# 行政院國家科學委員會專題研究計畫　成果報告

## 輸電變數對電穿孔經皮輸藥分子量阻斷值之影響

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# 行政院國家科學委員會生物處專題研究計畫研究成果報告

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計畫名稱：輸電變數對電穿孔經皮輸藥分子量阻斷值之影響

## 研究計畫成果中文摘要

**關鍵詞:　電穿孔、經皮輸藥、分子量阻斷值**

本計畫主要目的在於研究及評估不同輸電變數對電穿孔經皮輸藥分子量阻斷值之影響。此計畫乃是延續近年來主持人對電穿孔經皮輸藥系統之一系列研究。本計畫內容之執行成果除了提供電穿孔輸藥系統應用在大分子藥物(如peptide, protein)時重要參考，更可彌補在學術文獻上有關訊息之不足。因而本計畫之執行對此輸藥法實際應用及基礎理論之探討均將有相當助益。

本計畫所涵蓋之內容乃以 hairless rat 作為經皮穿透之模式皮，所選擇之大分子模式藥物乃是不同分子量之 FITC-dextrans，在不同的電穿孔條件下探討此些不同分子量模式藥物穿透速率及分子量阻斷值。研究結果顯示在使用電壓 300V 及脈衝時間 200ms 下，電穿孔經皮輸藥法可使分子量 38000 之 FITC-dextran 穿透且其穿透通量和分子量有線性關係存在。由上述研究所得之訊息可在基礎研究上將有助於闡明電穿孔輸藥對大分子藥物穿透之影響及限制，

# 計畫成果英文摘要

**Key words: electroporation, transdermal, molecular weight cut-off value**

The major purpose of this project is to assess the effects of various electrical factors on the molecular weight cut-off value for transdermal delivery of macromolecules under electroporation. The project is a continued effort of previous NSC project in exploring the feasibility of applying electroporation in transdermal drug delivery. This basic research may help to reveal the influence of various electric factors on the transdermal permeation of macromolecules under electroporation. The obtained information from this basic research may contribute to develop optimum transdermal electroporation devices for macromolecules.

In the present study, hairless rat skin was used as the permeation barrier. FITC-dextrans of increasing molecular weight was used as model molecules to study the effect of various electrical factors on permeation kinetics as well as molecular weight cut-off value. The results showed that the application of electroporation significantly increase the transdermal delivery of macromolecules. It also also indicated that the macromolecules with MW higher than 38000 can be delivered through skin via applying iontophoresis. Moreover, within the molecular weight range of 4400 to 38000, the transdermal flux for macromolecules was a function of molecular weight under the application of iontophoresis. The obtained information can be utilized to develop optimum transdermal electroporation devices for macromolecules.

## Introduction

Biotechnology produced macromolecules such as proteins or nucleic acids show great therapeutic promise, however, the delivery issue can be a significant impediment. The biotechnology products generally have low oral bioavailabilty and short biological half-lives. As a result, transdermal drug delivery can be an useful alternative to circumvent the delivery problem. The major purpose of this project is to assess the effects of electroporation technique on the transdermal characteristics of a series of FITC-dextrans. The FITC-dextrans are used as a molecular weight marker in this study. The obtained information can be utilized to develop optimum transdermal electroporation device for macromolecules.

## Materials and Mehtods

### Materials

FITC-dextrans with various molecular weights, including FD4400, FD10000, FD20000 and FD38000 were purchased from Sigma Chemical Company (Saint-Louis, MO. USA). All other chemicals and solvents were analytical grade and used as received.

### Preparation of Skin Membranes

Female hairless mice (~6 weeks old) were sacrificed by ether and full-thickness skin was excised from the dorsal region.

### Instruments and In Vitro Permeation Experiments

In vitro skin permeation experiments were performed using horizontal side-by-side diffusion cell. The donor medium was 8 ml of pH 7.4 citrate-phosphate buffer (8ml); the receptor medium was also 8 ml of pH 7.4 citrate-phosphate buffer. The available diffusion area between cells was 0.785 $cm^2$. The stirring rate and temperature were kept at 600 rpm and 37°C respectively. At appropriate intervals, aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh buffer. The amount of solutes were determined by the fluorescence detector.

Electroporation was applied using an exponential decay pulse generator (ECM630 Electro Cell Manipulator®, BTX Co., USA). The platinum electrodes (1×2 $cm^2$, 99.99%) were used and each located 3 cm from the skin. The anode was positioned in the donor compartment and the cathode was placed in the receptor

compartment. Unless otherwise noted, the electroporation protocol was 1 pulse per 30 s, applied for 10 min; the pulse voltage was 300 V and pulse length was 200 ms. The voltages were recorded as applied values but not transdermal values.

## Data Analysis

The flux was defined as amount of drug (nmole) permeated through 1 $cm^2$ of skin per unit time (hour); its value was defined as the amount of drug permeated divided by permeation area and time and was obtained from the linear portion of cumulative amount versus time profile.

## Results and Discussion

Figure 1 shows the cumulative amount versus time profiles for FITC-dextrans with various molecular weight. Since the transdermal permeation of those solutes via passive diffusion were quite low, it is obvious from Figure 1 that the application of electroporation significantly increase the transdermal delivery of macromolecules. Figure 1 demonstrates that, as the molecular weight of the solutes increased, the permeation rates decreased. The results also indicate that the macromolecules with molecular weight higher than 38000 can be delivered through skin via applying electroporation.

Figure 2 shows the relationship between the flux value and the molecular weight of FITC-dextran. A linear relationship can be obtained with r2 of 0.966. The results clearly indicate that, within the molecular weight range of 4400 to 38000, the transdermal flux for macromolecules is a function of molecular weight under the application of electroporation.

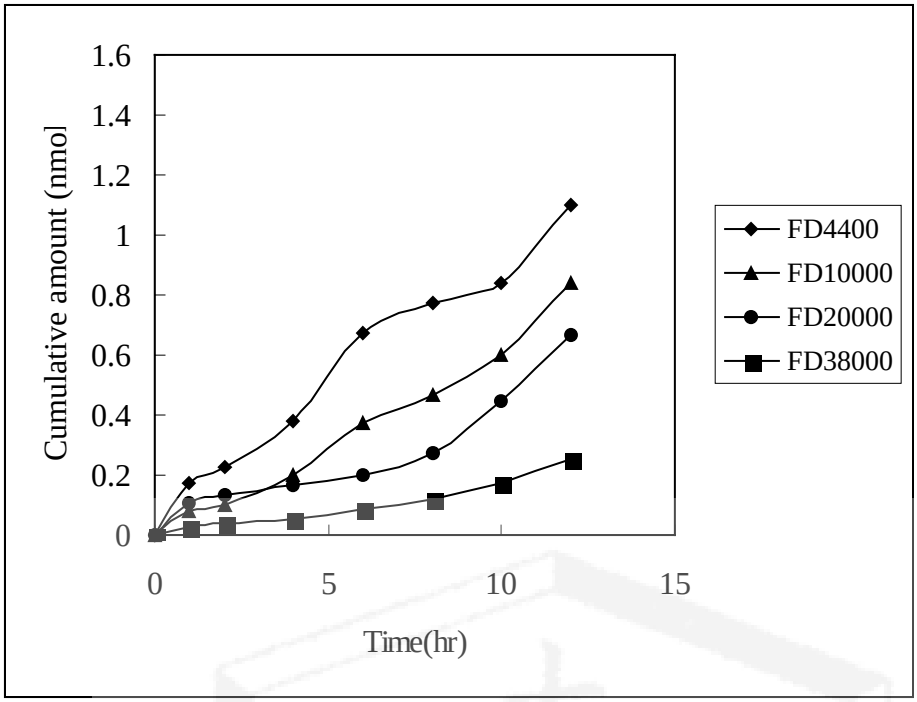


Figure 1: The cumulative amount versus time profiles for various FITC-dextrans

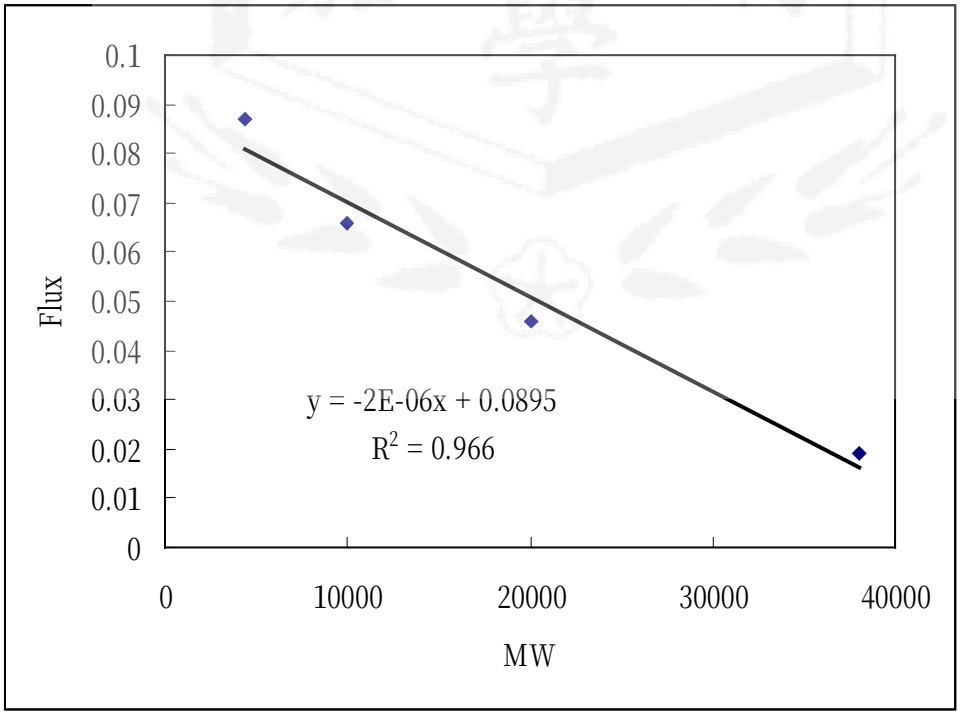


Figure 2: The relationship between flux (nmole/cm2*hour) and molecular weight (MW) of FITC-dextrans

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