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RESEARCH PAPERS

CHARACTERIZATION OF TRANSDERMAL DELIVERY OF NEFOPAM HYDROCHLORIDE UNDER IONTOPHORESIS

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ABSTRACT

In vitro iontophoretic delivery of nefopam hydrochloride was conducted to study the effects initial drug concentration, pH, ionic strength and viscosity of the donor solutions on the transdermal flux through a hairless mouse skin. Stability of nefopam hydrochloride under the experimental conditions was investigated. Type of electrode, current intensity, electric voltage and electrode distance were evaluated. Appropriate conditions were selected to minimize the potential degradation problems of nefopam hydrochloride during iontophoresis. Results show that the silver/silver chloride electrode provides better drug stability than the platinum electrode. Higher current intensity resulted in faster transdermal flux and therefore better drug permeability. The increase in the drug permeability appears to be proportionally increased as the current intensity increases in the range of 0.253 to 1.265 mA/cm^2. The iontophoretic transdermal delivery of nefopam hydrochloride was observed to increase as the drug concentration in the donor site was increased until it's close to the equilibrium concentration. The optimum pH to achieve the best iontophoresis under constant current was determined to be at pH 3.0. This may be due to the highest available charge density of nefopam was achieved at this pH to provide the best conductance. A decrease in the iontophoretic transdermal flux was encountered as an increase in

the solution ionic strength due to the increased competition of similar charged ions with the available current. The increase in the donor solution viscosity decreased the conductivity of the ions and hindered the trandermal flux of the drug under iontophoresis.
Key words: Iontophoresis; Hairless mouse skin; Nefopam hydrochloride; Transdermal; Permeability coefficient.

INTRODUCTION

The transdermal drug delivery has gained a lot of attention in recent years. It has many advantages over the traditional oral and injection routes of administration. However, it also has some disadvantages. One major obstacle to the development of transdermal delivery system is that the skin is extremely impermeable to hydrophilic compounds. Even for the hydrophobic compounds, the permeability of the drug to achieve an effective level in the body stream is very difficult (1). Iontophoresis which makes use of the external electrical energy to control and enhance the delivery of drugs through the skin is one of the many methods (2,3,4,5,6) being developed. The idea of iontophoresis was first introduced in early 19th century and well documented at the beginning of the 20th century. The use of iontophoresis in the 1930s and 1940s was frequent but not successful and declined in the subsequent years until recently. A renewed interest in this technique has been explored due to the development of broadened scopes of transdermal drug delivery. Although there are a number of published literatures describing the nature of the iontophoretic transport system relating to the membrane structure (7,8,9) and the transport mechanisms (10), little attention has been directed at studying the formulation factors in affecting the iontophoresis. Since there are a lot of influencing factors involved in the complexity of the iontophoretic drug delivery system, this study was conducted to investigate some of the important factors that could affect the iontophoretic performance. These factors evaluated include electrode type, current intensity, initial drug concentration, pH, ionic strength and viscosity of the drug solution.

METHODS

Materials

The following materials were used in this study: nefopam hydrochloride (Medichem, U.S.A., lot 10579); hydrochloric acid (E. Merck, West Germany, lot 80460); sodium hydroxide (E. Merck, West Germany, lot 284627-1088); 0.9% sodium chloride solution (Baxter Healthcare Copr., Deerfield, IL, U.S.A., lot C196600A); potassium chloride (E. Merck, West Germany, lot 37090); acetonitrile (Hau Fong Inc., Taiwan, lot OD25041010); methanol (Hau Fong Inc., Taiwan, lot DC25031246); sodium 1-pentanesulfonate (E. Merck, west

Germany, lot FCY01); polyethylene glycol 400 (The Vitarine Co., U.S.A., lot 138-4150); silver electrode (Aldrich Chemical Co., U.S.A., lot 08003-MW); platinum electrode (Chen Hsin Tang Inc., Taiwan, lot 47812). All materials were used as received.

Stability of Nefopam Hydrochloride under Iontophoresis

The chemical stability of nefopam hydrochloride in 0.9% sodium chloride solution (5.0 mg/mL) was employed. Stability study was conducted under iontophoresis at either a fixed current (0.2 mA) but different voltages (3.0, 5.0, 7.0 and 9.0 V) or at a constant voltage (2.0 V) but different currents (0.2, 0.4, 0.6, 0.8 and 1.0 mA) using the platinum electrodes. A side-by-side diffusion cell with controlled stirring of 600 rpm was used. The drug solution was placed in both donor and receptor cells without a membrane in between and the temperature was maintained at 32 $\pm$ 0.2 °C by circulating thermostated water through the jacketed cells. Both cationic and anionic electrodes were placed at 7.2 cm apart with equal distance from the center of the diffusion cells. Samples were taken at designated times and were frozen until analysis. Effects of the electrode type on the stability of nefopam hydrochloride was evaluated using both platinum and silver/silver chloride electrodes under a constant current of 0.2 mA and a voltage of 7.0 volts for 24 hours.

Preparation of The Hairless Mouse Skin

The full thickness abdominal skin was dissected from the male hairless mouse (obtained from the Animal Department, Tri-Services General hospital, Taipei, Taiwan, averaging 4-8 weeks old and body weights of 22.0 $\pm$ 2.0 grams). The exercised skin was cut into a size of 1.0 cm x 1.0 cm, then rinsed with 0.9% sodium chloride solution and immediately stored at -70 °C. These frozen skins were used within 2 weeks. The thickness of the skins ranged from 0.2 μm to 0.4 μm. These skins were removed from the freezer, thawed to room temperature before each use. Visual inspection of the skin for any defects before mounting onto the diffusion cell was carefully performed for each transdermal study.

Transdermal Studies

A side-by-side diffusion cells (Dong Horng Instruments Co., Ltd., Taipei, Taiwan, R.O.C.) with controlled stirring speed of 600 rpm in each half cell was used. An isothermal condition was maintained at 32 $\pm$ 0.2 °C by circulating the thermostated water through the jacketed cells. The hairless mouse skin was first sandwiched between two half cells with the epidermal side facing the donor cells and the dermal side facing the receptor cell. The donor cell contained 4.0 mL drug solution and the receptor cell contained 4.0 mL 0.9% sodium chloride solution. The silver/silver chloride electrode was used with anode in the donor cell and cathode in the receptor cell. The electrode was prepared by coating the

silver with silver chloride under the an electrolytic reaction. A 10 cm silver wire dipped in a 1000 mL 0.5M potassium chloride solution had undergone a electrolysis at an electric field of 5.0 volts and 1.0 mA for one hour. The following conditions were used throughout the entire study except otherwise designated: nefopam hydrochloride in 0.9% sodium chloride solution 1.0 mg/mL; electrode distance 7.2 cm, electric current 1.0 mA. Each iontophoretic run was carried out for 5 hour with 1.0 mL samples (with same volume replacement of 0.9% sodium chloride solution) taken every 30 minutes. The amount of drug passing through the skin was determined by high performance liquid chromatographic (HPLC) analysis of the sample concentration in the receptor cell and corrected for the replacement volume.

HPLC Analysis

The instrument consisted of a pump (Model ILC-6A, Shimadzu), a 20 μl loop injector (Model SIL-6A, Shimadzu) and a variable UV detector (Model SPD-6AV, Shimadzu) set at 266 nm. A NovaPak C_{18} column (3.9 mm x 150 mm with 5 μm packing, Waters Associates, Milford, MA) was used. The mobile phase was made of 20% (v/v) acetonitrile and 80% 0.5 M potassium phosphate monobasic solution containing 0.005M 1-pentanesulfonate and at a pH of 3.03. The mobile phase was delivered at a rate of 1.0 mL/min and the absorbance of the drug was recorded in an electronic integrator (Model C-R6A, Shimadzu) at a speed of 1.0 cm/min. Standard curves of nefopam hydrochloride in the concentration range of 0.1 mg/mL to 1.0 mg/mL were constructed each day. The correlation coefficient for the linearity of all the standard curves was found to be > 0.99. The between-day and within-day variation in the above concentration range were determined be less than 2%. The concentration of nefopam hydrochloride was determined from the chlormatogram by comparing the peak area of the sample with the peak area of the external standards from the standard curves.

Electrode Distance Effect Study

The electrode distance of 6.5, 7.2 and 7.5 cm was carried out to study the iontophoresis of nefopam hydrochloride under 0.2 mA current and 1.0 V voltage.

Initial Drug Concentration Effect Study

Various concentrations of nefopam hydrochloride solution ranging from 1.0 mg/mL to 20.0 mg/mL was evaluated under the iontophoresis.

pH Effect Study

Nefopam hydrochloride solutions adjusted to different pH (2.02, 3.03 and 4.06) were investigated under iontophoresis.

Ionic Strength Effect Study

Solutions of nefopam hydrochloride with different ionic strengths (0.124, 0.255, 0.345 and 0.457) were prepared by dissolving different amounts of sodium chloride in them. Iontophoresis of nefopam hydrochloride in different ionic strength solutions was evaluated.

Viscosity Effect Study

Solutions of nefopam hydrochloride (4.0 mg/mL) in 0.9% sodium chloride at different viscosity (1.254, 3.457 and 5.8992 cps) adjusted by the addition of polyethylene glycol 400 were used. Iontophoresis of these solutions was carried out under 0.2 mA current intensity.

RESULTS AND DISCUSSION

The effect of electrode type on the stability of nefopam hydrochloride under iontophresis is shown in Figure 1. Results show that under the same iontophoretic coditions, the silver/silver chloride provides better stability for nefopam hydrochloride solution than the platinum electrode. The first 2 hours of iontophoresis showed little difference between these two types of electrodes under the studied conditions. However, nefopam hydrochloride degraded faster under the platinum than the silver/silver chloride electrodes. The hydrolysis of water due to the presence of platinum under iontophoresis caused the microenvironmental pH change which resulted in affecting the drug stability. In this study, the silver/silver chloride electrodes provided resonably good stability for nefopam hydrochloride during 5 hours of iontophoresis and was selected as the choice.

Figure 2 shows the cumulative amount of nefopam hydrochloride in the receptor cell at each time interval following passive diffusion and under constant current (0.2-1.0 mA) conditions. A linear relationship was observed for all situations. The transdermal flux ($mg/cm^2/s$) is obtained from the slope of the plot for each condition assuming pseudo steady state conditions and is shown in Table 1. The permeability coefficient (P) which was defined as $P = Q/C_d$, where Q is the transderaml flux and C_d is the concentration of the drug in the donor cell. The relationship between the permeability coefficient of nefopam hydrochloride and the applied current intensity is depicted in Figure 3. A linear relatioship was observed indicating the proportionality of the increase in the permeability coefficient as the current density is increased.

Effect of Initial Drug Concentration

The permeability coefficient of nefopam hydrochloride under iontophoresis at different initial drug concentrations in the donor cells is listed in Table 2. The

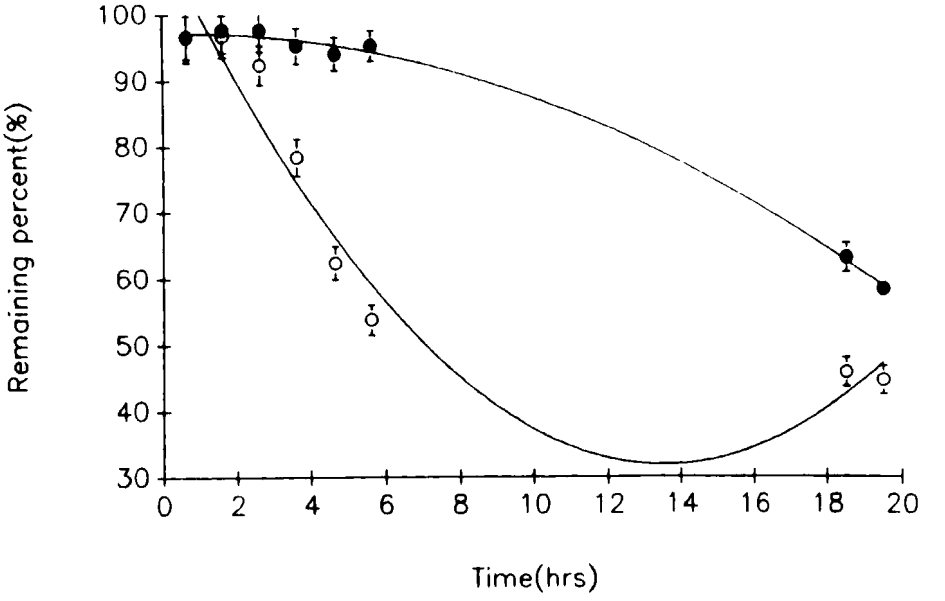

FIGURE 1.
Effect of electrode type on the stability of nefopam hydrochloride under iontophoresis. Key: (•) silver/silver nitrate electrode; (o) platinum electrode.

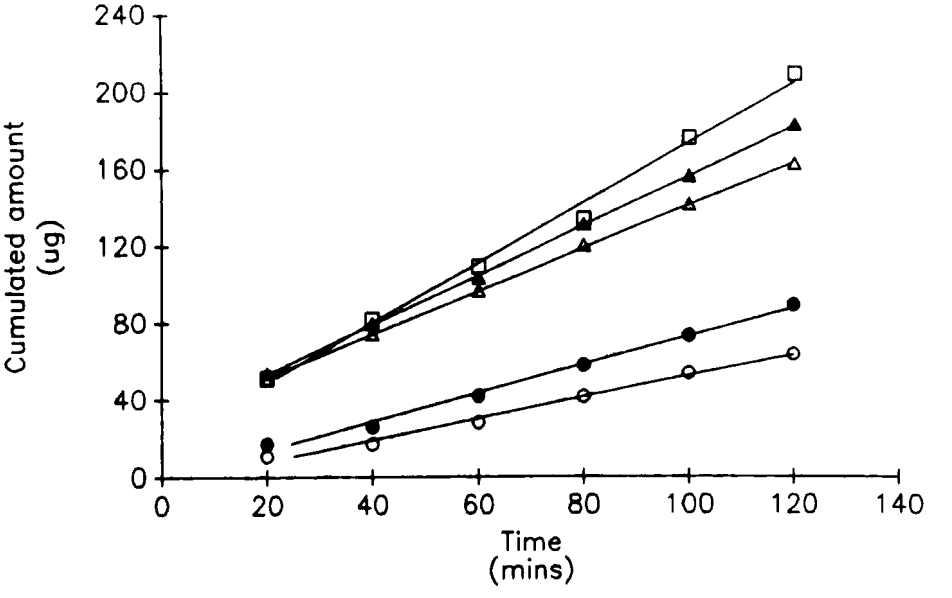

FIGURE 2.
Effect of current density on the amount of nefopam hydrochloride transported during iontophoresis. Key: (o) 0.253 mA/cm^2; (•) 0.507 mA/cm^2; (△) 0.759 mA/cm^2; (▲) 1.102 mA/cm^2; (□) 1.265 mA/cm^2.

TABLE 1.
Iontophoretic Transdermal Flux[a] and Permeability Coefficient[a] of Nefopam Hydrochloride under Various Current Densities

Current Density $mA.cm^{-2}$	Flux $10^5\ mg.cm^{-2}.s^{-1}$	Permeability Coefficient $10^{10}\ cm.s^{-1}$	Enhancement Factor
0	0.00646	0.004311	1
0.253	1.15±0.06	0.77±0.07	177.92
0.507	1.57±0.07	1.05±0.04	243.10
0.759	2.73±0.16	1.82±0.04	422.04
1.012	3.07±0.26	2.05±0.04	474.32
1.265	3.54±0.21	2.36±0.07	547.31

[a]Mean±S.D. n=3

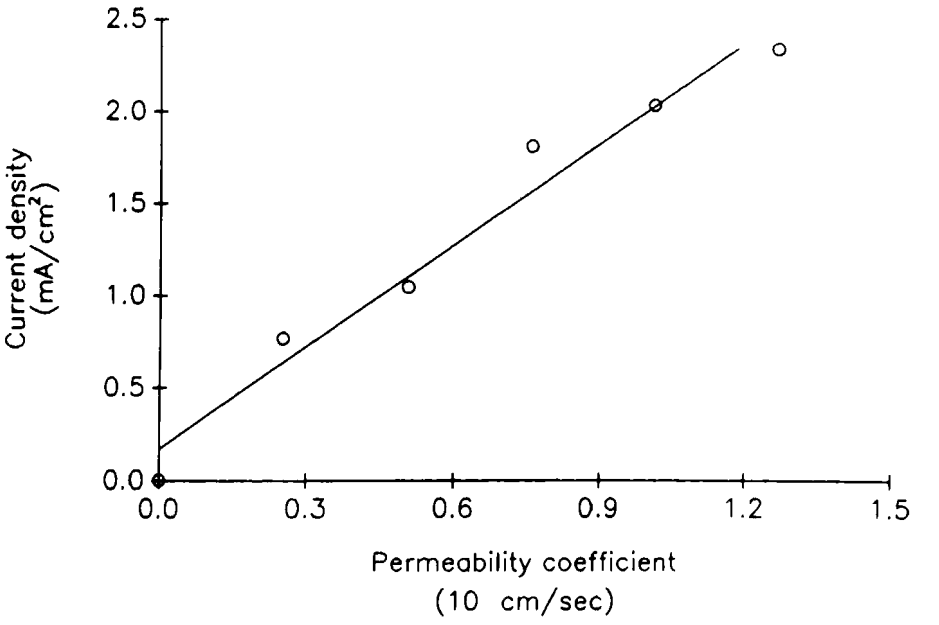

FIGURE 3.
The relationship between permeability coefficient and current density during the iontophoresis of nefopam hydrochloride.

TABLE 2.
Iontophoretic Transdermal Flux[a] and Permeability Coefficient[a] of Nefopam Hydrochloride under Various Initial Drug Concentrations

Initial Concentration mg/mL	Flux 10^5 mg.cm^{-2}.s^{-1}	Permeability Coefficient 10^{10} cm.s^{-1}	Enhancement Factor
1.00	0.38±0.03	0.26±0.03	58.92
2.00	0.43±0.05	0.29±0.02	66.02
4.00	0.51±0.09	0.34±0.01	78.29
10.00	1.13±0.13	0.75±0.02	174.35
15.00	3.91±0.28	2.60±0.28	603.08
20.00	1.61±0.12	1.08±0.03	249.08

[a]Mean±S.D. n=3

drug permeability was observed to increase as the initial drug concentration was increased until a maximun was reached where further increase in the initial drug concentration adversely affected the drug permeability. It was noted that the maximun permeability was reached when the initial drug concentration was close to its drug solubility limit (23.8 mg/mL at 32 °C). In this case, the initial drug concentration was about 63% of its eqilibrium solubility when the maximun permeability was reached. This phenomenum may be due to the high traffic environments surrounding the skin area which caused the hinderance of the transderaml flux.

Effect of pH

The effect of the pH in the donor drug solution on the transdermal flux of nefopam hydrochloride is shown in Table 3. Results show that a decrease in the pH increased the transderaml flux of nefopam hydrochloride in the pH range of 3.0 to 6.0). However, further decrease in the pH below 3.0 decreased the transderaml flux. Since nefopam hydrochloride has a pK_a of 8.93, 99% of nefopam is in the protonated form as the pH is below 5.93. As the pH decreases, more hydrogen ions are reacting with nefopam molecules to achieve higher positive charge density on the nefopam molecules. As a result of this, better conductivity of the solution provides faster transdermal flux under iontophoresis. Once the binding capacity of nefopam molecules is saturated with hydrogen ions (pH 3.0 in this study), futher increase in the amount of hydrogen ions will

TABLE 3.
Iontophoretic Transdermal Flux[a] and Permeability Coefficient[a] of Nefopam Hydrochloride in Various pH Drug Solutions

Solution pH	Flux 10^6 mg.cm^{-2}.s^{-1}	Permeability Coefficient 10^{11} cm.s^{-1}	Enhancement Factor
2.00	1.39±0.18	0.94±0.03	21.40
3.00	7.31±0.68	4.89±0.25	112.93
4.00	3.08±0.34	2.06±0.06	47.54
5.00	0.19±0.01	0.14±0.00	2.99
6.00	0.13±0.01	0.08±0.00	1.96

[a]Mean±S.D. n=3

TABLE 4.
Iontophoretic Transdermal Flux[a] and Permeability Corfficient[a] of Nefopam Hydrochloride in Drug Solutions of Different Ionic Strengths

Solution Ionic Strength	Flux 10^7 mg.cm^{-2}.s^{-1}	Permeability Coefficient 10^{12} cm.s^{-1}	Enhancement Factor
0.013	1.94±0.08	1.39±0.28	2.99
0.027	9.83±0.11	6.67±0.28	15.20
0.040	9.33±0.11	6.39±0.00	14.40
0.067	4.56±0.39	3.06±0.28	7.05

[a]Mean±S.D. n=3

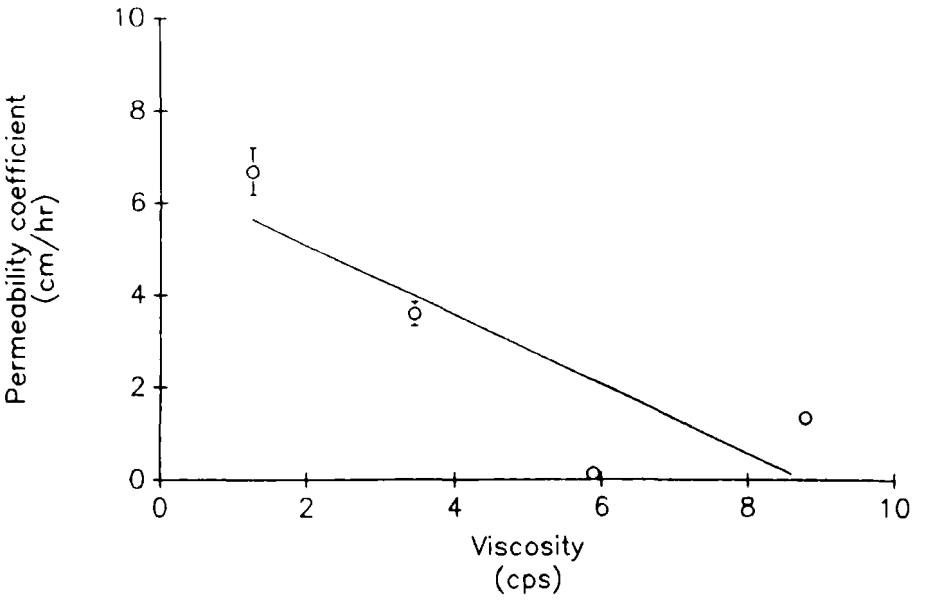

FIGURE 4.
Effect of viscosity on the amount of nefopam hydrochloride transported during iontophoresis.

increase the chances of competing ion species to the applied current which will therefore result in a decrease in the transdermal flux of nefopam.

Effect of Ionic Strength

The effect of ionic strength in the drug solution of donor cell on the transderaml flux of nefopam hydrochloride under constant current iontophoresis is listed in Table 4.

Results show that an increase of ionic strength in drug solution increases the competition of the available current from the added potassium ions and therefore decreases the transderaml flux of the nefopam.H^+.

Effect of Viscosity

Figure 4 shows the effect of solution viscosity in the donor cell on the iontophoretic transdermal flux of nefopam hydrochloride. An increase in the viscosity results in a decrease in the solution conductivity and the transdermal flux of nefopam hydrochloride is decreased as a result of that.

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