

**Research Article****EFFECT OF ENHANCERS AND IONTOPHORESIS ON CAPTOPRIL PERMEABILITY THROUGH EXCISED PIG SKIN****Ashish Jain<sup>1\*</sup>, Satish Nayak<sup>1</sup>, Vandana Soni<sup>2</sup>**<sup>1</sup> Bansal College of Pharmacy, Kokta, Anand Nagar, Bhopal-462021, India.<sup>2</sup> Department of Pharmaceutical Sciences, Dr H.S.Gour Vishwavidyalaya, Sagar 470003, M.P. India**ABSTRACT**

*In vitro* transdermal delivery of captopril across excised pig skin using various enhancers investigated. Permeation studies carried out at the modified franz diffusion cell. Steady state fluxes, permeability coefficients, diffusion coefficients, benefits and enhancement ratios by various enhancers were determined. Incorporation of various permeability enhancers like dimethyl sulfoxide, peppermint oil, menthol, oleic acid, sodium lauryl sulphate, poly ethylene glycol with pure drug solution showed synergistic effect when combined with iontophoresis. Dimethyl sulfoxide was the most active enhancer, and when combined with iontophoresis it was possible to deliver 103.940  $\mu\text{mol}/\text{cm}^2$  of drug at the end of 8 hours, it was 4.4 times enhancement as compare to delivery of drug without enhancers.

**Keywords:** Captopril, iontophoresis, transdermal, chemical enhancers.

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To accomplish and to maintain plasma drug concentration greater than the minimum therapeutic level with transdermal drug delivery, the barrier properties of the skin have to be overcome. A variety of capable approaches have been utilized to reduce skin barrier properties and to get goal of enhancement in transdermal permeation of drug [1]. Thanks to the development of some ground-breaking permeation enhancement techniques interest in transdermal delivery such as, The prodrug approach, Chemical Potential Adjustment, Ionic complex, Eutectic Systems, Encapsulating in Liposomes, High Velocity particles, Lowering of skin resistance by chemicals, Microneedle array, Abrasing the Skin, Phonophoresis, Sonophoresis, Electroporation, Magnetophoresis, Damaging the Stratum corneum by laser radiation and iontophoresis[2]. In present study permeation of drug facilitated by chemical enhancers and iontophoresis. Iontophoresis uses a small electrical current to enhance the transport of both ionic and nonionic molecules across the skin in controlled and programmable manner [3-4]. The enhancement of

drug due to this method results from a number of possible mechanisms including the ion-electric field interaction (electro repulsion), convective flow (electro-osmosis) and current-induced [5,6] increase in skin permeability. Chemical penetration enhancement refers to the process whereby chemical agents are used to modify the barrier properties of the stratum corneum and ultimately enhances the transdermal delivery of the drug substances [6]. Combinations of chemical permeation enhancer with iontophoresis as a potential mean for controlling and enhancing the transdermal delivery of drugs have been reported by many researchers [7-9]. In the present work we have studied the effect of various permeation enhancers and iontophoresis on transdermal delivery of captopril.

Captopril is an oral drug and a member of a class of drugs called angiotensin converting enzyme (ACE) inhibitors. ACE inhibitors are used for treating high blood pressure, heart failure, and for preventing kidney failure due to high blood pressure and diabetes. It has a short elimination half life and its plasma half life in man ranges from 1.6-1.9 h [10-12]. Moreover food may decrease oral absorption of Captopril by up to 25-40% [13-14]. The main problems associated with oral therapy include uneven bio-distribution throughout the body, a lack of drug targeting specificity, the necessity of a large dose to achieve high blood concentration and adverse side effects due to such high doses [15]. Captopril being an antihypertensive agent needs prolonged administration. Results of post market surveillance performed on Captopril had shown that 4.9% of the patients had to discontinue therapy because of the adverse effects [16]. The drug has low stability because of the oxidation, which converts the drug into Captopril disulphide. A recent study had shown that the oxidation rate of Captopril in dermal homogenates is significantly lower than that in intestinal homogenates [17]. This made Captopril is a good candidate for transdermal delivery.

## MATERIALS & METHODOLOGY

Captopril was a gift sample from Micro Lab Bangalore. Dimethyl sulfoxide [DMSO], Peppermint oil, Menthol, Oleic acid, sodium lauryl sulphate [SLS], Poly ethylene glycol [PEG], Sodium Chloride AR, octanol, isopropyl alcohol, Silver Chloride were obtained from SD Fine-Chem (Mumbai, India). Silver plates (purity 99.99%, 5 mm diameters) were obtained from a goldsmith shop at Bhopal, India. All the reagents/chemicals used were of analytical grade. Experiments were conducted with ultra pure water (resistivity, 18.2 MW cm) obtained from Milli-Q Academic System.

### *Equipment*

Iontophoretic DC source (digital display, current 0-10 mA, voltage 0-25 V) was purchased from C-tech Psu-2510/lab (Mumbai, India) and iontophoretic diffusion cell was fabricated by Navin Scientific Glass Product (Bangalore, India) as per given specifications. Silver/silver chloride electrode was prepared as per the standard procedure [18]. Silver wire (99.99% pure, 1.0 mm thickness) was used as connecting wire. UV Visible Spectrophotometer- Shimadzu UV-1700 PC Shimadzu Corporation, Japan was used for analysis.

### *Experimental Design:*

To evaluate the effect of chemical enhancers and iontophoresis on transdermal delivery of captopril, permeation of drug studied alone and with different permeation enhancers as well as using iontophoresis. Dimethyl sulfoxide, Peppermint oil, Menthol, Oleic acid, sodium lauryl sulphate, Poly ethylene glycol were used as permeation enhancers. Donor compartment contained 2 ml solution of drug having concentration 25mg/ml, with 1 % w/w concentration of different enhancers. Cathodal iontophoresis was carried out at current density 0.5 mA/cm<sup>2</sup>. To evaluate combine effect enhancers and iontophoresis, permeation of drug solution having enhancers carried out with influence of electric current.

### ***Preparation of skin membrane***

From a local abattoir, ear was obtained from freshly slaughtered pigs. The skin was removed carefully from the outer regions of the ear and separated from the underlying cartilage with a scalpel. After separating the full thickness skin, the fat adhering to the dermis side was removed using a scalpel and isopropyl alcohol. Finally the skin was washed with tap water and stored at refrigerator in aluminum foil packing and was used within two days [19].

### ***Procedure of passive permeation***

The *in vitro* passive permeation studies were conducted using vertical type Franz diffusion cell having a receptor compartment capacity of 10 ml. The excised skin was mounted between the half-cells with the dermis in contact with receptor fluid (0.9% NaCl) and was equilibrated for 1 h. The area available for diffusion was about 1.21cm<sup>2</sup>. The donor cell was covered with an aluminum foil to prevent the evaporation of vehicle. The fluid in the receptor compartment was maintained at 37±0.5 °C. Under these conditions, the temperature at the skin surface was approximately 32 °C. Different solution of Captopril ( each 2 ml) was placed in the donor compartment. The entire assembly was kept on a magnetic stirrer and the solution in the receiver compartment was stirred continuously using a magnetic bead. The sample solution was withdrawn from the receptor compartment at regular intervals and assayed for drug content [20].

### ***Permeation enhancement studies:***

Various enhancers considered for this study were dimethyl sulfoxide, peppermint oil, menthol, oleic acid, sodium lauryl sulphate, poly ethylene glycol. The donor compartment contained solution of drug with 1% w/w concentration of different enhancers. To know the combined effect of chemical enhancers and iontophoresis, permeation study carried out on drug solution containing enhancers using iontophoresis.

### ***Procedure of iontophoretic diffusion***

For iontophoresis diffusion cell was modified as suggested by Glikfield et al [21]. The apparatus essentially consisted of a glass molded large receiving chamber provided with two parallel ports on the topside and a sampling port on the side. Two upper chambers are made from open-ended cylindrical glass tubes, the outer diameters of which were equivalent to the inner diameter of the

parallel ports. The lower 10 mm of these tubes were slightly constricted to allow a clearance of 1 to 1.5 mm on the side. This ensured easy fitting. After the skin was tied at this constricted end, the effective diameter increased and became exactly equal to inner diameter of the extended ports. Once slipped into parallel ports, they stay attached by glass joints forming two separate chambers with skin at the base. Both the skin touched the receptor solution at the same depth and each chamber housed one electrode. Once the battery was switched on, current flowed through the skin placed in anodal compartment into receiving solution below and reached the cathodal electrode through the skin tied to cathodal end. Donor solution was filled in one of the top chambers depending on the polarity of the drug and the other serve as return electrode chamber. For our study, silver/silver chloride electrode was inserted into the donor compartment whereas silver plate was inserted into anodal chamber as return electrode. Direct current ( $0.5 \text{ mA cm}^{-2}$ ) was used throughout experiment. The receptor fluid (5 ml) was withdrawn at regular intervals and replaced with fresh solution to maintain sink condition. Samples were assayed by the U-V spectrophotometrically.

### Data analysis

The cumulative amount permeated was plotted against time, and the slope of the linear portion of the plot was estimated as the steady state flux. Permeability coefficient and diffusion coefficient were calculated using following formulas [22]:

$$KP = JSS / C_d \dots\dots\dots (1)$$

$$D = KP h / K \dots\dots\dots (2)$$

where  $K_p$  represents permeability coefficient,  $J_{ss}$  is the steady-state flux,  $C_d$  is the concentration of drug in donor compartment,  $D$  is the diffusion coefficient,  $K$  is the skin/vehicle partition coefficient and  $h$  the thickness of the skin. Flux enhancement was calculated by dividing enhanced steady state flux by the corresponding passive steady state flux.

## RESULT AND DISCUSSION

According to previous research of Wu et al [23] permeability of captopril had been evaluated by in a series of animal skins. Intrinsic skin permeability was found to be extremely low and it could not be detected in the rat plasma in an *in-vivo* experiment carried out by Hao et al [24]; This preliminary results indicate that skin permeation must be assisted with enhancers, so attempt had been taken up in this study.

Captopril, an orally effective angiotensin-I converting enzyme inhibitor, is widely used intreatment of hypertension and congestive heart failure but its oxidation in intestinal homogenates eliminates it with in 1.5 to 1.9 h[25-27] which is faster compared to its oxidation in dermal homogenates.[28] Consequently delivery of Captopril as transdermal may be the better dosage form. Skin permeability of a drug is strongly influenced by its physicochemical parameters. According to Doh and coworkers [29], drug candidates for transdermal delivery should have molecular weight around 200–500 Da. Captopril having molecular weight of 217.29 fits into the category. Physicochemical parameters of Captopril were investigated in our previous work, results showed good solubility in water (156.1mg/ml) and in 0.9%NaCl (145.57mg/ml)

but experimentally determined partition coefficient (0.335) indicates poor lipophilicity [30]. Which indicates drug have less affinity towards lipid as compare to aqueous phase, but as it is the intrinsic property of molecule we can not change. To enhance the permeation of drug we utilized chemical enhancers and iontophoresis out of various available enhancement techniques.

Although steady state fluxes generally use, but permeability and diffusion coefficient can we used for comparison purpose more efficiently [31]. Permeability and diffusion coefficients of captopril with different enhancers provided in Table 1, furthermore cumulative amount of permeation of drug with and without enhancers shown in figure 1 & figure 2. Permeability and diffusion coefficient increase in the order of Pure drug < PEG < SLS < Oleic acid < Menthol < Peppermint oil < DMSO. The permeability and diffusion coefficient of captopril with DMSO were found highest in our study, moreover with the iontophoresis synergistic effect was found. Results shows that overall permeability using enhancers significantly higher that of passive value Cumulative amount permeated at the end of 8<sup>th</sup> hours was recorded 103.940  $\mu\text{mol}/\text{cm}^2$  with combine effect of DMSO and iontophoresis it was 4.4 time higher than control. Enhancement ratio and benefits by enhancers are provided in Table 2.

**Table 1 Cumulative amount permeation, Permeability Coefficients, and Diffusion Coefficients of Captopril (n=3)**

Mode of Permeation/ Enhancer	Cumulative amount Permeated at 8 hour ( $\mu\text{mol}/\text{cm}^2$ ) (mean $\pm$ SD)	Permeability Coefficient (cm/hr)	Diffusion Coefficient ( $\text{cm}^2/\text{s}$ )
Passive (Pure Drug)	21.245 $\pm$ 1.285	0.0291	0.266*10 <sup>-5</sup>
DMSO	39.778 $\pm$ 1.231	0.0463	0.424*10 <sup>-5</sup>
Peppermint oil	34.993 $\pm$ 0.908	0.0397	0.363*10 <sup>-5</sup>
Menthol	32.786 $\pm$ 0.873	0.0381	0.348*10 <sup>-5</sup>
Oleic acid	30.699 $\pm$ 0.871	0.0353	0.323*10 <sup>-5</sup>
SLS	28.177 $\pm$ 0.916	0.0323	0.295*10 <sup>-5</sup>
PEG	24.861 $\pm$ 0.951	0.0297	0.272*10 <sup>-5</sup>
Iontophoresis	71.103 $\pm$ 3.485	0.0706	0.646*10 <sup>-5</sup>
Ionto + DMSO	103.940 $\pm$ 0.532	0.1293	1.183*10 <sup>-5</sup>
Ionto + Peppermint oil	98.221 $\pm$ 0.550	0.1247	1.141*10 <sup>-5</sup>

Ionto +Menthol	95.231± 0.358	0.1234	1.129*10 <sup>-5</sup>
Ionto +Oleic acid	93.430 ± 0.260	0.1212	1.109*10 <sup>-5</sup>
Ionto +SLS	88.554 ± 0.432	0.1147	1.050*10 <sup>-5</sup>
Ionto +PEG	82.799 ± 1.034	0.1072	0.981*10 <sup>-5</sup>

**Table 2 Benefit and Enhancement Ratios by Different Permeation Enhancers (n=3)**

Mode of Permeation/Enhancer	Steady state fluxes (μmol/cm <sup>2</sup> /hr) (mean ± SD)	Benefit by enhancers (μmol/cm <sup>2</sup> /hr)	Enhancement Ratio
Passive (Pure Drug)	3.345 ± 1.311	0	1
DMSO	5.328 ± 1.147	1.983	1.592
Peppermint oil	4.563 ± 1.025	1.217	1.364
Menthol	4.378 ± 1.020	1.033	1.308
Oleic acid	4.067 ± 0.802	0.721	1.215
SLS	3.716 ± 0.915	0.370	1.110
PEG	3.417 ± 0.955	0.072	1.021
Iontophoresis (Pure Drug)	8.127 ± 0.745	4.782	2.429
Ionto + DMSO	14.878 ± 0.707	11.533	4.447
Ionto + Peppermint oil	14.348± 0.478	11.003	4.289
Ionto +Menthol	14.202± 0.386	10.857	4.245
Ionto +Oleic acid	13.949± 0.317	10.603	4.170
Ionto +SLS	13.199± 0.336	9.853	3.945
Ionto +PEG	12.332± 0.915	8.987	3.686

These chemical substances temporarily diminishing the barrier of the skin and known as accelerants or sorption promoters can enhance drug flux. Dimethyl sulphoxides (DMSO) is one

of the earliest and most widely studied penetration enhancers. The mechanism of the sulphoxide penetration enhancers was suggest to change the intercellular keratin conformation, from  $\alpha$  helical to  $\beta$  sheet [32,33]. The possible mechanism of action of peppermint oil is due to opening of tight junctions. The oleic acid involving lipid perturbation via both conformational permutations and phase separation, with the latter effect predominating [34]. The oleic acid probably operates by penetrating into the lipid structure, with its polar end close to the lipid polar heads. Because of its bent structure, it then disrupts and increases the fluidity of the lipid region[35]. The solvation of keratin within the stratum corneum by competition with water for the hydrogen bond binding sites and the intercalation in the polar headgroups of the lipid bilayers by propylene glycol are postulated as mechanisms of action for the penetration enhancing effects of propylene glycol in the literature as well [36]. Anionic surfactant like SLS grossly swells the stratum cornea, uncoiling and extending the alpha-keratin helices and thereby opening up the protein- controlled polar pathway[37]. The change of dense barrier structure of the stratum cornea in presence of menthol [38] might have enhanced the flux of the captopril.

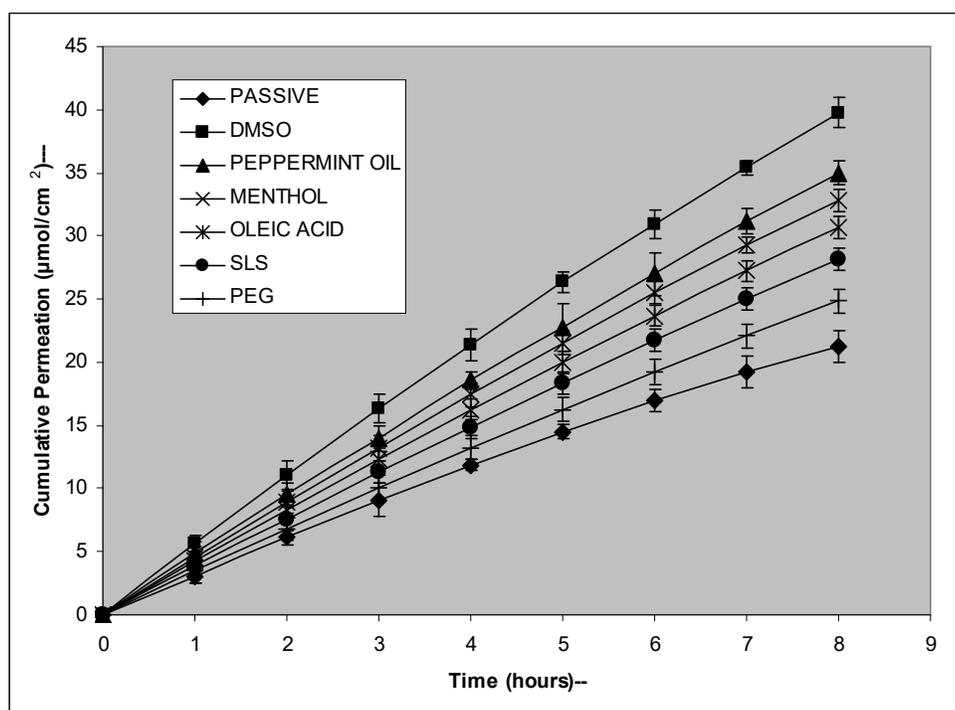


Figure 1: Cumulative Permeation Profile of captopril with different permeation enhancers (n=3)

In iontophoresis though ionic repulsion is the dominant force, convective flow of solutes toward the direction of current, influences the permeation rate. Permeability of skin also changes under influences of current [39]. The total flux of a solute during iontophoresis is the sum of fluxes due to electro-repulsion, convective flow, and passive diffusion [40]. Captopril (pKa 3.4) at pH 7.4 acquires a negative charge and was delivered from cathodal chamber. Since the isoelectric point of the skin varies between 3 and 4, at physiological pH, the volume flow was directed toward the

cathode. Hence at pH 7.4, only passive and electro-repulsive fluxes were likely to contribute to the overall permeation. Electro-osmotic flow may even oppose the permeation from the cathodal compartment [41].

Comparison of steady state fluxes with different enhancers and iontophoresis are shown in figure 3. Steady state fluxes increase in the following order Pure drug < PEG < SLS < Oleic acid < Menthol < Peppermint oil < DMSO < Iontophoresis < (Ionto+PEG) < (Ionto+SLS) < (Ionto+Oleic acid) < (Ionto+Menthol) < (Ionto+Peppermint oil) < (Ionto+DMSO). Steady state flux was found highest (14.878  $\mu\text{mol}/\text{cm}^2/\text{hr}$ ) with combine effect of DMSO and iontophoresis and benefit by this combine effect was 11.53  $\mu\text{mol}/\text{cm}^2/\text{hr}$ . Iontophoresis considerably increase permeation with all permeation enhancers.

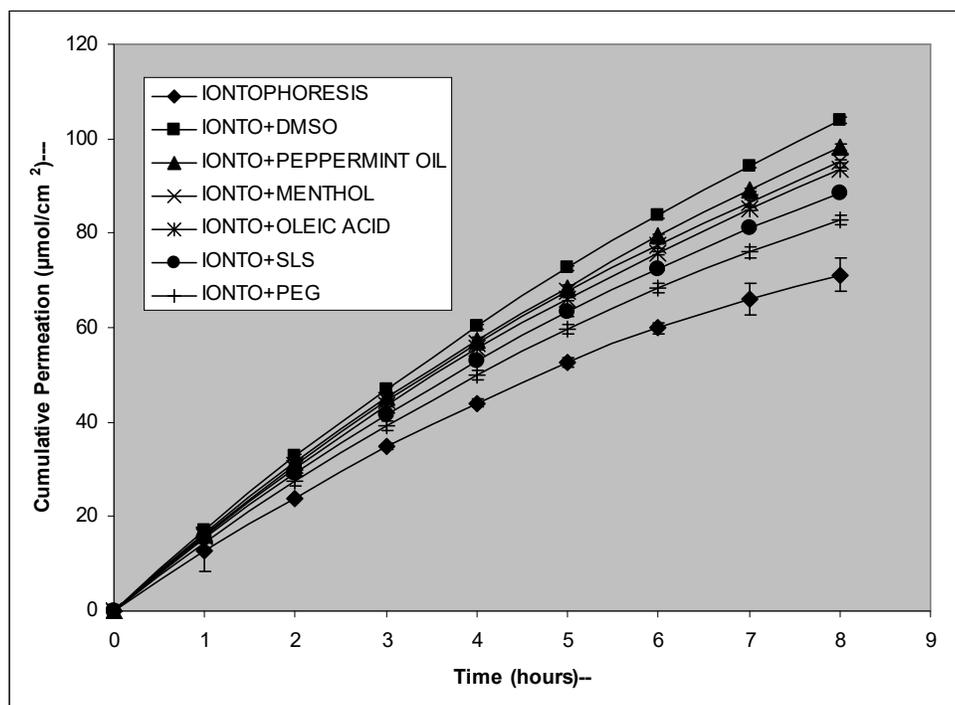


Figure 2: Cumulative Permeation Profile of captopril with different permeation enhancer and iontophoresis (n=3).

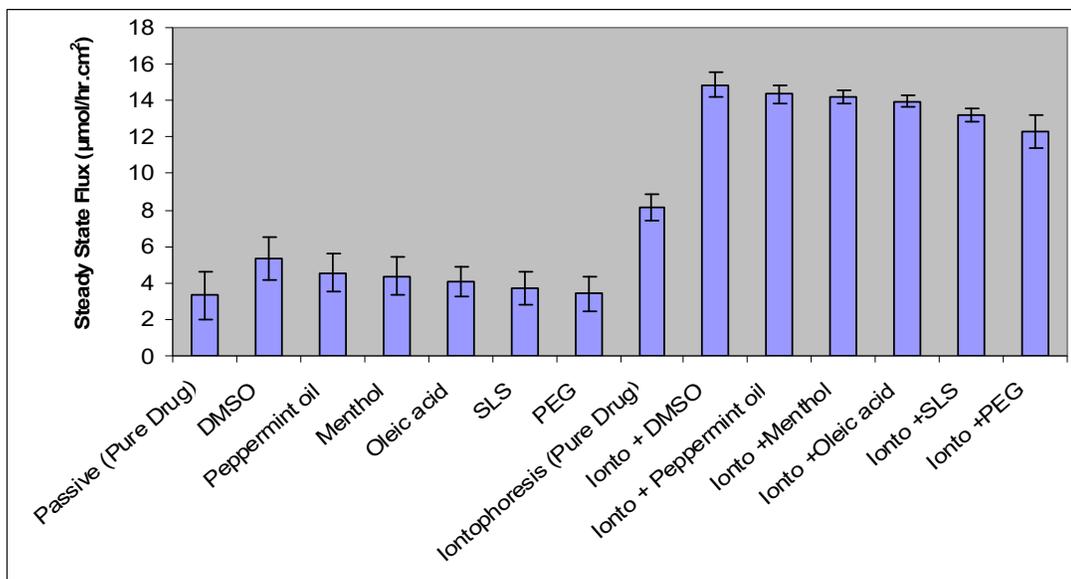


Figure 3 Comparison of steady state fluxes with different enhancers and iontophoresis (n=3).

## CONCLUSION

Optimization of transdermal delivery of drug is very complex process due to permeability limitations and others. *In-vitro* release studies are considered to be useful in pre-formulation step to predict the best permeability enhancement technique. In previous research intrinsic skin permeability of captopril was found to be extremely low and it could not be detected in the rat plasma, so attempt has been made with utilization of chemical enhancers and iontophoresis. Finally it was concluded that the combination strategy was very much successful in increasing the permeability of captopril. Transdermal delivery of captopril can be manipulated, controlled and optimized by use of various enhancers and iontophoresis.

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