Microneedle Assisted Transdermal Delivery of Levodopa

Buchi Naidu Nalluri, Sirivalli Kosuri, Sai Sri Anusha Valluru, Chandra Teja Uppuluri, Ashraf Sultana Shaik

Department of Pharmaceutics, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada-520010, AP, INDIA.

ABSTRACT

Objectives: The present study was designed to investigate the role of microneedle arrays (0.6 and 1.2 mm lengths) in enhancing the *in vitro* permeation of levodopa (LD) across pig ear skin. **Experimental:** *In vitro* skin permeation studies were carried out using Franz diffusion cells for a period of 24 h. A previously developed and validated RP-HPLC-PDA method was used to analyze the samples. Histological examination of skin samples treated with microneedles was carried out to confirm the penetration of microneedles into skin. **Results and Discussion:** Histological examination of the skin samples clearly showed the micro-conduits in the skin layers after micro-needle application. The penetration depth of micro-needles in skin was found to be about 40-50% of micro-needle length. It was found that increase in the donor volume, i.e., the chemical potential on donor side, increased the permeation of LD. A 9-fold increase in flux with a 16-fold decrease in lag time was observed with 1.2 mm micro-needle application when compared to passive permeation. **Conclusion:** Micro-needle assisted transdermal delivery of LD can be an ideal choice for the therapy of parkinson's disease for reducing off-episodes? and side effects pertaining to oral delivery. Further work needs to be done to effectively enhance the transdermal permeation of LD in order to achieve the clinically significant plasma levels.

Key words: Levodopa, Micro-needle arrays, Parkinson's disease, Permeation enhancement, Transdermal delivery.

INTRODUCTION

Parkinson's disease is a progressive degenerative disorder and generally affects older people. Levodopa (LD) has a beneficial effect in parkinson's disease as its efficacy exceeds that of any other drug used alone. It is inactive by itself, but is the immediate precursor of the transmitter dopamine. About 95% of an oral dose is decarboxylated by DOPA decarboxylase to dopamine in the peripheral tissues (mainly gut and liver) and 1-2% of administered LD reaches the brain and is taken up by dopaminergic neurons and converted to dopamine which is stored and released as a transmitter.[1] The most accepted clinically available route for LD administration in parkinson disease patients is the oral route. Presently, LD is administered orally and with oral administration, about 70% of the administered LD dose undergoes metabolism in the gastrointestinal tract and 30% reaches the systemic circulation of which 1-3% enters the brain (Figure 1).[2] For this reason, large oral doses of LD are required for adequate therapeutic effect and this may often be accompanied by nausea and other adverse reactions due to dopamine produced in extra-cerebral tissues. Considering the fluctuations in the plasma concentration due to the immediate absorption of LD in the proximal duodenum, following oral administration, excessive first-pass metabolism and pill burden, an optimal product and route of administration that would provide the advantage of rapid systemic LD administration with a consistent duration of action is of great interest.[3,4] Transdermal delivery of LD is expected to offer continuous input levels over an extended period resulting in an excellent patient compliance due to elimination of pre-systemic first-pass metabolism. However, LD is a hydrophilic drug with a very low log p(-4.7) and exhibits low transdermal penetration.[5] Overall, LD does not have ideal properties to permeate through the skin and hence optimization of

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Correspondence Address
Buchi Naidu Nalluri,
Department of Pharmaceutics, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada-520010, AP, INDIA.
Phone no: +91 9618394959
Facsimile no: +91-866-2493346
E-mail: buchinalluri@yahoo.com

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transdermal delivery of LD should be done by the use of suitable permeation enhancement technique. Few studies were published on increase in the permeation of LD using penetration enhancement techniques by the use of penetration enhancers and iontophoresis across rat skin.[6-7] In another investigation, Tiwary *et al.,* studied the role of sphingosine synthesis inhibition in transcutaneous delivery of LD and concluded that a decrease in the lag time was necessary to achieve the effective plasma concentration of LD.[8] However, therapeutically effective plasma levels of LD were not achieved in all these studies.

Micro-needle application is one of the most recent technologies available for delivering the macro molecules and hydrophilic drugs through skin.[9-13] Micro-needles are long enough to bypass the stratum corneum layer but short enough to avoid the damage of nerves located in the dermis, and offers painless drug delivery.[14] Stainless steel micro-needles offer good mechanical strength, the planar surface and geometrical properties of the micro-needles can enhance permeation through the viable epidermal layer of skin, via micro channel cavities created by microneedles.[15-17] Micro-channels are formed after insertion and removal of the micro-needles and then drug loaded patch can be applied for the penetration of the drug across the skin (poke and patch application). Various researchers studied on micro-needle insertion force and its correlation to parameters, such as tip radius, pain upon penetration and penetration depths, etc.[18-22] Hence, in the present investigation, commercially available stainless steel micro-needle arrays (AdminPatch®) with different needle lengths (0.6 mm, 1.2 mm) (Figure 2) were evaluated with respect to their efficiency on skin perforation and penetration enhancement of LD through pig ear skin with a special focus on the amount of LD permeated.

MATERIALS AND METHODS

Materials

Microneedle arrays were purchased from Admin Med, Sunnyvale, USA. Levodopa was a gift sample from Divis Labs, Hyderabad, India. Sodium chloride, propylene glycol, isopropyl alcohol, hydrochloric acid and light mineral oil were procured from Loba Chemie Pvt Ltd, Mumbai, India. *O*-phosphoric acid, methanol, acetonitrile, water were all of HPLC grade and procured from Merck Specialties Pvt Ltd, Mumbai, India. Hematoxylin and eosin stains were procured from Sigma Aldrich, India. Pig ears were obtained from local abattoirs.

Methods

Chromatographic Conditions

A reverse phase HPLC system was used for the analysis of LD in different samples.[23] Chromatographic separation was performed on a Shimadzu Prominence HPLC System equipped with LC 10 AT VP binary pumps, SPD-10A VP UV Detector. LC solution software was used to collect and process the data. Mobile phase consisting of 0.05% (v/v) *O*-phosphoric acid: acetonitrile (96:4 v/v) at used at a flow rate of 1 mL/min. The mobile phase was filtered through nylon disc filter of 0.45 µm (Millipore, USA) and sonicated for 3 min in ultrasonic bath before use. For quantitative analytical purpose 20 µL of the samples were injected and the eluents were monitored at 220 nm and the separation was achieved at an ambient temperature. The retention time for LD was 4.2 min.

Preparation of 0.9% (w/v) Sodium Chloride (Saline)

0.9 g of sodium chloride was accurately weighed and dissolved in 50 mL of distilled water. The volume was made up to 100 mL using the same.

Stability of LD in Saline

The stability studies of LD were performed in 0.9% (w/v) saline solution and saline solution containing 0.25% w/v sodium sulfite. The samples (20 µg/mL) were placed at 37°C in an orbital shaker for a period of 48 hr. The samples were withdrawn at different time points and analyzed by HPLC.

Skin Preparation

The pig ears were collected and processed as per the protocol approved by the Institutional Animal Ethics Committee (IAEC). Pig ears were collected from the local abattoirs (pigs aged about 6-7 months) and the ears were transported to the laboratory in a cooling box without previous treatment. Freezing of the skin was avoided during transport. In the laboratory, the pig ears were washed carefully with tap water. The hair was removed from the external part of pig ear using an electrical hair clipper. Then, carefully, the full-thickness skin from the external part of the pig ear was separated from the underlying cartilage using a scalpel and excess fat under the skin was removed to a thickness of 1.2 mm for all the skin samples. Dermis side was wiped with isopropyl alcohol cotton balls to remove residual adhering fat. The pieces of skin obtained were individually wrapped in plastic bags and stored in a deep freezer at -20°C till further use.

Skin Perforation by Micro-needle Arrays

Prior to the skin permeation experiments, the skin samples were taken from the freezer and brought to room temperature for about 30 min. After thawing, the skin surface was carefully washed with saline. The Admin Patch® differing in their micro-needle lengths (0.6 mm, 1.2 mm) was pressed over the skin surface (Figure 2). The micro-needles were periodically checked in between usage for potential damage of the needles under a stereo microscope.

Histological Examination and Calculation of Penetration Depth

The histological sections of the skin samples with and without micro-needle treatment were prepared and observed under the PZRM-700 microscope (Quasmo, Haryana, India) fitted with 10x objective. Skin samples were stained with hematoxylin and eosin for visualization of skin layers and to display a clear indentation of micro-needle penetration. The depth of penetration was also calculated with the help of Toup View 3.2 Software from AmScope FMA050 microscopic attachment (AmScope, Irvine, USA).

In vitro Skin Permeation Studies

The *in vitro* transdermal permeation studies were performed using a vertical type Franz diffusion cell apparatus equipped with a water circulation system, a water heater and an eight stage magnetic stirrer (Orchid Scientifics, Nasik, India). Franz diffusion cells with a diffusion area of 1.77 cm^2 and a receptor volume of 14 mL were used and saline was used as the receptor fluid. Pig ear skin was mounted between donor and receptor cells with stratum corneum surface facing towards the donor cell and these cells were clamped. The receptor medium was magnetically stirred for uniform drug distribution at a speed of 600 rpm. Care was taken to avoid any air bubbles between the underside of the skin (dermis) and receptor solution. The surface of the skin was maintained at 32°C using a circulating water bath. After equilibration for 30 min, 500 and or 1000 µL of donor solution (i.e., saturated solution of LD in light mineral oil) was applied on to the skin. Samples were withdrawn from the receptor fluid at regular intervals up to 24 h and replaced with the same volume of fresh saline. All the samples were stored at 4°C until analyzed by the HPLC method.

Evaluation of Skin Permeation Data

The cumulative permeation profiles were plotted as time versus cumulative amount of LD permeated (µmoL/cm^2) for passive and micro-needle (0.6 and 1.2 mm) treated skin permeation studies. The flux value for a given experiment was obtained from the slope of the steady state portion of the cumulative amount of LD permeated over time. Lag time was obtained from the X-intercept of the steady state portion of the cumulative amount of LD permeated versus time plot. Apparent permeability coefficient and diffusion coefficient values were computed from Fick's First Law of diffusion:

$$\frac{1}{A}\left(\frac{dM}{dt}\right) = J_s = K_p \Delta C$$

J_s is the steady-state flux (µmoL/cm^2/hr), M is the cumulative amount of LD permeating the skin (µmoL/cm^2), A is the area of the skin (1.77 cm^2), K_p is the apparent permeability coefficient (cm/hr), and ΔC is the difference in the concentration of LD in the donor and receiver compartments. Sink conditions were maintained in the receiver compartment throughout the experiment and hence ΔC was approximated to the drug concentration in the donor compartment.

LD Content in Skin

Drug disposition in the skin was measured at the end of the experiment. The exposed skin tissue to the donor solution was cut with a scalpel, rinsed with water and blotted with a paper towel in order to remove the adhered drug to the surface. The skin was minced and placed in a pre-weighed vial. LD was extracted from the skin by placing in 5 mL of acetonitrile and shaken (100 rpm) for 24 h at room temperature in an orbital shaker. Samples were analyzed by the HPLC method to determine the LD content after suitable dilution with saline.

Statistical Analysis of the Data

Results of the experimental data were subjected to one way ANOVA for statistical difference using SYSTAT 13 software (Systat Software, Inc. San Jose, USA). Results with p value less than 0.05 were considered to be statistically significant variance.

RESULTS AND DISCUSSION

Stability of LD in Saline

The stability of LD was investigated for a period of 48 hr and the samples were analyzed using HPLC. No significant degradation of LD was observed in saline and in saline with 0.25% w/v sodium sulfite during the stability time period. Hence, saline was selected as the receptor fluid.

Histological Examination and Calculation of Penetration Depth

Histological sections were prepared using the H&E stain and observed under Quasmo PZRM-700 microscope fitted with 10x objective (Figure 3a-3c).The existence of stratum corneum barrier breakage after micro-needle treatment was detectable in the histological sections and micro-needle arrays penetrated through the corneocytes without merely indenting them. Depth of penetration was calculated using Toup view software as 363.25 μm for 0.6 mm and 521.16 μm for 1.2 mm micro-needle arrays. The depth of penetration was found to be approximately 40-50% of the needle length.

In vitro Skin Permeation Studies

In vitro skin permeation studies were performed on vertical type Franz diffusion cells to determine the influence of micro-needles on the transdermal delivery of LD. Pig ear skin was used as a membrane replica between the donor and the receptor compartments. The pig ear has been used as a predictive model because it is relatively thin and highly vascularized and is the best alternative to human skin.[24-26] Moreover, the lipid content of pig skin is close to that of human skin.[27-28]

The passive permeation studies were initially carried out by charging donor compartment with LD saturated in light mineral oil of 0.5 mL and 1 mL volumes individually. Cumulative amounts of LD permeated at the end of 24 hr were found to be 0.208 ± 0.046 and 0.321 ± 0.027 μmoL/cm² respectively, with 0.5 and 1 mL donor volumes (Figure 4). A 1.6-fold increase in the permeation of LD was observed with 1 mL donor volume when compared to 0.5 mL ($p<0.05$).The increase in the permeation can be accredited to the increase in the amount of LD available for permeation through skin. Passive lag time values for LD were 17.122 ± 1.402 and 13.973 ± 0.753 hr respectively with 0.5 and 1 mL donor volumes. Passive flux values for LD obtained with 0.5 and 1 mL donor volumes were respectively 0.0051 ± 0.0012 and 0.0085 ± 0.0009 μmoL/cm²/hr. Owing to the high LD permeation with 1 mL donor volume, further studies were carried out using the same.

The area required for a transdermal system to achieve therapeutically effective levels of LD was calculated from the *in vitro* steady state flux (passive flux), steady state plasma concentration of LD and clearance values by using the following equation 1:

$$C_{ss} = (J_{ss}.A)/CL_T \quad (1)$$

Where, C_{ss} is the predicted steady state plasma concentration (ng/mL); J_{ss} is the steady state flux across skin; CL_T is the total body clearance obtained after i.v administration in humans.

Total body clearance of LD in humans is about 0.22 L/kg/hr, and the desired steady state concentration required to achieve the therapeutic effect was 1200 ng/mL.[29] Taking into consideration of the *in vitro* LD flux obtained with passive permeation studies, the area needed for a transdermal system (for example a patch) to maintain therapeutically effective LD plasma levels is 18410.996 and 11050.94 cm² for 0.5 and 1 mL donor volumes respectively. Considering such large area of the transdermal system, which may not be feasible in the realistic situation, an increase in the transdermal flux of LD may be required to reduce the area of the transdermal system and the lag time need to be shortened in order to achieve therapeutically effective levels of LD *in vivo* and quicker onset of action. However, LD is a hydrophilic drug and it is difficult to achieve significant permeation across the skin without the use of any penetration enhancement technique. The use of iontophoresis and permeation enhancers were reported for LD transdermal permeation enhancement through rat skin. However, therapeutically effective levels of LD were not achieved in these studies.[6-7]

With micro-needle application, cumulative amounts of LD permeated at the end of 24 hr were 1.184 ± 0.098 and 1.843 ± 0.111 μmoL/cm² respectively for 0.6 and 1.2 mm micro-needle lenghts. 0.6 mm micro-needle array has more number of micro-needles with less spacing as it possess one hundred eighty seven of 500 μm length micro-needles on a 1 cm² area which resulted in higher density of micro-needles (Figure 2). In spite of higher density, 0.6 mm micro-needles resulted lower permeation amounts of LD as they could not penetrate deep enough the skin layers due to the resistance by the skin's elastic nature and hence very small and shallow pores have been created through which the LD could permeate only to some extent (Figure 3b). Whereas, 1.2 mm micro-needle array possess forty three of 1100 μm length micro-needles on a 1 cm² area and has less number of micro-needles with increased spacing which resulted in decreased density when compared to 0.6 mm micro-needle arrays. When these arrays were used to pierce the stratum corneum, they have penetrated deeply into the skin layers because of their increased needle length and created pores of significant size and depth so that more amount of LD could permeate through the pores across the skin (Figure 3c**).**

The permeation profiles clearly indicated that the permeation of LD increased with micro-needle treatment when compared with passive permeation (Figure 4).

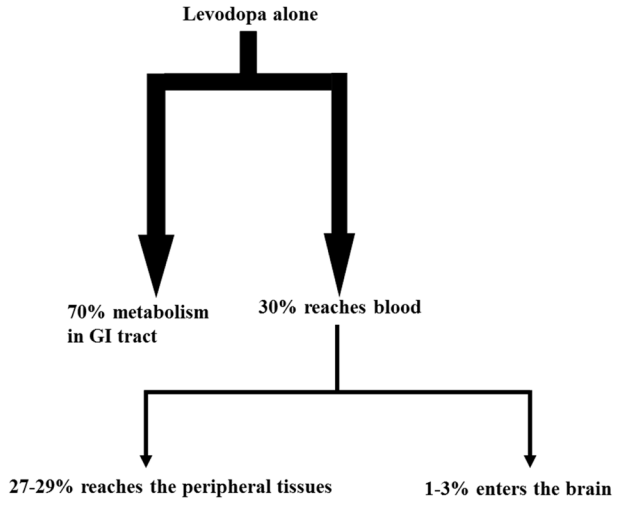

Figure 1: Availability of LD oral dose

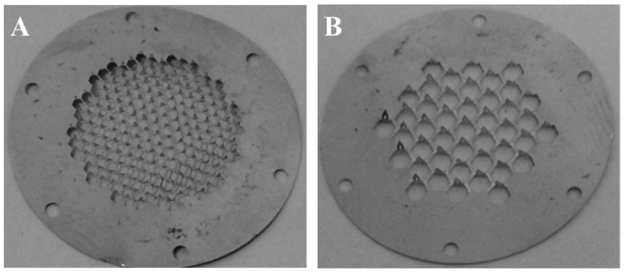

Figure 2: Photographs of micro-needle arrays, (A) 0.6 mm; (B) 1.2 mm

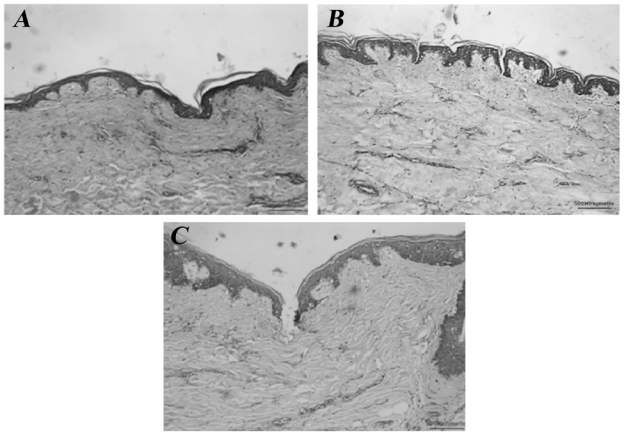

Figure 3: Histological sections of skin samples (A) control; (B) 0.6 mm treated; (C) 1.2 mm treated

A 5.7-fold increase in the cumulative amount of LD permeated at the end of 24 hr with 1.2 mm micro-needle array in comparision to passive permeation was observed ($p<0.05$). Similarly, a 1.56 fold increase in the cumulative amount of LD permeated at the end of 24 hr with 1.2 mm micro-needle array in comparison to 0.6 mm micro-needles was observed ($p<0.05$). Lag

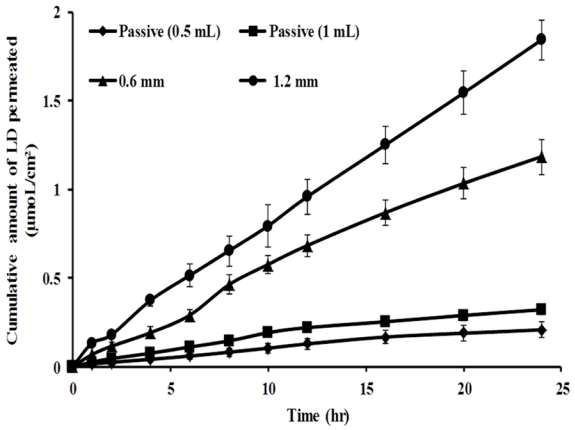

Figure 4: Comparative permeation profiles of LD with and without micro-needle treatments

time values for LD following the use of 0.6 mm and 1.2 mm micro-needle arrays were 5.990 ± 0.214 and 0.844 ± 0.569 hr respectively. A 2-fold and 16-fold decrease in lag time values were observed with 0.6 and 1.2 mm micro-needle arrays when compared with passive permeation across pig ear skin ($p<0.05$). The *in vitro* LD flux values obtained following the use of 0.6 mm and 1.2 mm micro-needle arrays were 0.0396 ± 0.0032 and 0.0741 ± 0.0010 µmoL/cm²/hr respectively. A 5-fold and 9-fold increase in LD flux was observed with 0.6 and 1.2 mm micro-needle arrays and were significantly higher when compared with passive permeation across pig ear skin ($p<0.05$). These results suggest that significant amounts of LD can be delivered through the skin with micro-needle application when compared to passive permeation.

With micro-needle application, several factors affect the transdermal penetration of the drugs across the skin such as micro-needle length, micro-needle density, no and insertion force and insertion time. Transdermal delivery of antibodies using maltose micro-needles was studied by Li *et al.,* and it was proved that there was an increase in human IgG delivery when the number of micro-needle arrays, micro-needle length and drug concentration in the donor chamber was increased.[30] Similarly, in our studies it was clearly evident that the LD permeation is dependent on micro-needle length and LD concentration in donor chamber.

LD content in the skin was also estimated after the permeation studies. Compared to passive permeation, LD content in the skin increased due to skin pretreatment with micro-needles (Table 1). The effective permeability coefficient and diffusion coefficient were also estimated (Table 1) and the data revealed that these two parameters

Table 1: Permeation Parameters for LD (Mean ± SD, n=3)

Permeation Parameter/ Variable	Skin Treatment			
	Passive (0.5 mL)	Passive (1 mL)	0.6 mm microneedles	1.2 mm microneedles
Apparent Permeability coefficient (cm/hr)	0.049 ± 0.012	0.082 ± 0.009	0.384 ± 0.031	0.719 ± 0.010
Diffusion coefficient (*10-6)(cm²/sec)	1.64 ± 0.40	2.74 ± 0.30	12.80 ± 1.05	23.96 ± 0.32
LD content in skin (µmoL/gm)	0.59 ± 0.06	1.14 ± 0.12	2.42 ± 0.57	4.37 ± 0.66

showed a significant increase with 1.2 mm micro-needle array compared to 0.6 mm and untreated skin samples ($p<0.05$).

Thus, in our investigation, we observed that as the micro-needle length increased, the permeation of LD increased significantly although same mode of insertion was applied for both the needle lengths. However, the area of the transdermal system required to be effective for micro-needle treated skin studies was calculated from eq. 1 and was found to be 2365.87 cm^2 and 1264.33 cm^2 respectively for 0.6 and 1.2 mm micro-needle arrays. In spite of micro-needle treatment, large area of the transdermal system is required to achieve effective therapeutic levels of LD with "poke and patch" technology and these results suggests that the *in vitro* LD flux obtained with micro-needle application need to be further increased using a suitable technique, for example, the use of prodrugs there by making LD more lipophilic and there by changing the solubility properties in donor vehicles.

CONCLUSION

In our investigation, micro-needles disrupted the stratum corneum, a barrier to transdermal penetration, by creating micro-conduits across the skin. It is clearly evident that micro-needles successfully penetrated the skin and significantly enhanced transdermal permeation of LD when compared to the passive permeation studies. However, from the *in vitro* flux data obtained, it was evident that large surface area of the transdermal system is required to achieve desired therapeutically effective LD levels which are not practically feasible with the obtained permeation parameters. Further studies on either co-administration of LD with carbidopa or LD prodrugs are of great interest in order to achieve the therapeutically effective LD levels. Hence, further work needs to be carried out in order to effectively permeate LD using a poke and patch application system for the successful treatment of parkinson's disease.

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SOURCE(S) OF SUPPORT

Nil

CONFLICT OF INTEREST

The author declare no conflict of interest.

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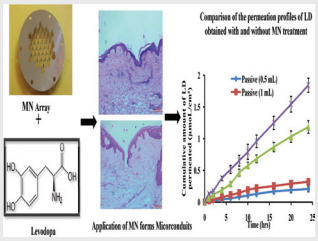
PICTORIAL ABSTRACT



ABBREVIATION USED

ANOVA : Analysis of Variance; **DOPA:** 3,4-dihydroxyphenylalanine; **H&E** : Hematoxylin and eosin; **LD:** Levodopa; **RP-HPLC-PDA:** Reverse Phase High Pressure Liquid Chromatography with Photo Diodearray Detector.

About Author

Prior to joining in KVSRCOPS, Dr. Nalluri worked as Postdoctoral fellow/Research Associate at University of Kentucky, USA and as Principal Scientist at Yaupon Therapeutics Inc. Dr. Nalluri obtained Ph.D. in pharmaceutical sciences from the College of Pharmaceutical Sciences, Andhra University (2002) and has won many awards throughout his academic career including UGC Research Fellowship (1997), best research paper award in pharmaceutics by IDMA, Mumbai (2000) and best PhD thesis in pharmaceutical sciences award (JJ Rao Gold Medal) by Andhra University (2003). He is PI of 3 research grants (funded by AICTE and DST) and published and presented more than 50 research papers.

Highlights of the paper

- Parkinson's disease is a progressive degenerative disorder and generally affects older people. Levodopa (LD) has a beneficial effect in parkinson's disease as its efficacy exceeds that of any other drug used alone.
- Transdermal delivery of LD is expected to offer continuous input levels over an extended period resulting in an excellent patient compliance due to elimination of pre-systemic first-pass metabolism. However, LD is a hydrophilic drug with a very low log p(-4.7) and exhibits low transdermal penetration.
- LD does not have ideal properties to permeate through the skin and hence optimization of transdermal delivery of LD should be done by the use of suitable permeation enhancement technique.
- Microneedle application is one of the most recent technologies available for delivering the macro molecules and hydrophilic drugs through skin. in the present investigation, commercially available stainless steel microneedle arrays (AdminPatch®) with different needle lengths (0.6 mm, 1.2 mm) were evaluated with respect to their efficiency on skin perforation and penetration enhancement of LD through pig ear skin with a special focus on the amount of LD permeated.
- Microneedles disrupted the stratum corneum, a barrier to transdermal penetration, by creating microconduits across the skin. It is clearly evident that microneedles successfully penetrated the skin and significantly enhanced transdermal permeation of LD when compared to the passive permeation studies.