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# THE EFFECTS OF FORMULATION VARIABLES ON IONTOPHORETIC TRANSDERMAL DELIVERY OF LEUPROLIDE TO HUMANS

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## ABSTRACT

Feasibility of applying iontophoresis to facilitate the transdermal permeation of leuprolide acetate was investigated. Because of the complexity of the factors involved in the process of iontophoresis, theoretical predictions of the combination effects from formulation variables are difficult. This study incorporated the formulation variables, drug levels and buffer concentrations, in a device prepared by Drug Delivery System, Inc., to assess the feasibility for leuprolide delivery.

Steady state serum leuprolide concentrations were achieved within 30 minutes of patch application, and were maintained for the duration of the study period. An increase in LH levels was observed for each formulation. The serum leuprolide concentrations were higher with lower drug concentration and more dilute buffer solutions. Increasing drug concentration in the patch appeared to inhibit delivery of leuprolide. A

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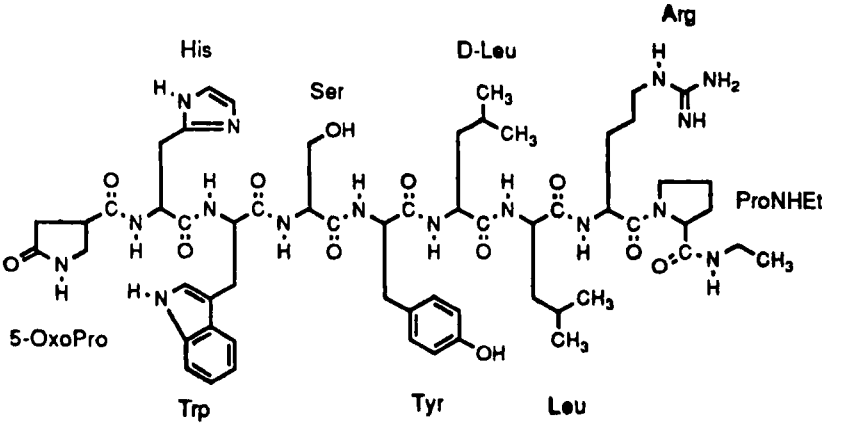
mean steady state serum concentration, 0.8 ng/ml, was achieved by a formulation composed of 10 mg/ml leuprolide acetate and 0.05 M acetate buffer at pH 5.0. Competitive reaction of ions possibly involved in the delivery mechanism will be discussed.

## INTRODUCTION

Leuprolide acetate is a synthetic nonapeptide analog of the naturally occurring gonadotropin releasing hormone (GnRH), also known as luteinizing hormone releasing hormone (LH-RH). The chemical name is defined as 5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-arginyl-N-ethyl-L-prolinamide acetate (salt) with the following structure:




The gonadotropins, follicle stimulating hormone (FSH), luteinizing hormone (LH), and chorionic gonadotropin (CG) are required for ovulation, spermatogenesis, and the biosynthesis of sex steroids. Leuprolide acetate, an LH-RH agonist, acts as a potent inhibitor of gonadotropin secretion when given continuously and in therapeutic doses. The clinical studies indicate that following an initial stimulation, chronic administration of leuprolide acetate results in suppression of ovarian and testicular steroidogenesis. This effect is reversible upon discontinuation of drug therapy. Leuprolide acetate is marketed as Lupron® for the palliative treatment of stage $D_2$ prostate cancer, and for the treatment of endometriosis.

The oral bioavailability of leuprolide is low, hence the drug is typically administered either subcutaneously in daily doses or monthly by intramuscular injection of a prolonged-release depot formulation. In humans, administration of leuprolide acetate results in an initial increase in circulating levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH), leading to a transient increase in levels of the gonadal steroids, testosterone and dihydrotestosterone in males (1,2); however, continuous administration of leuprolide acetate results in decreased levels of LH and FSH in all patients. In males testosterone is reduced to castrate levels. In females, estrogens are reduced to post-menopausal levels. These decreases occur within two to four weeks after initiation of treatment, and castrate levels of testosterone in prostatic cancer patients have been demonstrated for periods of follow-up exceeding five years (private communication with TAP Pharmaceuticals.)

For drugs that have poor oral bioavailability, the transdermal route often provides an alternate means of systemic delivery. Transdermal delivery avoids the problem of metabolism in the GI tract and hepatic extraction associated with oral delivery. However, the potential use of a transdermal delivery route for leuprolide (3,4) has been limited due to the low permeability of this molecule in the skin barrier. Iontophoretic drug delivery differs from passive delivery in that an applied electric potential gradient is employed to enhance the transdermal delivery of ionized drug molecules. The use of iontophoresis to enhance the transdermal delivery of ionic drugs for systemic therapy (5) has been reported. This study was undertaken to apply a low level of constant electrical current to the skin and evaluate the effects of formulation variables on this route administration of leuprolide acetate.

## EXPERIMENTAL

Materials - The following six formulations were prepared under a GMP facility.

| Formulation | leuprolide Concentration | Acetate Buffer |
|---|---|---|
| A | 10 mg/ml | 0.05 M |
| B | 10 mg/ml | 0.5 M |
| C | 25 mg/ml | 0.05 M |
| D | 25 mg/ml | 0.5 M |
| E | 50 mg/ml | 0.05 M |
| F | 50 mg/ml | 0.5 M |

Leuprolide Acetate was provided by TAP Pharmaceuticals, Deerfield, IL. Acetate buffers at pH 5.0, were prepared from acetic acid and sodium acetate following the buffer preparation procedure by Gomori (6). Formulations were very stable at room temperature at which the samples were stored before the study. The potency of leuprolide in solutions was determined by HPLC assay (7). All patches were provided by Drug Delivery Systems, Inc., New York. The patches contain an intrinsic power source supplied by a small disk battery, a resistance system to limit current, electronic conditioning components and drug reservoirs at positive and negative electrodes (8).

Human Study - The study was conducted at North Shore University Hospital, Manhassest, New York. Leuprolide was administered with an iontophoretic transdermal patch to eleven healthy male volunteers for a three-week study. There was a one-week washout between administrations. The study design is described as the following:

| | | |
|---|---|---|
| week 1 | Formulation A | Volunteer No. 1,2,3,4 & 5 |
| | Formulation B | Volunteer No. 6,7,8,9,10 & 11 |
| week 2 | Formulation C | Volunteer No. 1,2,3,4 & 5 |
| | Formulation D | Volunteer No. 6,7,8,9,10 & 11 |
| week 3 | Formulation E | Volunteer No. 1,2,3,4 & 5 |
| | Formulation F | Volunteer No. 6,7,8,9,10 & 11 |

The patches contained 0.4 ml of leuprolide solution which resulted in theoretical doses of 4 mg, 10 mg or 20 mg. The formulation was applied to the positive reservoir side of the delivery device and normal saline was applied to the negative reservoir. Patches were designed to deliver 0.2 mA of current. After obtaining a baseline blood sample, a patch was applied to the volar surface of the contralateral arm in each volunteer. All patches were held in place by a peripheral coating of tape and by wrapping with an elastic bandage. After application of the patch, blood samples were collected through heparin lock periodically over 10-12 hours. Serum was aspirated and immediately frozen for determination of LH (leuteinizing hormone), testosterone and leuprolide levels. Serum LH and testosterone concentrations were determined in the Nuclear Medicine Research Laboratory using the Amerlex LH RIA kit (Amersham Corp, Arlington Heights, IL.) and DSL testosterone RIA kit (Diagnostic Systems Laboratories, Webster, Texas). Serum Leuprolide concentrations were determined at University of Iowa School of Medicine using a radioimmunoassay.

After the completion of blood drawing for ten or twelve hours , the patch was removed and the skin was examined for evidence of injury.

Data analysis - An AUC was calculated for both LH and leuprolide concentrations using the trapezoidal rule to describe the overall area under serum concentration-time curve, using only the data out to 10 hours so all subjects would be comparable. A steady state serum leuprolide concentration was computed as an average of the readings from all the time points after 30 minutes of patch application.

## RESULTS

Serum testosterone concentrations - Mean serum testosterone concentrations are shown in Table I. A significant rise of testosterone concentration was observed only after application of Formulation A which consisted of drug level 10 mg/ml and buffer strength, 0.05M.

**Table I. Mean serum testosterone levels before and after iontophoretic transdermal administration of leuprolide acetate in men.**

| | Drug Conc. (mg/ml) | Buffer Conc. (M) | $T_{initial}$ (ng/dl) Mean ± SD | $T_{final}$ (ng/dl) Mean ± SD |
|---|---|---|---|---|
| A | 10 | 0.05 | 721 ± 59 | 819 ± 39 |
| B | 10 | 0.5 | 864 ± 277 | 765 ± 216 |
| C | 25 | 0.05 | 633 ± 146 | 727 ± 83 |
| D | 25 | 0.5 | 716 ± 174 | 704 ± 105 |
| E | 50 | 0.05 | 696 ± 97 | 705 ± 79 |
| F | 50 | 0.5 | 705 ± 263 | 719 ± 168 |



Serum LH concentrations - Figures 1, 2 and 3 show the mean serum LH response levels between two buffer concentrations after patch application of the formulations containing three drug levels 10 mg/ml, 25 mg/ml and 50 mg/ml. Elevations in serum LH concentrations were observed with all six formulations. The data of AUC, baseline LH concentrations and LH concentrations at 10 hours after patch application are summarized in Table II. The magnitude of the subject to subject variation in LH concentrations increased dramatically after the patch administrations, compared to the variation observed in the baseline concentrations. This may reflect variabilities in LH response among subjects after receiving a leuprolide treatment (9).

Serum Leuprolide concentrations - Figure 4, 5 and 6 show the mean serum leuprolide concentrations following patch administration of six

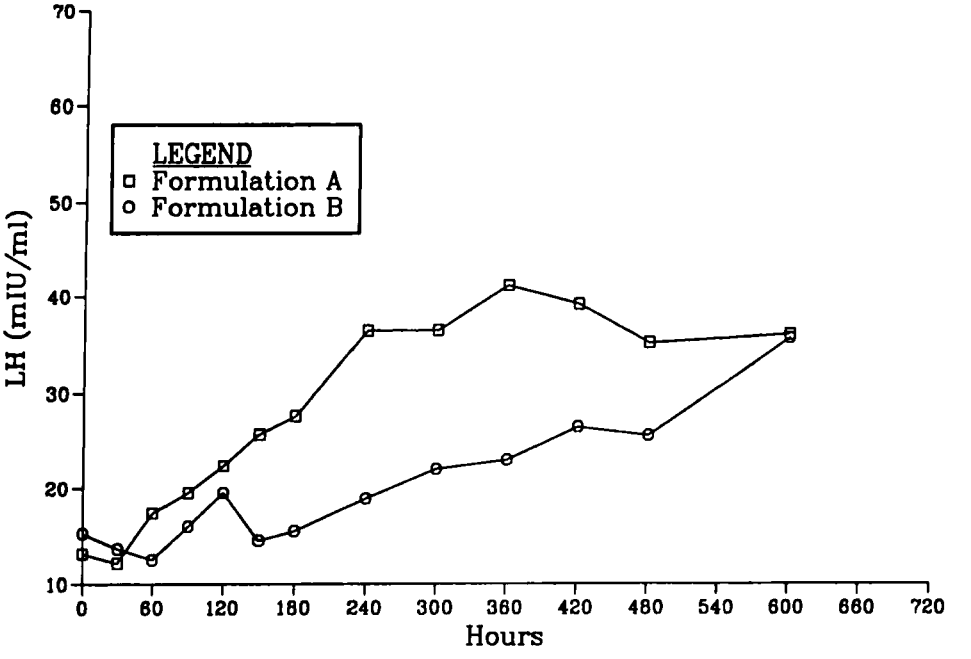



**Figure 1** Mean serum LH concentrations following iontophoretic transdermal administration of Formulation A and Formulation B.

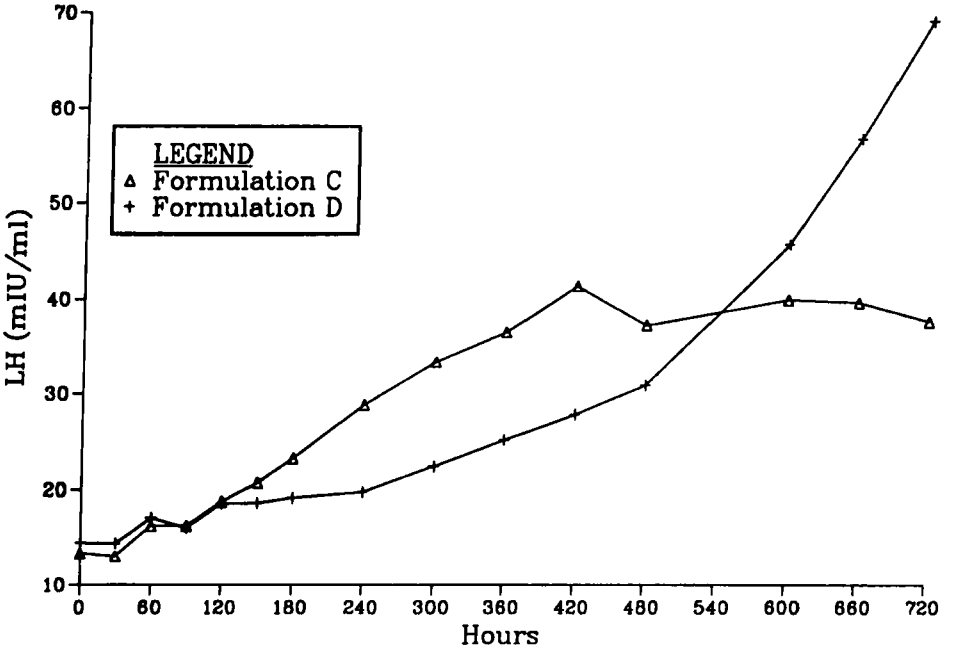



**Figure 2** Mean serum LH concentrations following iontophoretic transdermal administration of Formulation C and Formulation D.

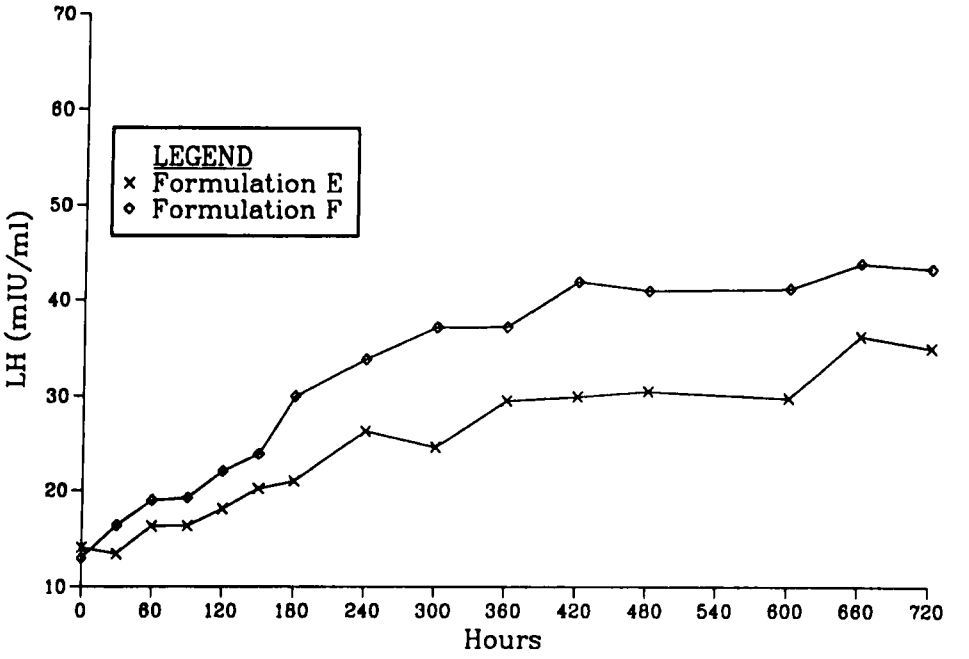



**Figure 3** Mean serum LH concentrations following iontophoretic transdermal administration of Formulation E and Formulation F.

formulations. A steady state level appears to be achieved within 30 to 60 minutes of application of the patch. Mean steady state concentrations and $AUC_{0\text{-}600\ min.}$ are shown in Table III. The data suggested that more transport of drug occurred with a combination of lower drug concentration and more dilute buffer. Higher drug concentrations appeared to inhibit the transport of leuprolide.

Adverse effects on skin - There were 4, 7 and 2 subjects noted to have mild erythema at the site of non-drug delivery electrode on the three study days respectively. This erythema resolved rapidly without sequelae.

## DISCUSSION

An important advantage of the iontophoretic drug delivery system is that a larger fraction of the drug contained within the reservoir can be

**Table II. AUC and mean serum LH concentrations before and after iontophoretic transdermal delivery of leuprolide to men with various formulations.**

| | Drug Conc. (mg/ml) | Buffer Conc. (M) | LH 0 min. (mIU/ml) Mean ± SD | LH 600 min. (mIU/ml) Mean ± SD | AUC 0-600 min. (min x mIU/ml) Mean ± SD |
|---|---|---|---|---|---|
| A | 10 | 0.05 | 13.1 ± 3.8 | 36.1 ± 21.4 | 18,896 ± 9,805 |
| B | 10 | 0.5 | 15.2 ± 4.4 | 35.7 ± 22.3 | 13,110 ± 6,154 |
| C | 25 | 0.05 | 13.4 ± 3.9 | 40.0 ± 11.9 | 17,974 ± 6,128 |
| D | 25 | 0.5 | 14.5 ± 4.3 | 45.7 ± 25.7 | 14,867 ± 6,628 |
| E | 50 | 0.05 | 14.0 ± 3.8 | 29.9 ± 10.5 | 14,870 ± 5,055 |
| F | 50 | 0.5 | 13.0 ± 4.9 | 41.2 ± 24.0 | 19,738 ± 10,607 |

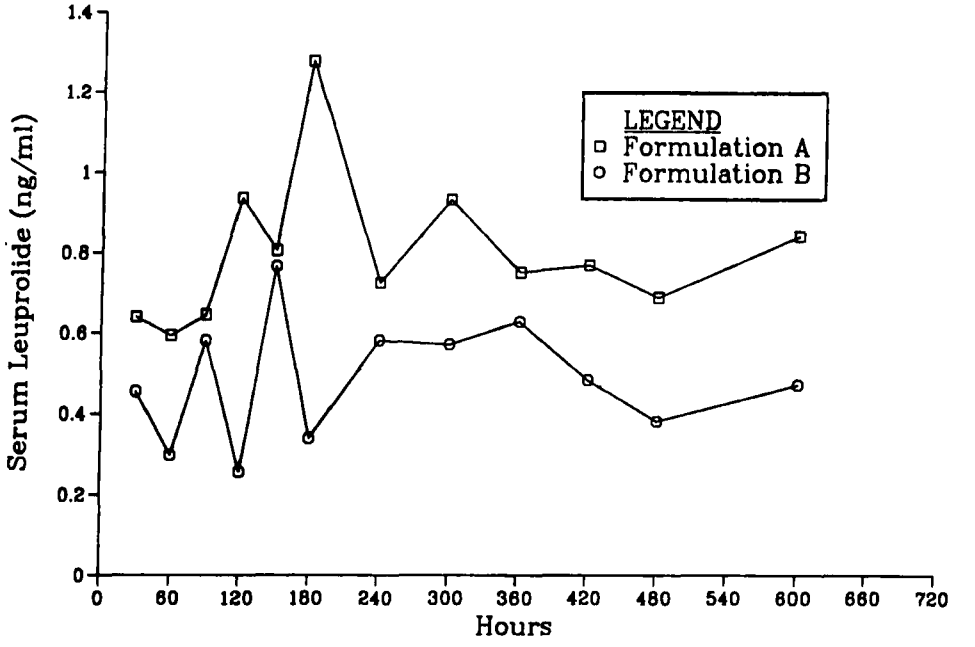



**Figure 4** Mean serum leuprolide concentrations following iontophoretic transdermal administration of Formulation A and Formulation B.

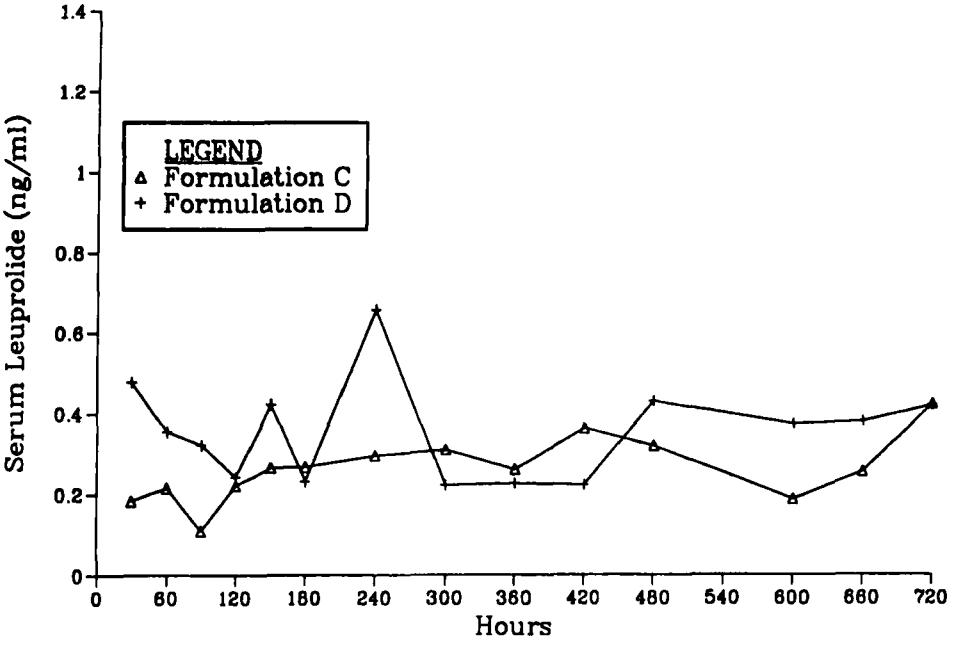



**Figure 5** Mean serum leuprolide concentrations following iontophoretic transdermal administration of Formulation C and Formulation D.

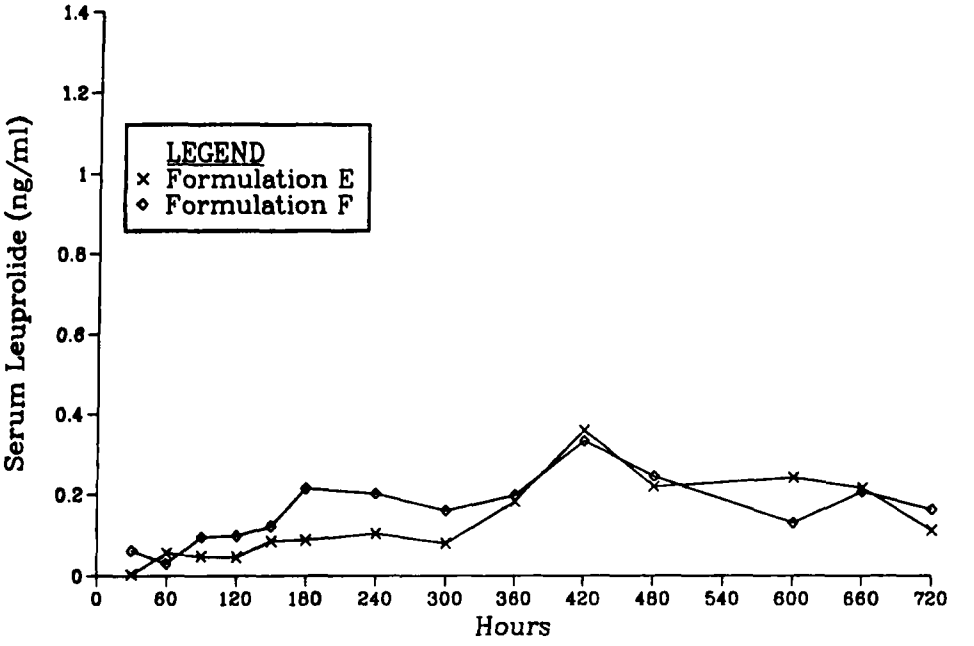



**Figure 6** Mean serum leuprolide concentrations following iontophoretic transdermal administration of Formulation E and Formulation F.

delivered since the driving force is supplied by the applied electric field and not solely by the concentration gradient, as in passive delivery systems. The driving force is maintained as long as there is sufficient battery capacity (10). This study showed that the most effective formulation (A) loading 4 mg of drug with 0.05 M acetate buffer produced a mean steady state serum leuprolide level of 0.8 ng/ml over 10 hours during the patch application.

In the iontophoresis, the physical characteristics of each ion, e.g. molecular weight, dipole moment, chemical composition, etc. will determine the speed at which each ion migrates. The efficiency of drug delivery is primarily determined by two factors: the extraneous ion concentration in the donor reservoir, and the mobility (cm/sec/v) of the drug ion in the skin relative to that of other ions migrating through the skin (e. g. chloride ions). The extraneous ion concentration in the donor reservoir should be minimized in order to maximize the delivery efficiency. It is known that in

**Table III. AUC and serum leuprolide steady state concentrations ($C_{ss}$) after iontophoretic transdermal delivery of leuprolide to men with various formulations.**

| | Drug Conc. (mg/ml) | Buffer Conc. (M) | $C_{ss}$. (ng/ml) Mean ± SD | AUC 0-600 min. (min x mIU/ml) Mean ± SD |
|---|---|---|---|---|
| A | 10 | 0.05 | 0.80 ± 0.41 | 470 ± 255 |
| B | 10 | 0.5 | 0.49 ± 0.29 | 285 ± 177 |
| C | 25 | 0.05 | 0.26 ± 0.08 | 156 ± 55 |
| D | 25 | 0.5 | 0.36 ± 0.21 | 206 ± 116 |
| E | 50 | 0.05 | 0.13 ± 0.12 | 89 ± 79 |
| F | 50 | 0.5 | 0.16 ± 0.12 | 105 ± 68 |

solution each ion is surrounded by ions with opposite charge, which exert a retarding effect on the motion of the ion that we wish to deliver iontophoretically into a tissue. The greater the ionic strength, the higher the concentration of extraneous ions, which will result in more competition for the electric current (11,12). An increase in ionic strength was reported to yield a reduction in the uptake of $^{32}P$ in tissue by iontophoresis (12). This work suggested that the use of a more concentrated buffer, 0.5 M versus 0.05 M, had a negative effect on the iontophoretic delivery of drug molecules.

An increased uptake of radioactive phosphorus by various tissues after iontophoresis was observed with an increase in the concentration of $^{32}P$ (12). On the contrary, the use of higher drug concentrations, 25 mg/ml and 50 mg/ml versus 10 mg/ml, appeared to reduce the amount of leuprolide delivered through the skin. This may support a previous discussion that the absorption of leuprolide may occur by the process of electroosmosis (3), in which the movement of water in an electrical current leads to the secondary flow of solutes. The effect of buffer strength on delivery of leuprolide was not observed when the drug concentrations were higher. This may be due to the delivery mechanism being dominated by an electroosmosis process. However, the comprehensive mechanism for transdermal delivery of leuprolide enhanced by electrical current requires further studies.

The pH is an important factor for drugs whose degree of ionization is pH dependent. For peptide and protein drugs, the pH of the solution can control the charge of the peptide and protein molecule based on their pK values. Thus the pH of the drug solution can be manipulated to deliver the drug either by cathode or anode. Leuprolide base has three ionizable sites, imidazolium nitrogen of histidine, phenolate hydroxyl of tyrosine and guanidinium nitrogen of arginine. The approximate pKa values of these three ionizable sites are 6, 9.5 and 11.5 respectively. The formulations used in this study were prepared at pH 5.0 for the purpose of obtaining maximum stability of leuprolide. However, we believe that the majority of the drug molecules carried two positive charges and a very small portion of the drug molecules carried one positive charge.

The ionic valence of a drug may significantly affect the delivery efficiency. Divalent ions may migrate more slowly (13) as a result of interacting more strongly with charged sites in the skin than do monovalent ions. There is a possibility that iontophoretical delivery of the leuprolide molecule would be more efficient with one positive charge at a pH as high as 7 or 8 than with two positive charges at pH 5.0. However, one should be aware of the fact that the chemical instability of leuprolide at a pH above 7 can cause a formulation concern.

When a steady direct current is used, it can cause skin polarization and may also induce skin irritation (14). Most cutaneous reactions observed in this study were at the negative electrode sites, rather than at the electrode sites containing drug. These reactions were presumed to be due to the pH changes at the non-drug delivery electrodes which lacked buffer capacity. However, the long term impact on cutaneous effects from this system would need further evaluation.



## ACKNOWLEDGEMENTS

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