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Role of AC Current-Iontophoresis in Enhancing Transport of Insulin through Different Membranes

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ABSTRACT

The use of needles for multiple injections of drugs, such as insulin for diabetes, can be painful. Several noninvasive methods exist for transdermal drug delivery. These include physical mechanisms such as iontophoresis. The present work focused on investigating the effect of AC current on the transport of insulin through different membranes. In order to achieve insulin transport, a homemade iontophoretic cell was designed and constructed. Three main skin membranes (chicken, rabbit and rat) and different concentrations from insulin (5, 25, 50 and 100 µIU/ml) in citrate-phosphate buffer (0.24 M, PH 3.6) were used in this study. Also, insulin concentration at 50IU/ml, 1KHz, different concentration of sodium chloride at (0.01, 0.05, 0.1 and 1 mole) and different times in chicken and rat skin were studied. The pulsing square wave electric field used was 10 Volts with 1, 2, 4 and 8 kHz pulse/sec for 30,45 and 60 minutes. Insulin concentrations in all studied membranes were determined using a ready for use immuoradiometric assay (IRMA). Results of this study revealed that transported insulin was increased by increasing insulin concentration, applied frequency and time. Flux rate of insulin increased with increasing concentration at different times, while the highest flux rate of insulin at most frequencies applied was after 30 minutes. There is a good correlation between insulin concentration and transported in different skin at low insulin concentration and low frequency after 30 minutes. The present work has demonstrated that the iontophoresis technique may provide a convenient means for the systemic delivery of insulin without the use of conventional methods.

Key words: Iontophoresis, AC current, Frequency, insulin, skin, rats, rabbit, chicken.

Introduction

The injection route of insulin deliver is favorable in terms of efficacy. However, it may result to some severe adverse conditions like, a burden of daily injections, physiological stress, pain, inconvenience and the localized deposition of insulin leads to a local hypertrophy and fat deposition at the injection sites (Kennedy, 1991). Therefore, the stress and discomfort of multiple daily injections provoked numerous attempts to develop a safe and an effective noninvasive route for insulin delivery (Divyen *et al.,* 2010). Systemic delivery of insulin is limited by the large size and charge, resulting in poor membrane permeability, regardless of their tendency to proteolytic degradation at most of the membrane interface. Transdermal delivery of peptides/proteins has been recognized as an attractive option, due to the fact that skin has less proteolytic enzymes (Pillai *et al.,* 1999). At the same time to overcome the permeability difficulties, several penetration enhancement strategies have evolved to expand the number of drugs delivered by transdermal route (Naik *et al.,* 2000; Barry, 2001). Transdermal drug delivery offers several advantages, including avoidance of irregular absorption, absence of gastric agitation, painlessness, noninvasiveness, as well as improvement in commitment of the patient to take the drug. With this mode of drug administration, there is no pre-systemic metabolism and it is possible to increase drug bioavailability and half-life (Kevin, 2016).

The skin which has increasingly become a route of the delivery for a wide range of drugs has generated a great deal of interest (Prausnitz, 2001). On the other hand, transdermal delivery is limited by the low permeability of skin caused mainly by stratum corneum, the skin's outermost layer (Prausnitz *et al.,* 1996). However, the permeability can be increased by various techniques such as the use of physical or electrical enhancers via electroporation or iontophoresis (Zakzewski *et al.,* 1998). Iontophoresis is a powerful technique to enhance percutaneous permeation of ionized drugs poorly absorbed by skin. It is

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basically an injection without the needle. Iontophoresis is a non-invasive technique, which uses electric current to deliver the charged or neutral molecule through biological membrane. It helps to increase the penetration of ionized drug. The technique utilizes a same small amount of current for the delivery of drug (Smirithi *et al.,* 2015).

Insulin transdermal iontophoretic delivery was first reported by Stephen *et al*. (1984), although this group failed in their attempt to administer regular soluble insulin to human volunteers (Stephen *et al.,* 1984). The authors believed that this might be due to the weakly ionized insulin form they were trying to deliver. They were able to deliver a highly ionized monomeric form of insulin to one experimental animal, a decline in blood glucose and an increase in serum insulin were observed. The effectiveness of passive transdermal versus electrically-enhanced delivery of insulin was studied in diabetic rats. The study showed that low levels of electrical current can induce changes in stratum corneum permeability that are sufficient to produce the transdermal absorption of physiologic doses of human insulin (Kanikkannan *et al.,* 1999). Also, biological activity of insulin emulgel alone and in combination with iontophoresis using Albino rabbits was investigated by Muhammad Akram et al. Their study showed that absorption of insulin through transdermal emulgel was greater in combination with iontophoresis to decrease blood glucose level (Muhammad *et al.,* 2013).

Direct current (DC) of on/off pulses and alternating current (AC) has been previously investigated for transdermal iontophoresis applications (Kinoshita *et al.,* 2003; Haga *et al.,* 2005). Li *et al.* (2003), has been suggested that pulsed DC and AC iontophoresis causes less skin irritation compared to traditional DC iontophoresis. Also, Yan *et al.* (2005) demonstrated that AC iontophoresis can significantly decreases skin electric resistance and enhance the transport of charged permeants across skin. Flux variability of neutral permeates during AC iontophoresis was also found to be less than that of conventional DC iontophoresis.

Taking advantage of these characteristics, the objective of this study was to examine the role of AC current at different frequencies, buffer type and its concentration on the in vitro iontophoretic transport of insulin with different concentrations through different membranes

Materials and Methods

Buffers

Citrate- sodium phosphate buffer at (0.24 M, PH 3.6) and sodium chloride buffer at different concentration of (0.01, 0.05, 0.1 and 1 M, PH 7.4) were freshly prepared and stored in refrigerator at $4C^{o}$ until used.

Insulin

Recombinant human insulin purchased from sigma – Aldrich Company (USA). In this work, concentration of insulin was varied from (5, 25, 50 and 100 μIU/ml) in citrate-phosphate buffer (0.24 M, PH 3.6) as a donor solution.

Skin Preparation

All experimental animals in this study were carried out in accordance with the ethical guidelines of the Medical Research Institute, Alexandria University, (Appendix 2 Guiding principles for Biomedical Research Involving Animals, 2011). Full thickness of abdominal skin from chicken, rabbit and male albino Wistar rats (8-10 weeks old and weighing 100-120 g) were shaved to remove all hairs and cleaned from fatty layer adhering to the dermis side. Every piece of skin was cut into equal pieces of ($15x3cm^2$), then washed three times with normal saline followed by distilled water and kept at $4C^0$ until used. This method was repeated for every piece of used skin.

Iontophoretic Cell Design

In order to achieve drug delivery (insulin), a homemade iontophoretic cell was designed and constructed Figure (1). A rectangular cell with two faces from Plexiglas with 15 cm width and 6.5cm length was constructed. The cell has two faces channels with a diameter of 2cm, one for donor insulin and the other for receiving transported insulin through different membrane. A manufactured silver/silver chloride electrode of 2cm x 0.2cm diameter was inserted in donor cell and receiver cell, then connected to

pulsing square wave power supply. In this work, AC function generator model CA1640 p-02 multi waveforms (sine, square, and triangle) with 5 digit frequency counter (up to 20 MHz) purchased from al-nekhely and brothers co. ltd Egypt. This function generator fitted with a power amplifier, capable to give multi an AC current which is able to enhance the entrance of insulin through the skin.

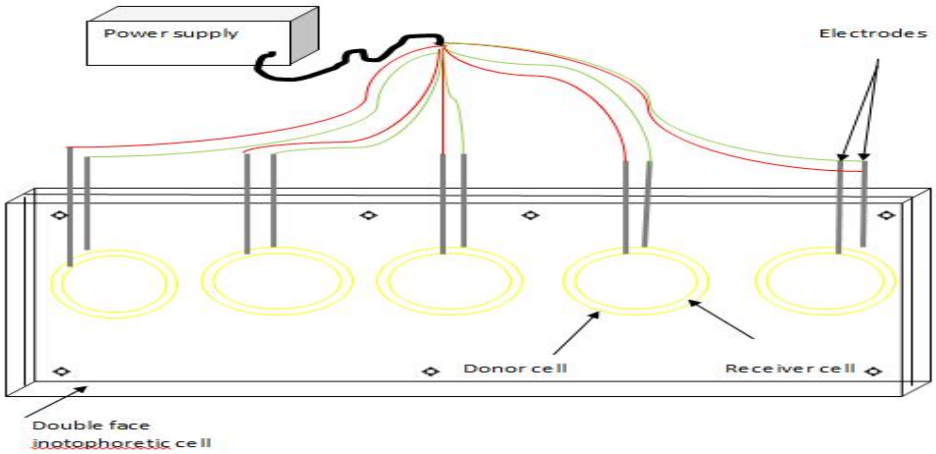

Fig. 1: Constructed iontophoretic cell

Membrane groups

Skin of (chicken, rabbit and rat) was divided into 2 main groups:
Group A was divided into 4 subgroup as follow:

1- 10 pieces of each membrane were subjected to pulsing square wave at a peak voltage of (10V), 1KHz, different concentration of insulin (5, 25, 50 and 100µIU/ml) in (citrate phosphate buffer 0.24 M, PH 3.6 as a donor and receiver solution) for different 3 times (30, 45 and 60 min).
2- 10 pieces of each membrane were subjected to the same condition at 2 KHz, 4 KHz and8 KHz.

Group B-

1-10 pieces of chicken and rat membrane were subjected to pulsing square wave at a peak voltage of (10V), 1KHz and 50µIU/ml with sodium chloride as a donor and receiver solution at 0.01, 0.05, 0.1 and 1 M) for different 3 times (30,45 and 60 min).

Methods

Experimental procedure

Each pieces of different skin membrane (chicken, rabbit and rat) was mounted tightly between two faces of iontophoretic cell where the *Stratum corneum* side facing the donor compartment.
In the donor compartment, 500 µl of insulin in citrate-sodium phosphate (0.24 M) at concentration of 5 IU/ ml was placed. Receptor compartment was filled with citrate-sodium phosphate only (0.24 M) as receiver solution. The anode was placed in the donor compartment and cathode in the receptor compartment. The electrodes were connected to the AC function generator at 1KHz except for control skin. Samples were periodically withdrawn from the receptor compartment after 30, 45, and 60 min. This procedure was repeated for different insulin concentrations (25, 50 and 100µIU), different frequencies (2, 4 and 8KHz) at different time (30, 45 and 60 min). Also, the same procedure was repeated with insulin concentration 50µIU/ml, 1KHz with different concentrations of sodium chloride (0.01, 0.05 ,0.1 and 1 mole) at different times in chicken and rat skin.

Determination of Insulin Levels in the transported solution

Insulin levels were determined using a ready-for-use immunoradiometric assay (IRMA) kit according to the manufacturer's protocol (Izotop, Hangerian). Briefly, the ^{125}I-labeled signal-antibody binds to an epitope of the insulin molecule spatially different from that recognized by the biotin capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples,

leading to the formation of a capture antibody-antigen-signal antibody complex, also referred to as "sandwich". During a 2-hour incubation period, immuno-complex is immobilized to the reactive surface of streptavidin-coated test tubes. Reaction mixture is then discarded, test tubes washed, and radioactivity is measured for 1 minute in a gamma counter (perkin Elmer, Finland). Thus, the concentration of insulin is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve, the unknown concentrations of insulin in the transported solution (µIU/ml) were determined. The average flux rate was calculated as the amount of insulin in µIU/ml transported across an area of 1 cm^2 divided by time in minutes, the treatment duration (transport/ treatment time).

Statistical analysis

Student – t – test and the correlation coefficient were applied to determine the best and suitable Iontophoretic electrical parameters, i.e. high transport, average flux rate of insulin through the skin membrane.

Results and Discussion

1-Effect of different frequencies on flux rate of insulin

The passive transport of insulin was very low and non-detected compared with iontophoretic permeation with all frequencies and different times. The change in flux rate of insulin as a function of increasing insulin concentration and times on chicken, rabbit and rat membrane using pulsed square wave at1, 2, 4 and 8 KHz are summarized in figures (2-4).

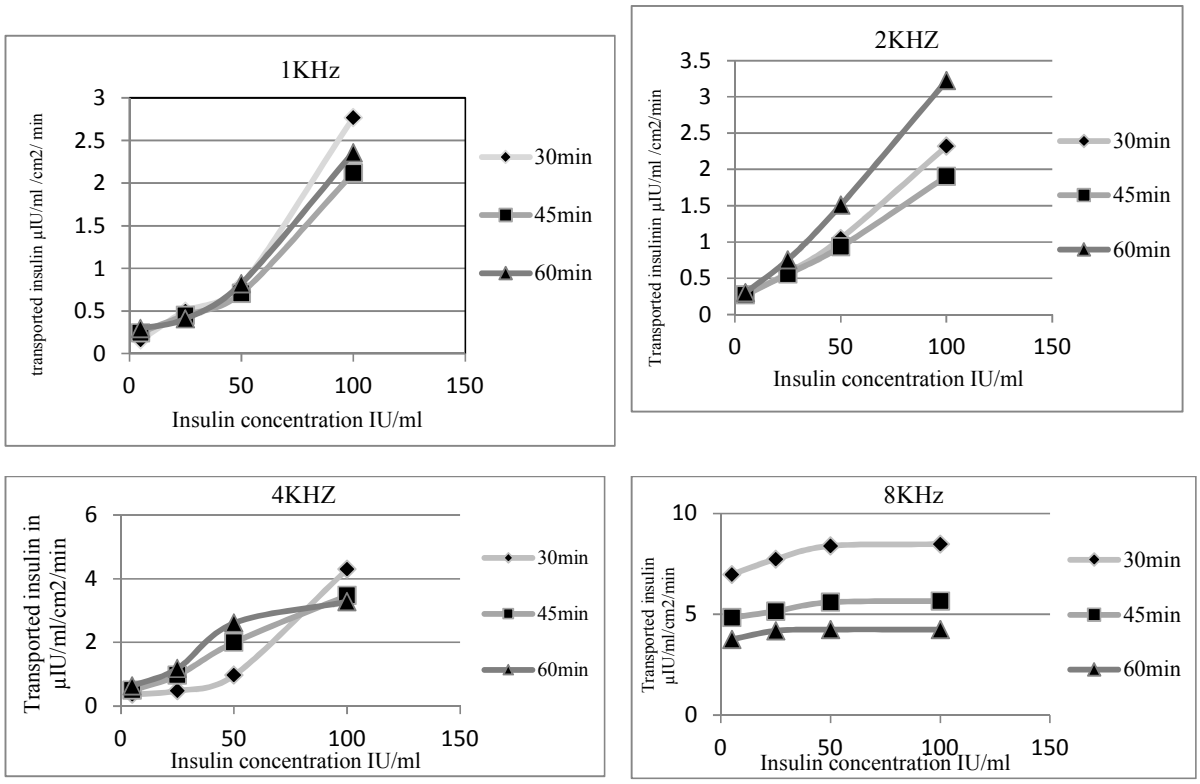

Fig. 2: Shows average flux rate of insulin through chicken membrane with different concentrations and different times at 1, 2, 4 and 8KHz.

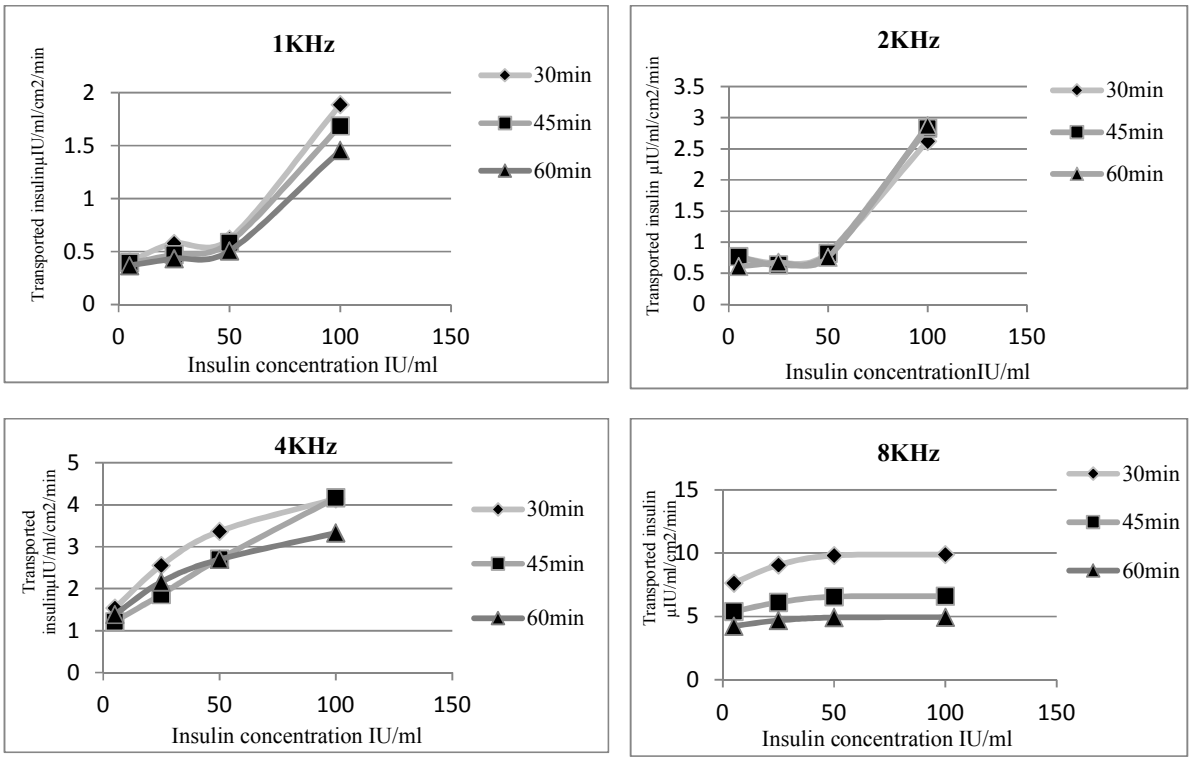

Fig. 3: Shows average flux rate of insulin through rabbit membrane with different concentrations and different times at 1, 2, 4 and 8KHz.

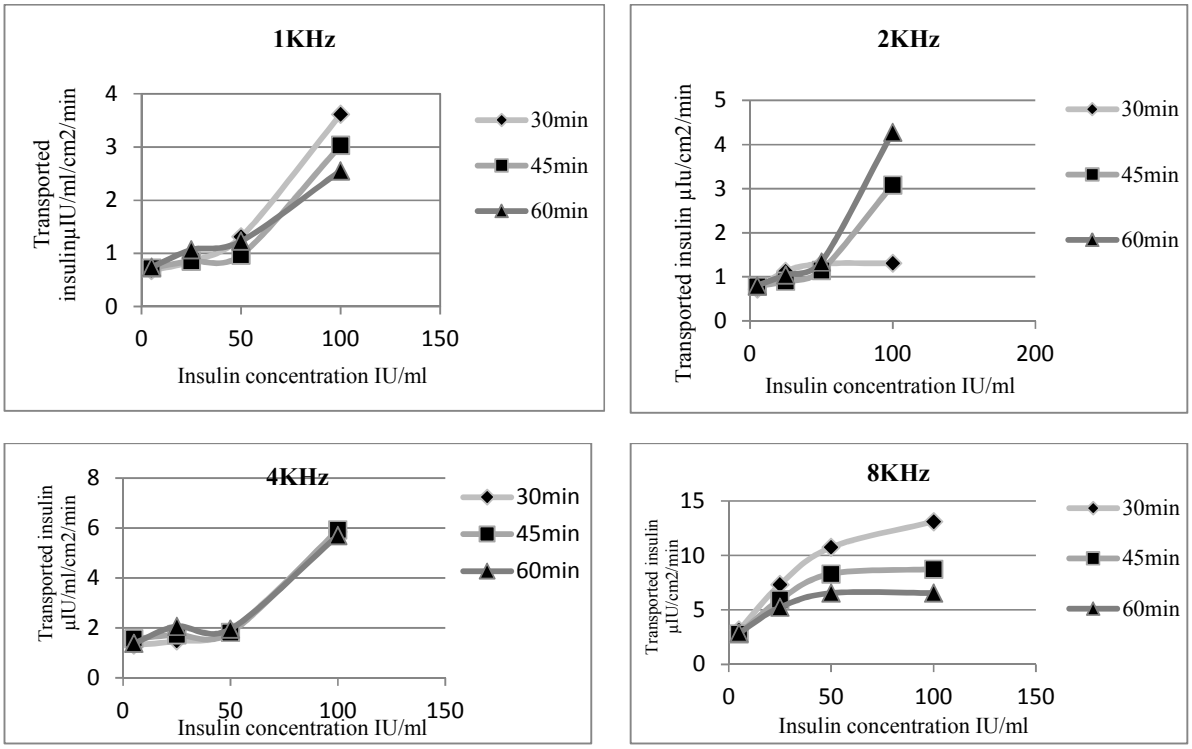

Fig. 4: Shows average flux rate of insulin through rat membrane with different concentrations and different times at 1, 2, 4 and 8KHz.

There was a disparate increase in flux rate with increase in insulin concentration and frequencies at different times. It was found that the iontophoretic flux rate enhancement increased with insulin concentration up to 100 IU/ml). Maximum enhancement was observed after 30 min for all membranes at 1, 8 KHz, while higher iontoforetic time resulted in very less enhancement. There are significant differences ($P > 0.05$) were found in skin permeation between different times at 1 KHz for chicken and rat skin and at 8KHz for all different membranes. On the other hand, there was no significant ($P > 0.05$) influence on skin permeation at 2, 4 KHz with different times except for 100 IU/ml insulin concentration for chicken and rat skin. Figure 5 shows comparison between insulin concentrations and transported through different membranes and different times at 1 KHz. It is clear that the skin of rat is the highest permeability for different insulin concentration followed by chicken membrane and then finally the skin of rabbits with different times. Table 1 depict linear relation between insulin concentration and transported as a function to different times at 1 KHz in rat skin where X is insulin concentration and Y is transported insulin with high correlation coefficient.

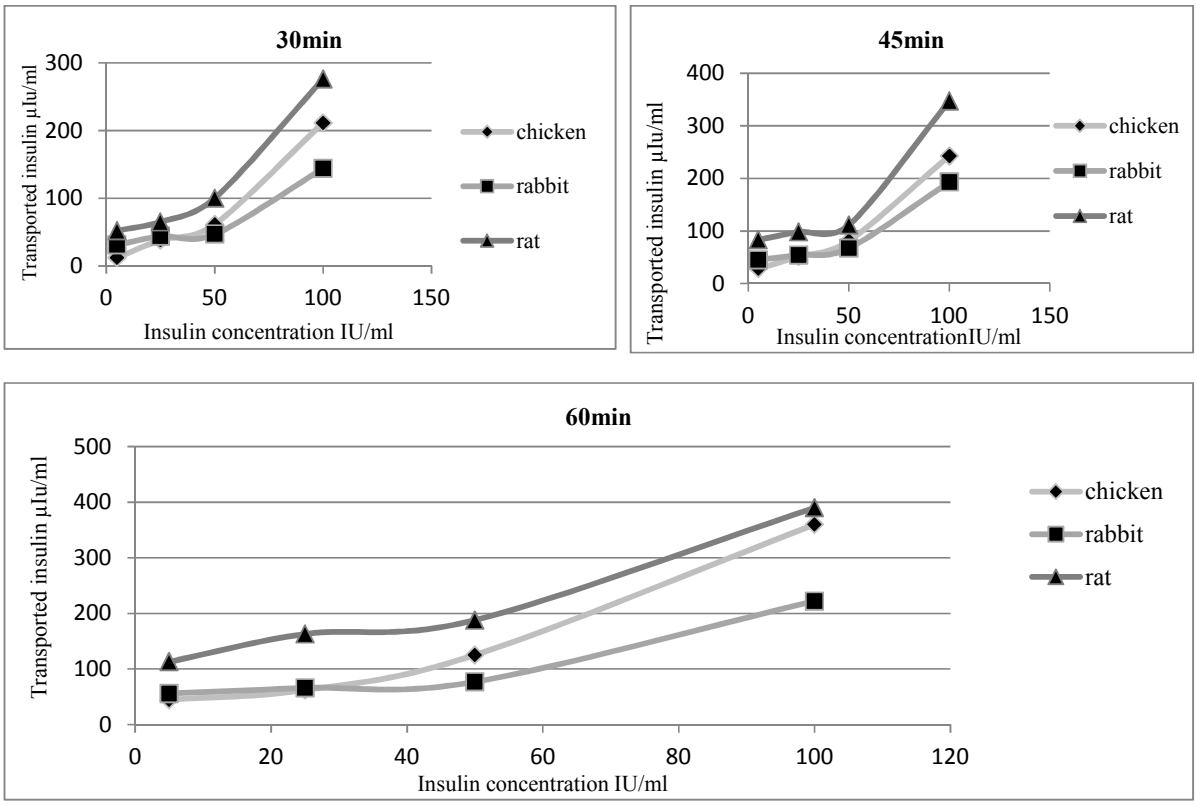

Fig. 5: Comparison between insulin concentration- transported through different membranes and different times at 1KHz.

Table 1: Linear relation between insulin concentration and transported as a function to different times at 1 KHz in rat skin.

Duration Time	Equation of Linear Relation	Correlation coefficient®
30 min	Y=0.0319x+ 0.1788	0.9624
45 min	Y=0.0248x+ 0.2778	0.9306
60 min	Y=0.019x+ 0.5459	0.9750

X= insulin concentration and Y= absorbed insulin.

2- Effect of sodium chloride concentrations on insulin flux rate

According to the previous results, 1 KHz and 50 IU/ml insulin concentration have been selected to study the effect of different concentrations of sodium chloride on insulin flux rate. A very low concentration of buffer ions would result in no significant ($P > 0.05$) influence on insulin flux rate with different times. On the other hand, flux enhancement was significantly high ($P < 0.05$) with 1M sodium chloride in rat skin especially after 30 min.

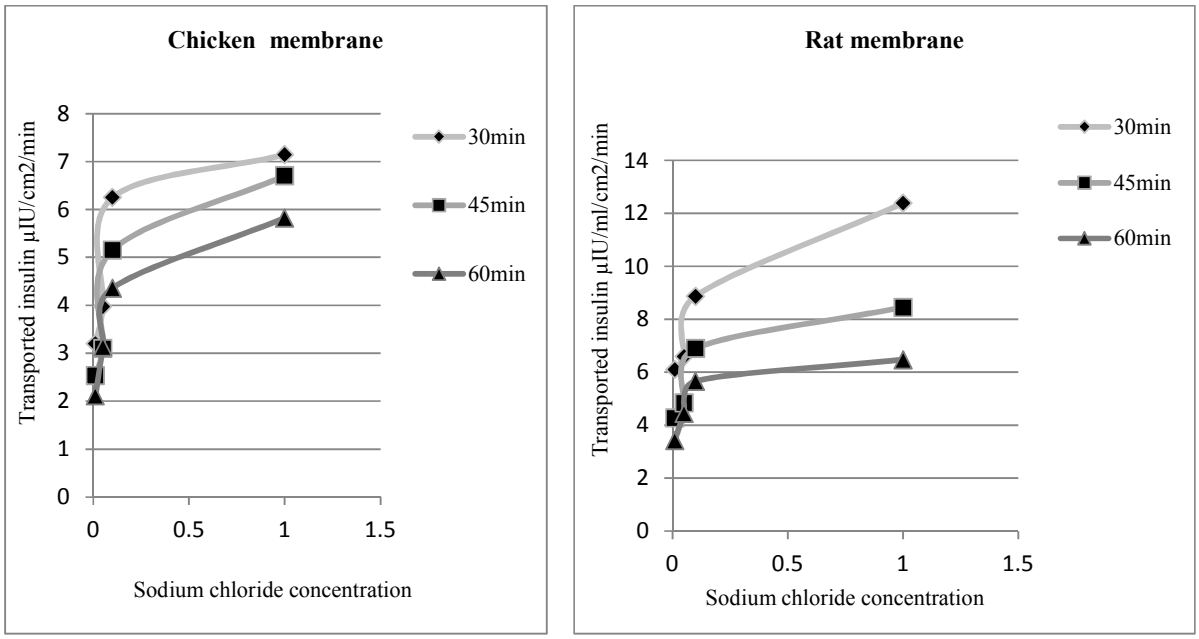

Fig. 6: Shows average flux rate of 50 IU/ml insulin concentration through chicken and rat membrane with different concentrations of sodium chloride and different times at 1KHz.

Table 2: Linear relation between sodium chloride concentration and transported insulin as a function to different times at 1 KHz in rat skin

Duration Time	Equation of Linear Relation	Correlation coefficient®
30 min	Y=5.6424x+ 6.853	0.9355
45 min	Y=3.4379x+ 5.1234	0.8511
60 min	Y=2.2092+ 4.3625	0.7814

X= sodium chloride concentration and Y= absorbed insulin.

Discussion

Skin serves as a port of entry into the body for drug administration to providing continuous transdermal infusion into the systemic circulation (Siddiqui *et al.,* 1985). The skin of an average adult body covers a surface area of ~ $2m^2$ and receives about one-third of the blood circulating through the body. For transdermal delivery of drugs, stratum corneum is the main barrier layer for permeation of drug (Gangarosa and James, 1995). So, to circumvent the stratum corneum and to increase the flux of high molecular weight drug like insulin through skin membrane, iontophoresis approach for enhancement of transport are used in this study. Iontophoresis is largely based on the stripping of stratum corneum to permit penetration and distribution of drug.

Previous study had proposed that all the drugs delivered through the skin with iontophoresis is removed by the subcutaneous circulation and distributed around the body (Charles and Arthur, 1995) [(20)]. Iontophoretic transport will occur through regions of low electrical resistance. These pathways may or may not be the same as those used during passive diffusion of solutes through the skin, as the application of an electrical potential may cause changes in the permeability of the skin and create new routes of permeation (Khan *et al.,* 2011).

There are several mechanisms that have been proposed for Iontophoretic action on skin depending on electric current, its frequency, and duration of application. However, it has been generally observed from this study, that flux rate of different insulin concentrations was enhanced by the application of pulsed alternative current as square wave with different frequencies at 1,2,4 and 8 KHz through skin of chicken, rabbit and rat as shown in figures(2-4) . On the other hand, pulsed square wave at 8 KHz decrease transport of insulin because during "off stage" of the current the skin gets depolarized and returns to the initial polarized state. However,

Bagniefski *et al.* (1990) showed that enhanced skin depolarization can decrease the efficiency of drug transport, if the frequency of pulsed current is high. Also, transport of insulin depends on the duration of current applied in iontophoretic drug delivery. Flux rate of insulin was increased with increasing

concentrations but decreased with increasing time in different membranes. Flux rate was higher after 30min than 45 and 60 min for 1, 4 and 8 KHz (figures 2-4).

Iontophoresis enhances drug delivery across the skin by two principal mechanisms: electrorepulsion and electroosmosis. Electrorepulsion is the direct effect of the applied electric field on a charged permeant. Electroosmosis, results from the fact that the skin supports a net negative charge at physiological pH. At a physiologic pH (around 7) the skin carries a negative charge, which enhances the migration of cations at the anode. This is greater migration seems to drag the solvent through the skin carrying with it any dissolved substance (Masada *et al.,* 1989; Charro and Guy, 1998). This is clear when donor insulin dissolved with sodium chloride, transported insulin increased with increasing sodium chloride concentration at low frequency as shown in figures (6).

Percutaneous absorption of insulin through different membranes subjected in this study may take place simultaneously by any combination of the three main pathways that include: transcellular, which involves the sequential partitioning of the solute ion between cells and intercellular lipids as it moves vertically down through the skin, intercellular, which involves the movement of solute ions through the lipid pathways between cells in the skin or transappendageal, which involves the movement of ions through skin appendages such as hair follicles, sebaceous cells and sweat ducts (Khan *et al.,* 2011). Ions prefer the routes of the least electrical resistance; in the SC this is believed to be via the pores. Some investigations indicate that these pores are sweat glands, others that transport occurs through both hair follicle and sweat glands (Madhulatha *et al.,* 2012). In iontophoresis, skin condition affects the penetrating properties of permeant. The vide differences in physical characteristics such as appendages per unit area, thickness and structural changes between different skin membranes used in this study. The average transported of drugs is in order of rat > chicken > rabbit as shown in figure (5).

Conclusion

It is believed to be practical alternative to parenteral therapy where the pain and discomfort associated with repeated injection therapy can be overcome by iontophoresis. It is difficult to improve the transport efficiency by increasing the concentration of the insulin only due to the limitations in terms of cost and physical stability. The study reported herein indicated that, Iontophoresis facilitated transport of insulin across different membranes under the influence of AC current as square wave at different frequencies and duration times. The most important of all, its low frequency and short duration time where no corrosion or irritation to the skin was observed. Iontophoretic system seems to be a potential alternative delivery system for high molecular weight drugs. It should be evident from this review that iontophores is hold a lot of promise for the future of drug delivery.

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