sodium iodide (Lot # 47016 and 07243) were obtained from EMD Chemicals, Inc. (Gibbstown, NJ).The following chemicals were purchased from Sigma (St. Louis, MO): Carboxymethyl cellulose(Lot # 106H1220); Dimethyl sulfoxide (DMSO)(Lot# 46081651);Ethyl acetate (Lot# 06546CJ); Hexansulfonate Sodium (approximately 98%; Lot #016K542);Limonene (Lot# 09002MH); andLinolenic acid (Lot # 03833GY and 117K1043). Acetonitrile (HPLC grade; Lot # A998SK-4) and Trifluroacetic acid (Lot # 095279) were obtained from Fisher Scientific (Pittsburgh, PA). Propylene glycol (Lot # E34607) and iodine (Lot # X36598 and K38591) werepurchased from Baker (Phillipsburg,NJ).Alcohol, USP (Lot # E1049 and 05H15GB) was from Pharmaco-AAPER (Brookfield, CT). Franz cell was a PermeGear Amie System (model V3, Serial number 30911) from PermeGear, Inc. (Hellertown, PA).

### Methods

#### Preparation of gel dosage form for diffusion study

7.5 mg of carboxymethyl cellulose sodium (CMC) was dissolved in 25 mL of water to prepare a 30 % solution of CMC. To prepare the final gel (50 IU of HINS/mL), 2.0 mL of the CMC solution were mixed with 2.0 mL of Humulin R® solution (100 IU/mL).

#### Preparation of Phosphate buffer

Phosphate buffer used in this experiment was a 10x dilution purchased from Fisher Scientific. In order to make a 100 mL of phosphate buffer, 10 mL of the original buffer was mixed with sufficient water to make 100 mL of 1x buffer. The pH of the diluted buffer was 7.4.

**High Performance Liquid Chromatography Assay for Insulin**

Quantification of HINS in solution was made by an HPLC assay. The main components of this system included C-18 column (5 µ of length 250 mm and ID 4.6 mm manufactured by Altima) with a guard column (Alltech Altima pre-guard C-18 3µ of length 53mm and ID 7 mm); ConstaMetric 4100 solvent delivery system; Waters 717 plus Autosampler; Waters 746 Data Module; and a UV detector (Waters 2487 Dual λ absorbance detector).

The mobile phase consisted of acetonitrile: water: trifluroacetic acid: hexanesulfonic acid-sodium salt (30:70:0.1:0.1). The flow rate was 1 ml/min, the wavelength was set at 215 nm, and the injection volume was 20 µL. Insulin absorbance in solution showed a linear profile throughout a concentration range of 0.5 to 50 IU of HINS/mL.

**Animals and Treatment**

Thirty male MDX strain mice (10 weeks old) were purchased from Charles River Laboratory (Wilmington, Massachusetts). After being sacrificed with $CO_2$ asphyxiation, the skin was removed from the abdominal area(each mouse provided enough skin for three Franz cell experiments). The hair was shaved off using a scalpel. The skin was then placed immediately in a chilled 0.9 % NaCl solution to keep it moist. The skin was then taken out of solution, dried by applying two pieces of artificial membranes on the skin. Six different permeation enhancers were chosen for testing: DMSO, ethyl acetate, iodide tincture, limonene, linolenic acid, and propylene glycol. In addition to the enhancers, four control vehicles were tested: Alcohol, USP; purified water; alcohol:water (50:50); and alcohol (50 mL)/sodium iodide (2.1 g)/water (enough to make 100 mL) mixture. Iodine tincture was prepared by mixing 1.8 g iodine and 2.1 g sodium iodide in 50 mL of alcohol. The mixture was then made to 100 mL with purified water. A solution of ethyl acetate in alcohol was prepared with a concentration-strength of 1:1. Limonene was dissolved in alcohol to prepare a final solution of 5% v/v. The alcohol used in all the solutions was Alcohol, USP.DMSO, Linolenic acid, and propylene glycol were applied directly on the skin without dilution. All solutions were made fresh on the day of the experiment.

Skin was treated with the permeation enhancer or control solutionsbyapplying the solution in a parallel motionon the skin surface using a cotton swab (Q tips). Each time the permeation enhancer was applied the cotton swab was dipped in the solution for the next application and passed over a new uncovered area until the entire surface was completely covered with the solution. Then the treated skin was mounted immediately (within 2-3 minutes)on a Franz cell prior to starting the experiment.

**Franz Diffusion Cell**

The Franz diffusion cell was made and named after its physician developer, Dr. Thomas J. Franz, over three decades ago. With the assistance of Mr. Paul Lehman, Dr. Franz characterized the use of this innovation for topical drug delivery.[17] The use of Franz diffusion cell in research and development has become a common practice for studying drug permeation through the skin.[18] Permeation experiments with a three-station Franz diffusion cell was performed using the full-thickness hair-less mouse skin (approximately 150 µm thick). Before starting the experiment, the diffusion cell was turned on and equilibrated for 30 minutes to reach a temperature of 37°C. The entire experiment was run isothermally by circulating warm water (37°C) in an outer jacket surrounding each diffusion cell. Franz diffusion cell consisted of a donor (1 mL) and a receptor compartment (5 mL). Hair-less mouse skin was mounted between the donor compartment and the receptor compartment. The area for diffusion between the two chambers was 0.6936 $cm^2$. The volume of the liquid (enhancer or control solutions) covering this area of the skin was approximately 70 µL. The two compartments were held together with a metal clamp. Receptor compartment was filled with 5 mL of phosphate buffer (pH 7.4). HINS containing gel was placed in the donor compartment and was covered with a Parafilm® M (SPI Supplies Division; West Chester, PA) layer to prevent dryness. Receptor solution was continuously stirred by means of a spinning bar magnet at 600 rpm. Receptor solution samples (0.3 mL each) were withdrawn through the sampling port at 5 min, 1 hr, 2 hrs, 3.5 hrs 5 hrs, and 7 hrs. An equal volume to the withdrawn sample of fresh buffer was added to maintain the volume in the receptor chamber at 5 mL. The withdrawing tool consisted of a 1-mL plastic syringe fitted with a narrow plastic tube to allow sampling from a point in the solution just above the magnetic stirring bar. Samples along with standard HINS solutions were stored in the refrigerator (4°C) until the time of analysis. The role of the standard HINS solutions was to ensure the stability of HINS during the storage period. No degradation was detected during the storage time.

**Calculation of the Permeability Coefficient**

The diffusion data was fitted to the following mathematical model for diffusion in order to estimate the permeability coefficient value:

$$M = k_p S C_d (t - t_L)$$

Where, M is the cumulative amount of HINS diffused, $k_p$ is the permeability coefficient, S is the area of diffusion (0.6963 $cm^2$), $C_d$ is the initial concentration of HINS in the donor compartment (50 IU/mL), t is the time, and $t_L$ is the lag time.[19] The lag time relates to the permeability coefficient by the following mathematical expression:

$$t_L = h/6\ k_p$$

Where, h is the thickness of the barrier membrane. The lag time can also be calculated by extending the straight-line segment of the cumulative amount diffused vs. time curve to the x-axis. The point of intersection with x-axis is $t_L$.[19]JMP® Statistical Discovery Software (SAS Institute, Cary, North Carolina) was used for estimating the $k_p$ value.

Statistical Analysis

Unless otherwise indicated, data are presented as mean ± standard deviation. Differences among the groups were analyzed using an ANOVA test, whereas individual mean values were tested using a one-sample Student t-test. A *p* value less than 0.05 was considered significant. JMP® Statistical Discovery Software (SAS Institute, Cary, North Carolina) was used for all the descriptive and inferential statistics analysis.

## Results and discussion

Diabetic patients, in particular those who suffer from Type 1 diabetes, are required to administer HINS multiple times daily. This daily and repeated injection of HINS is associated with inconvenience, pain, and discomfort. Thus, finding an alternative route of administration for delivering HINS, other than the parenteral route, is highly desirable. In this study, the diffusion profile of HINS through full-thickness hair-less mouse skin layer was investigated in vitro. We employed six different permeation enhancers along with control solvent systems to quantify the cumulative amount of HINS diffusing from a gel formulation through a layer of mouse skin. The compounded gel

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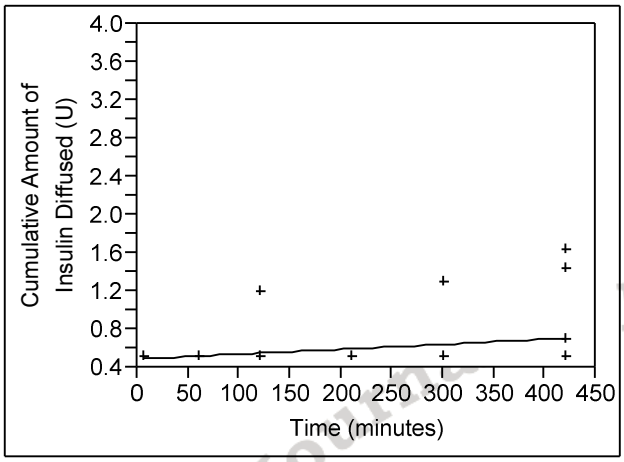


Figure1. The cumulative amount of insulin diffused over 7 hours for DMSO (—). Each data point is a single observation.

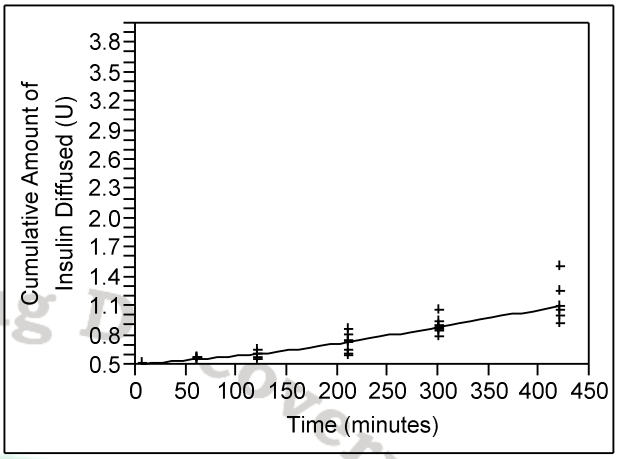


Figure2.The cumulative amount of insulin diffused over 7 hours for Linolenic Acid (—). Each data point is a single observation.

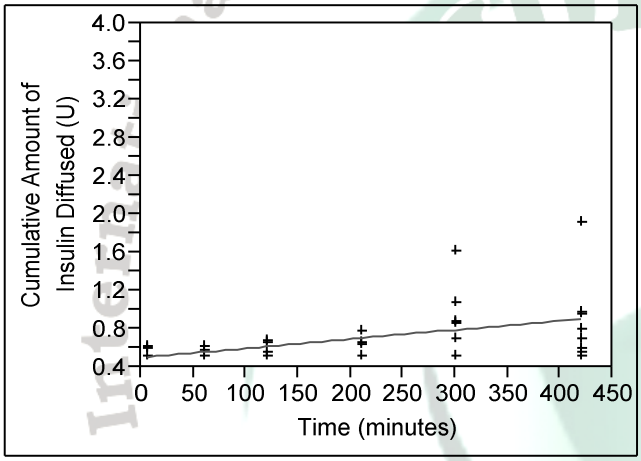


Figure 3. The cumulative amount of insulin diffused over 7 hours for propylene glycol (—). Each data point is a single observation.

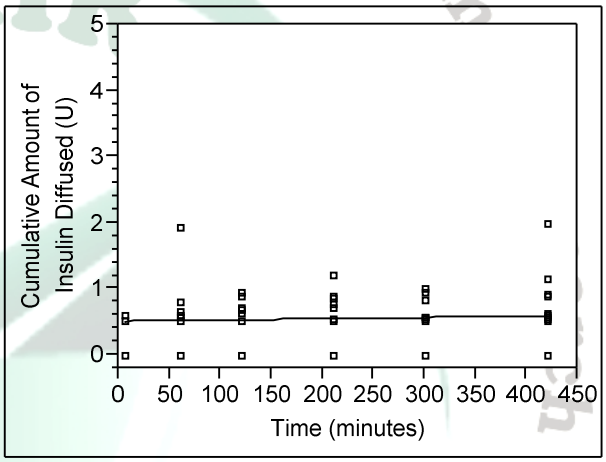


Figure 4. The cumulative amount of insulin diffused over 7 hours for water (—). Each data point is a single observation.

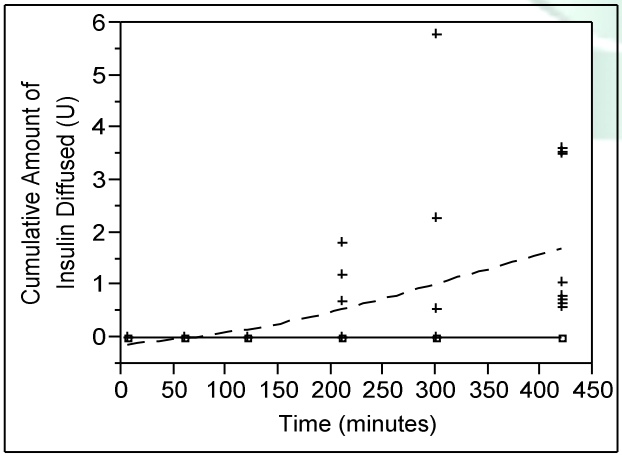


Figure 5. The cumulative amount of insulin diffused over 7 hours for ethyl acetate in alcohol (1:1) (----) and Alcohol, USP (—). Each data point is a single observation.

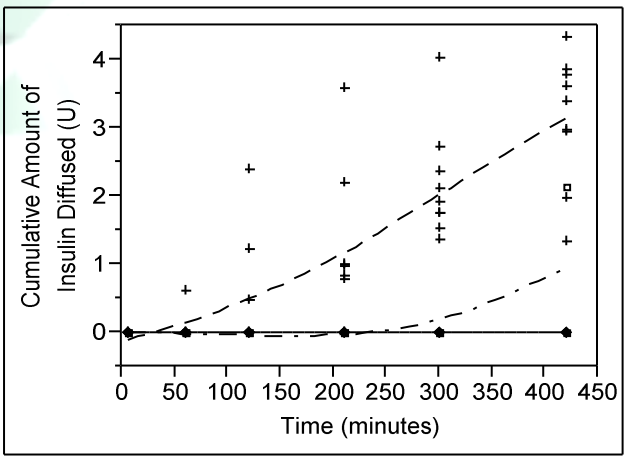


Figure 6. The cumulative amount of insulin diffused over 7 hours for iodine tincture (----), alcohol (50 mL)/sodium iodide (2.1 g)/water/100 mL solution (—), and alcohol:water (50:50) mixture (— • —). Each data point is a single observation.

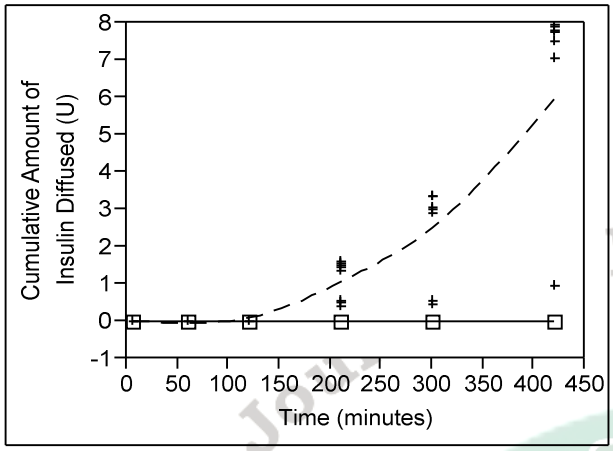


Figure 7. The cumulative amount of insulin diffused over 7 hours for limonene in alcohol (5%) (----) and Alcohol, USP (—). Each data point is a single observation.

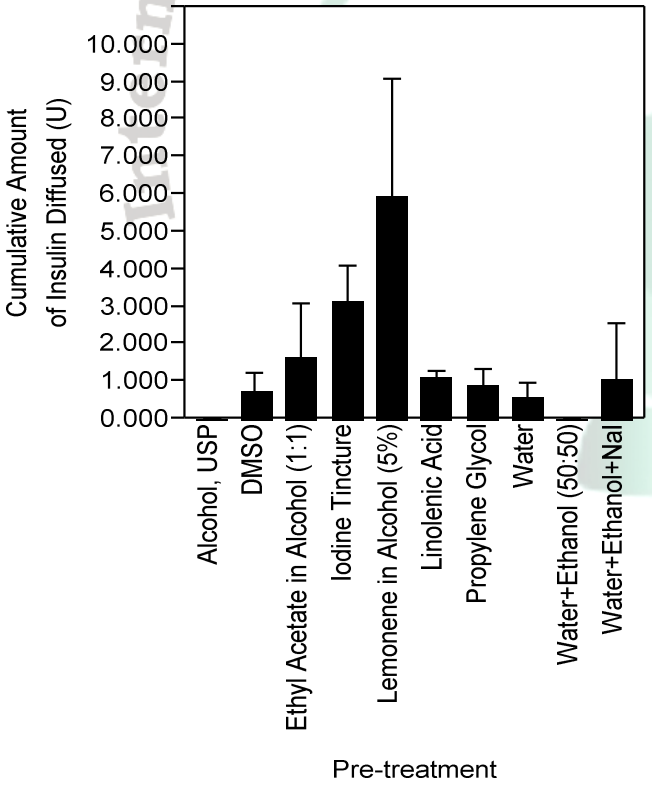


Figure 8. Cumulative amount of insulin (IU) diffused at 7-hour post-application. Error bars are onestandard deviation from the mean.

formulation contained carboxymethyl cellulose sodium (15% w/v) and HINS (50 IU/mL). In general, the use of gel as a transdermal delivery vehicle for drugs allows a better contact with the skin surface, permitting more efficient delivery.[20] Among the six enhancers used, no practical cumulative amount of HINS was detected with DMSO, linolenic acid, propylene glycol, or any control vehicle (Figures 1, 2, 3, and 4; Table 1). It is believed that DMSO produces a small, but reversible disruption of the lipid structures (mainly ceramides, cholesterol, and free fatty acids) within the stratum corneum.[21,22] The mode of action on drug diffusion enhancement of propylene glycol was described having a dual effect on lipid and keratin disruption of the stratum corneum.[23] It is believed that the degree of hydration of the stratum corneum plays a major role in the diffusion of solute through the skin. The higher the degree of hydration the better is the diffusion. This is perhaps due to the ability of water molecules to penetrate the skin and position themselves within the stratum corneum structure. This presence of water molecules within the membrane, however, was not found to cause any lipid or keratin disruption.[23] Pretreatment of the mouse skin with purified water enhanced the diffusion of HINS to some degree, albeit small (statistically significant at 7-hour; 0.12 IU/cm$^2$/hour; $p < 0.001$; Table 1); and for all practical reasoning, it is insignificant (Figure 4). The pretreatment with ethyl acetate (Figure 5), iodine tincture (Figure 6), and limonene (Figure 7) resulted in an increase in the cumulative amount of HINS diffused over a 7-hour period of approximately 3, 5, and 10 folds, respectively, when compared to that with water (Figure 8; Table 1). The use of alcohol alone or in a form of a hydroalcoholic solution had a negative effect on the diffusion of HINS. The presence of sodium iodide in a hydroalcoholic solution slightly improved the diffusion of HINS, however, not to the same extent as that of iodine tincture (about one-third of that of iodine tincture) (Figure 6). With respect to the enhancers used, limonene in alcohol (5%) showed the highest amount of HINS diffused (5.98 IU) and the lowest amount diffused was seen with DMSO (0.76 IU) (Figure 8; Table 1). It is interesting to note that the secretion rate of insulin by the pancreas in adults is in the range of 0.25 to 1.5 IU/hour, which places the delivery rate of insulin from our gel formulation in this normal range (1.23 IU/cm$^2$/hour) when limonene is pre-applied on the skin surface.[24]However, more in vivo research is needed to ascertain whether the two modes are physiologically equivalent. Among the three enhancers that showed any significant practical diffusion, limonene in alcohol (5%) showed the highest permeability coefficient for HINS through the mouse skin (9.47 x $10^{-6}$cm/sec) and the lowest was that with ethyl acetate in alcohol (1:1) (2.8 x $10^{-6}$ cm/sec); this difference was statistically significant ($p < 0.0001$). Rastogi and Singh (2003) observed a similar effect of limonene on the diffusion of insulin through porcine epidermis in a Franz diffusion cell model.[25]Ogiso et al. (1996) tested limonene as a diffusion enhancer for a gel formulation containing insulin applied on the skin of Wistar rat.[26] They reported a significant hypoglycemic effect lasting over 10 hours and a bioavailability of insulin from this formulation to be 20.7% ± 4.6%. The pretreatment with an alcoholic solution of limonene or linolenic acid was shown to produce an expansion in the stratum corneum and a more porous intracellular matrix,[27] with little interaction with the membrane lipids.[28] In our study, linolenic acid did not produce a significant effect on the diffusion of HINS through the skin. Iodine tincture was similar in its effect on diffusion rate to ethyl acetate in alcohol (1:1), despite its apparent higher permeability coefficient

value (4.4 x $10^{-6}$ cm/sec) (Table 2). The time for HINS to achieve a uniform concentration gradient within the mouse skin layer ($t_L$) was the highest with limonene (142 minutes), followed by ethyl acetate (121 minutes), and the lowest with iodine tincture (78.6 minutes) (Table 3). This is significant, because $t_L$is the time point where the drug's diffusion through the membrane reaches equilibrium(i.e., becomes linear, according to Fick's law for diffusion.) In the case of iodine tincture, it was suggested that iodine deactivated HINS degradation mechanisms within the skin (e.g., reduction in glutathione concentration), so that more HINS was available for permeating the skin layers.[29] Pretreatment with an iodine solution was also shown to produce hypoglycemia in streptozocin-diabetic rats upon applying 0.5 mL of Humulin® R to the hair-less abdominal area of skin (50 IU; diffusion area of 1.33 $cm^2$);a 90% decrease in blood glucose was observed after 4 hours post HINS treatment.[29] For ethyl acetate in alcohol (1:1) solution, the mechanism of action by which this solvent mixture acted was perhaps due to a lipid-extraction effect on the skin.[30] In general, for enhancing the diffusion of drugs through the skin, mechanisms that increase the partition, the diffusion, and/or the solubility of the drug may be utilized in that regard.[31] Some mechanisms of action of the permeation enhancers were suggested to involve dilation of the intercellular lipid spaces within the stratum corneum, an increase in the fluidity of cellular membrane lipids, and/or the removal intercellular lipids, thus reducing the diffusional resistance and facilitating the transdermal diffusion of drugs.[32,33] These mechanisms collectively or individually could have influenced the partitioning, the diffusion, and/or the solubility of HINS in and through the stratum corneum. In this in vitro study, the results suggest that pre-treatment of the skin with limonene in alcohol (5%) solution has the potential for delivering HINS transdermally within a normal, therapeutic range.

## Conclusion

The diffusion of HINS through mouse skin was facilitated by pretreatment with limonene in alcohol (5%), Iodine tincture, or ethyl acetate in alcohol (1:1). The highest amount of HINS diffused was seen with limonene in alcohol (5%) at a rate of 1.23 IU/$cm^2$/hour, which mimics the pancreatic insulin secretion. HINS was prepared in a simple gel formulation which allowed intimate contact with the skin surface. The application of this gel formulation on an area of the skin pretreated with a limonene solution in alcohol (5%) has the potential to deliver HINS transdermally.

## References

1) Quattrin, T. Inhaled insulin: a novel and non-invasive way for insulin administration? Curr Drug Saf 2006;1(2):151-158.
2) Brain, JD. Inhalation, deposition, and fate of insulin and other therapeutic proteins. Diabetes TechnolTher 2007;9(Suppl 1):S4-S15.
3) Bernstein, G. Delivery of insulin to the buccal mucosa utilizing the RapidMist system. Expert Opin Drug Deliv 2008;5(9):1047-1055.
4) Yadav, N; Morris, G; Harding, SE; Ang, S; and Adams, GG. Various non-injectable delivery systems for the treatment of diabetes mellitus. Endocr Metab Immune Disord Drug Targets 2009;9(1):1-13.
5) Heinemann, L; Pfützner, A; and Heise, T. Alternative routes of administration as an approach to improve insulin therapy: update on dermal, oral, nasal, and pulmonary insulin delivery. Curr Pharm Des 2001;7(14):1327-1351.
6) Prausnitz, MR and Langer, R. Transdermal drug delivery. Nat Biotechnol 2008;26(11):1261-1268.
7) Werle, M; Mkhlof, A; and Takeuchi, H. Oral protein delivery: a patent review of academic and industrial approaches. Recent Pat Drug Deliv Formul 2009;3(2):94-104.
8) Ramesan, RM and Sharma, CP. Challenges and advances in nanoparticle-based oral insulin delivery. Expert Rev Med Devices 2009;6(6):665-676.
9) Liedtke, RK; Suwelack, K; and Karzel, K. Effect of preoral and transdermal insulin preparations on blood glucose concentration in mice. Arzneimitte Iforschung 1990;40(8):880-883.
10) Batheja, P; Thakur, R; and Michniak, B. Transdermal iontophoresis. Expert Opin Drug Deliv 2006;3(1):127-138.
11) Tokumoto, S; Higo, N; Sugibayashi, K. Effect of electroporation and pH on the iontophoretic transdermal delivery of human insulin. Int J Pharm 2006;326(1-2):13-19.
12) Dixit, N; Bali, V; Baboota, S; Ahuja, A; and Ali, J. Iontophoresis – an approach for controlled drug delivery: a review. Curr Drug Seliv 2007;4(1):1-10.
13) Rao, R and Nanda, S. Sonophoresis: recent advancements and future trends. J Pharm Pharmacol 2009;61(6):689-705.
14) Chen, H; Zhu, H; Zheng, J; Mou, D; Wan, J; Zhang, J; Shi, T; Zhao, Y; Xu, H; and Yang, X. Iontophoresis-driven penetration of nanovesicles through microneedle-induced skin microchannels for enhancing transdermal delivery of insulin. J Control Release 2009;139(1):63-72.
15) Roxhed, N; Samel, B; Nordquist, L; Griss, P; and Stemme, G. Painless drug delivery through microneedle-based transdermal patches featuring active infusion. IEEE Trans Biomed Eng 2008;55(3):1063-1071.
16) Ruchalski, C and Mancano, MA. Hormones and hormone antagonists. In Remington: The science and practice of pharmacy; 21st Edition; Lippincott Williams & Wilkins, 2006; p. 1437-1473.
17) Raney, S; Lehman, P; and Franz, T. 30th anniversary of the Franz cell finite dose model: the crystal ball of topical drug development. Drug Delivery Technology 2008;8(7):32-37.
18) Bucks, D. Rational & design of studies to screen & identify promising topical formulations. Drug Delivery Technology 2008;8(8):44-47.
19) Amiji, MM. Mass Transport. In Applied Physical Pharmacy, Mansoor M. Amiji and Beverly J. Sandmann; McGraw-Hill, New York; 2003; p. 285-309.
20) Pillai, O and Panchagnula, R. Transdermal delivery of insulin from poloxamer gel: ex vivo and in vivo skin permeation studies in rat using iontophoresis and chemical enhancers. J Control Release 2003;89(1):127-140.
21) Al-Saidan, SM; Winfield, AJ; and Selkirk, AB. Effect of preheating on the permeability of neonatal rat stratum corneum of alkanols. J Invest Dermatol 1987;89(4):430-433.

Table 1.Cumulative amount of insulin diffused at 7-hour post-administration.

| Enhancer Type | n[a] | Cumulative Amountof Insulin Diffused (IU) (Mean ± S.D.) | Overall rate IU/cm$^2$/hour | *p*Value[b] |
|---|---|---|---|---|
| Alcohol, USP | 3 | 0 ± 0 | 0 | — |
| DMSO | 9 | 0.76 ± 0.45 | 0.16 | 0.0009 |
| Ethyl Acetate | 9 | 1.67 ± 1.42 | 0.34 | 0.0076 |
| Iodine Tincture | 9 | 3.13 ± 0.96 | 0.64 | < 0.0001 |
| Limonene | 8 | 5.98 ± 3.11 | 1.23 | 0.0010 |
| Linolenic Acid | 9 | 1.10 ± 0.19 | 0.22 | < 0.0001 |
| Propylene Glycol | 9 | 0.89 ± 0.42 | 0.18 | 0.0002 |
| Purified Water | 29 | 0.60 ± 0.34 | 0.12 | < 0.0001 |
| Water:Alcohol (50:50) | 2 | 0 ± 0 | 0 | — |
| Alcohol/Water/NaI | 2 | 1.07 ± 1.50 | 0.22 | 0.5000 |

[a] n is the number of replicates. Each replicate was a single Franz cell.
[b] Tested whether the mean of the cumulative amount of insulin was zero for each enhancer type.

Table 2. The permeability coefficient ($k_p$) (cm/sec) of human insulin through hair-less mouse skin pre-treated with permeability enhancers.

| Permeability Enhancer | n | $k_p$ (cm/sec) (x $10^{-6}$) | 95% C.I. (x $10^{-6}$) | *p*Value[a] |
|---|---|---|---|---|
| Ethyl Acetate in Alcohol (1:1) | 9 | 2.78 | [0.99 – 4.58] | 0.0034 |
| Iodine Tincture | 9 | 4.42 | [3.06 – 5.79] | <0.0001 |
| Limonene in Alcohol (5%)[b] | 8 | 9.47 | [6.80 – 12.12] | <0.0001 |

[a] Tested whether the value of $k_p$ was zero for each enhancer type. A zero value for $k_p$signified no permeation.
[b] Different from Iodine Tincture and Ethyl Acetate in Alcohol (1:1) ($p$< 0.0001). There was no statistical difference between Iodine Tincture and Ethyl Acetate in Alcohol (1:1).

Table 3. Lag time ($t_L$) in minutes of human insulin through hair-less mouse skin in the presence of permeability enhancers.

| Permeability Enhancer[a] | n | $t_L$ (minutes) |
|---|---|---|
| Ethyl Acetate in Alcohol (1:1) | 9 | 121.0 |
| Iodine Tincture | 9 | 78.6 |
| Lemonene in Alcohol (5%) | 8 | 142.0 |

[a] Tested whether the value of $k_p$ was zero for each enhancer type. A zero value for $k_p$signified no permeation.
[b] Different from Iodine Tincture and Ethyl Acetate in Alcohol (1:1) ($p$< 0.0001). There was no statistical difference between Iodine Tincture and Ethyl Acetate in Alcohol (1:1).

22) ten Grotenhuis, E; Demel, RA; Ponec, M; Boer, DR; van Miltenburg, JC; and Bouwstra, JA. Phase behavior of stratum corneum lipids in mixed Langmuir-Blodgett monolayers. Biophys J 1996;71:1389-1399.
23) Cornwell, PA; Barry, BW; Stoddart, CP; and Bouwstra; JA. Wide-angle X-ray diffraction of human stratum corneum: effects of hydration and terpene enhancer treatment. J Pharm Pharmacol 1994;46(12):938-950.
24) Sevast'ianov, VI; Salomatina, LA; Kuznetsova, EG; Iskovleva, NV; and Shumakov, VI. Transdermal delivery of insulin. Med Tekh 2003;2:21-
25) Rastogi, SK and Singh, J. Effect of chemical penetration enhancer and iontophoresis on the in vitro percutaneous absorption enhancement of insulin through porcine epidermis. Pharm DevTechnol 2005;10(1):97-104.
26) Ogiso, T; Nishioka, S; and Iwaki, M. Dissociation of insulin oligomers and enhancement of percutaneous absorption of insulin. Biol Pharm Bull 1996;19(8):1049-1054.
27) Bhatia, KS and Singh, J. Effect of Linolenic acid/ethanol or limonene/ethanol and iontophoresis on the in vitro percutaneous absorption of LHRH and ultrastructure of human epidermis.Int J Pharmaceut 1999;180:235-250.
28) Yamane, MA; Williams, AC; and Barry, BW. Terpene penetration enhancers in propylene glycol/water co-solvent systems: effectiveness and mechanism of action. J Pharm Pharmacol 1995;47(12A):978-989.
29) Sintov, AC and Wormser, U. Topical iodine facilitates transdermal delivery of insulin. J Control Rel 2007;118:185-188.
30) Rastogi, SK and Singh, J. Transepidermal transport enhancement of insulin by lipid extraction and iontophoresis. Pharm Res 2002;19(4):427-433.
31) Hadgraft, J. Modulation of the barrier function of the skin. Skin PharmacolAppl Skin Physiol 2001;14(Suppl 1):72-81.
32) Choi, EH; Lee, SH; Ahn, SK; and Hwang, SM. The pretreatment effect of chemical skin penetration enhancers in trandermal drug delivery using iontophoresis. Skin Pharmacol Appl Skin Physiol 1999;12960;326-335.
33) Ogiso, T. and Tanino, T. Transdermal delivery of drugs and enhancement of percutaneous absorption. Yakugaku Zasshi 2000;120(4):328-338.