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RESEARCH PAPER



# Topical Delivery of Cyclosporin A Coevaporate Using Electroporation Technique

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**ABSTRACT**

*The immunosuppressive drug cyclosporin A (CSA) is useful in treating psoriasis. However, the systemic use of CSA is fraught with the problem of toxicities. The best way to treat psoriasis would be to deliver CSA topically but no such formulation is currently available. The highly lipophilic nature and the large molecular weight of CSA are the main hurdles in developing an efficient topical formulation. Attempts were made in our laboratory to deliver CSA topically using electroporation technique. First, the aqueous dissolution of CSA was improved by preparing a coevaporate using polyvinyl ethyl methyl ether maleic acid copolymer, which resulted in a 9.5-fold increase in the aqueous solubility of CSA. Subsequently, the aqueous solution of the coevaporate was used as the donor solution to deliver CSA transdermally in a rat skin model, using single and multiple pulse electroporation. Use of single pulse mode at a field strength 200 V/cm- and 10-msec pulse interval, resulted in delivering 87 ng of CSA per 0.87 $cm^2$ of the rat skin. This was a significant increase by a factor of 8.5 in the delivery of CSA, when compared to the passive diffusion. The amount of CSA delivered to the skin using electroporation may be further enhanced by increasing the thermodynamic activity of CSA in the donor solution. The other electroporation variables which need to be further optimized include field strength, pulse length, and number of pulses.*

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## INTRODUCTION

Psoriasis is a common chronic skin disease characterized by recurrent exacerbations and remissions of thickened, erythematous, scaling lesions. About 3 million people in the United States are affected by psoriasis, and annually it consumes about $2 billion from the health care funds (1). Among the variety of drugs used to treat psoriasis, cyclosporin A (CSA) has been reported to be quite effective (2). Due to the potentially higher risk of unacceptable side effects such as nephrotoxicity and hepatotoxicity, the systemic use of CSA is not desirable (3). Intralesional injection of CSA has been found effective in treating psoriasis, but the injection is reported to be very painful (4,5). Localizing CSA in the skin may be the best solution to minimize the problem of systemic toxicity, but no such formulation exists at present. This paper describes our attempt to deliver CSA topically from CSA–polymer coevaporate using electroporation technique.

In electroporation, high-intensity electric fields (about 100–300 volts) are pulsed for millisecond (msec) durations to create transient changes in the skin in order to facilitate drug transport (6,7). Although the use of electroporation in transdermal delivery is presently at its early stage of development, this technique has been in use for many years in cell biology to transport macromolecules such as DNA and proteins (8,9). Electroporation under appropriate conditions may be considered safe for clinical use. Electrochemotherapy, which is the use of electric pulse along with chemotherapy, has been reported to be well tolerated in patients (10). The mechanism for electroporation-aided transmembrane delivery of molecules reported in the literature attributes increased membrane permeability to the creation of transient aqueous pores (6,11). This implies that for the transmembrane delivery of molecules using electroporation, an aqueous donor solution of the substance is required. Attempts have been made by different groups of researchers to transdermally deliver water-soluble drug molecules using electroporation as the driving force (7,12,13). Our effort is aimed at delivering the poorly water-soluble peptide CSA transdermally using electroporation.

CSA is a neutral, undecapeptide (MW 1203 daltons) that has a poor solubility in water. In our attempt to deliver CSA using electroporation, we first improved its aqueous dissolution, by preparing a coevaporate of CSA using a water-soluble copolymer, polyvinyl methyl ether–maleic acid (PVME/MA). The aqueous solution of the coevaporate was subsequently used as the donor solution to deliver CSA transdermally using electroporation in in vitro experiments using hairless rat skin.

## MATERIALS

Cyclosporin A and D were gifts from Sandoz Pharma AG, Switzerland, and were used as received. $^3$H-Labeled CSA was procured from Amersham Life Sciences, Ontario, Canada. Polyvinyl methyl ether–maleic acid copolymer (Gantrez S-97®) was obtained as a free sample from International Specialty Products Inc. (NJ, USA) and was used as received. The tissue digesting solvent, Solvable®, and scintillation cocktail were obtained from Dupont NEN Research Products (MA, USA). All other chemicals and reagents were of analytical and high-performance liquid chromatography (HPLC) grade.

## METHODS

### Analysis of CSA

The concentrations of CSA from aqueous test samples were determined using a HPLC procedure described in the USP XXII (14) after modifying it to suit our laboratory conditions. Essentially, it comprised a HP-1050 pump (Hewlett Packard, Waldbronn, FRG) equipped with a variable ultraviolet (UV) detector set at 210 nm, a column heating compartment maintained at 70°C, and an autosampler. The different HPLC modules were controlled and monitored using the ChemStation® software supplied by Hewlett Packard. The mobile phase consisted of 75% acetonitrile in water delivered isocratically at 1.5 ml/min. Cyclosporin D (CDS, 4.75 μg/ml in mobile phase) was used as the internal standard and all measurements were based on CSA/CDS peak height ratio.

In the transdermal experiments using hairless rat skin as the model membrane, the concentrations of CSA in the skin and receiver compartment were determined by measuring the radioactivity of $^3$H-labeled CSA using a scintillation counter (Beckman LS 5000 TD, CA, USA).

### Preparation of CSA–PVME/MA Coevaporate

CSA and PVME/MA in different weight proportions (w/w CSA:PVME/MA; 1:5, 1:8, and 1:10) were

weighed and dissolved separately in distilled ethanol. The two solutions were mixed and the solvent was evaporated using a flash evaporator, set at 100 rpm and heated using a water bath maintained at 50°C. The coevaporates were further dried in a desiccator under vacuum, pulverized and sifted through a #70 sieve (pore size 212 μm).

### Equilibrium Solubility Determination

The equilibrium solubilities of the coevaporate samples and untreated CSA were determined in deionized water at room temperature (25°C). In this experiment, an excess amount of the test sample was shaken in deionized water using an electrical shaker for 20 hr, which was estimated as the time required to reach equilibrium solubility. The samples were then centrifuged, filtered through a 0.45-μm PVDF filter (0.45 μm, Millipore, Yonezawa, Japan) and analyzed by HPLC to determine the amount of CSA dissolved in water. The experiments were performed in triplicate and the average values reported.

### Dissolution Test

The dissolution tests were done using a Vanderkamp R 600 (Vankel Industries Inc., NJ, USA) modified USP type II dissolution apparatus set to 50 rpm. One liter of deionized water maintained at 37°C was used as the dissolution medium. The coevaporate powder test sample, equivalent to 2.5 mg of CSA, was placed in a nylon bag and tied to the paddle. This was done to prevent the loss of any undissolved solid particle during sampling. Aliquots of 1 ml were withdrawn periodically and centrifuged at 13,200 rpm for 5 min. A portion of the supernatant (500 μl) was spiked with the internal standard (CSD, 25 μl) and injected into the HPLC for analysis. The volume of the dissolution medium was maintained constant by replenishing the aliquot sample by fresh medium, equilibrated to 37°C. Cumulative amount of CSA released at each time point was determined by averaging results from 4 determinations.

The controls included untreated CSA and the physical admixture of the CSA and PVME/MA (1:8). One of the factors contributing to poor dissolution is inadequate wetting. To determine the role of a wetting agent in improving the aqueous dissolution of CSA, a control experiment was done where 5 mg of CSA was mixed with 2 mg of a mixture of Span 80 and Tween 20 in a w/w ratio 7:3 (HLB 8.0) and the mixture was used as the test sample for a dissolution test in water.

### In Vitro Transdermal Delivery of CSA Using Electroporation

Freshly excised skin from a hairless rat (Harlan Sprague Dawley, IN, USA) was used as the model skin for the transdermal experiments. The skin sample was sandwiched between two custom-made side-by-side diffusion cells made from Delrin (Fig. 1). The effective surface area exposed for transdermal delivery was 1.3 $cm^2$. After the experiment, the skin sample was punched out using a biopsy punch (area 0.87 $cm^2$) and used for analysis. Gene Pulser® II (Bio Rad, CA, USA) with modified electrodes (Ag/AgCl) was used as the source for generating electroporation pulse. While preparing the coevaporate to be used in the transdermal study, CSA was spiked with $^3H$ CSA so as to enable drug quantification using a scintillation counter. The donor (0.8 ml) solution consisted of coevaporate solution in PBS and the receiver (0.8 ml) contained PBS solution. A concentration (64 μg of CSA/ml) lower than the equilibrium solubility of CSA was used as the donor solution to avoid any chance of drug precipitation. The skin sample was assembled in the diffusion cell with the stratum corneum facing the donor solution. After adding the respective donor and receiver solutions, the skin was allowed to equilibrate for 1 hr prior to initiation of electroporation pulse. The electrodes were then inserted in the diffusion cell as shown in Fig. 1 and the electrical pulse was delivered. After waiting for 4 hr, which was considered as the optimum waiting period after the electroporation pulse, the skin sample was removed from the diffusion cell assembly and washed with PBS to remove any loosely adhered drug solution. Since our main interest was to determine the amount of drug that actually crossed the principal barrier of the skin, the

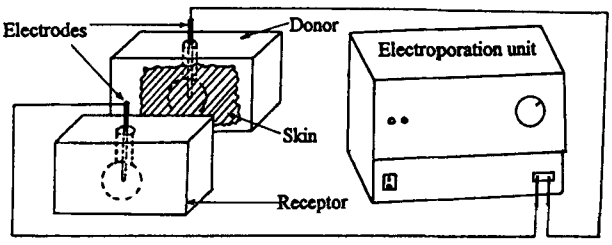


**Figure 1.** Electroporation device for in vitro experiments.

stratum corneum, the skin was stripped 10 times with Scotch® tape (3M, Ontario, Canada) to remove the stratum corneum. This would also remove any adsorbed CSA from the surface of stratum corneum. The remaining part of the skin was then digested using Solvable® solvent. Scintillation cocktail was added to the dissolved skin and the concentration of CSA was determined using a scintillation counter. Aliquot samples were taken from the donor (50 μl) and receiver (250 μl) compartments at the end of the experiment and treated with the scintillation cocktail for determining the amount of CSA. Passive diffusion delivery of CSA from the coevaporate solution was used as the control in which no electroporation pulse was delivered. The field strength ($F$) and pulse length ($\tau$) were monitored from the digital display of the electroporation unit. Field strength is the applied voltage divided by the distance between the electrodes, and pulse length is the time interval for 63% of the initial voltage to decay. The experiments were done using both single- and multiple-pulse electroporation modes. The single-pulse electroporation experiments were conducted at 150 and 200 V/cm, and the pulse length ($\tau$) varied between 5 and 10 msec. In the multiple-pulse mode, 25 pulses with an interval of 1 min between pulses was used. The $F$ and $\tau$ were similar to those used in single-pulse mode.

## RESULTS AND DISCUSSION

### HPLC Analysis

The HPLC chromatograms of the coevaporates were comparable to those of untreated CSA and there were no extra peaks. This indicated that the CSA in the coevaporate maintained its chemical integrity. The chromatogram exhibited well-resolved peaks for CSA (retention time = 3.4 min) and CSD (retention time = 4 min). The calibration curve produced using spiked CSA, having concentrations ranging from 0.5 to 5 μg/ml, resulted in a straight line. The equation for the straight line and its statistical evaluations are as follows:

$$\text{Peak height ratio} = 0.025 + 0.026 \times \text{concentration of CSA}$$

$$R^2 = 0.99,\ CV = 4.9\%,\ n = 5$$

### Equilibrium Solubility

The equilibrium solubilities of CSA from the untreated sample and from CSA-PVME/MA coevaporates are shown in Table 1. The coevaporate had 7 to 9.5 times increased solubility as compared to untreated CSA.

*Table 1*

*Equilibrium Solubility (at 25°C) of Untreated CSA and CSA from Coevaporate*

| Sample | Solubility of CSA, μg/ml ± *SD* ($n$ = 3) |
|---|---|
| CSA (untreated) | 9.25 ± 0.21 |
| 1:5 Coevaporate | 63.10 ± 7.87 |
| 1:8 Coevaporate | 88.43 ± 1.68 |
| 1:10 Coevaporate | 82.17 ± 3.18 |

### Dissolution Test

CSA from the coevaporates showed a significantly improved aqueous dissolution (Fig. 2). The cumulative CSA released from the coevaporates 1:5, 1:8, and 1:10 (w/w CSA:PVME/MA) were respectively, 82, 94, and 85%.

To elucidate the kinetics of drug release from the coevaporates, the percentage of CSA remaining to be released from the coevaporate as a function of time was fitted separately to the following equations:

$$[Q_\infty - Q_t] = Q_\infty - K_0 \cdot t \qquad \text{Zero-order}$$

$$\ln [Q_\infty - Q_t] = \ln Q_\infty - K_1 \cdot t \qquad \text{First-order}$$

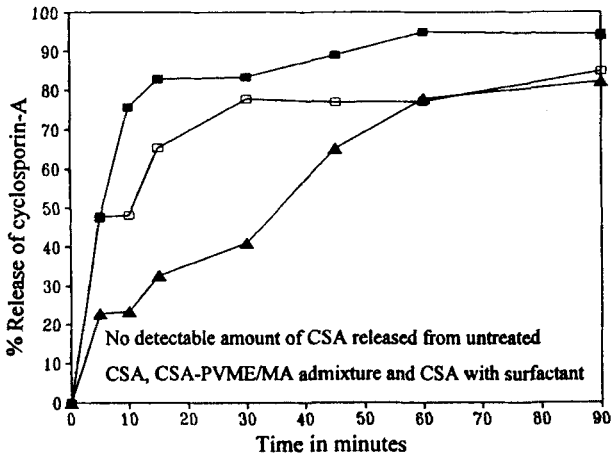


**Figure 2.** Dissolution profiles of powder samples of CSA:PVME/MA coevaporates. Coevaporates: ▲ 1:5, ■ 1:8, □ 1:10.

In the above equations, $Q_t$ and $Q_\infty$ represent, respectively, the percentage of CSA released at any time $t$ and at infinite time, under the experimental conditions. $K_0$ and $K_1$ are respectively, the zero- and first-order rate constants. The correlation coefficients ($R^2$) for the two equations were considered as the indicator for the order of release. For the 3 formulations 1:5, 1:8, and 1:10, the values of $R^2$ were, respectively, 0.95, 0.53, and 0.58 for the zero-order equation and 0.96, 0.84, and 0.72 for the first-order equation. Since $R^2$ was closer to 1 when fitted to the first-order equation, the release of CSA from the coevaporates could be considered to follow first-order kinetics.

As the concentration of PVME/MA was increased from 83.3% (in 1:5) to 88.9 (in 1:8), there was a significant increase ($p < 0.05$) in the cumulative amount of CSA released. However, further increase in the polymer concentration to 90.9% in the 1:10 coevaporate resulted in a decrease in the cumulative amount released. PVME/MA is a water-soluble polymer, which at a higher concentration forms a viscous gel. It is envisaged that the reduced cumulative release from the 1:10 coevaporate could be due to the increased diffusional barrier at the higher polymer concentration. This trend was also observed with the coevaporates in the equilibrium solubility studies.

The control experiments using untreated CSA, CSA:PVME/MA admixture, and CSA wetted with Span and Tween, did not result in any improvement in the dissolution of CSA. The amounts of CSA released in both instances were below the detection sensitivity of our analytical method. This clearly demonstrated that the hydrophobicity of CSA could not be overcome by simply improving the wetting of CSA particles. The lack of dissolution of the physical admixture of CSA and PVME/MA might suggest that the polymer per se has little or no direct solvent effect on the solubilization of CSA in water. It appears that CSA and PVME/MA in the coevaporate are associated in a unique way, which is essential for improving the aqueous dissolution of CSA. Other possible contributing factors for the improved dissolution of CSA could be the formation of a molecular dispersion, and improved wetting and the resultant dissolution of CSA in the microenvironment of the coevaporate. PVME/MA has a hydrophobic polyvinyl backbone and a maleic acid hydrophilic part. Therefore, it is also possible that PVME/MA acts as an amphipathic molecule and facilitates the dissolution of the hydrophobic drug, CSA. Elsewhere, we have reported similar work, where the aqueous dissolution of a highly lipophilic drug, clofazimine, was enhanced with the anhydride polymer of PVME/MA (15).

## In Vitro Transdermal Delivery of CSA Using Electroporation

The delivery of CSA using electroporation is shown in Table 2. Although the scintillation counter measured the radioactivities (dpm) for the samples with very low drug content, it was not possible to differentiate among the CSA concentrations below the 10 ng level. Hence, the CSA concentrations equivalent to or below 10 ng are shown as $<10$ ng in Table 2.

CSA was confined to the dermis and only a small amount of CSA was transported across the skin to the receiver side, which could be perceived as a desirable feature for treating psoriasis topically. This could primarily be due to the high lipophilicity of CSA. The amount of drug delivered through the passive diffusion was very low (below 10 ng). However, with the use of electroporation, there was a significant increase ($p < 0.05$) in the amount of CSA delivered to the skin. Compared to the passive diffusion, there was a 6- to 8.5-fold increase in the delivery of CSA to the skin with the use

***Table 2***

*Transdermal Delivery of CSA from Coevaporate Using Single- and Multiple-Elctroporation Pulse*

| Field Strength (V/cm) | Pulse Length (msec) | Number of Pulses | CSA in 0.87 $cm^2$ of Skin (ng) ± *SD* | CSA in 0.8 ml of Receiver (ng) ± *SD* | *n* |
|---|---|---|---|---|---|
| Passive | — | — | <10 | <10 | 15 |
| 150 | 10 | 1 | 60 ± 20 | <10 | 3 |
| 150 | 10 | 25 | 23 ± 5.7 | 17 ± 11 | 3 |
| 200 | 10 | 1 | 87 ± 15 | <10 | 3 |
| 200 | 5 | 25 | 37 ± 5.8 | 40 ± 17 | 3 |
| 200 | 10 | 25 | 50 ± 10 | 27 ± 12 | 3 |

of single electroporation pulse at 150 and 200 V/cm ($\tau$ = 10 msec). However, with the use of multiple-pulse mode at the two field strengths of 150 and 200 V/cm, a smaller accumulation of CSA in the skin was observed compared to that in single pulse. In the multiple-pulse mode, detectable quantities of CSA were also transported across the skin to the receiver. The total amount of CSA delivered (which is the sum of CSA in skin and CSA in the receiver) at 150 and 200 V/cm ($\tau$ = 10 msec) with single- and multiple-pulse modes was not significantly different ($p > 0.05$). The total amount of CSA delivered at 200 V/cm using multiple-pulse modes with $\tau$ = 5 and $\tau$ = 10 msec was also similar ($p > 0.05$). This is contrary to the literature reports where an increase in pulse length resulted in increased drug delivery (7,16). This may be attributed to the lack of adequate thermodynamic activity of CSA in the donor compartment. The transient pores created in the skin during electroporation lead to increased diffusion of molecules from the donor to the receiver; and since the diffusion is a concentration-dependent phenomena, a 7-fold increase in the concentration of CSA in the donor coevaporate solution may not be sufficient to maintain a sufficiently high thermodynamic activity of the CSA. It is recognized that altogether a 6- to 8.5-fold increase in dermal delivery of CSA compared to passive delivery was obtained with the use of electroporation, it may not be a therapeutically significant quantity. Other means of solubilizing CSA to achieve a higher donor concentration of CSA are currently being explored. It may be speculated that with the creation of transient pores in the stratum corneum during electroporation, some amount of PVME/MA, despite a molecular weight of 70,000, could also get access to the skin. No attempt has been made to quantify the amount of PVME/MA transported during the transdermal experiments. It should be noted that PVME/MA is a nontoxic polymer and is used safely as a pharmaceutical excipient (17,18).

## CONCLUSIONS

The coevaporate prepared using PVME/MA resulted in a significant increase in the dissolution of CSA in water. The CSA to polymer w/w ratio 1:8 seemed to have the optimum dissolution profile in water. It had over 90% dissolution in 60 min and showed a 9.5-fold increased equilibrium solubility in water compared to untreated CSA. Aqueous solution of 1:8 coevaporate was used to deliver CSA transdermally, using electroporation pulse in hairless rat skin in an in vitro diffusion cell assembly. Use of a single-pulse mode at 200 V/cm, $\tau$ = 10 msec resulted in delivering 87 ng of CSA in 0.87 $cm^2$ of dermis. Topical delivery of CSA confined to the dermal area could be advantageous in treating psoriasis without having any unwanted systemic side effects of the drug.

## ACKNOWLEDGEMENTS

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