Multiple daily injections of insulin for diabetes cause many hazards for diabetic patients. Oral non-invasive insulin delivery could be more convenient and less painful than parenteral route. In past decades transdermal iontophoresis had been studied for insulin delivery across the skin with or without chemical permeation enhancers. However, the results of these studies were not efficacious and serum insulin levels were not therapeutically effective. In the present study an advanced technology “gut iontophoresis” for insulin delivery across the gut wall was compared with traditional oral insulin delivery in the form of nanoparticles. In vitro application of electric current to the intestinal membrane could enhance the flux of insulin nanoparticles (3.4 fold enhancement of insulin transport) from the donor to the receptor compartment in the Franz cell. In vivo iontophoresis of insulin nanoparticles through the gut wall would produce intense hypoglycemia (57% glycemia drop in 3 h) without damage of the intestinal tissues. Cell viability assay indicated that 50–500 µg/mL nanoparticles had no toxic effect on Caco-2 cells. Nanoparticles gut iontophoresis could be a promising non-invasive technique for oral insulin delivery.

Key words  insulin; nanoparticle; gut iontophoresis; oral delivery

INTRODUCTION

In the last few decades, nanotechnology has been applied for different routes of drug administration, which has allowed for overcoming the problems and meeting the shortage of current conventional dosage forms. Insulin is a hormone peptide in nature composed of 51 amino acids in two peptide chains with molecular weight about 5.7 kDa. Insulin was widely used for treatment of insulin dependent diabetes mellitus as a replacement therapy in which insulin is administered subcutaneously to compensate the shortage of insulin secretion in diabetic patients.

Insulin delivery has occupied an important area in the medical field and dosage form design. Actually, most of therapeutic peptides like insulin are administered parenteral because of rapid hydrolysis and enzymatic degradation after oral administration.

Modification in the chemical structure of peptides or nanencapsulation in biocompatible, biodegradable polymers made them more stable against degradation by gastrointestinal enzymes. Oral mucoadhesive nanoparticles for insulin delivery provided an intimate contact with the mucosa which made them a useful vehicle to overcome enzymatic barriers to oral insulin delivery. Natural, biodegradable polymers such as (alginate, Chitosan and dextran sulfate) have shown a superior protective ability against proteolytic enzymes.

Iontophoresis is an effective noninvasive technique for drug delivery using small current potential difference for short periods of time (0.5 mA/cm²) for 20–30 min. In iontophoresis, the used electrical current (direct or pulsed) pushes drug molecules across the barriers on which the working electrode is placed. This electrode can be positive or negative according to the surface charge of the drug molecules and the other electrode is placed elsewhere to complete the electrical circuit.

Simply, cationic drug in the anode reservoir transports toward the cathode electrode across the circuit after electric current application. Before it reaches the cathode, the drug is partitioned into different tissues and absorbed by blood circulation away from the site of permeation.

Iontophoretic drug transport occurs by different mechanisms like diffusion, migration, electroosmosis or electroporation. Electroosmosis involves the movement of fluid without concentration gradient and occur in the same direction of counter ions when a voltage difference is applied across charged and porous membrane.

Transdermal iontophoretic delivery of insulin showed positive results and caused a reduction in blood glucose levels in animal models. However, in human the challenge was difficult because of higher amounts of insulin was required for therapeutic effect and the longer pathway that insulin should take across the human skin to reach the blood stream.

In this study, the challenge was to investigate the potential of insulin nanoparticles permeation across the gut wall by using a small electric current for short periods of time without causing damage to the intestinal cells and whether delivered insulin was biologically active and caused a hypoglycemic effect in diabetic rats.

The application of iontophoresis at the gut wall “gut iontophoresis” is a novel procedure for insulin delivery into the intestine then to the blood stream.

MATERIALS AND METHODS

Materials Recombinant human insulin dry powder (Sigma-Aldrich Chemie, France), sodium alginate (Al-Shark Al-Awsat Co., Cairo, Egypt) and chitosan (50kDa) (75% deacetylation) (Gama Group Co., Cairo, Egypt), dextran sulphate (Al-Shark Al-Awsat Co.), calcium chloride dehydrate

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(Gama Group Co.). Hematoxylin and eosin solutions were bought from Sigma-Aldrich (St. Louis, MO, U.S.A.). Amicon ultra 15 (Ultracel–100 K) centrifuge tube with 100 kDa cut off. Enzyme-linked immunosorbent assay (ELISA) test kit, Merckodia, Uppsala, Sweden. Glucometer (Accu-check, Active, Germany). Franz diffusion cell (Keshary Chien type). Tens 7000 (A battery operated pulse generator) was manufactured for Roscoe medical and 100 kΩ potentiometers were bought from local stores. Dialysis membrane bag with molecular cut-off of 12 kDa (Gama Group Co.). Dulbecco’s modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, t-glutamine and alamar blue (AB) were purchased from Nile Center For Experimental Research (Mansoura, Egypt). Caco-2 (human colon cancer cell line, HTB-37) was purchased from Vacsera (cell culture unit; Giza, Egypt). All other reagents used were of analytical grade.

Male Sprague-Dawley rats weighing 190–210 g (21 d age) from Breeding unit of the Egyptian organization for biological products and vaccines (Helwan, Egypt).

Preparation of Insulin Loaded Nanoparticles Optimum insulin loaded nanoparticles were prepared by ionotropic pregelation followed by polyelectrolyte complexation of biomaterials carrying opposite charges under controlled pH conditions. A standard formulation was prepared at room temperature under magnetic stirring at 800 rpm. Ionotropic pregelation involves dropwise extrusion of 7.5 mL of 0.2% (w/v) calcium chloride solution into 117.5 mL of a solution at pH 4.9 containing 0.06% (w/v) alginic sodium salt, 0.02% (w/v) dextran sulphate and 7 mg of insulin (equivalent to 200 IU insulin). Polyelectrolyte complexation occurred by dropwise addition of 25 mL of 0.014% (w/v) Chitosan (dissolved in 1% acetic acid) for stabilization of pre-gel nuclei into nanoparticles at pH 4.6. The nanoparticle suspension was then centrifuged at 4°C in the Amicon–Ultra-15 at 15000 rpm for 20 min to separate free polymers from nanoparticles. Nanoparticles in the dialysis tube were evaluated for their size and zeta potential. The solution collected in the outer tube was analyzed for loading efficacy.  

Cytotoxicity Assay of Insulin Loaded Nanoparticles Caco-2 cell lines were used to evaluate the effect of nanoparticles on the target sites in the colon. Cells were cultured with DMEM in the presence of 2 mM t-glutamine and antibiotic (100 U/mL penicillin and 100 U/mL streptomycin) and 10% (v/v) FBS. This culture was maintained under standard conditions (5% CO₂ in 95% air and 37°C). Cells were seeded in 96-well microplate with cell density 5×10⁴/mL (100 µL/well) and incubated for 24h. After 24h, the medium was replaced with fresh medium with nanoparticles at concentrations 50, 100, 200, and 500 µg/mL and the control occurred as FBS-free medium.  

The cells were incubated for 24h under the same conditions. After the incubation period, the incubating medium was removed and 10% (v/v) alamar blue was added (100 µL/well) then incubated for 4h (37°C, 5% CO₂ in 95% air). Cell viability was tested by reading the absorbance at 570 nm with microplate reader.

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\% \text{ Viability} = \frac{\text{Absorbance of experimental group}}{\text{Absorbance of control}} \times 100
\]

In Vitro Insulin Release from Insulin Loaded Nanoparticles Quantity of dried nanoparticles equivalent to about 2 mg of insulin was then re-dispersed in 5 mL of ultrapure water and placed in a dialysis membrane bag with molecular cut-off of 12 kDa, tied and placed into 50 mL of dissolution media. The entire system was kept at 37 ± 0.5°C with continuous magnetic stirring (100 rpm) and the study was carried out in three dissolution media: acidic medium, pH 1.2 (simulated gastric fluid) and phosphate-buffered solution (PBS), pH 6.8 and 7.4 (simulated intestinal fluids). 

At appropriate time intervals, 3 mL of the release medium was removed and 3 mL fresh medium was added into the system to maintain sink conditions.

As the control, 6 mg insulin powder was finely dispersed in 300 µL distilled water to form a suspension, 100 µL aliquots (i.e., an equivalent of 2 mg insulin) of which were mixed with 5 mL of ultrapure water and placed in the dialysis bag under the same conditions of insulin loaded nanoparticles. The withdrawn samples were centrifuged at 15000 rpm for 15 min to remove the precipitate before insulin determination by ELISA.

Preparation of Intestinal Membrane for In Vitro Permeation Study An intestinal segment was excised from the rat (ether anesthetized by inhalation for about 5 min) abdominal cavity. It was rinsed with deionized water and fixed on a glass rod. The dermal layer of the tissue was rubbed carefully with a scalpel to reveal the intestinal mucosa.

In Vitro Permeation Study by Franz Diffusion Cell (Passive Transport) Franz diffusion cell as shown in Fig. 1 composed of two chambers, the donor and the receptor chambers with a diffusion area of about 1.4 cm². The excised intestinal segment was mounted between the compartments in 37°C and mixed gas environment of 95% air and 5% CO₂. Magnetic stirrer was added to the receptor compartment and filled with phosphate buffer saline, pH 7.4 as the receptor medium.

Insulin loaded nanoparticles (containing 500 µg/mL insulin) were suspended in deionized water, and then added to the donor compartment (200 µL) for in vitro permeation test. One hundred microliter samples were taken from the receptor medium at different time intervals and replaced with the same volume. The amount of permeated drug was examined by ELISA.

In Vitro Permeation Study by Franz Diffusion Cell under Iontophoresis The Franz cell was prepared as described in passive diffusion and then the iontophoretic device
was used to supply an electrical current to the intestinal membrane fixed between the two compartments. The cathode was connected to the donor chamber and the anode was connected to the receptor chamber with a distance of 1 cm away from the membrane. The current applied to the cell was adjusted to 50 µA using a potentiometer.

The current was applied to the cell for one hour as pulsed current (10 min current on) followed by (10 min recovery/off period).

After one hour, the electrical current was switched off and then at predetermined time intervals 1 mL was removed from the receptor chamber and replaced with the same volume for 3 h. The samples were centrifuged at 5000 rpm for 10 min and then the supernatant was analyzed for insulin content by ELISA.

**In Vivo Study of Gut Iontophoresis Using Insulin Nanoparticles** The animal studies were performed in accordance with the research ethics committee for experimental and clinical studies at Faculty of Pharmacy, Cairo University (Cairo, Egypt), PI (1383).

Adult male Sprague-Dawley rats weighing 190–210 g were rendered diabetic by a single intraperitoneal injection of streptozotocin (STZ) (45 mg/kg in pH 4.5 citrate buffer) (9 mg STZ in 0.5 mL citrate buffer pH 4.5 for each rat). After 2 weeks, rats with fasted blood glucose levels above 300 mg/dL were selected for experiments. These rats were fasted 12 h before and 24 h during the experiment, but were allowed water ad libitum, then the rats were divided into 4 groups (n = 6).

The rats of the first group under anesthesia (Fig. 2) were prepared for surgery and the abdomen was incised to expose the intestine. Two small batches about 2 cm² containing insulin nanoparticles of 501U/kg insulin were connected to the wires of the iontophoretic device.

The electric current (40 µA) was conducted for 2 min followed by 4 min for recovery for one hour, therefore the total current for iontophoresis was 20 min.

At the end of the experiment, the rats were sacrificed and the intestinal sections under and around the batches were appropriately cut for histological examination to analyze iontophoresis damage if present.

For the second group, insulin nanoparticles containing 501U/kg insulin were administered intragastrically by oral gavage. Control group tests were performed for insulin nanoparticles without electric current (passive diffusion) with the same insulin content, the last group was administered subcutaneous insulin of 1 IU/kg.

Blood samples (0.2 mL) were collected from the tail vein every 30 min during experiment and 3 h after the experiment. Sample was separated in two volumes, one to determine plasma glucose level and the other for insulin determination.

**Histological Examination of Intestinal Tissues after Iontophoresis** Histological morphology was tested before and after application of electric current using a light microscope. Cross sections of intestinal tissues were fixed in 10% buffered formalin, dehydrated in ethanol and then embedded in paraffin. The sections were rehydrated and stained with hematoxylin and eosin.

**Statistical Analysis** In vitro statistical analysis was performed using independent sample t-test. The cumulative hypoglycemic effect and the cumulative amount of insulin delivered to plasma were calculated for each rat, and a one-way ANOVA used to evaluate treatment differences. All statistical analyses were performed with the SPSS software package (SPSS for Windows 14.0, SPSS, U.S.A.).

**RESULTS AND DISCUSSION**

**Characterization of Insulin Loaded Nanoparticles** Insulin nanoparticles with mean volume diameters of about 360 ± 21 nm were prepared with ionotropic pre-gelation polyelectrolyte complexation technique.

As shown in Fig. 3, transmission electron microscopy (TEM) images of insulin nanoparticles were spherical in shape with insulin distributed on the surface of nanoparticles. Zeta potential of insulin nanoparticles was −28 ± 5.8 and entrapment efficiency of about 83 ± 26%.

**The Cytotoxicity of Insulin Nanoparticles** As illustrated in Fig. 4, insulin nanoparticles showed no evidence of toxicity response toward cell lines and there was no significant difference (p < 0.05) with respect to the cell viability compared to the control after 24 h in the presence of alamar blue as a redox indicator.

These results may be due to that polymers in the nanoparticulate system are natural in origin and biocompatible in nature. These results are in agreement with previous studies that indicated the lack of toxicity and the induction of proliferation rate of the cells compared to the control which could be due to the mitochondrial activity of the cells.
In Vitro Insulin Release from Insulin Nanoparticles

At Acidic Medium (pH 1.2)
The nanoparticles showed burst drug release in acidic medium about 13% (w/w) of initial drug amount ($p < 0.05$) after 2h (Fig. 5). The nanoparticles showed burst drug release in acidic medium about 13% (w/w) of initial drug amount ($p < 0.05$) after 2h (Fig. 5). The nanoparticles showed burst drug release in acidic medium about 13% (w/w) of initial drug amount ($p < 0.05$) after 2h (Fig. 5). The nanoparticles showed burst drug release in acidic medium about 13% (w/w) of initial drug amount ($p < 0.05$) after 2h (Fig. 5). The nanoparticles showed burst drug release in acidic medium about 13% (w/w) of initial drug amount ($p < 0.05$) after 2h (Fig. 5). The nanoparticles showed burst drug release in acidic medium about 13% (w/w) of initial drug amount ($p < 0.05$) after 2h (Fig. 5). The nanoparticles showed burst drug release in acidic medium about 13% (w/w) of initial drug amount ($p < 0.05$) after 2h (Fig. 5). The nanoparticles showed burst drug release in acidic medium about 13% (w/w) of initial drug amount ($p < 0.05$) after 2h (Fig. 5).

At Alkaline Medium (pH 6.8)
The nanoparticles showed the release of insulin about 44% ($p < 0.05$) of initial insulin amount after 6h (Fig. 5). Alginate polymer chain had been swollen in pH greater than 6.5 (pH of the duodenum) and obtains more porosity in the structure of nanoparticle resulting in more insulin release. At pH 6.8 the NH3+ groups of chitosan get deprotonated to yield uncharged NH2 groups and so their electrostatic interactions were reduced with COO groups of alginate chains.24)

At Alkaline Medium (pH 7.4)
The disintegration of insulin nanoparticles occurred mainly at this pH (pH of the ileum) where the absorption of insulin was performed (Fig. 5). The disintegration may be due to ion exchange between Na+ ions from the phosphate buffer and Ca2+ ions from the egg-box cavities of polyguluronate blocks of alginate. The nanoparticles showed the maximum release of insulin about 74% ($p < 0.05$) after 10h. Free non-encapsulated insulin was observed with around 90% degradation when immersed in phosphate buffer pH 7.4 after 10h, this may be due to absence of any polymer protection.13,25)

In Vitro Passive Permeation Study of Insulin Nanoparticles

Upon the application of electrical stimulus on the nanoparticulate system consisting of ionizable hydrogels or electroresponsive polymers as (alginate and chitosan), phase transition occurs influencing the swelling behavior of the polymers. There are forces affecting the conversion between the two phases as hydrogen bonding and van der Waals interaction. In the first phase, the electrical stimulus increases the attraction forces and the matrix expels the water (shrinking phase).26) In the second phase, the electrical stimulus increases the repulsion forces and the matrix absorbs the water (swelling phase). This phase transition occurred by the electrical stimuli results in a change in physicochemical properties of the nanoparticulate system and plays a great role in the drug release (on/off release behavior).26)

Insulin loaded nanoparticles were studied for in vitro permeation through excised intestinal membrane.2) The permeation flux was calculated as the slope divided by the membrane surface area:

$$J = (dQ/dt) \cdot 1/A$$

Where ($J$) is the permeation flux ($\mu g/cm^2/h$), ($dQ/dt$) is the amount of insulin passed per unit time and $A$ is the surface...
The passive permeation flux of insulin nanoparticles (µg/cm²/h) within the first 15 min as shown in Fig. 6 was 0.24 µg/cm²/h occurred without using electric current, and then after 3 h 4.85 µg of insulin was transported to the receptor medium.\(^{28}\)

Low amount of insulin nanoparticles was permeated across the intestinal membrane by pure or passive diffusion through paracellular pathway.\(^{27}\) The occurrence of such a simple diffusion was regulated by concentration gradient and this behavior was affected by several factors which have to be examined before application. These factors include physico-chemical properties of insulin nanoparticles such as (zeta potential and nanoparticle size) and also the relative affinity of the membrane for insulin nanoparticles.\(^{28}\)

In vitro passive permeation study showed that the nanosize range of the formulation would help in penetration into the intestinal membrane, since higher concentrations of drug were quantified in the membrane when nanoparticles were applied. The dynamic properties of nanoparticles allied with their small size would help in the interaction with the intestinal membrane and may cause certain disorder of the intestinal membrane, consequently facilitating permeation.\(^{29}\)

In Vitro Permeation Study of Insulin Nanoparticles Using Iontophoresis In Iontophoresis, charges with the same signal repel and charges with opposite charges attract which would facilitate the permeation of ionized particles across the intestinal membrane. And also, the electric current would cause a slight reversible disturbance in the membrane structure which would open the pores of the membrane to enhance the permeation of the ions.\(^{30}\)

An iontophoresis physical technique was used with nanocarriers to increase a synergistic permeation of insulin into/through the membrane. In other words, iontophoresis would cause disturbance in the intestinal membrane and also could push insulin nanoparticles into the receptor medium.\(^{29}\)

Mucus layer is a semipermeable barrier consists mainly of mucin which obstructs the diffusion of insulin. This behavior is due to the electrostatic repulsion between negatively charged sialic acid residues of mucin and insulin molecules. Chitosan coated nanoparticles exhibited mucoadhesion characteristic due to electrostatic attraction between negatively charged mucin and positively charged chitosan. Furthermore, these nanoparticles able to cause reversible opening of tight junctions (TJs) and increase paracellular transport through intestinal epithelia.\(^{31}\)

There was a significant difference (\(p < 0.05\)) between insulin transport through the membrane with and without using electric current. By using electric current, insulin nanoparticles transport within the first 15 min was 1.47 ± 0.04 µg of insulin as shown in Fig. 6 and then after 3 h 16.5 ± 0.40 µg of insulin was permeated across the intestinal membrane.\(^{32}\)

Moreover, the negative zeta potential of insulin nanoparticles would contribute to a better interaction of the nanoparticles with the intestinal membrane and to a higher capacity of the cathodic iontophoresis.\(^{29}\)

In Vivo Permeation Study of Insulin Nanoparticles As shown in Fig. 7, cathodic gut iontophoresis with an electric current of 40 µA using insulin nanoparticles containing 50IU insulin demonstrated a significant reduction (\(p \leq 0.05\)) in blood glucose level after 2 h about 31% of initial value (69 ± 1.63%) and then after 3 h the reduction was about 57% of initial value (43 ± 1.52%).\(^{33}\) In contrast, oral insulin nanoparticles with 50IU insulin showed only 12% (88 ± 0.17%) reduction and followed by 22% reduction after 3 h (78 ± 1.86%).\(^{3}\)

Passive diffusion of insulin nanoparticles by iontophoretic device without using electric current showed a slight reduction about 3% of initial blood glucose level (97 ± 0.58%) after 2 h then after 3 h 8% reduction (92 ± 2.31%) was observed.\(^{3}\)

Maximum reduction in blood glucose level was observed after subcutaneous injection of insulin 1IU/kg about 42% of initial value (58 ± 1.48%) in 2h, however there was a recovery of blood glucose level to about 32% of initial value (68 ± 0.63%) after 3h.\(^{3}\)

The paracellular transport is a major pathway for absorption of oral insulin nanoparticles and passive diffusion of insulin nanoparticles. However, the hypoglycemic effect of passive diffusion was less than oral insulin nanoparticles. This result may be due to tightly compact insulin nanoparticles in passive diffusion which reduced surface area of nanoparticles at the absorption sites and the paracellular transport became identified as limiting the absorption rate. Also, the absorption rate was reduced due to the saturation of the junctions of the intercellular spaces especially in the absence of the permeation enhancers as positive iontophoresis which caused disturbances in the intestinal membrane.\(^{34}\)
As shown in Fig. 8, the highest increase in serum insulin levels was observed after subcutaneous injection about 1.65 ± 0.040 ng/mL after 2 h that reduced to 1.05 ± 0.04 ng/mL after 3 h. Insulinemia levels after gut iontophoresis showed a significant increase (p ≤ 0.05) to about 1.17 ± 0.04 ng/mL after 2 h and then 1.22 ± 0.034 ng/mL after 3 h. Oral insulin nanoparticles showed an increase in serum insulin of about 0.88 ± 0.033 and 0.92 ± 0.023 ng/mL after 2 and 3 h, respectively. Passive diffusion of insulin nanoparticles showed non-significant increase in serum insulin levels.35)

From the above results, there was a good correlation and synergism between the mucoadhesion characteristics of insulin nanoparticles and the electrical gradient established by gut iontophoresis. This electrical gradient would potentiate the permeation of the drug formulation across pathways that already exist within the intestinal barrier without causing notable damage.36) While the mucoadhesion properties of nanoparticles would increase the residence time of the formulation at the absorptive sites which would explain the sustained hypoglycemic effect of the formulation.35,36)

The application of this technology requires modification of conventional oral drug delivery systems to micro-electronic devices that can perfectly target absorption sites in the gastrointestinal tract and in a controlled manner.37) The design of this iontophoretic device can be formulated as an enteric

Fig. 8. Serum Insulin Levels after Oral Administration of Insulin Nanoparticles (50IU Insulin), Subcutaneous Insulin (11U/kg), Gut Iontophoresis of Insulin Nanoparticles and Passive Diffusion

Each value represented as mean ± S.E.

Fig. 9. Photomicrographs of Intestinal Tissues after Hematoxylin and Eosin Staining

L: lumen of intestine; M: mucosa of the intestine; SM: smooth muscle (A) before iontophoresis; (B) after iontophoresis.
coated capsule containing mucoadhesive insulin nanoparticles and an electronic microchip circuit. The release of the electric circuit and nanoparticles occurs in the intestine in which the aqueous medium will initiate the gut iontophoresis followed by disturbance in intestinal pathways and then mucoadhesion of nanoparticles facilitates insulin release in a controlled manner.\(^{19}\)

As discussed in previous studies, oral glucose tolerance test (OGTT) is a challenge performed to ensure that oral insulin nanoparticles have the ability to suppress blood glucose levels sufficiently under feeding conditions. In this test, there was multiple administration of oral insulin nanoparticles (50IU/kg) 3 times daily followed by feeding of the diabetic rats for 30 min after drug administration. The results indicated that oral insulin nanoparticles significantly suppressed the postprandial increase in blood glucose levels and showed a continuous hypoglycemic activity.\(^{39,40}\)

Consequently, gut iontophoresis as a new technique for delivering oral insulin nanoparticles would give the postprandial hypoglycemic action. Furthermore, this method would give a more rapid hypoglycemic response than conventional oral insulin nanoparticles as mentioned before.

**Histological Examination of Intestinal Tissues after Iontophoresis** As shown in Fig. 9, the morphology of intestinal tissues in sections where patches were applied showed no difference or cellular damage before and after application of electric current.\(^{18}\) There were no damages in the intestinal lumen (lamina propria and intestinal gland) and in the thickness of smooth muscles.\(^{18}\)

**CONCLUSION**

Gut iontophoresis using insulin nanoparticles as a model drug was found to effectively enhance insulin delivery \textit{in vitro} and \textit{in vivo}. In Franz cell, there was a significant difference between insulin transport through the membrane with and without using electric current. Cytotoxicity test showed no toxic effects of insulin nanoparticles. \textit{In vivo}, there was a remarkable hypoglycemia in rats after surgical application of insulin nanoparticles within the iontophoresic circuit on rat small intestine. After histological examination, we found no structural damage in intestinal tissues after iontophoresis. Application of micro-electrical drug delivery systems has been developed a challengeable and controlled oral dosage form that can deliver drugs in gastrointestinal tract in a controlled manner.

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**Conflict of Interest** The authors declare no conflict of interest.

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