J. Pharm. Pharmacol. 1998, 50: 635–640
Received December 8, 1997
Accepted February 18, 1998

Transdermal Iontophoretic Delivery of Hydrocortisone from Cyclodextrin Solutions

SHU-LUN CHANG AND AJAY K. BANGA

Department of Pharmacal Sciences, School of Pharmacy, Auburn University, AL 36849-5503, USA

Abstract

Enhanced skin penetration of hydrocortisone can be desirable for treatment of several diseases. Transdermal iontophoretic delivery of hydrocortisone solubilized in an aqueous solution of hydroxypropyl-β-cyclodextrin (HP-β-CyD) was investigated and compared with chemical enhancement of co-solvent formulations.

The passive permeation of hydrocortisone through human cadaver skin was higher when delivered from propylene glycol than when delivered after solubilization in an aqueous solution of HP-β-CyD. However, the iontophoretic delivery of the 1% hydrocortisone–9% HP-β-CyD solution was higher than the amount delivered passively by the 1% hydrocortisone-propylene glycol formulation, even if oleic acid was used as a chemical enhancer. Iontophoretic delivery of 1% hydrocortisone with 3% or 15% HP-β-CyD was lower than that of the 9% HP-β-CyD solution.

These data suggest that free hydrocortisone rather than complexes is predominantly delivered iontophoretically through the skin and the HP-β-CyD complex serves as a carrier to replenish depletion of hydrocortisone. HP-β-CyD prevents hydrocortisone from forming a skin reservoir. Iontophoresis provides better enhancement of transdermal delivery of hydrocortisone than the chemical approach when just sufficient HP-β-CyD is added to solubilize the hydrocortisone.

Hydrocortisone is an anti-inflammatory corticosteroid used to treat skin diseases (Tauber 1994). Enhanced penetration of hydrocortisone into the skin might be useful for various topical or systemic diseases (Fuhrman et al 1997) and chemical enhancers (Michniak et al 1994) including cyclodextrin derivatives (Arima et al 1990; Vollmer et al 1994) have been used for this purpose (Loftsson & Siguroardottir 1994; Loftsson et al 1994; Preiss et al 1995). Because cyclodextrins form inclusion complexes with drugs, it has been suggested that they might act as enhancers by interacting with components of the skin (Szejtli 1994).

A physical mechanism for enhancing percutaneous absorption is the use of a mild current to drive drug molecules into the skin, a process called iontophoresis (Banga & Chien 1988). Iontophoresis provides a driving force for charged molecules by electrorepulsion. During iontophoresis, a flow of water from anode to cathode occurs when the skin is negatively charged. This is called electroosmosis (Pikal 1992; Lin et al 1997). Solubilized neutral molecules, such as hydrocortisone, can be delivered by taking advantage of this phenomenon. Iontophoretic delivery of hydrocortisone in the presence of surfactant has been investigated (Wang et al 1993). The reported flux of hydrocortisone was low because of the relatively poor aqueous solubility of hydrocortisone and the limited solubilization capacity of surfactants. Salt forms such as hydrocortisone sodium succinate and hydrocortisone sodium phosphate result in higher iontophoretic flux but these salt forms tend to be unstable (Seth et al 1994). The salt forms of hydrocortisone have ester linkages at C21 which might undergo hydrolysis during iontophoresis, especially when the pH of donor and receptor solutions are changed, for example by the use of platinum electrodes. The problems resulting from the use of salt forms can be avoided by using HP-β-CyD to solubilize hydrocortisone.

In this study we chose hydroxypropyl-β-cyclodextrin (HP-β-CyD) because of its excellent solubility in water and the stability of its complexes. The mechanism of enhancement of HP-β-CyD on passive and iontophoretic transdermal delivery of hydrocortisone was investigated. The skin reservoir

Correspondence: A. K. Banga, Department of Pharmacal Sciences, 401 Pharmacy Building, Auburn University, Auburn, AL 36849-5503, USA.

effect of the HP-β-CyD–hydrocortisone complex is also discussed.

Materials and Methods

Materials

Hydrocortisone was obtained from Sigma (St Louis, MO) and tritium-labelled hydrocortisone from DuPont NEN (Boston, MA). 2-Hydroxypropyl-β-cyclodextrin (degree of substitution 0·6) was a gift from Wacker Chemie (Munich, Germany). Scintillation fluid (Ultima-Gold) and Solvable tissue and gel solubilizer were obtained from Packard (Meriden, CT).

Human cadaver skin was obtained from the National Disease Research Interchange (Philadelphia, PA). The skin was frozen within 12 h of death and supplied as dermatomed (250 μm) or full-thickness skin. When required, epidermis was separated from full-thickness skin by heating in a water bath at 60 °C for 45 s, rubbing with two spatulas and then teasing off the epidermis from the underlying dermis by means of forceps. Once received, the skin was stored at −80 °C and then thawed just before use. Oleic acid, *N*-(2-hydroxyethyl)-1-piperazine-*N*′-(2-ethanesulphonic acid) (HEPES), sodium chloride, sodium azide, monobasic and dibasic sodium phosphate, and propylene glycol were obtained from Fisher Scientific (Pittsburgh, PA). Tetrabutylammonium hydroxide was from Aldrich (Milwaukee, WI). Silver-silver chloride electrodes were purchased from In Vivo Metric (Healdsburg, CA).

Transdermal iontophoretic permeation studies

Valia-Chien cells were modified to furnish two ports on each half-cell. One port served as the sampling port and the other accommodated the electrode. Unless otherwise specified, excised dermatomed human skin was thawed and mounted between the two half-cells. Experiments were performed in triplicate. An external water bath maintained the temperature of the water circulating in the jackets at 37 °C, and the solutions in both compartments were stirred continuously with star-headed magnetic stirrers. Silver-silver chloride electrodes were used because these do not cause electrolysis of water. A silver wire was used as the anode and a silver-silver chloride wire was used as the cathode. For all experiments the anode was placed in the donor solution which contained 4 mL 1% hydrocortisone with HP-β-CyD in 25 mM HEPES buffer consisting of HEPES, sodium chloride, and sodium azide. The pH was adjusted to 7·4 by addition of tetrabutylammonium hydroxide. The receptor phase was filled with 4 mL phosphate-buffered saline at pH 7·4. Unless otherwise specified a current of 0·5 mA cm^{-2} was applied from a commercially available power supply for 24 h. Sampling was continued for approximately 72 h. When 1·0-mL samples were withdrawn they were replaced by 1·0 mL fresh phosphate-buffered saline. At the end of some experiments the skin was removed and digested with Solvable tissue solubilizer. A ratio of 5 mL solubilizer per g skin was used and the mixture was incubated at 50 °C until the skin had dissolved (typically 2 days).

All data are presented as mean±the standard deviation of the mean, unless otherwise specified. Comparisons between two groups were by use of Student's *t*-test, $P < 0.005$ being regarded as indicative of significance.

Analytical techniques

The residual radioactivity in the skin or the radioactivity in the receptor samples was assayed by liquid scintillation counting (LSC). For LSC analysis samples (0·5 mL) were treated with Ultima-Gold scintillation fluid (2 mL) and the radioactivity measured. Hydrocortisone in the receptor compartment was also analysed by UV spectroscopy at 242 nm; the absorbance of the compound, with or without HP-β-CyD, was found to be linearly dependent on concentration in the range 0·8–40 μg mL^{-1}. A colorimetric assay for HP-β-CyD was also tried. The assay takes advantage of the phenomenon that the absorbance of a guest molecule might be altered by complexation with cyclodextrin, i.e. the absorbance of complexed material can be shielded. Therefore, the amount of HP-β-CyD was expected to be quantitated indirectly by evaluating the absorbance of the uncomplexed material at the wavelength of maximum absorption λ_{max}. Phenolphthalein was mixed with HP-β-CyD in hydroalcoholic solution and sodium carbonate solution was added immediately before assay at 550 nm.

Differential scanning calorimetry

The endothermic–exothermic change a material undergoes during phase transition can be detected by differential scanning calorimetry (DSC). It is characteristic of pure substances and of some homogeneous mixtures. Complexation of hydrocortisone with HP-β-CyD was investigated by DSC performed with a TA instruments (Newcastle, DE) DSC-2910 equipped with a TA instruments computer. Scans were first run separately for pure hydrocortisone and pure HP-β-CyD, then for a physical mixture of both. Hydrocortisone and HP-β-CyD were then dissolved in water in the ratio of 1 : 9 and the solution was lyophilized. The lyophi-

lized powder was then analysed by DSC. Samples were heated from 30 °C to 260 °C at a scan rate of 10 ° min^{-1}.

Results and Discussion

This study compared the effectiveness of a HP-β-CyD formulation delivered by iontophoresis as an active enhancement mechanism for the topical or transdermal delivery of hydrocortisone with that of a passive approach in which the permeation at the same concentration of hydrocortisone was promoted by use of chemical enhancers. HP-β-CyD was added in increments to 1% hydrocortisone suspension. It was found that a minimum of 9% HP-β-CyD was required to give a clear solution. Tritium-labelled hydrocortisone was added to these hydrocortisone–HP-β-CyD solutions which were then used as donor solutions in iontophoresis studies.

The formation of hydrocortisone–cyclodextrin complexes was demonstrated by DSC studies. Figure 1 shows the DSC profiles of pure hydrocortisone, pure cyclodextrin, a physical mixture of hydrocortisone and HP-β-CyD, and hydrocortisone–HP-β-CyD complex. The endothermic peak of pure hydrocortisone occurred at 225 °C whereas for pure HP-β-CyD there was no peak up to 260 °C. The physical mixture still shows the hydrocortisone peak at approximately 225 °C, but this peak is lost in the lyophilized powder, suggesting that complexation has taken place. Preiss et al (1994) used a similar approach and also found that an inclusion complex of hydrocortisone with HP-β-CyD does not give a peak for the melting of hydrocortisone.

The passive permeation of 1% hydrocortisone–9% HP-β-CyD solution was very low (Figure 2), much lower than the passive permeation of 1% hydrocortisone in propylene glycol. This seems to suggest that HP-β-CyD is not complexing with skin

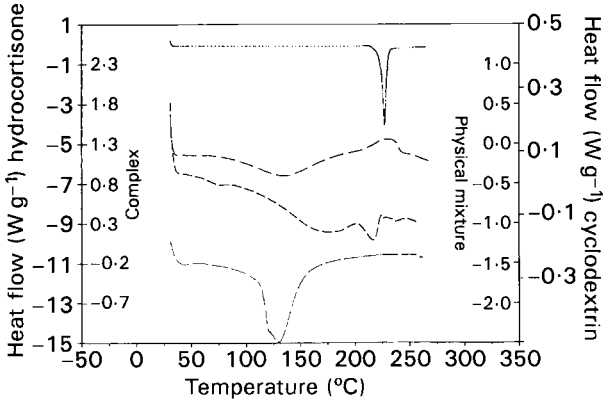

Figure 1. Differential scanning calorimetry profile of hydrocortisone, HP-β-CyD, physical mixture and complex (from top to bottom).

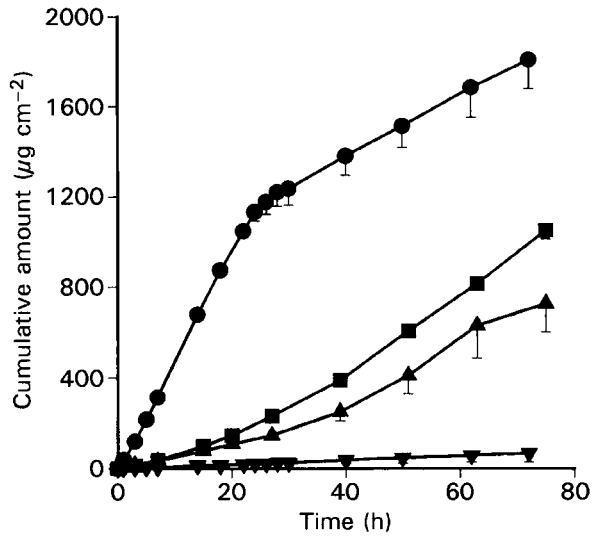

Figure 2. Comparison of the transdermal permeation of 1% hydrocortisone either by passive delivery from propylene glycol (▲), propylene glycololeic acid (■) or 9% HP-β-CyD (▼), or by iontophoretic delivery from 9% HP-β-CyD (●).

constituents in a non-specific manner because this should have led to higher passive permeation. Another possibility is that the highly hydrophilic HP-β-CyD is unable to partition into the skin.

The iontophoretic delivery of hydrocortisone as 1% hydrocortisone–9% HP-β-CyD was much higher than could be achieved by passive permeation of hydrocortisone from non-aqueous vehicles, even if a chemical enhancer such as oleic acid was used (Figure 2). The much greater transport of the hydrocortisone–HP-β-CyD complex by iontophoresis might be because of the transport of the entire complex across the skin. Alternatively, the complex might serve as a carrier from which hydrocortisone dissociates and is iontophoretically transported.

To explore these possibilities further, iontophoretic delivery of a 1% hydrocortisone–3% HP-β-CyD suspension and 1% hydrocortisone–15% HP-β-CyD solution was investigated. The 15% concentration provided excess cyclodextrin whereas 3% was insufficient to solubilize the hydrocortisone and resulted in a suspension. These data were compared with those from investigation of 1% hydrocortisone–9% HP-β-CyD. The cumulative amounts of hydrocortisone which had permeated after 72 h were significantly ($P < 0.005$) different, in the order 9% > 3% > 15% (Figure 3). The stoichiometry of the complex between hydrocortisone and HP-β-CyD has been determined to be 1 : 3 (Preiss et al 1994). On the basis of this ratio and the aqueous solubility of hydrocortisone there should be 0·28 mg mL^{-1} of free form, 2·7 mg mL^{-1}

of complex form, and 7·0 mg mL^{-1} of solid hydrocortisone in the 3% HP-β-CyD solution. However, almost all the hydrocortisone is present as the complexed form in the 15% HP-β-CyD solution and little free hydrocortisone is available because the excess HP-β-CyD forces the equilibrium towards the complexed form. This suggests that high complexation of hydrocortisone does not enhance iontophoretic transdermal delivery. To verify this by direct comparison, the concentration of hydrocortisone was reduced to 0·02% which is close to its aqueous solubility in the absence of HP-β-CyD. The iontophoretic delivery of 0·02% hydrocortisone alone was found to be more efficient than in the presence of 9% HP-β-CyD (Figure 4), confirming that free hydrocortisone was delivered more readily by iontophoresis than its HP-β-CyD complex. These data suggested that HP-β-CyD could be an aqueous carrier with a relatively hydrophobic cavity in which the less soluble compound was loaded and released when the equilibrium of dissociation shifted owing to the depletion of hydrocortisone by transdermal delivery. Iontophoresis did not enhance the penetration of the hydrocortisone–HP-β-CyD complex, which was possibly too bulky to permeate the skin.

Although the trans-appendageal route might possibly accommodate the complex, any potential enhancement via this pathway does not, apparently, predominate over that by the trans-cellular or intercellular routes, which would only allow passage of free hydrocortisone. This partly explains why iontophoresis predominantly enhances the permeation of the free form of hydrocortisone. However, excess HP-β-CyD might retard the release of hydrocortisone from the complexes while insufficient HP-β-CyD fails to solubilize all the hydrocortisone. Nevertheless, just sufficient HP-β-CyD can solubilize hydrocortisone and maintain an optimum dissociation equilibrium which continuously replenishes free hydrocortisone for delivery.

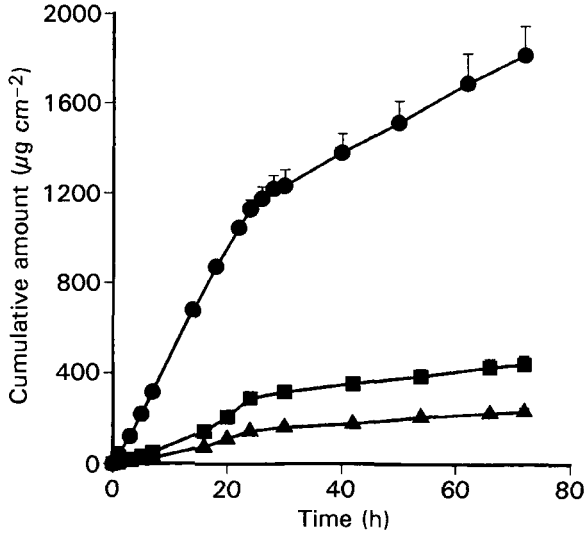

Figure 3. Iontophoretic delivery of 1% hydrocortisone with 3% (■), 9% (●) and 15% (▲) HP-β-CyD in HEPES buffer at pH 7·4. The current was stopped after 24 h.

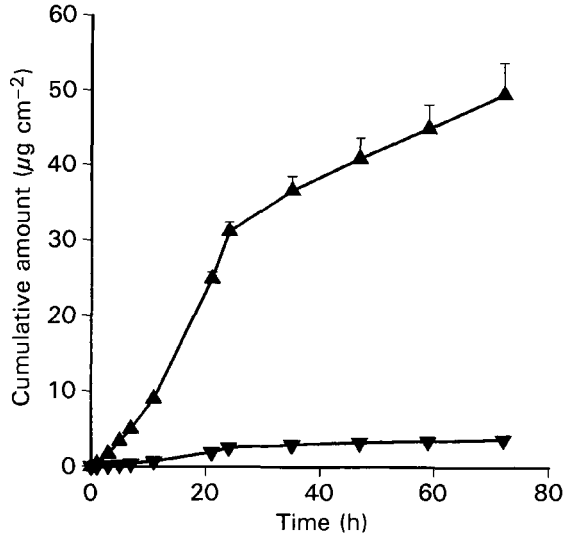

Figure 4. The cumulative permeation of 0·02% hydrocortisone with (▼) or without (▲) 9% HP-β-CyD under iontophoresis. A current of 0·5 mA cm^{-2} was applied for 24 h.

Continued permeation of hydrocortisone was observed beyond the termination of the current (Figures 3, 4). The post-iontophoretic flux of hydrocortisone with 9% HP-β-CyD was 13·83±0·15 μg cm^{-2} h^{-1}, approximately 4- and 8-times higher than with 3% and 15% HP-β-CyD, respectively (Table 1). It was not clear whether this is because of reservoir formation or because the integrity of the skin is compromised.

To identify the cause of the continued permeation, another experiment was performed in which the donor solution was removed when the current was stopped but sampling from the receptor was continued. Full-thickness skin was used for this study because the histological site of the skin where reservoir might form was not clear. It was observed (data not shown) that permeation continued beyond iontophoresis but with reduced flux, suggesting that a reservoir was formed. The amount of

Table 1. The flux rate of 1% hydrocortisone (μg cm^{-2} h^{-1}).

HP-β-CyD (%)	Iontophoresis	Post-iontophoresis	Passive
3	11·58±0·78	3·00±0·07	
9	48·23±0·51	13·83±0·15	1·02±0·02
15	5·99±0·36	1·79±0·10	

All data are presented as mean±s.e.

Table 2. Formation of a reservoir of hydrocortisone in full-thickness skin and in epidermal skin.

	Amount of hydrocortisone ($\mu g\,cm^{-2}$)	
	Full-thickness skin	Epidermal skin
Cumulative permeation	243·42±98·32	15·08±4·65
Residual amount in skin	42·40±6·51	5·89±1·45

The donor solution contained 1% hydrocortisone and 9% HP-β-CyD. All data are presented as mean±s.e.

Table 3. Formation of a reservoir of hydrocortisone with different concentrations of HP-β-CyD and hydrocortisone.

Donor solutions of hydrocortisone	Amount of hydrocortisone ($\mu g\,cm^{-2}$)
0·02% Hydrocortisone alone	645·50±23·48
0·02% Hydrocortisone with 9% HP-β-CyD	36·07±4·15
1% Hydrocortisone with 3% HP-β-CyD	41·97±3·86
1% Hydrocortisone with 15% HP-β-CyD	15·86±1·70

All data are presented as mean±s.d.

hydrocortisone remaining in the skin at the end of these studies was also measured. It was found that not all the amount in the skin had been released during the study period, both for full-thickness skin and for epidermis (Table 2). The amount of hydrocortisone remaining in the skin was measured for each group (Table 3). The skin used with 0·02% hydrocortisone alone contained the highest amount of the drug, suggesting that excess complexation of HP-β-CyD prevents hydrocortisone from forming a reservoir in the skin and supporting the hypothesis that hydrocortisone is predominantly transported as the free molecule.

Iontophoresis data were also generated for unlabelled hydrocortisone only and analysed by UV spectrophotometry. UV analysis of iontophoresis data for hydrocortisone–HP-β-CyD solution resulted in a similar profile (data not shown) to the LSC data. To answer the question whether HP-β-CyD permeated through the skin, the assay of this molecule in the receptor phase was desired. Unfortunately, radiolabelled HP-β-CyD is not commercially available, therefore other assay methods were tried. The colorimetric assay discussed above was investigated but could not be used because the data obtained from phenolphthalein–HP-β-CyD standard solutions at $\lambda_{max} = 550\,nm$ did not show a linear relationship between response and concentration. An attempt was made to detect the presence of HP-β-CyD in the receptor phase and the skin by differential scanning calorimetry. The triplicate receptor phase was combined and lyophilized to concentrate the hydrocortisone in the receptor phase and the resulting powder assayed by DSC. However, no peak was observed, perhaps because the quantity was too low to be in the sensitivity range of the instrument. However, our indirect evidence as discussed earlier suggests that hydrocortisone was probably transported as the free form rather than as a complex.

In summary, HP-β-CyD enhances iontophoretic transdermal delivery of hydrocortisone if just sufficient (9%) is used to solubilize the hydrocortisone (1%). However, delivery was low if insufficient (3%) or excess (15%) HP-β-CyD was used. The enhancement observed with 9% HP-β-CyD might result from high availability of free hydrocortisone in solution as a result of dissociation of the complex or renewal of amounts depleted by transdermal transport. This enhancement was higher than that observed with passive permeation of hydrocortisone from non-aqueous vehicles such as propylene glycol, even if oleic acid was used as an enhancer.

References

Arima, H., Adachi, H., Irie, T., Uekama, K. (1990) Improved drug delivery through the skin by hydrophilic β-cyclodextrins: enhancement of the anti-inflammatory effect of 4-biphenylacetic acid in rats. Drug Invest. 2: 155–161

Banga, A. K., Chien, Y. W. (1988) Iontophoretic delivery of drugs: fundamentals, developments and biomedical applications. J. Contr. Rel. 7: 1–24

Fuhrman, L. C., Michniak, B. B., Behl, C. R., Malick, A. W. (1997) Effect of novel penetration enhancers on the transdermal delivery of hydrocortisone: an in vitro species comparison. J. Contr. Rel. 45: 199–206

Lin, R. Y., Ou, Y. C., Chen, W. Y. (1997) The role of electroosmotic flow on in vitro transdermal iontophoresis. J. Contr. Rel. 43: 23–33

Loftsson, T., Siguroardottir, A. M. (1994) The effect of polyvinylpyrrolidone and hydroxypropyl methylcellulose on HP beta CD complexation of hydrocortisone and its permeability through hairless mouse skin. Eur. J. Pharm. Sci. 2: 297–301

Loftsson, T., Frioriksdottir, H., Ingvarsdottir, G., Jonsdottir, B., Siguroardottir, A. M. (1994) The influence of 2-hydroxypropyl-beta-cyclodextrin on diffusion rates and transdermal delivery of hydrocortisone. Drug Dev. Ind. Pharm. 20: 1699–1708

Michniak, B. B., Player, M. R., Chapman, J. M., Sowell, J. W. (1994) Azone analogues as penetration enhancers: effect of different vehicles on hydrocortisone acetate skin permeation and retention. J. Contr. Rel. 32: 147–154

Pikal, M. J. (1992) The role of electroosmotic flow in transdermal iontophoresis. Adv. Drug Del. Rev. 9: 201–237

Preiss, A., Mehnert, W., Fromming, K. H. (1994) Complexation of hydrocortisone with beta-cyclodextrin and hydroxypropyl-beta-cyclodextrin. Arch. Pharm. 327: 729–734

Preiss, A., Mehnert, W., Fromming, K. H. (1995) Penetration of hydrocortisone into excised human skin under the influence of cyclodextrins. Pharmazie 50: 121–126

Seth, S. C., Allen, L. V., Pinnamaraju, P. (1994) Stability of hydrocortisone salts during iontophoresis. Int. J. Pharm. 106: 7–14

Szejtli, J. (1994) Medicinal applications of cyclodextrins. Med. Res. Rev. 14: 353–386

Tauber, U. (1994) Dermatocorticosteroids: structure, activity, pharmacokinetics. Eur. J. Dermatol. 4: 419–429

Vollmer, U., Muller, B. W., Peeters, J., Mesens, J., Wilffert, B., Peters, T. (1994) A study of the percutaneous absorption-enhancing effects of cyclodextrin derivatives in rats. J. Pharm. Pharmacol. 46: 19–22

Wang, Y., Allen, L. V., Li, L. C., Tu, Y. H. (1993) Iontophoresis of hydrocortisone across hairless mouse skin: investigation of skin alteration. J. Pharm. Sci. 82: 1140–1144