Design and development of insulin emulgel formulation for transdermal drug delivery and its evaluation

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Abstract: The objective of the present study was to formulate an insulin emulgel, selection of an optimize formulation through *in vitro* drug release kinetics and finally evaluate its hypoglycemic activity in animal model.
Insulin emulgel was prepared using Emu Oil as penetration enhancer with the combination of Carbomer or Hydroxypropyl Methylcellulose (HPMC) as gelling agent and Polysorbate 80 as emulsifier. The response of gelling agent type (Carbomer or HPMC) and concentration of other two variables penetration enhancer and emulsifier were studied using 2^3 factorial design during *in vitro* drug release through excised rat skin. Biological activity of emulgel formulation was also investigated using Albino rabbits alone and in combination with iontophoresis. The in vivo efficacy of insulin emulgel was assessed by measuring the blood glucose level at start of the experiment and after every 15 minutes interval for 120 minutes.
Total eight formulations were studied. F4 formulation showed maximum insulin permeation flux (4.88±0.09 µg/cm^2/hour) through excised rat skin. Insulin permeation from these formulations was found to follow the Korsmeyer-Peppas model (r^2 =0.975 to 0.998) during 24 hour with non-Fickian mechanism. Formulation F4 was further investigated in Albino rabbits. For the first group (treated with insulin emulgel alone) the blood glucose level decreased from initial value 250±10mg/dl to 185±7mg/dl at 120 minutes and for the second group (treated with insulin emulgel plus iontophoresis) the blood glucose level decreased to 125±5mg/dl in 120 minutes ($P<0.05$).
It was observed that absorption of insulin through transdermal emulgel was greater in combination with iontophoresis to decrease blood glucose level. On the basis of this study, it has been shown that application of insulin emulgel iontophoretically can be used as alternative (acceptable & painless) to injectable insulin subject to further studies on large animals.

Keywords: Insulin delivery, factorial design, Emu Oil, transdermal emulgel, drug release kinetics and iontophoresis.

INTRODUCTION

The development of drug delivery methods for the drug products having poor absorption and enzymatic degradation during oral delivery and poor patient compliance while given parenterally has been a challenge for the pharmaceutical community. Transdermal drug delivery is an attractive system that meets all above challenges along with the controlled delivery of drugs and also it is non-invasive, having reduced side effects, improved bioavailability and easy termination of drug therapy. Despite of these advantages, transdermal drug delivery is severely limited because of stratum corneum which has very low permeability. The stratum corneum is first layer from outside (defined as brick and mortar type structure) and mainly constituted of dead cells called corneocytes "bricks" which are scattered in a lamellar lipid bilayer "mortar" and latter is responsible for barrier properties of stratus corneum (Elias, 1983; Pirot *et al*., 1997).

Drug delivery through skin is usually take place either from sweat ducts, hair follicles, sebaceous glands or directly by diffusing through stratum corneum. Various scientists worked on relative importance of drug delivery through hair follicles or sebaceous glands versus stratum corneum in the literature (Flynn, 1985; Kasting *et al*., 1992; Potts and Guy, 1992) and however there is a need to develop experimental setup to find out mechanism of drug delivery by the each route.

Various dosage forms like ointment, emulsion and gel are applied for transdermal delivery of the drugs. As emulsions are concerned, both types of emulsions (O/W & W/O) are used as vehicles in transdermal drug delivery system (Eccleston, 1992) and also they have certain degree of elegance and are easily washable. Gel formulations are favorable based on their properties like easily removable, thixotropic behavior, emollient nature, compatibility and miscibility with other ingredients (Klich, 1992). Emulgels are formed by combine formulation of both emulsion and gel in a single dosage form and its excellent patient compliance is based on above mentioned merits of both.

Significant efforts have been devoted in the literature to minimize skin impermeability and enhance delivery of drugs through transdermal route. Various methods have been described to overcome the barrier properties of skin for transdermal drug delivery and can be classified in two

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main groups which are either passive (penetration enhancer, supersaturated system, prodrugs or metabolic approaches, liposomes and other vesicles) or active methods (Electroporation, Iontophoresis, Ultrasound "Sonophoresis and Phonophoresis", Laser Radiation and Photomechanical Waves, Radio-Frequency, Magnetophoresis, Temperature "Thermophoresis", Microneedle-Based Devices, Skin Puncture and Perforation, Needleless Injection, Suction Ablation, Application of Pressure, Skin Stretching, Skin Abrasion) (Kewal and John, 2008). These methods share a common goal to permeabilize the skin by creating nanometer-scale disruptions of stratum corneum structure. Despite progress using these techniques, it remains a significant challenge to deliver macromolecules into the skin.

Insulin is a peptide hormone (produced in the pancreas by the islets of Langerhans) composed of 51 amino acids (two amino acid chains connected to one another by disulfide linkages) having a MW of 5808 Daltons. There is variation in insulin structure obtained from different sources (animal species) and also differs in hypoglycemic activity in human because of the structure configuration. Integrity of insulin molecule must be maintained for biological activity (Sanger, 1960 and Nicols, 1960).

Emu Oil is composed of fatty acids obtained from the emu. Emu is a bird known as *Dromaius Novae-Hollandiae,* (Snowden and Whitehouse, 1997) which is a native of Australia. It has various medicinal applications like muscle and joint pain treatment and also in skin disorder like eczema and burn because of its excellent skin penetration ability. As per International Emu Oil Standards, emu oil contains following fatty acids in high proportion like oleic, palmitic linoleic, stearic acid and it also contains small fraction of linolenic, palmitoleic, myristic acids. Emu oil has high proportion of oleic acid among all of the fatty acids (American Emu Association, 1997 and Minnaar, 1997).

In the present study an attempt was made to investigate transdermal delivery of insulin from emulgel formulation. Emu oil was selected as penetration enhancer because of it's high Oleic acid contents and capability to penetrate the skin owing to its non-phosphorous composition. Optimize formulation containing penetration enhancer, emulsifier and gelling agent was selected during *in vitro* testing and further studied its pharmacodynamic response in Albino rabbits. The combination effect of iontophoresis and insulin emulgel formulation was also studied.

MATERIALS AND METHODS

Materials

Bovine insulin (≥ 27 units per mg) and Isopropyl Myristate were obtained from Sigma-Aldrich, Inc. Emu Oil was purchased from Uniquely Emu Products, Inc. Methyl Paraben and Propyl Paraben were obtained from Ueno Fine Chemicals Industry Ltd. Carbomer was obtained from Corel Pharma Chem. Hydroxypropyl methylcellulose and Propylene Glycol was obtained from Dow Chemical Company. Polysorbate 80 was purchased from Croda International. Other chemicals and reagents used in the experiments were met the quality of analytical grade.

Design of experiment

Insulin emulgel formulation was developed for *in vitro* permeation study with the help of systematic formulation approach. Experiments were performed according to 2-level factorial design to study the effect of three formulation variables on *in vitro* permeation of insulin emulgel. The concentration of Emu oil (X_1) & Polysorbate 80 (X_2) were two numeric factors and type of gelling agent HPMC or Carbomer (X_3) was categoric factor (independent variables). The studied response was the permeation of insulin (Y) from emulgel formulation (dependent variable). All eight possible experimental trials were performed (Bolton, 1990; Franz *et al*., 1988).

Independent & dependent variables along with their levels, qualitative composition and matrix of the factorial design are shown in table 1.

Preparation of Insulin Emulgel

Insulin Emulgel was prepared by dissolving Methyl Paraben & Propyl Paraben in hot purified water following by dispersing Carbomer (for formulation F1 – F4) or Hydroxypropyl methylcellulose (for formulation F5 – F8) with continuous mixing. Solution was allowed to cool at room temperature with continuous mixing. Emu oil, Isopropyl Myristate, Propylene Glycol and Polysorbate 80 were mixed together and transfer into Carbomer or Hydroxypropyl methylcellulose solution with continuous stirring and finally Insulin was added and mixed well. Sodium Hydroxide was added drop wise with gentle stirring till smooth emulgel was obtained at a pH of 6.5 to 7.0. Final volume was adjusted using Purified water and mixed well.

Preparation of skin membrane

Albino rats were selected for permeation study. They were sacrificed using anesthetic ether. Then the skin was excised and it was cleaned of fatty layer, adhering to the dermis side, and finally cut into small pieces (Scott *et al*, 1986).

In vitro study design

Franz diffusion cell (orifice diameter 20mm equal to surface area 3.14cm^2) was used to measure the penetration of insulin in emulgel formulations *in vitro*. The prepared skin membrane of rat was arranged at the receptor compartment of Franz diffusion cell, where the stratum corneum side was kept towards donor compartment. Saline phosphate buffer pH 7.4 was added in receptor

compartment, placed over magnetic stirrer and the temperature was kept at 37 ± 0.5°C and observed during the whole experiment. One gram of insulin emulgel formulation was placed on the skin membrane surface. At appropriate time intervals (1, 2, 3, 4, 5, 6, 9, 12, 18 & 24 hr), 1 ml sample was taken from the diffusion cell and same quantity was replaced with buffer solution (Hayton and Chen, 1982). The amount of permeated drug was measured using UV Visible spectrophotometer (Shimadzu UV-1601, Japan) by measuring absorbance at λ_{Max} 214 nm.

Data Analysis

Amount of insulin from emulgel formulations permeated during *in vitro* studies was evaluated by applying following mathematical models:

- Zero order ($C = k_0 t$)
- Higuchi's model ($Q = k_H t^{1/2}$)
- Korsmeyer- peppas model ($M_t / M_\infty = bt^n$)

If the value of n is less than 0.5, it indicates drug follow the release mechanism called Fickian diffusion and if its values lies in between 0.5 – 1.0 then it follows anomalous or non-Fickian transport (Patel *et al*., 2007).

Permeation flux

During *in vitro* studies, amount of Insulin penetrated through rat skin was plotted against sampling time. Relation between both variables was studied by regression. Regression analysis was applied to derive slop and intercept of linear portion and finally slope was divided by exposed area of skin to determined permeation flux.

$$\text{Permeation flux } (\mu g/cm^2/hour) = \frac{\text{Quantity of insulin penetrated in unit time } (\mu g/hour)}{\text{Area of skin membrane } (cm^2)}$$

HPLC analytical method (Insulin emulgel formulation)

Insulin content in the emulgel formulation was determined by HPLC during stability studies. 1 gm of each sample was taken in 10ml volumetric flask, mixed with 0.1 N HCl for 10 minutes and then centrifuge at 13,000RPM for 10 minutes. The supernatant layer was collected separately and analyzed through HPLC system. 10 mg working standard was weighed and dissolved in 100ml volumetric flask containing 0.1N HCl and mix for 10minutes. The configuration of HPLC system used was a pump (Model 1500, Lab Alliance), variable wavelength UV-Vis detector (Model 500, Lab Alliance) controlled by HPLC integration software manufactured by Birds Chemotec. Analysis was done at wavelength 214 nm and separation was obtained by using mobile phase which was consisted of 45:55 v/v 1.6% aqueous solution of Sodium sulphate & acetonitrile with pH 2.3 (adjusted by phosphoric acid) and flow rate of 1ml/min using analytical column ($C_{18,}$ 10 µm, 4.6 x 250 mm).

In vivo study design (Hypoglycemic activity in diabetic rabbits)

Animal selection: 18 Albino rabbits (1.5-1.6 Kg) were used in experiment. The experimental protocol for hypoglycemic activity *in vivo* (using animal model) was approved by Institutional Animal Ethical Committee, University of Karachi. The rabbits were divided into two groups

Group I: Insulin emulgel was applied lightly by rubbing on thigh area of rabbit for approx: 2 minutes.

Group II: Insulin emulgel was applied in combination with iontophoresis.

Induction of Hyperglycemia: All the rabbits were anesthetized by using Ketamine (100mg/Kg) and Xylazine (10mg/Kg). Ketamine/Xylazine were used to cause acute but sustained hyperglycemia (Saha *et al*., 2005).

Current density during iontophoresis

Insulin emulgel was applied in combination with iontophoresis at current density 0.5mA/cm^2 (Brand and Iversen, 1996). Only direct current was used throughout the experiment.

Preparation of electrodes

Electrodes (Silver/Silver Chloride) were fabricated using silver wire (0.5mm in diameter). Two wires were used; first these were cleaned by rubbing with abrasive paper and then wiped with alcohol. The wires were mounted in a beaker containing 0.1 M HCl in such a way that the most of the lengths of wires were immersed, without touching each other. One wire was connected to positive terminal and other one was connected to negative terminal of a 1.5V battery. Current was allowed to flow, after few minutes wire connected to the positive terminal becomes coated with a layer of silver chloride and H_2 gas bubbles were liberated from the other wire. Process was continued till uniform dark grayish purple color coating layer was produced (David *et al*., 1995).

Blood glucose monitoring

Prior to beginning the experiments, each rabbit was checked for glucose level by taking blood sample from ear vein. Optium Omega blood glucose monitoring system (Abbott Laboratories) was used for the purpose and data recorded in measuring unit (mg/dl). During experiments, blood glucose level monitoring was continued after every 15 minutes interval for upto 120 minutes.

Stability studies

Insulin Emulgel formulation was primary packed in clean, dry, airtight, light and moisture resistant container and stored at 2-8°C for stability studies. Samples were withdrawn at predetermined intervals (1, 2, 3 and 6

months) and evaluated for physical appearance (visually inspected for any change in color and odor), drug content and pH.

STATISTICAL ANALYSIS

All the observations were made in thrice ± the standard deviation (SD) and difference between both groups were analyzed using one way ANOVA (one-way analysis of variance). It was considered significant with $P<0.05$.

RESULTS

Insulin emulgel formulations were prepared using 2^3 factorial design (table 1) where three independent variables were selected (Emu oil was used as penetration enhancer = X_1, Polysorbate 80 as emulsifier = X_2 and Carbomer or Hydroxyproyl methylcellulose as gelling agent = X_3) to check one response (insulin permeation = Y). Total eight formulations were derived, prepared and investigated through *in vitro* testing from excised rat skin using Franz diffusion cell. Quantitative composition of formulations and response data is presented in table 2. Interaction of independent variables on quality of response was studied by using trial version of Design Expert® Software (Version 8.07.1, State-Ease Inc., Minneapolis, MN) and interaction graphs along with response surface plot and observed & predicted value are presented in fig. 2.

Each formulation was applied and amount of insulin permeated through skin membrane over 24 hour was plotted against the sampling time (as shown in fig. 1). Permeation flux (μg/cm^2/hr) was calculated for all formulations (table 2). Highest permeation flux 4.88 ± 0.09μg/cm^2/hr was observed for formulation F4 which contains maximum concentration of Emu Oil (7.5%) and Polysorbate 80 (5.0%) and Carbomer as gelling agent. Formulation F8 also contains same quantities of Emu Oil and Polysorbate 80 except gelling agent which was HPMC but permeation flux was noted 4.38 ± 0.07μg/cm^2/hr.

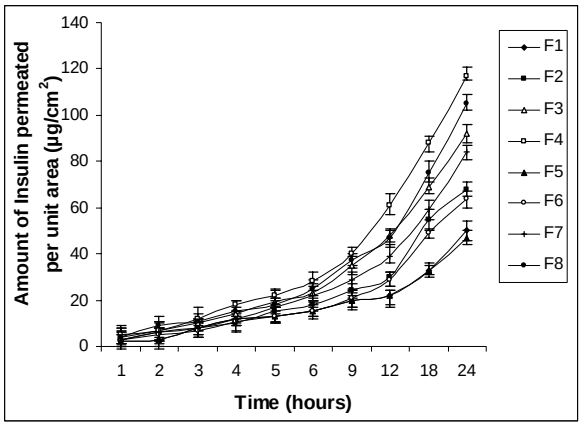

Fig. 1: *In vitro* insulin permeation profile through excised rat skin per unit area from Insulin Emulgel Formulations (mean ± SD, n = 3).

Transdermal delivery of insulin from emulgel formulation (F4) was further studied in rabbits. There were two groups

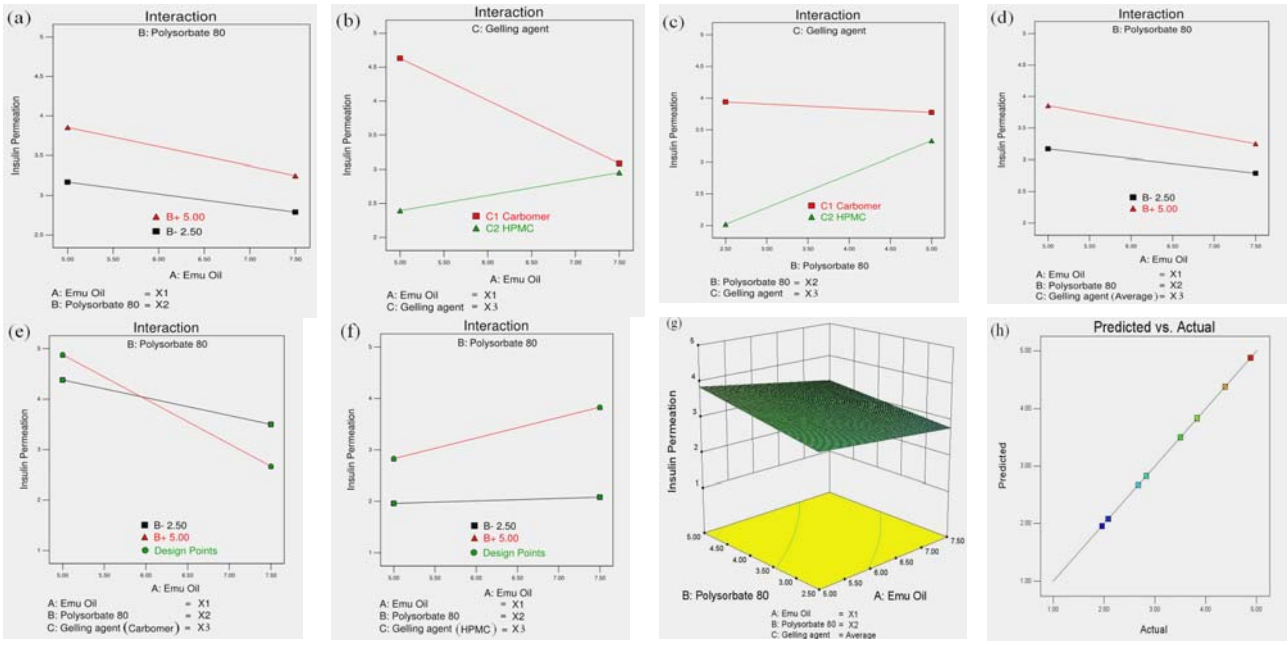

Fig. 2: (a) Interaction of X_1 & X_2 factors (b) Interaction of X_1 & X_3 factors (c) Interaction of X_2 & X_3 factors (d) Interaction of X_1, X_2 & X_3 factors (e) Interaction of X_1, X_2 & X_3 (X_3= Carbomer) factors (g) Interaction of X_1, X_2 & X_3 (X_3= HPMC) (g) Response surface plot showing the effect of X_1 & X_2 factors (h) Linear plot between observed and predicted value of response.

Table 1: 2^3 factorial design (factors and their levels), qualitative compositions and the matrix of factorial design

A 2^3 factorial design factors and level	Factors		Type of Factors	Response (Y)	Levels	
					(−)	(+)
	X_1 = Penetration enhancers (Emu Oil)		Numeric	Insulin Permeation	5.0 %	7.5 %
	X_2 = Emulsifier (Polysorbate 80)		Numeric		2.5 %	5.0 %
	X_3 = Gelling agent:	(HPMC)	Categoric		------	2.0 %
		(Carbomer)			1.5 %	------

Qualitative composition of formulation (factors & levels)	Formulation Code	Composition					
		Coded Values			Actual Values		
		X_1	X_2	X_3	X_1	X_2	X_3
	F1	−	−	−	5.0 %	2.5 %	1.5 %
	F2	+	−	−	7.5 %	2.5 %	1.5 %
	F3	−	+	−	5.0 %	5.0 %	1.5 %
	F4	+	+	−	7.5 %	5.0 %	1.5 %
	F5	−	−	+	5.0 %	2.5 %	2.0 %
	F6	+	−	+	7.5 %	2.5 %	2.0 %
	F7	−	+	+	5.0 %	5.0 %	2.0 %
	F8	+	+	+	7.5 %	5.0 %	2.0 %

The matrix of Factorial Design

Formulation Code	X_1	X_2	X_3	$X_2 X_1$	$X_1 X_3$	$X_2 X_3$	$X_1X_2X_3$
F1	−	−	−	+	+	+	−
F2	+	−	−	−	−	+	+
F3	−	+	−	−	+	−	+
F4	+	+	−	+	−	−	−
F5	−	−	+	+	−	−	+
F6	+	−	+	−	+	−	−
F7	−	+	+	−	−	+	+
F8	+	+	+	+	+	+	+

of Rabbits and first group was treated with insulin emulgel alone and in the second group insulin emulgel was applied in combination with iontophoresis. Absorption of insulin in both groups was observed as function of change in blood glucose level during 120 minutes of experiment. The results were graphed as shown in fig. 3. Before induction of hyperglycemia, blood glucose level was determined in all rabbits which was approximately 110±10 mg/dl. Hyperglycemia was induced by using Ketamine (100mg/Kg) and Xylaxine (10mg/Kg) anesthesia as these drugs produced acute and sustained hyperglycemia (Saha *et al*., 2005). The average blood glucose level was noted 250 ± 10 mg/dl after induction of hyperglycemia. For the first group (treated with insulin emulgel) blood glucose level was decreased to 185±7mg/dl as compared with initial value during 120 minutes observation.

However, in the second group (treated with insulin emulgel plus iontophoresis) reduction in blood glucose level was observed that is 125mg±5mg/dl as compared to initial value 250 ± 10 mg/dl. There was an average decreased in blood glucose level at the rate of 62.5 mg/dl per hour in the second group as compared to first group 32.5 mg/dl per hour. After completing the experiments, rabbit's skin was observed for any damage or visible lesion. Visual examination did not indicate any change at skin surface. For statistical analysis of results between two groups, one way ANOVA was applied. At the start of experiment (after 15 min) there was marginal difference in blood glucose level. However with the passage of time during 120 minutes experiment blood glucose level was significantly reduced in second group as compared to first group ($P < 0.05$).

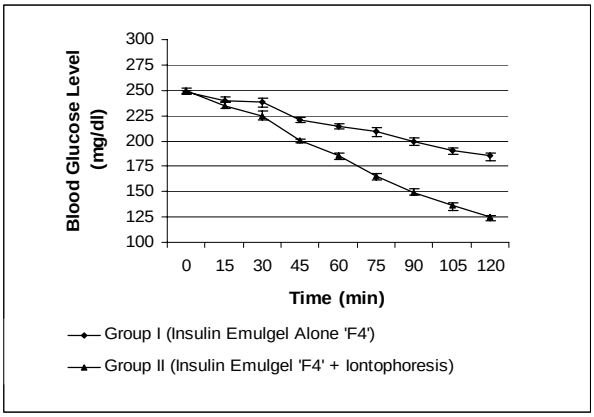

Fig. 3: Change in the Blood glucose level versus time period up to 120 minutes during the application of Insulin Emulgel alone and Insulin Emulgel + Iontophoresis (mean ± SD, n= 3).

DISCUSSION

Formulation development

Emulgel formulations are topically applied which compromises on safe drug administration because upon sudden change in body condition it can be easily removed from skin surface and also promising in case of medicaments like insulin which decomposes in stomach therefore not effective by oral route and painful delivery while administrating through injection. In the present work, Emu oil was studied as penetration enhancer because of its high oleic acid contents 47.4% and it also contains other fatty acids like 22.0% Palmitic acid, 15.2% Linoleic Acid, 9.6% Stearic Acid, 3.5% Palmitoleic Acid, 0.9% Linolenic Acid and 0.4 % Myristic Acid. The penetration enhancing activities of fatty acids have been well documented in literature. Oleic acid is substance in this group which has been most investigated (Trommer and Neubert, 2006). Based on spectroscopic investigations of deuterated oleic acid, it was indicated that oleic acid at high concentration form a separate phase within lipid bilayer followed by providing permeability defects in the same and facilitate the permeation of hydrophilic molecules through stratus corneum (Ongpipattankul *et al*., 1991). Jiang and Zhou studied the effect of oleic acid on stratum corneum lipids of rat using electron microscopy and it was found that oleic acid enhance permeability through its ability to perturb the stratum corneum and lacunae formation (Jiang and Zhou, 2003). It was also studied by Larrucea *et al* that combination of oleic acid with propylene glycol in Carbomer gel enhanced the absorption of tenoxicam (Larrucea *et al*., 2001).

Emulsifiers are usually used in emulsion to stabilize the formulation by the formation of micelles and also have potential for the solubilization of the stratum corneum lipids, therefore they also imparts their role as penetration enhancers (Trommer and Neubert, 2006). Based on physical parameters of prepared emulgel formulations, it was observed that those formulations containing carbomer have smooth and aesthetically good texture as compared to others (containing hydroxypropyl methylcellulose as gelling agent) however, remaining parameters were found same like pH and phase separation stability.

The aqueous emulgel formulation could support microbial growth therefore preservatives were added namely methyl paraben and propyl paraben. However it was reported that nonionic surfactant like Polysorbate 80 (because of micellization) reduce preservative efficacy of paraben (methyl paraben and propyl paraben) (Aoki *et al*., 1956; Patel and Kostenbauder, 1958). To resolve this incompatibility, 10% propylene glycol was added in the formulations as per reported by Poprzan and deNavarre that the presence of propylene glycol (in 10% concentration) not only prevents the interaction between parabens and polysorbate but also potentiates their preservative activity (Poprzan and deNavarre, 1959). Hence, all these factors were considered in the development of present formulations.

Isopropyl Myristate is a nongreasy emollient and absorbed readily by the skin. In the current formulations, Isopropyl Myristate was used as a co-permeation enhancer in combination with Emu oil. Fang *et al* were studied the penetration enhancing activity of Isopropyl Myristate in transdermal formulation alone and in conjunction with iontophoresis and ultrasound (Fang *et al*., 1997).

Factorial design

The results of analysis showed that for obtaining a maximum insulin flux, insulin emulgel should be prepared using a higher concentration of Emu oil and Polysorbate 80 and Carbomer as gelling agent. The systematic formulation approach helped in understanding the effect of formulation independent variables.

The concentration of Emu oil (X_1), concentration of Polysorbate 80 (X_2), and type of gelling agent HPMC or Carbomer (X_3) were chosen as independent factors. The Polynomial equation as given below obtained to correlate the factors X_1, X_2 and X_3 and their interactions with the insulin permeation (Y) (Banker and Rhodes, 2007).

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_1X_2 + \beta_5X_1X_3 + \beta_6X_2X_3 + \beta_7X_1X_2X_3$$

Where Y is the measured response, X_1, X_2 & X_3 represents coded level of the factors, β_1 to β_7 are coefficients calculated from observed experimental values of Y (Coefficients with one factor express the effect of that particular factor while the coefficients with more than one factor represent the interaction between those factors) and β_0 represents the intercept of arithmetic mean of quantitative outcomes of eight experiments.

To assess the reliability of the above-described equations, a check point batch was experimented that varied the independent variables and estimated the dependent variable. The experimental values of check point batch was compared with the predicted values gained from equations, fig. 2(h) represented the observed response value compared with that of predicted values indicating the correctness of model.

Interactions among independent variables were studies by the help of Design Expert® Software and interaction between two factors X_1X_2, X_1X_3 & X_2X_3 are shown in figs. 2a, b and c respectively. Increased concentration of Emu Oil and Polysorbate 80 resulted in higher insulin permeation as shown in fig. 2a and also evident by the surface of fig. 2g that showed parallel ascent in the

Table 2: Quantitative composition of insulin emulgel formulation (% w/w) & Permeation flux through excised rat skin from insulin emulgel formulations.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Insulin	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Emu Oil	5.0	7.5	5.0	7.5	5.0	7.5	5.0	7.5
Polysorbate 80	2.5	2.5	5.0	5.0	2.5	2.5	5.0	5.0
Carbomer	1.5	1.5	1.5	1.5	-----	-----	-----	-----
HPMC*	-----	-----	-----	-----	2.0	2.0	2.0	2.0
Isopropyl Myristate	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Propylene Glycol	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Methyl Paraben	0.033	0.033	0.033	0.033	0.033	0.033	0.033	0.033
Propyl Paraben	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.017
Purified Water q.s to	100	100	100	100	100	100	100	100
Permeation flux † ($\mu g/cm^2/hr$)	2.08 ± 0.04	2.83 ± 0.07	3.83 ± 0.06	4.88 ± 0.09	1.96 ± 0.11	2.67 ± 0.08	3.50 ± 0.06	4.38 ± 0.07

*Hydroxypropyl methylcellulose, † mean ± standard deviation, n = 3

Table 3: *In vitro* skin permeation data results of insulin emulgel formulations.

Formulation	r^2 values			Diffusional exponent (n)
	Zero Order	Higuchi	Korsmeyer-Peppas	
F1	0.969	0.819	0.975	0.902
F2	0.891	0.773	0.991	0.934
F3	0.998	0.788	0.998	0.707
F4	0.896	0.769	0.997	0.850
F5	0.967	0.842	0.981	0.860
F6	0.994	0.766	0.996	0.760
F7	0.851	0.769	0.997	0.754
F8	0.995	0.760	0.998	0.879

Table 4: Stability studies of Insulin Emulgel (Stability condition: at 2 – 8°C)

Formulation	Sampling Intervals (months)	Appearance	pH (6.5 – 7.0)	Drug Content (%)
Insulin Emulgel (F4)	0	Translucent smooth gel.	6.80	99.95
	1	No Change	6.78	99.90
	2	No Change	6.75	99.58
	3	No Change	6.75	99.15
	6	No Change	6.70	98.95

response surface with higher concentration of Emu Oil (X_1) and Polysorbate (X_2). Effect of Carbomer on insulin permeation was more while interacting either with Emu Oil=X_1 or Polysorbate 80 =X_2 as compared to HPMC as shown in figs. 2(b) and 2(c). A higher concentration of both numeric factor (Emu Oil=X_1 and Polysorbate 80=X_2) with categoric factory (Carbomer=X_3) enhanced insulin permeation. Figs. 2e and f show the interaction among all three factors $X_1X_2X_3$=Carbomer and $X_1X_2X_3$=HPMC respectively.

The pronounced effect of Carbomer over HPMC can be explained on the fact that HPMC swells in water and slow down the release of drug content because of forming complex matrix while Carbomer did not impart any hindrance in drug release.

Insulin permeation through excised rat skin (in vitro studies)

Drug permeation studies (*in vitro*) are helpful in the prediction of skin permeation in vivo. Insulin is large molecule where passive diffusion is difficult; however, literature is available where various efforts were made for transdermal delivery of insulin with the help of chemical penetration enhancer. It was reported that trypsin acts as biochemical enhancer for transdermal delivery of insulin. Bovine insulin was applied to assess *in vivo* hypoglycemic effect in rats with and without trypsin pretreatment and concluded that trypsin changes stratum corneum structure from alpha to beta form and deceases its electric resistance thereby enhancing insulin permeation by decreasing stratum corneum barrier properties (Li *et al*., 2008). In the present work, combine

effect of Emu Oil, Isopropyl Myristate and Polysorbate 80 as penetration enhancers were studied. Formulation prepared with Carbomer (F1-F4) showed improved permeation flux as compared to those prepared using HPMC (F5-F8) as gelling agent (table 2). The presence of hydroxyproyl methylcellulose may be one of the factors in decreased permeation flux in the formulations (F5-F8) as it is supported by the observations of Karakatsani *et al* where they studied the effect of different penetration enhancers on transdermal delivery of diltiazem HCl which was prepared in hydroxypropyl methylcellulose gel and it was observed that penetration enhancers increase drug flux at reduced viscosity "with low HPMC content" (Karakatsani *et al*., 2010).

Increase in insulin permeation was observed as surfactant quantity was increased owing to its ability to fluidize the lipid matrix of stratum corneum (F3, F4 and F7, F8 showed more permeation flux as compared to F1, F2 and F5, F6) and it indicates that surfactant can loosen or fluidize the lipid matrix of stratum corneum (main barrier in transdermal drug delivery system). In the same manner at the maximum quantity of both factors "emu oil and polysorbate 80" highest flux was observed (F4 and F8 showed more permeation flux as compared to F1, F2, F3 and F5, F6, F7). Suitable mathematical model is necessary to apply to predict and evaluate *in vitro* permeation behavior from these insulin emulgel formulations. Permeation data results were evaluated kinetically by the following mathematical models like zero order, Higuchi and Korsmeyer-Peppas model. The results are shown in Table 3. Respective correlation coefficients were compared for each formulation for each model and it was found that all the formulation followed Korsmeyer-Peppas model. Permeation mechanism was found by determining diffusion exponent value (n) in Korsmeyer-Peppas model which was between 0.707 - 0.934 and it indicates that drug permeation from these formulation following non-Fickian diffusion mechanism.

Penetration enhancers facilitate absorption of active moiety through skin mainly by three mechanisms either by interacting with intracellular protein, by disrupting stratum corneum structure or through partitioning of drug into stratum corneum (Barry, 1983; Nayak *et al*., 2010; Pathan and Setty, 2009). It has been reported that oleic acid accumulates in the lipid bilayers of stratum corneum, reduces its resistance for drug penetration and therefore increase its penetration ability (Barry and Bennett, 1987; Goodman and Berry, 1988). Isopropyl myristate accumulates and disrupt stratum corneum structure because of its chain structure and enhance drug permeation (Sato *et al*., 1988).

The result of (F4) formulation indicates that fatty acid of emu oil, polysorbate and isopropyl myristate were in a sufficient quantity to form a complex with excised rat skin to show maximum flux of insulin. Mollgaard reported synergistic effect of isopropyl myristate in transdermal drug delivery with propylene glycol (Mollgaard, 1989). Hence, in the present work propylene glycol had dual function in these formulations first to prevent interaction between parabens with Polysorbate 80 and second to synergize the penetration enhancing effect with Isopropyl Myristate.

Hypoglycemic activity of insulin emulgel in diabetic rabbits

The final objective of the study was to evaluate the hypoglycemic activity of optimize formulation in animal model. Formulation F4 showed maximum permeation flux through *in vitro* studies. This formulation was applied in diabetic rabbits alone and also in combination with iontophoresis. Various attempts were made in the literature to enhance transdermal delivery of insulin through iontophoresis alone or in combination with chemical penetration enhancers. Pillai and Panchagnula (2004) studied the effect of fatty acid and iontophoresis on transdermal delivery of insulin. It was reported that fatty acids showed synergistic enhancement when combined with iontophoresis (Pillai and Panchagnula, 2004). Zakzewski *et al* also studied passive transdermal delivery of insulin versus application with iontophoresis in diabetic rat and found maximum flux when depilatory lotion was applied along with iontophoresis (Zakzewski *et al*., 1998).

As shown in results when insulin emulgel formulation (F4) was applied alone on diabetic rabbits, blood glucose level was decreased by 185±7mg/dl with reference to initial values 250 ± 10mg/dl. Although this effect showed insulin permeation but quantitatively it is too small to lower the blood glucose level normal hence useless. Iontophoresis was applied in combination with the formulation to potentiate insulin permeation enough to bring blood glucose level near to normal.

Isoelectric point of Insulin is 5.3 therefore it has negative charge at physiological pH. In the present study cathodal iontophoresis was used to deliver insulin at pH 6.8 and current density 0.5 mA/cm^2. It was also evident by Kari work where cathodal iontophoresis was used in insulin delivery in New Zealand white rabbits (Kari, 1986).

Skin permeation of charged drug molecules through iontophoresis is governed by two mechanisms: electrorepulsion and electroosmosis. The drug delivery by iontophoresis is expressed by the formula as follows:

$$J_{total} = J_{electroreplusion} + J_{electroosmosis}$$

When both groups were compared, there was difference of 60 mg/dl blood glucose level over 120 min experiment. Statistical difference ($P < 0.05$) was found in one way ANOVA analysis between two groups.

Stability studies

For the protein/peptide pharmaceutical products, real time stability data is required for assessing shelf life as per United States Food and Drug Administration. Accelerated stability studies only used in formulation screening and for product shipping at room temperature (Singh, 1999). Insulin emulgel formulation was tested by HPLC method and the results of stability studies are given in Table 4. The stability study was conducted at refrigeration condition (recommended storage condition for insulin). There was no considerable change in drug content of insulin emulgel after 6 month analysis as compared to initial results. Other parameters considered in stability testing are physical appearance & pH. There was no change observed in color & odour and also there was no any phase separation. Similarly pH was also observed during stability studies and found within a range (6.5-7.0) during 6 month testing.

CONCLUSION

Emu oil in the presence of polysorbate 80 and isopropyl myristate has good penetration enhancing ability and also synergistic effect was shown in combination with iontophoresis. Based on satisfactory results found during *in vitro* drug release through excised rat skin and *in vivo* study in small animal model, it is suggested to conduct this study in large animal to make it a pleasant and painless alternative for injectable insulin.

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