

# Electroporation Parameters for Successful Transdermal Delivery of Insulin

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This work investigates the effects of electroporation parameters on the transdermal delivery of insulin. Electroporation (EP) is known to induce temporal pores in the membrane, which are expected to enhance the diffusion of insulin through rabbits' skin. For such purpose, 5 different formulations of insulin and enhancers are applied to rabbit groups (5 rabbits each) with induced hyperglycemia in the presence of electroporative pulses. The blood sugar level (BSL) is followed up to 5-hour duration starting from the administration of the hyperglycemia-inducing factor. The effect of different electroporation parameters on BSL of rabbits is examined and compared with control groups. Results show that the increase in the number of pulses (from 15 up to 60 successive pulses) at an insulin concentration of 50 IU/mL, the increase in insulin concentration (from 50 to 70 IU/mL), and the decrease in applied field strength (from 200 to 100 V/cm) result in a significant decrease in BSL compared with control. Among all of the investigated formulations, the best performance is recorded for the insulin solution + EP (without enhancers) in almost all of the studied experimental conditions.

*Keywords:* electroporation, insulin, hyperglycemia, transdermal delivery

## INTRODUCTION

Insulin is the most clinically significant protein that is a frequent topic of investigation in the present delivery literature. The simple, convenient, and inexpensive method of insulin delivery that has long been adopted is the needle-based administration.<sup>1,2</sup> This is an invasive procedure that is painful and may cause compliance problems for elderly patients.<sup>3</sup> Moreover, the injection of insulin subcutaneously has the risk of pain, inconvenience, hyperinsulinemia, and localized deposits of insulin. The latter may result in local hypertrophy and

fat deposits at the sites of injection.<sup>4</sup> Therefore, providing a noninvasive delivery mechanism for insulin represents an essential need.

Transdermal delivery is a promising alternative to oral delivery of pharmaceuticals such as insulin. It has been reported that 1000 years ago, people have placed substances on the skin for therapeutic uses. Now a days, a variety of topical formulations is introduced for the local treatment of disease.<sup>5</sup>

Transdermal drug delivery systems are expected to influence the way of administration of traditional drugs and new pharmaceuticals.<sup>2</sup> The transdermal method of drug administration makes use of the relatively large and readily accessible skin surface area (1–2 m<sup>2</sup>) for absorption.<sup>5,6</sup> It benefits from the skin tolerance for the application of a patch-like device that is considered localized and noninvasive.<sup>7,8</sup> The transdermal means has the advantage of being a highly efficient route for delivery of protein and peptide drugs, which avoid the effects of gastric degradation and hepatic first-pass metabolism.<sup>7,9</sup> Transdermal drug delivery systems also have the potential for

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sustained release and controlled input kinetics, which are essential for drugs with narrow therapeutic indices. Therefore, transdermal administration offers slow sustained drug delivery over long periods rather than being a means to achieve rapid bolus-type drug inputs.<sup>10</sup>

The transdermal delivery of insulin has been studied by many groups. In the absence of a special enhancer, the high molecular weight of insulin (~5.7 kg/mol) reduces its permeation through the skin. The rate of drug delivery has been enhanced by a number of active transdermal methods using physical<sup>11–14</sup> or chemical interventions.<sup>15,16</sup>

Chemical permeation enhancers are widely used to enhance the transport of drugs across the skin.<sup>8,10</sup> It has been reported that the addition of chemical enhancers (hydrocarbons, sulfoxides, fatty acids, surfactants, esters, and alcohols) can enhance drug flux by a temporary alteration of the barrier properties of the stratum corneum (SC).<sup>7,16</sup>

Among the physical means that are introduced to increase the throughput of small and macromolecules across the skin are ionophoresis (which used low-level electrical current to deliver drugs through the skin),<sup>6,13,17</sup> sonophoresis (which used low-frequency ultrasonic energy to create microchannels in the skin for drug delivery),<sup>14,18</sup> laser ablation (which, in SC, allowed drugs to penetrate the skin),<sup>19,20</sup> microneedles (which penetrated the outer layers of the skin, providing pathways for drug delivery),<sup>21,22</sup> and electroporation (which used high-intensity electric pulses to increase the permeability of cell membranes).<sup>12,15</sup>

The increase in the permeability of the skin using electroporation (EP) enables the transfer of drugs across the skin into the circulatory system. There are a limited number of drug molecules that can be transported through the epidermis using electroporation. Fortunately, the insulin molecule is one of these drug molecules.<sup>23–27</sup>

EP involves the creation of transient aqueous pathways across lipid bilayer membranes by applying short high-voltage pulses, which results in a large increase in the transdermal transport of molecules, mainly due to the electrophoretic movement and diffusion through the newly created aqueous pathways. These were observed *ex vivo* in human skin and *in vivo* in hairless rats.<sup>28</sup>

## MATERIALS AND METHODS

### Materials

Recombinant crystalline human insulin is purchased from Vacsera, Egypt, with purity greater than 98.8%. Hydrochloric acid (vapor density 1.3 vs. air vapor

pressure 3.23 psi at 21.1°C, purity ≥ 99%), Carbopol ultrez 10 (transition temperature 106°C, purity ≥99%), and triethanolamine [(HOCH<sub>2</sub>CH<sub>2</sub>)<sub>3</sub>N, MW 149.19, purity ≥99%] are purchased from Sigma-Aldrich (Chema-Tec Co, Egypt). Ketamine (50 mg/mL) is purchased from Sigma-Tec Pharmaceutical Industries (Egypt). Xyla-Ject (xylazine 20 mg/mL) is purchased from Adwia Co. (10th of Ramadan City, Egypt).

The chemical penetration enhancers povidone-iodine 10% ointment (equivalent to 1% iodine) is purchased from Sedico Co (Egypt). Oleic acid [0.89 g/mL, purity ≥99%] is purchased from Sigma-Aldrich (Chema-Tec Co, Egypt), and castor oil (purity ≥99%) is purchased from Pharmaoverseas Co (Egypt).

### Methods

#### *Preparation of insulin solution*

Native insulin (20 mg) is dissolved in 10 mL of phosphate buffer to give a solution of 50 IU/mL, pH 3.0, and then preserved at 4°C.

#### *Preparation of hydrogel-containing insulin*

The Carbopol gel 0.5% is prepared by the following procedure<sup>29</sup>; Carbopol resin 0.5 g is dispersed in distilled water (100 mL). The native insulin is dissolved in the distilled water to give a solution of 50 IU/mL, pH 4. The mixture is stirred until thickening occurs and then neutralized by drop-wise addition of 50% (wt/wt) triethanolamine, until a transparent gel appears. The amount of triethanolamine is adjusted to achieve a gel with the desired pH. Gels are stored for 24 hours at room temperature to stabilize before use.

#### *Electroporation*

Electroporation is performed using an electroporator (MicroPulser; Bio-Rad). This type delivers exponential-decaying capacitive discharge pulses. The pulse duration  $t_p$  can vary from 1 to 4 milliseconds. Electrodes contact wires are 2 parallel stainless steel rods with a thickness of 0.5 cm and a length of 2.7 cm at a distance of 2 cm from each other.

One electrode limitation is that the distance between the 2 electrodes has to be large when compared with their thickness.<sup>30</sup>

#### *Animal experiments*

All animal procedures are performed in accordance with protocols approved by the Institutional Ethical Committee at the Hashemite University. Female New Zealand white rabbits weighing between 1.5 and 2.5 kg are obtained from the local market and divided mainly into 6 experimental groups (5 rabbits each). Each animal is preanesthetized intramuscularly with

a combination of ketamine hydrochloride (40 mg/kg) and xylazine (10 mg/kg) before electroporation.

Xylazine is used to induce anesthesia and temporary but sustained hyperglycemia in rabbits. This increased the initial blood glucose level of rabbits to become much higher than normal. The purpose of inducing hyperglycemia in rabbits is to investigate the feasibility of transdermal delivery of insulin using EP in reducing a high blood glucose level (~284.25 mg/dL) to normal (~104 mg/dL).<sup>31,32</sup>

The hair of rabbits' thigh selected area is shaved, and a depilatory agent (ONE; Eva Cosmetics Co, Egypt) is gently applied to the skin to remove any remaining hair. A rabbit's skin is washed gently with distilled water at least 3 days before EP to avoid skin barrier disruption. Before the application of an insulin formulation and EP, the rabbit is placed in the lateral recumbent position.

At the beginning of the experiment, blood drop samples (~0.3 mL each) are collected from the ear vein of each rabbit for the determination of baseline glucose level. The blood glucose level (mg/dL) is determined using One Touch Ultra Easy (LifeScan, Inc, Milpitas, CA) blood glucose monitoring device.<sup>4</sup>

In each experiment, 2 blood samples are taken at 0.15, 1, 2, 3, 4, and 5 hours. The electroporation protocol involved application of electroporative pulses (from  $n_p = 15$  up to  $n_p = 60$  successive pulses), each of duration  $t_p = 1$  milliseconds up to  $t_p = 4$  milliseconds, at field strengths from  $E_{(appl)} = 200$  V/cm to  $E_{(appl)} = 100$  V/cm and an interpulse time of 1 second.

For each rabbit, the entire experiment lasted a total of 5 hours. Before the application of pulses, insulin gel formulations are uniformly spread in the shaved area between the 2 electrodes. For the experiment involving insulin solution alone, 1 mL of insulin is added, drop by drop, to the shaved area of rabbit's thigh in between the 2 electrodes.

When using enhancer compounds (castor oil, oleic acid, and iodine), the enhancer compound is added for about 2 hours and then washed off before the application of an insulin formulation on to the skin.<sup>33</sup> All experiments are performed at a room temperature of about 22°C.

It should be reported that the present EP protocol with insulin seems to be substantially safe and painless to rabbit's skin. The repeated treatments on the same area for 3 consecutive days have shown no gross changes on the skin surface. Apparently, there are signs of complete recovery within 1 hour after EP.

### Statistical analysis

The area under time versus blood sugar level (AUBSL) curves is calculated using Origin 6.0 software for all

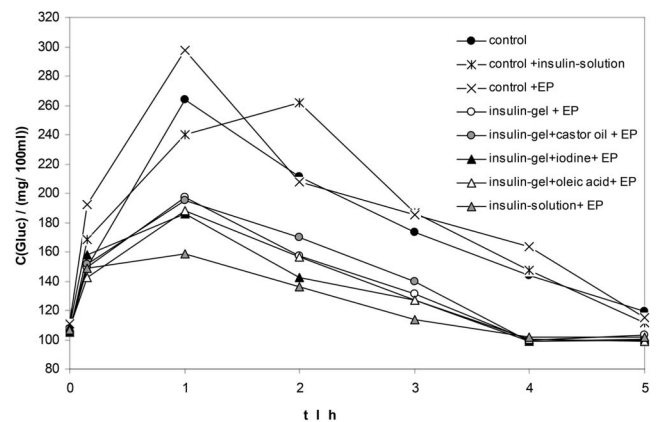
formulations at different experimental conditions. Data are analyzed using the Duncan's multiple range test offered by SPSS software version 17.0. The differences are considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Electroporative pulses enable control of blood sugar level by transdermal administration of insulin

Figure 1 presents the effect of skin administration of suggested 5 different formulations of insulin in the presence of electroporative pulses on the mean blood sugar level (BSL) of rabbit groups (5 rabbits each) with induced hyperglycemia.

The performance of these formulations is compared with 3 different control groups of rabbits (5 rabbits each). The first control group is not given any insulin formulation or exposed to EP (control). A second control group is treated with insulin solution in the absence of EP (control + insulin solution). The third control group is exposed to EP in the absence of insulin (control + EP). For almost all groups, the maximum recorded BSL is at 1 hour after the administration of the hyperglycemia-inducing agent



**FIGURE 1.** The change in the blood glucose level of rabbits during the 5 hours of the experiment for control groups (control, control + EP, and control + insulin solution) and the 5 different formulations of insulin (insulin gel + EP, insulin gel + castor oil + EP, insulin gel + iodine + EP, insulin gel + oleic acid + EP, and insulin solution + EP) in the presence of electroporative pulses (EP). The electroporation protocol involved application of 60 pulses, at field strength of 100 V/cm, and pulse duration of 2 milliseconds with an interpulses time of 1 second. The insulin concentration is 70 IU/mL. Each data point represents the mean value for readings from 5 rabbits.  $C(\text{Gluc})/(\text{g}/\text{mL})$  is glucose concentration,  $t/\text{h}$  is the time in hours.

**Table 1.** AUBSL curve values for all of insulin formulations + EP and controls.

Formulation	AUBSL curve values at 100 V/cm/60 pulses/2 ms, insulin concentration (70 IU/mL)
Control	914.400 <sup>d</sup>
Control + insulin solution	976.215 <sup>e</sup>
Control + EP	992.465 <sup>e</sup>
Insulin gel + EP	704.605 <sup>b,c</sup>
Insulin gel + castor oil + EP	722.430 <sup>c</sup>
Insulin gel + iodine + EP	677.185 <sup>b</sup>
Insulin gel + oleic acid + EP	686.045 <sup>b,c</sup>
Insulin solution + EP	631.585 <sup>a</sup>

Significantly different ( $P < 0.05$ ) AUBSL curve values are given different letters (a–e).

(xylazine). The different insulin formulations and EP are applied 15 minutes after the injection of xylazine. It can be observed that all of the insulin formulations + EP successfully decreased the BSL of treated rabbits compared with different controls all over the monitoring period (5 hours). After 4 hours, the BSL of rabbits treated with different formulations of insulin + EP become very close to the normal BSL levels (at 0 hours) and are considerably lower than the BSL of control rabbits. This low BSL level continued to be maintained up to the end of the monitoring period. Despite all insulin formulations + EP are able to cause a reduction in the BSL over a 5-hour period after administration of xylazine, the performance of individual formulations + EP seems to be considerably different. Figure 1 shows that insulin solution + EP formulation is probably able to maintain lower BSL all over the monitoring period compared with the other formulations.

To obtain a quantitative measure of the performance of different formulations over the 5-hour period after xylazine administration, the area under the BSL versus time curves is calculated for all insulin formulations + EP and control.

To find out whether the variation in between the different controls and those in between the different formulations + EP are significant, Duncan multiple range test is performed for all groups.

Table 1 shows that all formulations + EP are significantly different ( $P < 0.05$ ) from controls and that they all maintain a lower AUBSL curves compared with controls (note that significantly different AUBSL curve values are given different letters a–e). In between the different formulations, Table 1 shows that insulin solution + EP (letter a) has significantly lower AUBSL curve compared with all other formulations.

The superior performance of insulin solution compared with the other formulations could be attributed to 3 main factors. The first is that although the association between a gel and insulin may offer a reservoir to hold insulin in contact with the skin for a long period, yet it has been reported that this kind of association may affect the skin penetrability of the complex.<sup>34</sup> Second, after the penetration of skin, while insulin solution can act promptly, the insulin gel complex would result in a gradual release of insulin.<sup>35,36</sup> Finally, insulin solution has the highest conductivity (10 mS/cm) compared with the other formulations.

Insulin gel + iodine + EP (b) is insignificantly different ( $P > 0.05$ ) from each of insulin gel + oleic acid + EP (b, c) and insulin gel + EP (b, c).

The latter 2 formulations + EP are both insignificantly different from each other and insignificantly different from insulin gel + castor oil + EP (c). Although the control (d) is significantly different from control + EP (e) and control + insulin solution (e), yet all controls have significantly higher AUBSL curves compared with any of the investigated formulations.

**Table 2.** AUBSL curve values for all insulin (50 IU/mL) formulations + EP and control at different number of electro-porative pulses.

Number of pulses [100 V/cm, insulin concentration (50 IU/mL)]	AUBSL curves for insulin formulations					
	Control	Insulin gel + EP	Insulin gel + castor oil + EP	Insulin gel + iodine + EP	Insulin gel + oleic acid + EP	Insulin solution + EP
15	1084.850 <sup>c</sup>	1013.6742 <sup>d</sup>	972.915 <sup>c</sup>	972.505 <sup>c</sup>	1000.710 <sup>d</sup>	1033.945 <sup>c</sup>
25	1026.230 <sup>b,c</sup>	979.215 <sup>d</sup>	954.165 <sup>c</sup>	961.737 <sup>c</sup>	909.865 <sup>c</sup>	780.015 <sup>b</sup>
40	944.945 <sup>a</sup>	911.900 <sup>c</sup>	836.550 <sup>b</sup>	861.785 <sup>b</sup>	813.420 <sup>b</sup>	764.820 <sup>b</sup>
50	947.555 <sup>a</sup>	816.575 <sup>b</sup>	798.845 <sup>a,b</sup>	853.975 <sup>b</sup>	824.180 <sup>b</sup>	773.685 <sup>b</sup>
60	1000.540 <sup>a,b</sup>	742.437 <sup>a</sup>	764.920 <sup>a</sup>	784.250 <sup>a</sup>	733.945 <sup>a</sup>	651.320 <sup>a</sup>

Significantly different ( $P < 0.05$ ) AUBSL curve values are given different letters (a–d).

**Table 3.** AUBSL curve values for all insulin (70 IU/mL) formulations + EP and control at different number of electro-porative pulses.

Number of pulses [100 V/cm, (insulin concentration is 70 IU/mL)]	AUBSL curves for insulin formulations					
	Control	Insulin gel + EP	Insulin gel + castor oil + EP	Insulin gel + iodine + EP	Insulin gel + oleic acid + EP	Insulin solution + EP
15	965.490 <sup>b</sup>	797.290 <sup>a</sup>	746.630 <sup>a,b</sup>	831.360 <sup>b</sup>	799.280 <sup>a</sup>	747.765 <sup>b</sup>
25	937.535 <sup>a,b</sup>	755.775 <sup>a</sup>	714.110 <sup>a,b</sup>	772.160 <sup>a</sup>	804.170 <sup>a</sup>	780.185 <sup>b</sup>
40	880.210 <sup>a</sup>	765.995 <sup>a</sup>	738.775 <sup>a,b</sup>	774.595 <sup>a</sup>	762.885 <sup>a</sup>	832.795 <sup>c</sup>
50	909.820 <sup>a,b</sup>	768.415 <sup>a</sup>	755.850 <sup>b</sup>	796.090 <sup>a,b</sup>	789.710 <sup>a</sup>	760.345 <sup>b</sup>
60	978.250 <sup>b</sup>	765.425 <sup>a</sup>	704.730 <sup>a</sup>	791.200 <sup>a,b</sup>	805.005 <sup>a</sup>	531.365 <sup>a</sup>

Significantly different ( $P < 0.05$ ) AUBSL curve values are given different letters (a–c).

### Effect of number of electro-porative pulses on the BSL of rabbits treated using electro-porative transdermal insulin administration

Table 2 shows that at insulin concentration of 50 IU/mL, for all of the investigated formulations, the AUBSL curve values are significantly lower at 60 pulses compared with lower number of pulses and that there seem to be a significant gradual increase in the AUBSL values with the decrease in the number of pulses down to 15 pulses. It has been reported elsewhere<sup>2</sup> that the increase in the number of electro-porative pulses would slightly decrease the skin resistance.

The present results show that there is a significant increase in the penetration of different insulin formulations with the increase in the applied number of electro-porative pulses at low concentration of insulin (50 IU/mL). At a higher insulin concentration (70 IU/mL), the effect of number of pulses seems to be completely absent (Table 3) except for insulin solution + EP at 60 pulses, which record a significant decrease in the AUBSL curves compared with all other number of pulses applied to the same formulation.

### Effect of insulin concentration on the control of BSL by electro-porative transdermal administration of insulin

On comparing the effect of changing insulin concentration on the performance of individual formulations, it is found that all formulations have significantly ( $P < 0.05$ ) lower values of AUBSL curves at 70 IU/mL compared with 50 IU/mL (Table 4).

The decrease in the AUBSL curves in case of 70 IU/mL compared with 50 IU/mL is an expected result probably because of the increase in the number of insulin molecules available to penetrate the rabbits' skin on the application of electro-porative pulses. Denet et al reported that the increase in the concentration of drug in the presence of electro-poration can result in an increase in the diffusion of drug through cell membrane.

### Effect of pulse duration on the electro-porative transdermal administration of insulin

Table 5 shows that the effect of pulse duration on the electro-porative administration of insulin (70 IU/mL) is almost negligible. The only significant difference (at 4 milliseconds) is reported for insulin gel + castor oil + EP.

**Table 4.** AUBSL curve values for all insulin formulations + EP and control at 2 concentrations (50 and 70 IU/mL) of insulin.

Insulin concentration (100 V/cm/60 pulses), IU/mL	AUBSL curves for insulin formulations					
	Control	Insulin gel + EP	Insulin gel + castor oil + EP	Insulin gel + iodine + EP	Insulin gel + oleic acid + EP	Insulin solution + EP
50	1067.050 <sup>a</sup>	818.575 <sup>a</sup>	798.090 <sup>b</sup>	881.505 <sup>a</sup>	805.005 <sup>b</sup>	747.005 <sup>b</sup>
70	978.250 <sup>a</sup>	834.480 <sup>a</sup>	686.469 <sup>a</sup>	815.510 <sup>a</sup>	719.225 <sup>a</sup>	549.543 <sup>a</sup>

Significantly different ( $P < 0.05$ ) AUBSL curve values are given different letters (a, b).

**Table 5.** AUBSL curve values for all the insulin (70 IU/mL) formulations + EP and control at different electroporative pulse durations.

At 200 V/cm/60 pulses, insulin concentration (70 IU/mL), ms	AUBSL curves for insulin formulations					
	Control	Insulin gel + EP	Insulin gel + castor oil + EP	Insulin gel + iodine + EP	Insulin gel + oleic acid + EP	Insulin solution + EP
1	869.180 <sup>a</sup>	791.880 <sup>a</sup>	793.755 <sup>a</sup>	780.395 <sup>b</sup>	791.770 <sup>a</sup>	701.160 <sup>a,b</sup>
2	912.620 <sup>a,b</sup>	791.900 <sup>a</sup>	790.515 <sup>a</sup>	789.395 <sup>b</sup>	771.300 <sup>a</sup>	726.880 <sup>c</sup>
3	867.165 <sup>a</sup>	770.687 <sup>a</sup>	757.580 <sup>a</sup>	787.670 <sup>b</sup>	764.530 <sup>a</sup>	719.105 <sup>b,c</sup>
4	934.495 <sup>a</sup>	751.857 <sup>a</sup>	755.270 <sup>a</sup>	747.270 <sup>a</sup>	752.800 <sup>a</sup>	694.770 <sup>a</sup>

Significantly different ( $P < 0.05$ ) AUBSL curve values are given different letters (a–c).

A similar result is also obtained using insulin concentration of 50 IU/mL (data not shown); this result is probably related to the shape of the exponential pulse used in this work. The pulse decays very fast, which makes the increase in pulse duration of minor effect.<sup>28</sup> This is different from the case of square pulses where the threshold voltage depends on the pulse duration and an increase in the electroporative pulse duration leads to a decrease in the critical voltage. Therefore, longer square electric pulses would probably produce larger pores.<sup>12,37,38</sup>

#### Effect of applied field strength on the electroporative transdermal administration of insulin

Table 6 shows that a significant ( $P < 0.05$ ) difference from control at 100 V/cm compared with 150 and 200 V/cm only exists for insulin gel + iodine + EP, insulin gel + oleic acid + EP, and insulin solution + EP formulations. This is a favorable result as it is always recommended to decrease the field strength to save the treated skin<sup>30,27</sup> and to reduce the associated pain and muscle stimulation effects of electroporative pulses that constrain the electric field within the SC.<sup>39</sup>

The superiority of the lower field strength (100 V/cm) over higher field strengths may be attributed to the possible effects of high-voltage pulses on the structure and function of insulin molecule, which suggests

that insulin may have penetrated more efficiently, yet with considerably lower functionality.

## CONCLUSIONS

It has been shown that electroporative pulses can be used as a means to control BSL in rabbits with induced hyperglycemia by enhancing insulin transport through rabbit's skin. The investigated different insulin formulations + EP can efficiently lower BSL compared with control. The insulin solution + EP formulation shows the best performance compared with all other formulations. Using insulin concentration of 50 IU/mL, the increase in the number of applied electroporation pulses to 60 successive pulses, for almost all formulations, enhances transdermal insulin delivery compared with control. This trend is almost absent at insulin concentration of 70 IU/mL with an exception of the insulin solution + EP, which shows a remarkable enhancement in transdermal delivery of insulin at 60 successive pulses compared with all other number of pulses. The increase in the concentration of drug in the presence of electroporation has been shown to improve the transdermal delivery of insulin. Low-field strength results in better transdermal insulin delivery. Insulin may have penetrated more efficiently

**Table 6.** AUBSL curve values for all insulin (70 IU/mL) formulations + EP from control at different field strengths.

Different field strength [60 pulses/insulin concentration (70 IU/mL)], V/cm	AUBSL curves for insulin formulations					
	Control	Insulin gel + EP	Insulin gel + castor oil + EP	Insulin gel + iodine + EP	Insulin gel + oleic acid + EP	Insulin solution + EP
100	914.400 <sup>a</sup>	704.605 <sup>a</sup>	722.430 <sup>a</sup>	677.185 <sup>a</sup>	710.045 <sup>a</sup>	633.690 <sup>a</sup>
150	882.350 <sup>a</sup>	744.760 <sup>b</sup>	760.925 <sup>a</sup>	769.740 <sup>b</sup>	770.355 <sup>b</sup>	723.493 <sup>b</sup>
200	867.165 <sup>a</sup>	733.165 <sup>a,b</sup>	746.415 <sup>a</sup>	779.995 <sup>b</sup>	759.710 <sup>b</sup>	719.105 <sup>b</sup>

Significantly different ( $P < 0.05$ ) AUBSL curve values are given different letters (a–c).

at higher field strengths, yet the possible effects of high-voltage pulses on the structure and function of insulin molecule probably resulted in that the delivered insulin at higher pulse voltages is not completely functional. The effect of pulse duration on the electroporative administration of insulin is almost negligible.

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