Journal of Drug Targeting

http://informahealthcare.com/drt
ISSN: 1061-186X (print), 1029-2330 (electronic)

J Drug Target, Early Online: 1–13
 DOI: 10.3109/1061186X.2012.757768

informa
healthcare

ORIGINAL ARTICLE

Controlled delivery of ropinirole hydrochloride through skin using modulated iontophoresis and microneedles

Neha D. Singh and Ajay K. Banga

College of Pharmacy and Health Sciences, Mercer University, Atlanta, GA, USA

Abstract

The objective of this study was to investigate the effect of modulated current application using iontophoresis- and microneedle-mediated delivery on transdermal permeation of ropinirole hydrochloride. AdminPatch® microneedles and microchannels formed by them were characterized by scanning electron microscopy, dye staining and confocal microscopy. *In vitro* permeation studies were carried out using Franz diffusion cells, and skin extraction was used to quantify drug in underlying skin. Effect of microneedle pore density and ions in donor formulation was studied. Active enhancement techniques, continuous iontophoresis ($74.13 \pm 2.20\,\mu g/cm^2$) and microneedles ($66.97 \pm 10.39\,\mu g/cm^2$), significantly increased the permeation of drug with respect to passive delivery ($8.25 \pm 2.41\,\mu g/cm^2$). Modulated iontophoresis could control the amount of drug delivered at a given time point with the highest flux being $5.12 \pm 1.70\,\mu g/cm^2/h$ (5–7 h) and $5.99 \pm 0.81\,\mu g/cm^2/h$ (20–22 h). Combination of modulated iontophoresis and microneedles ($46.50 \pm 6.46\,\mu g/cm^2$) showed significantly higher delivery of ropinirole hydrochloride compared to modulated iontophoresis alone ($84.91 \pm 9.21\,\mu g/cm^2$). Modulated iontophoresis can help in maintaining precise control over ropinirole hydrochloride delivery for dose titration in Parkinson's disease therapy and deliver therapeutic amounts over a suitable patch area and time.

Keywords

Dose titration, iontophoresis, modulated delivery, Parkinson's disease, ropinirole, skin microporation, transdermal

History

Received 17 August 2012
Revised 2 December 2012
Accepted 9 December 2012
Published online 11 January 2013

Introduction

Parkinson's Disease Foundation (PDF) estimates that around 7–10 million of world population is affected by Parkinson's disease [1]. Diagnosis of the disease pathophysiology reveals degeneration of dopamine cells mainly in the substantia nigra region of the brain due to genetic or environmental factors and leads to progressive worsening of the motor functions [2,3]. Parkinson's disease is marked by symptoms such as tremor, bradykinesia, rigidity and postural instability which disrupt the daily life of a patient [4,5]. Levodopa is the first line of therapy in Parkinson's disease and considered a ''gold standard'' in the treatment regimen. However, as this disease worsens with time, long-term therapy with levodopa gives rise to side effects like dyskinesia and fluctuation in motor responses [6].

Ropinirole is a D2 receptor agonist and acts by dopaminergic stimulation of the central and peripheral receptors to offer symptomatic relief in Parkinson's disease. Ropinirole hydrochloride is available as an oral tablet, both immediate and extended release, prescribed to alleviate the limitations of levodopa therapy [7]. The dose range administered to the patients increases from 0.25 up to 25 mg per day as the disease progresses thus emphasizing one of the most integral aspects of Parkinson's disease management, i.e. dose titration [8,9]. Inter-patient variability due to differences in age, sex and tolerance as well as intra-patient variability resulting from constant neurodegeneration makes customized dosing regimens the most feasible approach [10]. Since patients are prescribed multiple oral medications it makes compliance an important concern and stresses the need for a less frequent/prolonged administration [11].

Transdermal route of delivery bypasses first pass metabolism (bioavailability of ropinirole is 50%) and can achieve stable plasma levels when applied once daily. Rotigotine, another dopamine agonist, which is formulated as a patch for passive delivery offers the advantage of once daily use and prolonged effect [12,13]; also a patch can be withdrawn at the occurrence of side effects like nausea, dizziness, hallucinations and orthostatic hypotension associated with peripheral receptor stimulation of ropinirole hydrochloride [14,15]. However, like oral administration a patch formulation limits the use to specified doses and does not allow the physician to make adjustments to the dose tailored to the needs of a patient's condition. Ropinirole, which is also a small molecular weight dopamine agonist, will face a similar challenge when administered by transdermal route. Ropinirole base is unstable; hence, formulation of a passive transdermal system is difficult [16]. A topical formulation or patch of specific strength will limit the use to a narrow dose range and not according to

Address for correspondence: Ajay K. Banga, College of Pharmacy and Health Sciences, Mercer University, Atlanta, GA 30341, USA. Tel: 678-547-6243. Fax: 678-547-6423. E-mail: banga_ak@mercer.edu

disease progression in a patient. Transdermal administration of ropinirole can be compared to extended release tablets as in both the cases dose flexibility is reduced. The lag time associated with passive delivery will be higher which leads to delay in alleviating symptoms. Active transdermal techniques, on the other hand, can help overcoming these limitations.

Iontophoresis is an active enhancement technique where low current intensities are applied to the skin for topical or transdermal delivery of charged or neutral molecules. It works on the principle of electrorepulsion, i.e. like repels like and electroosmosis where neutral molecules are transported from anode to cathode along with the bulk solvent flow [17–19]. A programmed iontophoretic system can allow precise and controlled delivery of therapeutic agent. Transdermal delivery of dopamine agonists like ropinirole hydrochloride, apomorphine, rotigotine and 5-OH-DPAT using iontophoresis has been studied due the advantages offered by this technique [20–25]. Therapeutic levels of drug are achieved when current is applied; however, the level decreases in the post-iontophoretic phase. However, modulation of the current applied can help in delivering the required dose and thereafter in maintaining it based on the disease progression of the patient.

Microneedle-mediated transdermal delivery is a minimally invasive technique used for hydrophilic and large molecular weight compounds [26]. Different methods and fabrication techniques have been described in literature for fabrication of microneedles made from materials like stainless steel, glass, polyethylene glycol, carboxymethyl cellulose and maltose to name a few, as the type used depends on the application [27]. Length and density of pores are important parameters which determine the efficiency of microchannel formation as well as the safety concerns. Microchannels remain open up to duration of 72 h under occluded conditions in vivo and this can be helpful for delivering drug for prolonged duration at the microporated site [28]. Combination of iontophoresis and microneedles has been investigated and encouraging results have been reported for large molecular weight compounds [29,30].

In this study the enhancement of transdermal permeation of ropinirole hydrochloride across porcine ear skin model using iontophoresis and microneedles was investigated. Continuous iontophoresis (4 h) and its combination with microneedles were also studied. However, the main objective was to see if a predictable dose could be delivered if the duration of iontophoresis was modulated, i.e. 4 h were split into 2 h application time at two intervals. Modulated iontophoresis was thus applied and the control over delivery of drug delivered at a given time point was assessed. We also studied the effect of combination of modulated iontophoresis and microneedle on the delivery profile of the drug at later time points. Although other studies in literature have used iontophoresis for delivery of antiparkinson's agents, this is the first study which uses modulated iontophoresis and its combination with microneedles to achieve the desired delivery at a given time point in accordance with the dose titration requirement. Effect of microneedle pore density and presence of ions in solution on delivery of ropinirole hydrochloride delivery were also studied. Skin extraction method was used to quantify drug in underlying skin.

Materials and methods

Materials

Ropinirole hydrochloride was obtained from Hangzhou Uniwise International Co. Ltd, Hangzhou, China. AdminPatch® array 600-μm microneedles (1-cm^2 patch area) were purchased from AdminMed. Sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic and ammonium acetate buffer were obtained from Fisher Scientific (Pittsburg, PA). Acetonitrile and centrifuge tubes for skin extraction studies were obtained from MedSupply Partners (Atlanta, GA). Silver wire and silver chloride used for preparing iontophoresis electrodes were obtained from Sigma Aldrich (St. Louis, MO). 3 M Transpore™ tape for tape stripping was purchased from 3 M (St. Paul, MN). Calcein (Fluoresoft®, 0.35%) used for imaging micropores was obtained from Holles Laboratories, Inc. (Cohasset, MA). Methylene blue used as the hydrophilic dye for characterizing micropores was obtained from Eastman Kodak Co. (Rochester, NY). Fluospheres® (0.2 μm) for confocal imaging studies was purchased from Invitrogen™ (Carlsbad, CA). All the solutions used in this study and HPLC solvents for analyses were prepared using deionized water. Porcine ears were obtained from slaughter house.

Methods

Skin preparation

Porcine ear skin was freshly excised, cleaned thoroughly stored at −80 °C until further use. On the day of experiment, skin was thawed to room temperature, thoroughly cleaned with deionized water to remove subcutaneous fat and hair was carefully cut using scissors. Skin pieces were cut into required sizes and mounted onto vertical Franz diffusion cells.

Microneedle insertion

A custom-made syringe applicator was used for microneedle insertion into the skin to help in uniform insertion of 1-cm^2 AdminPatch®. The microneedle patch was stuck onto the circular end of the plunger of a 5-ml syringe using a double sided tape. The barrel provided an easy grip on the entire assembly so that uniform force could be applied from all sides while inserting the microneedles in the skin. Skin was stretched and microneedles was inserted for 1 min prior to a study.

Characterization of microneedle dimensions by scanning electron microscopy

Dimensions of AdminPatch® microneedles were confirmed using a Hitachi S-3700N variable pressure scanning electron microscope (Hitachi High-Technologies, Maidenhead Berkshire, UK). Microneedle array was placed in an emission-field scanning electron microscopy (SEM) with an accelerated voltage of 15 kV. Images were taken from various distances and angle to measure the microneedle height and tip sharpness.

Characterization of microchannels created by AdminPatch® microneedles

Microporated skin was treated with hydrophilic dyes methylene blue and calcein to visualize pores created by

AdminPatch® microneedles. Microneedles were inserted into the skin pieces using a custom-made applicator. Methylene blue solution (1% w/v solution of water) and fluorescent dye calcein were placed onto the microporated skin for duration of 1 min. Methylene blue dye was removed from the skin using kimwipes and alcohol swabs. Proscope HR video microscope (Bodelin Technologies, Lake Oswego, OR) was used to capture images of the stained micropores. For calcein dye, the skin was cleaned with kimwipes to remove excess dye and images were immediately taken using a fluorescent camera (Nikon camera integrated with a macrolens and 525-nm long-pass filter). The images from calcein dye studies were further analyzed by Fluoropore software which measures fluorescent intensity around each pore and calculates a value called as pore permeability index (PPI).

Confocal microscopy was performed to measure the depth of microchannels formed by AdminPatch® microneedles. Porcine ear skin was microporated followed by application of FluoSpheres® for 40 s. Excess solution was cleared by kimwipes, the skin piece was mounted onto a slide and observed under Zeiss confocal microscope. Images were obtained using X–Z sectioning at excitation/emission wavelength of 495/515 nm, respectively.

Preparation of electrodes

Silver wire was manually coiled into circles and used as anode. Silver chloride coated onto a thin silver wire served as the cathode. To briefly describe this procedure, silver chloride was melted by heating in a crucible. Silver wire was coiled at one end and dipped into the solution to ensure sufficient and uniform coating over the surface.

Permeation studies

In vitro studies ($n \geq 3$) were carried out on porcine ear skin using vertical Franz diffusion cells. The diffusion cells were surrounded by a water jacket and temperature of the water bath was maintained at $37 \pm 1\,^{\circ}C$. The receptor compartment was washed prior to each experiment and filled with a 5-ml phosphate buffered saline (50 mM) which was used as the receptor buffer. Skin samples for passive and iontophoresis treatment were mounted onto the Franz diffusion cells (effective surface area of $0.64\,cm^2$) such that the dermal side faced receptor compartment and donor was placed on the epidermal side. For microneedle and combination studies skin samples were microporated according to the procedure mentioned and then mounted onto the Franz cells in a manner similar to passive and iontophoresis. Donor cells were placed on the skin and the entire assembly was maintained together using clamps. Donor formulation (500 µl) was then placed on the skin surface. Donor formulation consisted of 1 mg/ml solution of ropinirole hydrochloride in phosphate buffer and 75 mM sodium chloride. Anodal iontophoresis was used for studying iontophoretic delivery of ropinirole hydrochloride across skin. Silver wire coil (anode) was immersed in the donor compartment and it was ensured that the coil did not touch skin surface and sides of donor compartment. Silver chloride wire (cathode) was placed in the receptor chamber arm. Current supply (Keithley Instruments, Cleveland, OH) was maintained at $0.2\,mA/cm^2$ for a total duration of 4 h; however, the time points of current application were varied for continuous versus modulation experiments. For combination studies, microporated skin was first mounted onto Franz diffusion cell; donor was placed on the skin surface followed by application of anodal iontophoresis. Passive permeation study was performed on untreated skin as a control for enhancement using active techniques iontophoresis and microneedles. Samples were withdrawn from receptor compartment (500 µl) at predetermined time points and equal volume of fresh receptor buffer was added immediately. Samples were analyzed using a validated HPLC assay.

Continuous versus modulated iontophoresis

Iontophoresis was applied for a total duration of 4 h; however, the pattern of application was changed. Continuous iontophoresis was applied at a stretch from 0 to 4 h. Modulated iontophoresis was applied in intervals, from 0 to 2 h followed by 3 h of passive diffusion and then again from 5 to 7 h. Samples were taken up to 24 h and passive diffusion of ropinirole hydrochloride through the skin post-iontophoresis was also studied. In another experiment modulated iontophoresis was applied from 0 to 2 h and 20 to 22 h and sampling was carried until 32 h.

Effect of microneedle pore density

Skin was microporated with microneedles one time, two times and three times over the same area ($0.64\,cm^2$) to study the effect of increasing pore density on delivery of ropinirole hydrochloride, when using microneedles alone. The effect of increasing pore density was also studied for combination approach where skin was microporated once and twice followed by application of iontophoresis over the same area.

Effect of ions in solution

Donor formulations consisting of phosphate buffer (50 mM) and distilled water were compared for studying the effect of ions on iontophoresis-mediated delivery of ropinirole hydrochloride. Sodium chloride (75 mM) was added to both donors. pH of the donors was noted using a MI-410 combination pH electrode (Microelectrodes, Inc., Bedford, NH).

Skin extraction

Drug present in the skin was quantified using a skin extraction assay. Following permeation study, the donor formulation was pipetted from the skin surface. The mounted skin was cleaned using Q-tips and receptor solution three times to ensure removal of excess donor formulation. The skin piece was then dabbed with kimwipes to dry the remaining moisture. The permeation area was then tape stripped with 3 M Transpore tape one time to clean any excess formulation resulting in overestimation of ropinirole hydrochloride. Skin pieces were then minced using a pair of scissors into centrifuge tubes. Extraction solvent (3 ml of distilled water) was added to each centrifuge tube and the samples were sonicated using Fisher Scientific mechanical ultrasonic cleaner FS110 for 20 min. Overnight extraction was carried out by placing the samples on a roller shaker (New Brunswick Scientific Co. Inc., Edison, NJ) at a speed of 200 rpm. Samples were again

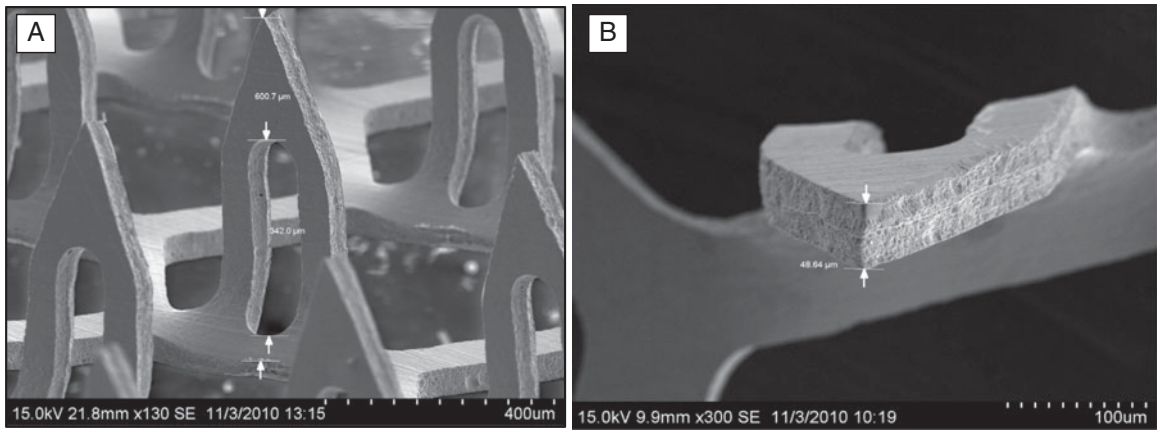

Figure 1. Scanning electron microscopy images of AdminPatch® microneedles: (A) an array of microneedles each having length of 600 µm and (B) magnified image of microneedle tip measuring 48.64 µm.

sonicated for 20 min the next day followed by centrifugation (Sorvall RT6000 refrigerated centrifuge) at 4000 rpm for 10 min to separate the supernatant extract from skin pieces. The extract was filtered, diluted as required and analyzed using HPLC method.

Recovery studies

Extraction efficiency was determined *in vitro* in porcine ear skin using recovery study. Ropinirole hydrochloride standard solutions (200, 500 and 1000 µg/ml) were made and 50 µl of each solution was injected into porcine ear skin ($n = 3$). Injected samples were incubated for 4 h and the skin extraction procedure mentioned in detail above was followed. Amount of drug extracted was quantified using a standard curve and the extraction recovery was found to be $78.45 \pm 4.56\%$. This recovery value was used when quantifying drug for skin samples from *in vitro* permeation experiments (total amount of drug calculated from high-performance liquid chromatography (HPLC) quantification was divided by 0.7845 to accurately predict drug in skin).

Quantitative analysis

Ropinirole hydrochloride was quantified using HPLC by using modified assay from literature. Analysis was performed on Perkin Elmer System (Waltham, MA) with a UV detector operating at 245 nm. The column used was Phenomenex Gemini-NX 5 µ C18 110 A; 250×4.6 mm. Mobile phase consisted of ammonium acetate buffer, 50 mM pH 7.8: acetonitrile (50:50%, v/v). Isocratic elution was performed at flow rate of 0.6 ml/min after injecting 10 µl of sample. The total run time was 7 min and the retention time of ropinirole hydrochloride was around 5 min. Standards were prepared in the range of 0.1–100 µg and the assay was sensitive to detection within this range.

Statistical analysis

Statistical significance was determined using analysis of variance (ANOVA) and Student's *t*-test. Tukey's honestly significant difference post hoc test was performed for specific comparison.

Results

Characterization of microneedle dimensions by scanning electron microscopy

AdminPatch comprises of a 1-cm^2 circular patch with 187 microneedles. Figure 1(A) shows an array of microneedles viewed by SEM. The magnified image shows that the height of the microneedles from the base is 600 µm; at the center of the microneedle is a hollow groove measuring 342 µm. The microneedles had a tip width of 48.64 µm (Figure 1B) which helped in successfully breaching the stratum corneum for delivery of ropinirole hydrochloride.

Characterization of microchannels created by AdminPatch® microneedles

Porcine ear skin treated with AdminPatch® microneedles was stained by methylene blue dye indicating that the microneedles created micropores on the skin (Figure 2A). Since the microneedle patch has an area of 1 cm^2, pores were formed over this area, i.e. about 187 microchannels/cm^2. Calcein imaging further confirmed the effectiveness of microporation by AdminPatch® microneedles. Images were analyzed using the Fluoropore software (Figure 2B) for uniformity of calcein distributed around each pore by designating each pore a number called as PPI. The mean value of PPI calculated for a total of 116 pores was 23.6 ± 11.08. The histogram shown in Figure 2(B) depicts the narrow distribution of PPI values for the uniformly created micropores with the mean lying around 23.6. Confocal microscopy was performed to visualize the depth of microchannels formed by tracing the path of Fluospheres® which were placed on the porcine skin following treatment with microneedles. Skin surrounding the microporated area served as a control since no diffusion of particles was seen through these regions. Figure 3 shows an image of the pore on the surface of the skin and the subsequent pathway taken by the microparticles through the microchannels up to an average depth of 160 ± 25 µm. AdminPatch® microneedles with a length of 600 µm thus created microchannels which could reach a depth of 160 ± 25 µm in the skin. The diameter of individual

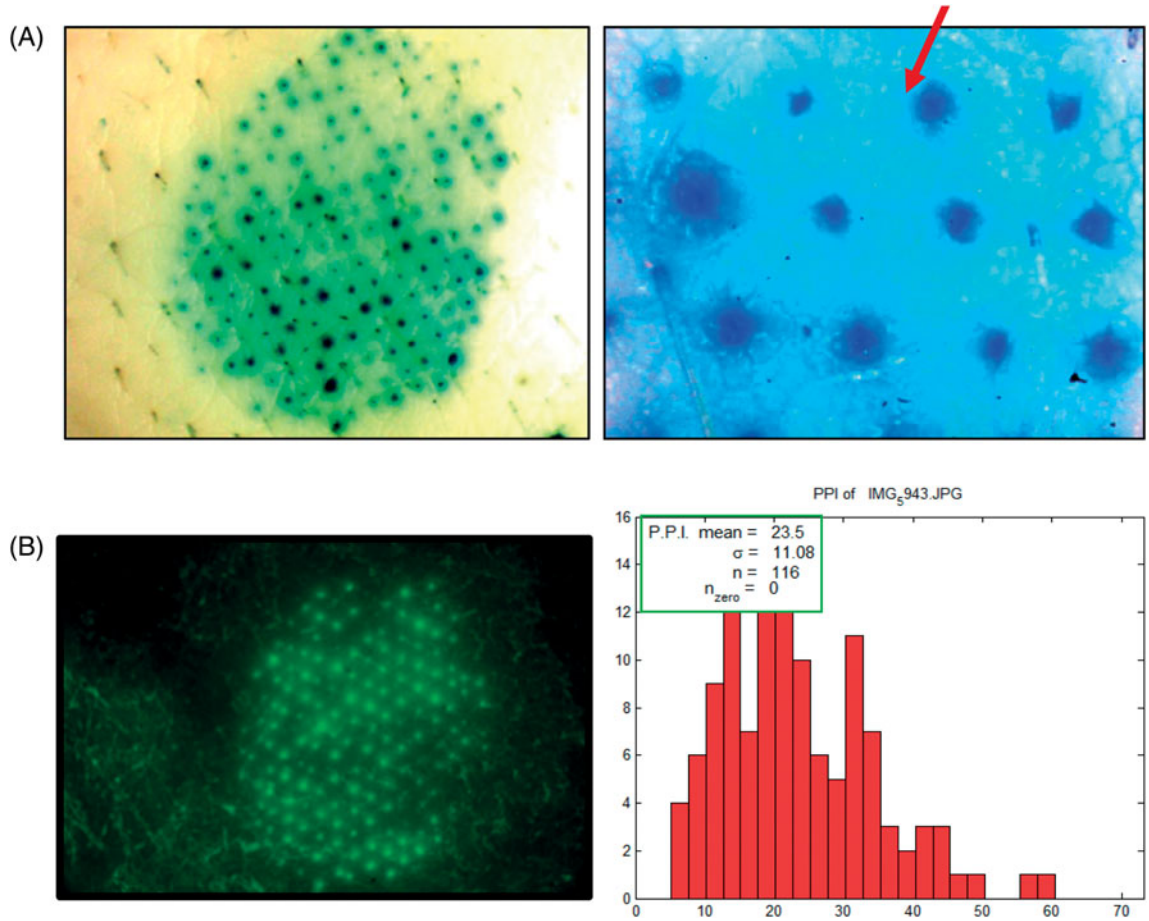

Figure 2. Dye binding studies for visualization and characterization of micropores. (A) Staining by methylene blue dye confirms formation of microchannels, arrow indicates magnified single microchannel. (B) Calcein imaging for calculation of pore permeability index.

micropore was measured on the surface of the skin and the average was calculated to be $86.6 \pm 13.24\,\mu m$.

Passive versus active enhancement techniques

In this study, the effect of active enhancement techniques on delivery of ropinirole hydrochloride through porcine ear skin was investigated. Delivery through intact skin, i.e. passive permeation served as a control. Transdermal delivery of ropinirole hydrochloride increased significantly from $8.25 \pm 2.41\,\mu g/cm^2$ for passive delivery to $74.13 \pm 2.20\,\mu g/cm^2$ and $66.97 \pm 10.39\,\mu g/cm^2$ for continuous iontophoresis ($0.2\,mA/cm^2$ for 4 h) and AdminPatch® microneedles treated skin, respectively (Figure 4).

Continuous versus modulated iontophoresis

Delivery of ropinirole hydrochloride through the skin was compared using continuous iontophoresis and modulated iontophoresis to study the control on dose delivered over a given time point. Continuous iontophoresis delivered $74.13 \pm 2.20\,\mu g/cm^2$ of ropinirole hydrochloride which was significantly higher than $46.50 \pm 6.46\,\mu g/cm^2$ of ropinirole hydrochloride delivered by modulated iontophoresis at the end of 24 h as seen in Figure 5(A). A plot of average flux versus time (Figure 5B) shows that amount of ropinirole hydrochloride delivered at a particular time point depends on the current applied. Continuous iontophoresis resulted in a steady rise of drug delivered when the current was applied and highest flux was $8.21 \pm 3.06\,\mu g/cm^2/h$ at the end of 4 h after which the flux decreased gradually. In case of modulated iontophoresis (0–2 + 5–7 h) increase in flux was observed concurrent with the application of iontophoresis, i.e. $2.48 \pm 1.11\,\mu g/cm^2/h$ at 3 h and $5.12 \pm 1.70\,\mu g/cm^2/h$ at 8 h; after termination of iontophoresis at 7 h, the flux seen was $2.99 \pm 1.42\,\mu g/cm^2/h$ and it continued to rise till 8 h time point. The pattern of modulation was varied and iontophoresis was applied from 0 to 2 h followed by 20–22 h to investigate effect on permeation due to change in intervals of modulated iontophoresis; the total amount of ropinirole hydrochloride delivered was $60.03 \pm 17.12\,\mu g/cm^2$ at the end of 24 h. Sampling was continued till 32 h in this case to observe post-iontophoretic permeation and the average cumulative amount delivered was $81.04 \pm 19.87\,\mu g/cm^2$ (Figure 5A). Increase in flux was observed each time current was applied, at the end of 2 h and again next day at 22 h, the average flux being $4.33 \pm 2.16\,\mu g/cm^2/h$ and $5.99 \pm 0.86\,\mu g/cm^2/h$, respectively. The flux decreased gradually in the next 8 h, as seen for continuous and modulated iontophoresis (0–2 + 5–7 h), and the average flux at 32 h was $2.29 \pm 0.47\,\mu g/cm^2/h$.

Effect of microneedle pore density

The number of microchannels created by the microneedles might decide the efficiency of transdermal delivery.

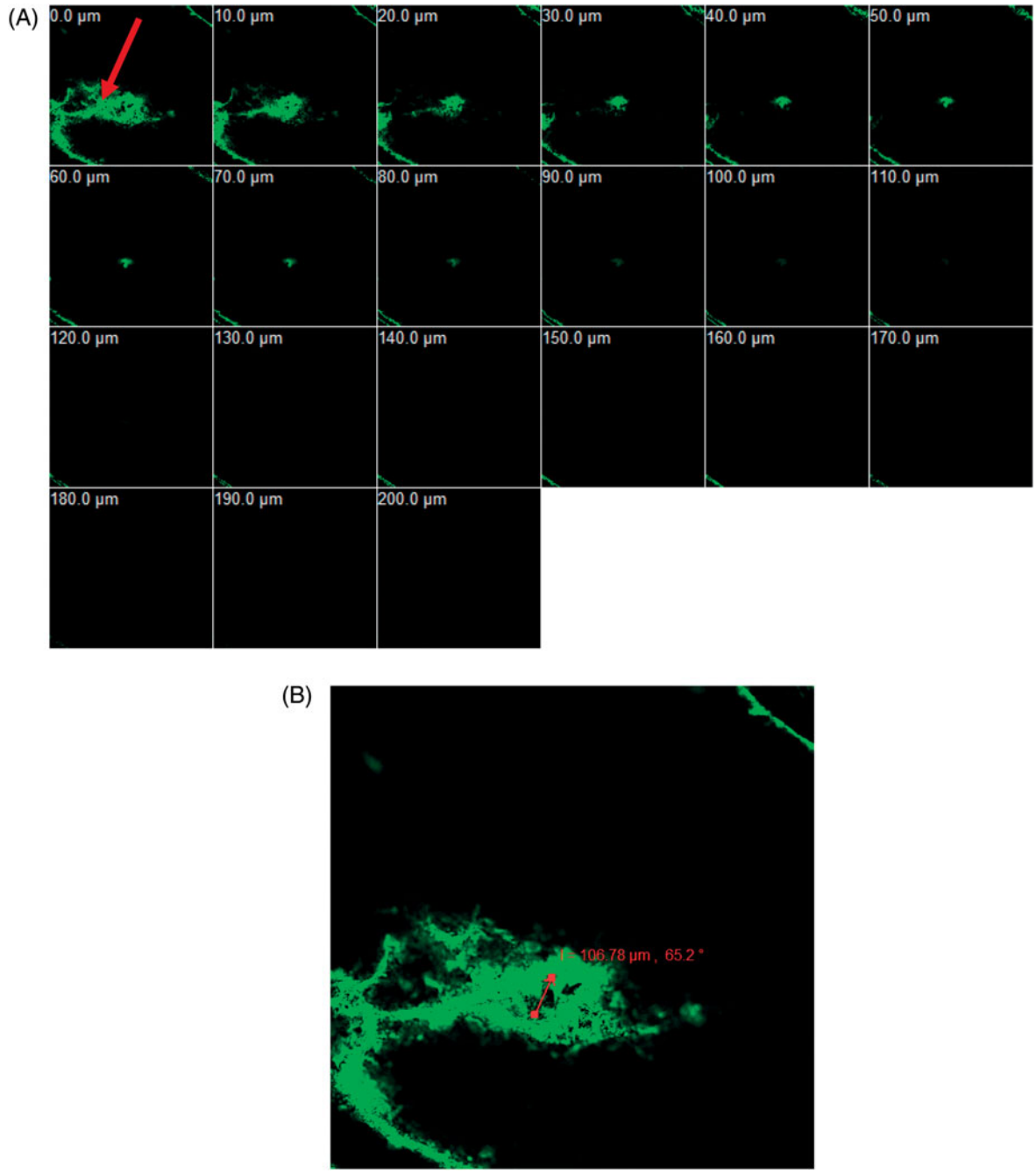

Figure 3. (A) Confocal microscopy traces the pathway of 0.2-µm fluorescent particles. The depth of microchannels is investigated using this technique, in this case the average penetration was 160 ± 25 µm. Red arrow indicates the pore on surface of the skin. (B) Confocal image showing diameter of a single pore at the surface of the skin, average diameter was calculated as 86.6 ± 13.24 µm ($n = 5$).

This study was designed to investigate the effect of increase in microneedle pore density over a given area on delivery of ropinirole hydrochloride through the skin. Microporation of the skin three times lead to significantly higher amount of ropinirole hydrochloride transdermal delivery of 123.62 ± 15.32 µg/cm^2 compared to one time and two times which resulted in transdermal delivery of 66.99 ± 10.36 µg/cm^2 and 75.87 ± 8.07 µg/cm^2 (Figure 6A).

Effect of combination therapy (iontophoresis and microneedles)

Iontophoresis and microneedles, both active enhancement techniques, resulted in higher transdermal permeation of ropinirole hydrochloride through the skin. A combination approach of microneedle pretreatment followed by application of iontophoresis was used to determine any synergistic effect or improvement in delivery as compared to individual therapy. In the first study, skin was microporated and continuous iontophoresis (0–4 h) was applied. Figure 6(B) demonstrates that the combination of microneedles (porated twice over the same area) and continuous iontophoresis showed significantly higher delivery of ropinirole hydrochloride with respect to iontophoresis alone. However, no significant difference was seen between combination of microneedles and iontophoresis for one microporation versus two microporations over the same area and

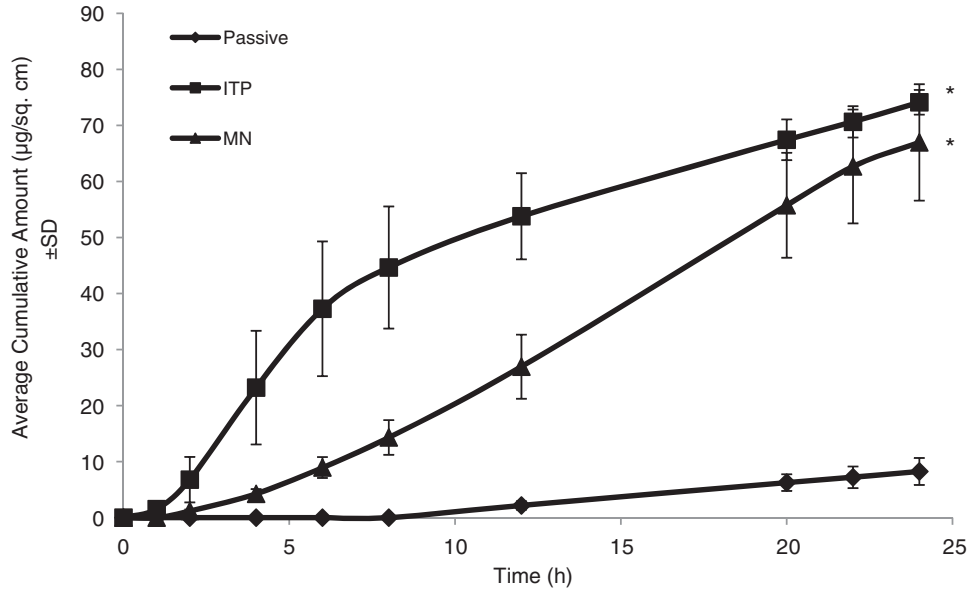

Figure 4. Effect of active enhancement techniques, continuous iontophoresis and microneedles, on *in vitro* transdermal permeation of ropinirole hydrochloride (current application for 4 h at 0.2 mA/cm^2). * indicates significant difference from passive delivery ($p < 0.05$).

combination of microneedles and iontophoresis (microporated once) versus iontophoresis alone.

Combination of microneedle pretreatment and modulated iontophoresis was also studied (Figure 7A). The total amount of ropinirole hydrochloride delivered at the end of 24 h was 84.91 ± 9.21 µg/cm^2 and did not show significant difference compared to the combination of microneedle pretreatment and continuous iontophoresis which delivered 100.90 ± 22.17 µg/cm^2 (Figure 6). The trend shown by modulated iontophoresis was repeated and delivery of ropinirole hydrochloride, at a given time point, could be controlled by the application of iontophoresis. Combination with continuous iontophoresis resulted in highest flux of 8.90 ± 0.33 µg/cm^2/h at end of 4 h while in case of modulated iontophoresis the flux was 3.63 ± 0.25 µg/cm^2/h at 3 h and 6.94 ± 0.55 µg/cm^2/h at end of 8 h (Figure 7B).

Effect of ions in solution

Presence of ions can decide the efficiency of iontophoretic delivery of a drug; hence, transdermal permeation of ropinirole hydrochloride was investigated in absence of competing ions, i.e. buffer solution to study its effect on delivery through the skin and changes in pH of donor formulation. The use of water to formulate the donor solution lead to twofold increase in delivery of ropinirole hydrochloride from 46.50 ± 6.46 to 95.4 ± 6.97 µg/cm^2 and significantly higher amount of drug was delivered at the end of 24 h (Figure 8). A sharp rise in flux was registered with application of current and the average flux at 2 h and 7 h was fivefold higher than donor formulation containing phosphate buffer. The rise of flux was faster when current was applied during the second interval; highest flux was noted to be 12.37 ± 4.18 µg/cm^2/h at 7 h while for phosphate buffer it was significantly lower with 5.12 ± 1.70 µg/cm^2/h delivered at 8 h ($p < 0.05$). The pH of donor formulation remained constant when phosphate buffer was used (7.32 ± 0.02); however, in presence of water the initial donor pH was 6.4 which changed to 7.3 ± 0.8 at the end of 24 h.

Quantification of drug in skin

The amount of ropinirole hydrochloride in skin was quantified to determine the accumulation of drug in skin, Figure 9. Microneedle treatment, continuous iontophoresis and combination of microneedles with continuous iontophoresis resulted in significantly higher permeation ($p < 0.05$) of ropinirole hydrochloride than the amount deposited in the underlying skin. Modulated iontophoresis alone and its combination therapy with microneedle pretreatment, demonstrated lack of significance between transdermal permeation and deposition of ropinirole hydrochloride in underlying skin. Passive delivery and microneedle treated skin showed significantly lower amount ($p < 0.05$) of ropinirole hydrochloride in the skin compared to combination therapy of modulated iontophoresis and microneedles.

Discussion

Transdermal delivery of ropinirole hydrochloride, using physical enhancement techniques, iontophoresis and microneedles, offers a promising approach for administering precise and controlled amount of drug in comparison to existing oral therapy. Ropinirole hydrochloride is available as immediate release and extended release oral tablet in the dosage range of 0.25 mg three times a day up to 25 mg once a day; depending on the disease progression and patient compliance the dose is titrated [8,31]. Transdermal delivery of ropinirole has been investigated in the past using patches (matrix and reservoir) which were formulated with ropinirole base as well as ropinirole hydrochloride. Ropinirole base is unstable, has low water solubility and the patches have shown occurrence of crystals during storage. Low water solubility may not be concern for passive delivery but crystallization of ropinirole in patches can have implications on efficacy and shelf-life

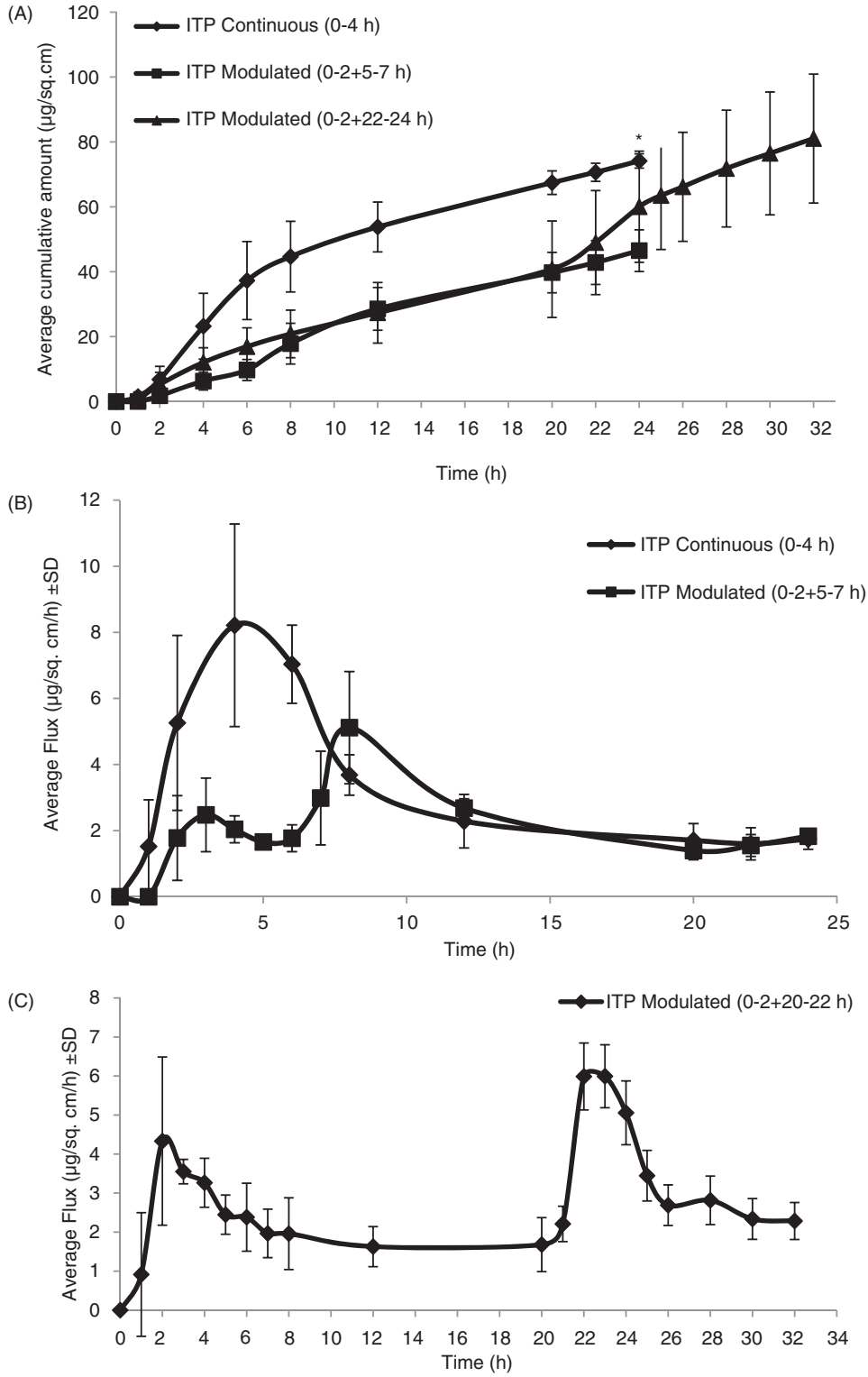

Figure 5. Effect of modulated versus continuous iontophoresis on transdermal delivery of ropinirole hydrochloride. (A) Permeation profile showing transdermal delivery, (B) flux profile comparing amount delivered over each time point, and (C) flux profile of modulated iontophoresis (0–2 + 20–22 h) over a period of 32 h. * indicates significant difference from other groups ($p < 0.05$).

stability of the product [16,32]. Ropinirole hydrochloride, i.e. a salt form of the drug, was chosen for the study as active enhancement techniques microneedles and iontophoresis require the drug to be hydrophilic (water solubility 133 mg/ml) and in ionized form respectively for successful delivery [19,33]. Dose titrations form an integral part of Parkinson's therapy, this emphasizes the need for ability to vary the amount of drug delivered over a period of time with accuracy [15]. Iontophoresis acts on the drug molecule when current is applied and thus helps in exercising control over the delivery over the given time period [17]. Microneedles, on the other hand, create microchannels which can deliver the drug through the skin up to a period of 72 h when occluded by a patch containing the drug, hence

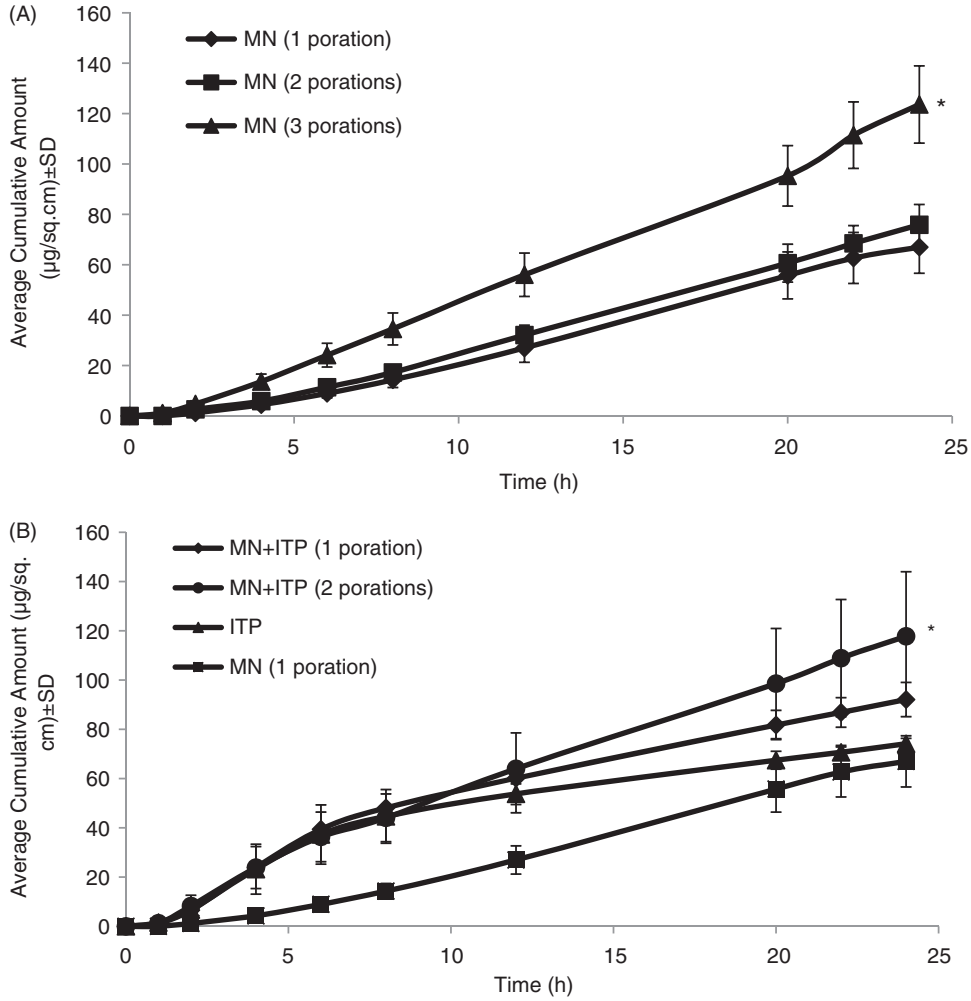

Figure 6. Effect of microneedle pore density on transdermal delivery of ropinirole hydrochloride. (A) Permeation profile depicting transdermal delivery using microneedles alone. (B) Permeation profile representing transdermal delivery using combination of microneedles and continuous iontophoresis. * indicates significant difference from combination of MN+ITP (2 poration) versus ITP alone and MN alone ($p < 0.05$).

maintaining the amount of drug in the body at therapeutic levels [28].

As mentioned earlier AdminPatch® microneedles were used for microporation studies. Characterization of microneedles and microchannels formed by them was done to confirm that stratum corneum was breached without breaking the metal microneedles which should not chip in the skin to leave behind metal particles. Figure 1(A) shows that the skin porated with 600 µm needles was stained by hydrophilic dye methylene blue; however, the surrounding skin (with intact barrier function) was unstained. This indicated the formation of hydrophilic channels which enable permeation of the dye. Calcein imaging and PPI validated the results from methylene blue study and the uniformity of pores was established. We have performed similar studies for maltose microneedles and these parameters helped in predicting the efficiency of microneedles for further studies [28,34]. Microneedles are designed to disrupt the epidermis for transdermal delivery; however, any contact with the dermis is undesirable as this layer comprises of nerves which are sensitive to needles; pain associated with hypodermic needles is due to this reason [35–37]. Confocal microscopy showed that the microneedles successfully created microchannels. Figure 3 depicts the pathway of Fluospheres through the microchannels and it is observed that the microneedles cross the epidermis while not reaching the dermis to a depth of 160 ± 25 µm. AdminPatch® microneedles with a height of 600 µm thus could bypass the stratum corneum.

The influence of anodal iontophoresis and microneedle on delivery of ropinirole hydrochloride was studied and passive delivery acted as the control. Ropinirole hydrochloride is a small molecule and has a $\log P$ of 3.32 [25]. However, for hydrochloride salt the partition between organic and buffer phase is determined by the degree of ionization of the molecule at a particular pH and pKa, i.e. $\log D$ which can be calculated according to Equation (1) [38]:

$$\log D = \log P - \log(1 + 10 \wedge (pKa - pH)) \qquad (1)$$

The $\log D$ value of a compound reflects its ease to ionize at a given pH condition and hence the effectiveness of electrorepulsion transport by iontophoresis. Ropinirole hydrochloride has a $\log D$ of 1.1 (calculated from equation 1) at pH 7.4 which indicates that it is well ionized and can undergo iontophoretic transport through the skin. The drug has a pKa of 9.2; hence, at pH of 7.4 it will be positively charged and application of anodal iontophoresis will actively transport ropinirole by electrorepulsion. Microneedle-assisted delivery of drugs is favored when the drug exists in dissolved form in a

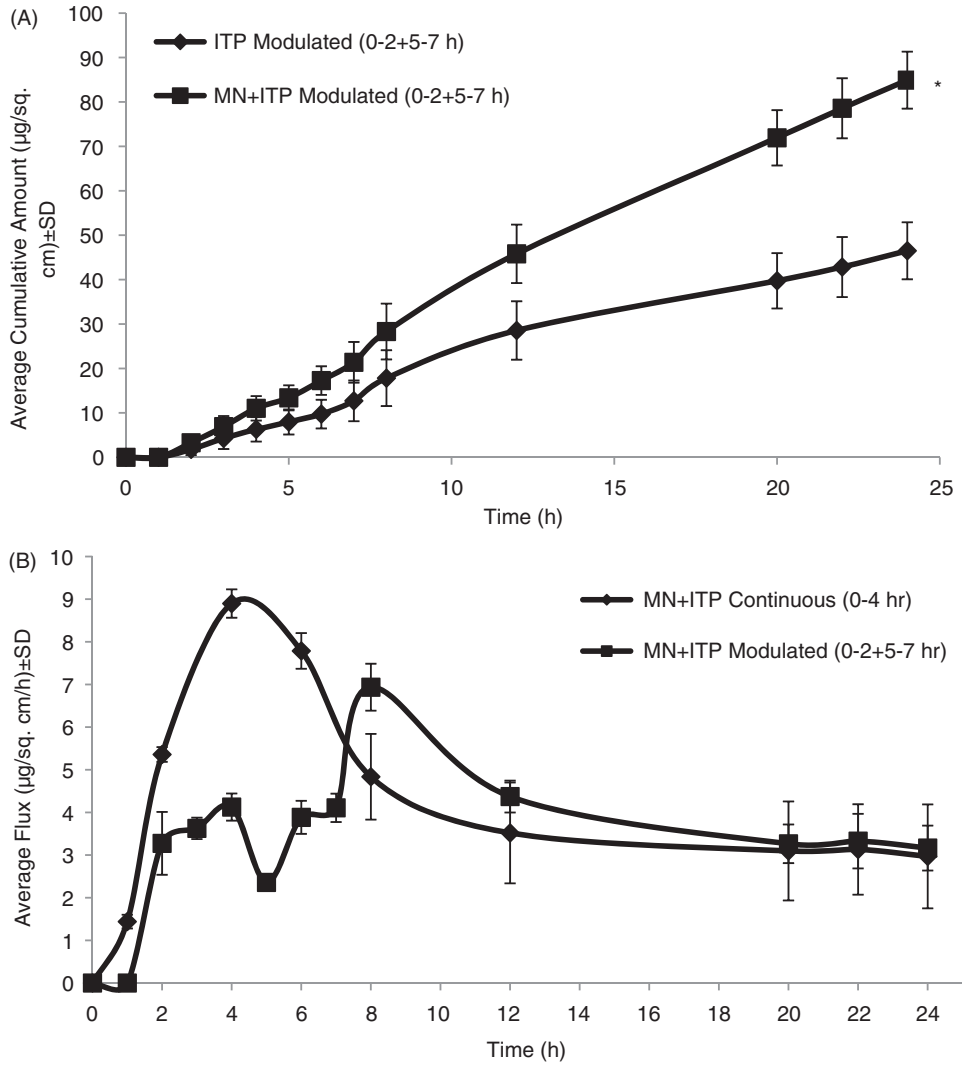

Figure 7. Effect of combination therapy of modulated iontophoresis and microneedle on transdermal delivery of ropinirole hydrochloride. (A) Permeation profile depicting transdermal delivery. (B) Flux profile comparing amount delivered at a given time point for combination therapy using modulated versus continuous iontophoresis. * indicates significant difference from other groups ($p < 0.05$).

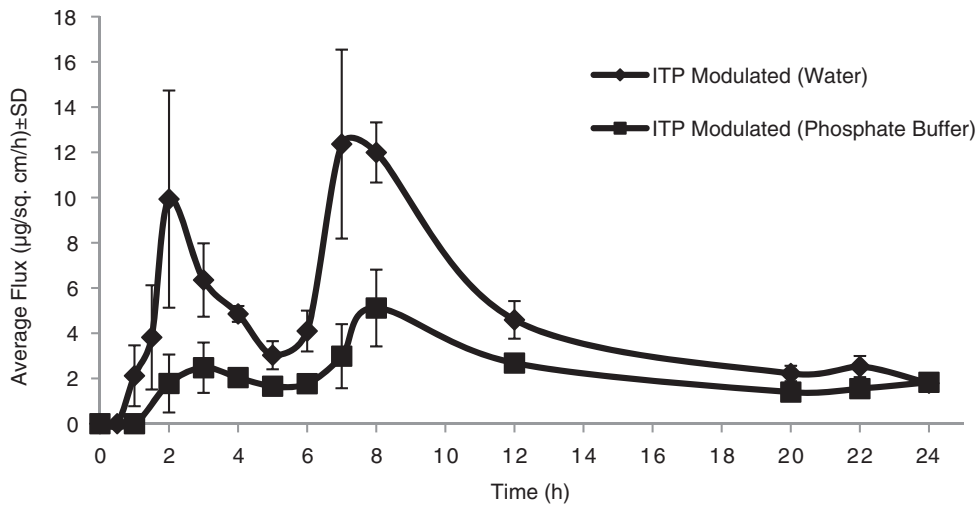

Figure 8. Effect of ions in donor formulation on transdermal delivery of ropinirole hydrochloride. Flux profile depicting amount of drug delivered at each time point for modulated iontophoresis.

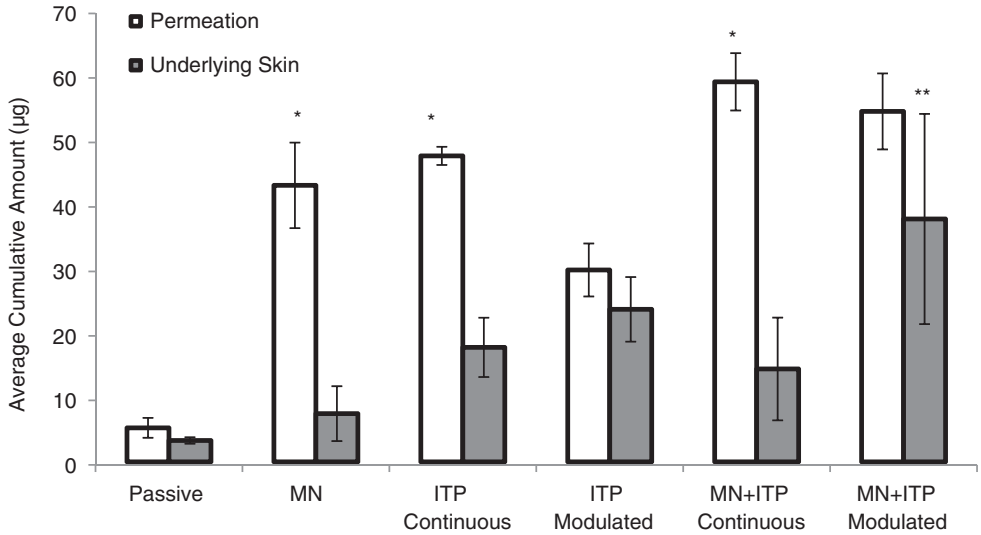

Figure 9. Graphical representation of drug quantified in skin versus transdermal delivery. * indicates transdermal permeation is higher than drug quantified in underlying skin for that group ($p < 0.05$), ** indicates significant difference from passive and microneedle group for drug quantified in underlying skin ($p < 0.05$).

hydrophilic vehicle. High water solubility of hydrochloride salt form of the drug facilitates its passage through the aqueous microchannels formed after microporation [39]. Iontophoresis acts directly on the drug while microneedle is a minimally invasive active technique which enables drug delivery by altering the pathway of drug through the skin [40]. Iontophoresis (1.22 ± 0.20 h) hence showed a faster onset of action as the drug is pushed through the skin in the presence of current, and lag time of the drug is noticeably reduced as compared to passive (7.67 ± 0.29 h) and microneedle (4.08 ± 0.38 h) delivery. A more lipophilic molecule partitions slower into the receptor compartment compared to a hydrophilic molecule which leads to delayed iontophoretic effect and greater lag time or likelihood of depot formation in skin. This was reported when anodal iontophoresis was studied for two different Parkinson's agents, rotigotine ($\log P$ of 4.03) and 5-OH-DPAT ($\log P$ of 2.19), as the later drug showed faster initiation and termination of permeation when current was switched on and off respectively [21–23]. Ropinirole hydrochloride has a $\log P$ of 3.32 and thus is moderately lipophilic which may explain both a gradual decrease in iontophoretic flux as well as slower onset of microneedle delivery as the drug might not instantaneously partition from the skin to the receptor compartment.

Parkinson's disease is a progressive disorder which makes dose adjustment an important aspect of therapy. Ropinirole hydrochloride offers symptomatic relief and hence continuous dosing at required intervals to avoid recurrence of tremors is needed in addition to the dose titration [41]. Iontophoresis helps in controlled drug delivery; modulated iontophoresis takes this a step further and allows in delivering a known amount of drug at a given time point. This approach has not yet been widely investigated and is especially useful for drugs which require customized delivery or for therapeutic agents like proteins where simulation of *in vivo* conditions is required. The flux at 2 h for continuous iontophoresis (0–4 h), modulated iontophoresis (0–2 + 5–7 h) and modulated iontophoresis (0–2 + 20–22 h) was 5.26 ± 2.65, 1.78 ± 1.28 and 4.33 ± 2.16 µg/cm^2/h, respectively, which was not different statistically indicating that initial delivery was same. Continuous iontophoresis showed a cumulative effect over a period of 4 h as current was applied without a break and hence higher permeation was achieved over a period of 24 h. Modulated iontophoresis showed a reproducible profile, when current was applied the second time in both cases from 5–7 and 20–22 h, highest flux was seen at 8 h (5.12 ± 1.70 µg/cm^2/h) and 23 h (5.99 ± 0.81 µg/cm^2/h) and was not significantly different (Figure 5). The post-iontophoretic flux dropped after 2 h once iontophoresis was stopped; hence, the highest flux was achieved after a slight delay at the next interval and then dropped gradually. Considering that 5.5 µg/cm^2/h is delivered from a 10-cm^2 area with a donor concentration of 1 mg/ml, the total amount of ropinirole hydrochloride delivered would be 55 µg/h. Hence, depending on the duration of current application more drug will be delivered with increasing time as seen for continuous iontophoresis and ropinirole hydrochloride delivered over a period of 4 h will be more than 220 µg which equals the lowest therapeutic dose of oral ropinirole hydrochloride, i.e. 0.25 mg. However, transdermal delivery bypasses first-pass metabolism (oral bioavailability is 50%) and ropinirole hydrochloride will directly reach systemic circulation. This will reduce the transdermal dose needed for therapeutic effect. In this study, a low iontophoretic current (0.2 mA/cm^2) and concentration of donor (1 mg/ml) were used; hence, there is scope for using higher current intensity and concentration which may help achieve a better delivery profile at a given time point. The post-iontophoretic flux reduced gradually and reached a value of 1.5–2 µg/cm^2/h (15–20 µg/h from a 10-cm^2 patch) over a period of 24 h. This indicates that a constant level of dopaminergic stimulation would be maintained to prevent the on–off effect associated with recurrence of symptoms in Parkinson's disease [15].

Combination therapy with continuous iontophoresis and microneedles did not show any synergistic effect on permeation of ropinirole hydrochloride through the skin; similar

trend has been reported in the literature for other small molecular weight compounds including deuterium oxide, methylene blue, theophylline and fluorescein sodium [29,42]. On the other hand, large molecular weight drugs/therapeutic agents show increased delivery as compared to individual treatment [30]. Small molecular weight drugs can be easily transported by iontophoresis through shunt pathways in the skin; however, this does not apply to high molecular weight compounds. Micron-sized pores formed by microneedles can deliver molecules as large in size as a monoclonal antibody, vaccines, proteins and peptides. For small molecular weight compound, additional pathways created by combination therapy may not significantly drive higher amounts of drug through them as compared to individual active techniques [35]. The reason for this can be an efficient transport of drug through existing pathways when using iontophoresis and microneedles alone so that further enhancement of delivery cannot be seen at same concentration and microneedle pore density. However, exceptions to this have been reported [43] and further work is needed to establish mechanism behind the same. Higher flux was seen with iontophoresis at initial time points which was concurrent with the application of current, whereas microneedles achieved greater flux at later time points after a gradual increase in delivery at start of the experiment. Combination therapy on the other hand showed increase in flux when iontophoresis was applied and also helped in maintaining higher flux at later time points as compared to iontophoresis alone. Modulation of iontophoresis during combination therapy, however, resulted in significantly higher permeation of ropinirole hydrochloride with respect to modulated iontophoresis ($p < 0.05$). The presence of microchannels may be a reason for better delivery by modulated combination therapy. When using modulated iontophoresis alone, the delivery only depends on application of current and flux remains low in the absence of iontophoresis whereas for modulated combination therapy drug continues permeating through the microchannels in the absence of current, maintaining the flux until it rises at next iontophoretic interval. This can avoid fluctuations in steady state levels of ropinirole hydrochloride associated with appearance of side-effects during ''off'' period of therapy [15].

The permeation of ropinirole hydrochloride was significantly increased when the skin was porated thrice over the same area compared to once and twice. This can be attributed to the increase in number of microchannels formed over the exposed permeation area. However, no significant difference was seen between one time and two time poration. It has been reported before that the amount of drug delivered increases with the number of pores formed over an area and our results confirm this [35,44]; however, the increase may not be linear and depends on the total number of pores formed over that area as seen in the case of one poration versus two porations. Combination of iontophoresis and skin microporated twice over the same area lead to significantly higher permeation of ropinirole hydrochloride compared to combination of iontophoresis and skin microporated once which confirms that microneedle pore density does affect permeation of ropinirole hydrochloride.

The efficiency of iontophoretic transport depends on the transport number of the charged drug species being delivered under the electrode [45]. In presence of small molecular weight charged competing ions the transport number of drug decreases thus affecting the flux [46].

$$J = I \times t \div F \times z, \tag{2}$$

where J equals flux, I is the current applied, F is Faraday's constant and z is the valency of drug.

Our finding confirms these results as the use of water in presence of 75 mM sodium chloride leads to twofold higher delivery. The rise of flux was faster and when current was applied during the second interval; highest flux was noted to be $12.37 \pm 4.18\ \mu g/cm^2/h$ at 7 h while for phosphate buffer it was significantly lower with $5.12 \pm 1.70\ \mu g/cm^2/h$ delivered at 8 h ($p < 0.05$). The pH of donor formulation without buffer showed a drift, even though it was in acceptable range for this experiment it emphasizes the need for finding the right balance between eliminating the competition from other ions (buffer) and maintaining the stability of formulation.

Quantification of ropinirole hydrochloride in the skin established that stratum corneum was a barrier to the delivery of this drug since negligible amounts were detected in the skin after passive delivery. The application of microneedles and continuous iontophoresis (both alone and in combination) efficiently delivered ropinirole hydrochloride into the receptor compartment; however, modulated iontophoresis showed a trend of depositing equal amount of drug in the skin [47,48]. The reason behind this can be duration and continuity of iontophoretic application. In case of continuous iontophoresis, 4 h is sufficient for drug to partition from skin into the receptor under the influence of current; on the other hand, modulated iontophoresis is applied only for 2 h at both intervals (0–2 + 5–7 h). Hence the drug might be still partitioning into the receptor when the current is stopped hindering any further delivery. Ropinirole hydrochloride has a log P of 3.32, as mentioned earlier and this may further affect effective transport from skin to the receptor in the absence of current.

Conclusion

Iontophoresis has been used for delivering dopamine agonists but application of modulated iontophoresis to suit dose titration schedules in Parkinson's therapy has been used for the first time. Modulated iontophoresis showed a predictable flux profile at two different intervals and amount of drug delivered at a time point could be controlled. Combination therapy of modulated iontophoresis with microneedles lead to increased delivery of ropinirole hydrochloride; higher flux was maintained even at later time points which is desirable for preventing recurrence of symptoms associated with Parkinson's disease. Optimization of microneedle density and ions present in solution further helped in improving transdermal delivery of ropinirole hydrochloride. Therapeutic amounts of ropinirole hydrochloride can be delivered with precision by applying the right current density over a stipulated time and patch area to allow customized treatment of Parkinson's disease.

Declaration of interest

The authors report no declarations of interest.

References

1. Parkinson's Disease Foundation I. Statistics on Parkinson's. 2012 ed. 2012.
2. Schapira AH. Neurobiology and treatment of Parkinson's disease. Trends Pharmacol Sci 2009;30:41–7.
3. Warner TT, Schapira AH. Genetic and environmental factors in the cause of Parkinson's disease. Ann Neurol 2003;53:S16–23; discussion S23–5.
4. Samii A, Nutt JG, Ransom BR. Parkinson's disease. Lancet 2004;363:1783–93.
5. Mamikonyan E, Siderowf AD, Duda JE, et al. Long-term follow-up of impulse control disorders in Parkinson's disease. Mov Disord 2008;23:75–80.
6. Obeso JA, Olanow CW, Nutt JG. Levodopa motor complications in Parkinson's disease. Trends Neurosci 2000;23:S2–7.
7. Im JH, Ha JH, Cho IS. Lee MC. 2003. Ropinirole as an adjunct to levodopa in the treatment of Parkinson's disease: a 16-week bromocriptine controlled study. J Neurol 2003;250:90–6.
8. Adler CH, Sethi KD, Hauser RA, et al. Ropinirole for the treatment of early Parkinson's disease. The Ropinirole Study Group. Neurology 1997;49:393–9.
9. Rascol O, Brooks DJ, Brunt ER, et al. Ropinirole in the treatment of early Parkinson's disease: a 6-month interim report of a 5-year levodopa-controlled study. 056 Study Group. Mov Disord 1998;13:39–45.
10. Levy G. Predicting effective drug concentrations for individual patients. Determinants of pharmacodynamic variability. Clin Pharmacokinet 1998;34:323–33.
11. Nashatizadeh MM, Lyons KE, Pahwa R. A review of ropinirole prolonged release in Parkinson's disease. Clin Interv Aging 2009;4:179–86.
12. Kim HJ, Jeon BS, Lee WY, et al. Overnight switch from ropinirole to transdermal rotigotine patch in patients with Parkinson disease. BMC Neurol 2011;11:100.
13. Sanford M, Scott LJ. Rotigotine transdermal patch: a review of its use in the treatment of Parkinson's disease. CNS Drugs 2011;25:699–719.
14. Schrag AE, Brooks DJ, Brunt E, et al. The safety of ropinirole, a selective nonergoline dopamine agonist, in patients with Parkinson's disease. Clin Neuropharmacol 1998;21:169–75.
15. Pahwa R, Stacy MA, Factor SA, et al. Ropinirole 24-hour prolonged release: randomized, controlled study in advanced Parkinson disease. Neurology 2007;68:1108–15.
16. Uchida N, Takagi Y, Takada Y. Adhesive patch-containing package bag and method for storing adhesive patch. European Patent Application 2012;13/265:418.
17. Banga AK. Electrically assisted transdermal and topical drug delivery. Bristol, PA: Taylor and Francis; 1998.
18. Pikal MJ. The role of electroosmotic flow in transdermal iontophoresis. Adv Drug Deliv Rev 2001;46:281–305.
19. Kalia YN, Naik A, Garrison J, Guy RH. Iontophoretic drug delivery. Adv Drug Deliv Rev 2004;56:619–58.
20. Vemulapalli V, Yang Y, Siddoju S, et al. In vitro and in vivo iontophoretic transdermal delivery of an anti-parkinsonian agent. Int J Pharm 2011;420:20–5.
21. Nugroho AK, Li G, Grossklaus A, et al. Transdermal iontophoresis of rotigotine: influence of concentration, temperature and current density in human skin in vitro. J Control Release 2004;96:159–67.
22. Nugroho AK, Li GL, Danhof M., et al. Transdermal iontophoresis of rotigotine across human stratum corneum in vitro: influence of pH and NaCl concentration. Pharm Res 2004;21:844–50.
23. Nugroho AK, Li L, Dijkstra D, et al. Transdermal iontophoresis of the dopamine agonist 5-OH-DPAT in human skin in vitro. J Control Release 2005;103:393–403.
24. Van Der Geest R, Danhof M, Bodde HE. Iontophoretic delivery of apomorphine. I: in vitro optimization and validation. Pharm Res 1997;14:1798–803.
25. Luzardo-Alvarez A, Delgado-Charro MB, Blanco-Mendez J. Iontophoretic delivery of ropinirole hydrochloride: effect of current density and vehicle formulation. Pharm Res 2001;18:1714–20.
26. Prausnitz MR. Microneedles for transdermal drug delivery. Adv Drug Deliv Rev 2004;56:581–7.
27. Singh N, Kalluri H, Herwadkar A, et al. Transcending the skin barrier to deliver peptides and proteins using active technologies. Crit Rev Ther Drug Carrier Syst 2012;29:265–98.
28. Kalluri H. Banga AK. Formation and closure of microchannels in skin following microporation. Pharm Res 2011;28:82–94.
29. Wu XM, Todo H, Sugibayashi K. Enhancement of skin permeation of high molecular compounds by a combination of microneedle pretreatment and iontophoresis. J Control Release 2007;118:189–95.
30. Katikaneni S, Badkar A, Nema S, Banga AK. Molecular charge mediated transport of a 13 kD protein across microporated skin. Int J Pharm 2009;378:93–100.
31. Matheson AJ, Spencer CM. Ropinirole: a review of its use in the management of Parkinson's disease. Drugs 2000;60:115–37.
32. Beier C, Wilhelm M. Transdermal therapeutic system (reservoir-tts) for using pramipexole and ropinirole. 2004. 10/485043.
33. Banga AK. Microporation applications for enhancing drug delivery. Expert Opin Drug Deliv 2009;6:343–54.
34. Kolli CS, Banga AK. Characterization of solid maltose microneedles and their use for transdermal delivery. Pharm Res 2008;25:104–13.
35. Li G, Badkar A, Nema S, et al. In vitro transdermal delivery of therapeutic antibodies using maltose microneedles. Int J Pharm 2009;368:109–15.
36. Badran MM, Kuntsche J, Fahr A. Skin penetration enhancement by a microneedle device (Dermaroller) in vitro: dependency on needle size and applied formulation. Eur J Pharm Sci 2009;36:511–23.
37. Kaushik S, Hord AH, Denson DD, et al. Lack of pain associated with microfabricated microneedles. Anesth Analg 2001;92:502–4.
38. Kah M. Brown CD. LogD: lipophilicity for ionisable compounds. Chemosphere 2008;72:1401–8.
39. Banks SL, Pinninti RR, Gill HS, et al. Flux across [corrected] microneedle-treated skin is increased by increasing charge of naltrexone and naltrexol in vitro. Pharm Res 2008;25:1677–85.
40. Banga AK. Transdermal and intradermal delivery of therapeutic agents: Application of physical technologies. London: Taylor and Francis; 2011.
41. Steiger M. Constant dopaminergic stimulation by transdermal delivery of dopaminergic drugs: a new treatment paradigm in Parkinson's disease. Eur J Neurol 2008;15:6–15.
42. Garland MJ, Caffarel-Salvador E, Migalska K. Dissolving polymeric microneedle arrays for electrically assisted transdermal drug delivery. J Control Release 2012;159:52–9.
43. Vemulapalli V, Yang Y, Friden PM, Banga AK. Synergistic effect of iontophoresis and soluble microneedles for transdermal delivery of methotrexate. J Pharm Pharmacol 2008;60:27–33.
44. Badkar AV, Smith AM, Eppstein JA, Banga AK. Transdermal delivery of interferon alpha-2B using microporation and iontophoresis in hairless rats. Pharm Res 2007;24:1389–95.
45. Mudry B, Guy RH, Delgado-Charro MB. Transport numbers in transdermal iontophoresis. Biophys J 2006;90:2822–30.
46. Phipps JB, Gyory JR. Transdermal ion migration. Adv Drug Deliv Rev 1992;9:137–76.
47. Fatouros DG, Groenink HW, De Graaff AM. Visualization studies of human skin in vitro/in vivo under the influence of an electrical field. Eur J Pharm Sci 2006;29:160–70.
48. Calatayud-Pascual MA. Balaguer-Fernandez C, Serna-Jimenez CE, Effect of iontophoresis on in vitro transdermal absorption of almotriptan. Int J Pharm 2011;416:189–94.