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IN-VITRO STUDIES ON CORTICOSTERONE PERMEATION THROUGH HAIRLESS MOUSE SKIN: (PART I)

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لقد ازداد الاتجاه إلى استعمال محفزات النفاذ في المستحضرات الجلدية حيث تقلل هذه الإضافات من اتحاد العقار مع الجلد و تزيد من مرور ثنائي الجزئيات خلال الجلد. و قد استهدف هذا البحث تقييم تأثير كل من عديد جزيئات الفينيل بيروليدون وهيدروكسى بروبيل بيتا دكسترين الحلقي بالإضافة إلي محفزات النفاذ الكيمائية على ذوبان و نفاذية عقار الكورتيكوستيرون عبر جلد الفأر.

أظهرت النتائج أن تسخين عقار الكورتيكوستيرون مع عديد جزيئات الفينيل بيروليدون إلى ١٢١ درجة مئوية يؤدى إلى وجود حالة فوق التشبع كنتيجة لقدرة الأخير على أضعاف تكون بلورات العقار و زيادة نفاذيتة خلال الجلد. كما وضح من النتائج أن هيدروكسى بيتا الدكسترين الحلقي ليس له تأثير على نفاذية عقار الكورتيكوستيرون و لكن يمكن استخدامه كمادة مذيبة لمحفزات النفاذية ذات القابلية العالية للدهون مثل أوكتيل و ثنائي دوسيل البيروليدون و بالتالي زيادة فاعلية هذه المحفزات على النفاذية.

The use of penetration enhancer adjuvants was emerged as a growing trend in transdermal drug delivery. These adjuvants may reduce the capacity for drug binding to skin and promote the permeation of bimolecules through the skin. The main objective of this study was to evaluate the effect of polyvinylpyrrolidone (PVP) and hydroxypropyl–beta-cyclodextrin (HP-β-CD) as well as certain chemical enhancers on solubility of unlabeled corticosterone (CS) and its transdermal permeation through skin. The results revealed that autoclaving CS with PVP maintained supersaturated state through inhibition of crystal growth and in turn increased its flux across hairless mouse skin. The data showed also that HP-β-CD had no effect on CS permeation but could be used as solubilizing agent for highly lipophilic skin permeation enhancers octyl pyrrolidone (OP) and dodecyl pyrrolidone (DDP). It also prevented depletion of such lipophilic enhancers into skin, increased by this way the permeation enhancement of these adjuvants.

INTRODUCTION

The potential of using the skin as an alternative route for systemic administration of active drugs had attracted considerable interest in recent years. The transdermal delivery of drugs for systemic use is limited by the low permeability of stratum corneum compared to other biological tissues. One generalized approach to overcome the barrier properties of the skin for drugs and bimolecules was dependent on the incorporation of chemical compounds such as skin-penetration enhancers, accelerates, or sorption promoters into transdermal delivery systems.[1] The use of drug-polymer interactions to improve the bioavailability of drug of low solubility following oral administration has been reported since the discovery of their remarkable dissolution-enhancing property.[2] Polyvinyl-pyrolidone (PVP) was one of the most water-soluble polymers and low toxicity that had been extensively used as excipient in a wide variety of pharmaceutical formulations. In solution, PVP has the ability to form complexes with a wide range of drugs and the ability to prevent and /or retard crystal growth.[3] The use of penetration enhancers was also a growing trend in transdermal drug delivery because of their ability to increase the penetration of solutes through stratum corneum via fluidization of lipids. Dimethyl sulfoxide (DMSO) was the most common enhancer early demonstrated by Stoughton and Fritsch

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(1964).[4] Many other compounds promote percutaneous absorption including dimethyl acetamide, dimethyl formamide, various pyrrolidones, alkyl sulfoxides and azones. Propylene glycol showed a marked enhancement effect when used in combination with various fatty acids and alcohols because of lipid fluidization mechanism as evidenced by DSC studies.[5] Surfactants (ionics and nonionics) were also suggested as a class of enhancers that open up aqueous channels in stratum corneum by interacting with proteins to increase the permeability of polar compounds but did not affect compounds penetrating through the lipophilic pathway.[5]

The following investigation was carried out to determine the effect of polyvinylpyrrolidone and hydroxypropyl–beta-cyclodextrin as well as the two chemical enhancers (octyl-and dodecyl pyrrolidones) on the solubility and *in-vitro* permeation of corticosterone as a lipophilic drug through hairless mouse skin.

EXPERIMENTAL

Materials

Unlabeled corticosterone (CS), Sigma Chemical Company. [^{3}H]Corticosterone (^{3}H-CS) at > 95% purity was purchased from American Radiolabeled Chemicals, Inc., St. Louis, MO., U.S.A., and tested for purity by methods suggested by the supplier. Polyvinylpyrrolidone (PVP) K 30 (intrinsic viscosity 80-100) of molecular weight 360,000 was purchased from Sigma. Chemical Company, St. Louis, Mo. Octylpyrrolidone (OP) and dodecylpyrrolidone (DDP) and sodium azide were purchased from Sigma Chemical Company, St. Louis, Mo. Acetonitrile (HPLC grade) / water ratio of 85; 15 was used as mobile phase at a flow rate of 2.0 ml/min. Hydroxypropyl–beta-cyclodextrin (HP-β-CD) was kindly supplied from Roquette Freres, France. Phosphate buffered saline (PBS) tablets, pH 7.4, were obtained from Sigma Chemical Company, St. Louis, MO., and used as received.

Equipment

Horizontal two-chamber side-by-side diffusion cell system, Scientific Glass Inc., California, U.S.A. Liquid scintillation counter, Model 1900 TR (TRI-CARB TM liquid scintillation analyzers), Packard Instrument Company, U.S.A. Liquid scintillation cocktail, Ultima Gold, high flash-point universal LSC–Cocktail, Parked, Meriden, CT., U.S.A. Scintillation vials, Kimble Glass Inc., Vineland, New Jersey, U.S.A. HPLC system consisted of a Beckman pump, a HP1050 series auto-sampler HPLC, with a variable wavelength UV absorbency detector and a 15cm x 4.6 mm Discovery ® C18 column. Packing particle size 4.0-4.3 mm (Supelco, Bellefonte, PA). Autoclave, HIRAYAMA, Amerex Instruments INC. Lafyette, California, U.S.A. Thermostatic water bath, model YB-521, American Scientific Products, McGaw Park, Illinois, U.S.A. Screw capped Pyrex culture tubes, diameter, 13 mm; length 100 mm; VWR Scientific, Philadelphia, PA. Para-film, laboratory film, American National Can, Chicago, Illinois, U.S.A. Millipore® 0.22 μm filter, 25 mm diameter, Millipore Corporation, Bedford, MA, U.S.A. Series of micropipettes, Eppendorf 2000, variable volume, Brinkmann, VWR Scientific Products, Willard, Ohio, U.S.A

Animals- Female hairless mice (strain SKH-HRI, 8-12 weeks old) were obtained from Charles River, Wilmington, MA.

Methodology

Solubility studies of polyvinylpyrrolidone and enhancers on corticosterone in presence or absences of HP-β-CD

Excess amount of CS recrystallized from absolute ethanol (pure form) was added to aqueous solutions containing 0.25 and 1% PVP with or without 5% HP-β-CD. Some of the suspensions were directly equilibrated at 37° for 3 days and others were first heated in an autoclave to 121° for 20 min,[6] and then allowed to equilibrate for at least 3 days at room temperature. The same experiments were carried out with both octyl pyrrolidone (OP) and dodecyl pyrrolidone (DDP) as enhancers with or without 5% HP-β-CD. The solubility experiments with enhancers were equilibrated at 37° for 3 days. After equilibration, the suspensions were centrifuged, filtered through 0.22 μm filter and then analyzed for CS concentration by HPLC. The supernatants were

then used in the following studies of CS transport across hairless mouse skin.

Effect of polyvinylpyrrolidone and enhancers on CS permeability in presence or absences of HP-β-CD

The transport experiments were conducted through hairless mouse skin with the following: (a) PVP, (b) PVP/ HP-β-CD. (c) chemical enhancers in absence and in presence of HP-β-CD supernatant solutions (Table 1). These solutions were mixed with ^{3}H-CS to measure the effect of PVP and the enhancers on the drug influx in presence or absences of HP-β-CD. The ^{3}H-CS was used because the amount of non-radioactive CS fluxed through the skin into receiver chamber was too small to be detected by HPLC. It was previously proved that radioactive CS (^{3}H-CS) reflected the behavior of non-radioactive CS.[7] The permeability experiments were carried out at 37° using two-chamber side-by-side diffusion cell system, with each compartment having 2-ml volume and an effective diffusional area 0.78 cm^2. The experiments were conducted with hairless mouse skin membrane sandwiched between the two half-cells. The experiments were carried out with saturated and super-saturated CS solutions mixed with ^{3}H-CS. The apparent permeability coefficient and the flux of ^{3}H-CS was determined by measuring the radioactivity then used to calculate the flux of non-radioactive CS. The calculation of non-radioactive CS flux from that of ^{3}H-CS was depended on conversion of the dpm (disintegration per minute) content in the donor chamber (C_D) to its equivalent of mg/ml, then divide the flux (slope of curve) by the new value of C_D.[8]

$$P_T = P_L = \frac{1}{AC_D} \cdot \frac{dQ}{dt}$$

The permeability experiments, were carried in absence and in presence of 5% HP-β-CD to determine the effect of enhancers on the apparent permeability coefficient of ^{3}H-CS. For Octylpyrrolidone (OP) the permeability

Table 1: Effect of polyvinylpyrrolidone and the enhancers in absence or presence of HP-β-CD on the solubility of corticosterone (CS), permeability coefficient and the flux of ^{3}H-CS in CS saturated solution through hairless mouse skin.

Condition of experiment (^{3}H-CS added to saturated CS solution in:)	Solubility (mg/ml)	P of ^{3}H-CS (cm/sec) x10^{-7}	Flux of ^{3}H-CS (dpm/min.cm^2) x10^3	Flux of CS (mg/min.cm^2) x10^{-4}
PBS at 37°	0.23 ± 0.01	2.6 ± 0.3	3.86 ± 0.01	3.8 ± 0.2
0.25% PVP at 37°	0.23 ± 0.009	208 ± 0.2	3.92 ± 0.08	3.85 ± 0.1
1% PVP at 37°	0.23 ± 0.01	2.9 ± 0.1	3.87 ± 0.03	3.79 ± 0.2
0.25% PVP at 37° after 121° treatment	0.78 ± 0.05	2.9 ± 0.2	3.66 ± 0.04	12.3 ± 0.2
1% PVP at 37° after 121° treatment	0.79 ± 0.02	2.8 ± 0.3	3.9 ± 0.01	12.9 ± 0.3
5% HP-β-CD at 37°	2.36 ± 0.04	0.23 ± 0.3	0.38 ± 0.24	3.8 ± 0.4
5% HP-β-CD + 0.25% PVP at 37°	2.4 ± 0.01	0.25 ± 0.2	0.38 ± 0.28	3.76 ± 0.3
5% HP-β-CD + 0.25% PVP at 37° after 121° treatment	7.8 ± 0.02	0.27 ± 0.1	0.41 ± 0.05	13.3 ± 0.2
2.3 x 10^{-3} M octyl-pyrrolidone	0.23 ± 0.01	23.0 ± 0.2	36.8 ± 0.2	38.1 ± 0.2
2.3 x 10^{-3} M octyl-pyrrolidone/ 5% HPβCD	2.36 ± 0.01	2.3 ± 0.2	0.38 ± 0.01	3.78 ± 0.2
2.3 x 10^{-2} M octyl-pyrrolidone/ 5% HPβCD	2.38 ± 0.02	24.1 ± 0.3	3.76 ± 0.09	38.1 ± 0.4
2.3 x 10^{-4} M octyl-pyrrolidone/ 5% HPβCD	2.4 ± 0.03	22.2 ± 0.1	3.69 ± 0.02	37.2 ± 0.2

experiment was carried out after equilibrating the skin with OP for 2 hr prior to addition of the drug. In the equilibration process, the enhancer solution in both chambers was replaced every 15 min with fresh OP solution. The transport experiments were conducted with 2.3 x 10^{-3} M of OP solution in PBS (phosphate buffer saline) and 10 times this concentration (2.3 x 10^{-2} M) with 5 % HP-β-CD in both diffusion cell chambers. The permeability coefficient of ^{3}H- CS was determined in OP and OP/ HP-β-CD solutions to validate the calculation method of free OP concentration.

The permeability experiments testing the effect of dodecyl-pyrrolidone (DDP) solubilized in 5% HP-β-CD on the apparent permeability coefficient of ^{3}H-CS with hairless mouse skin can not be carried out with DDP, due to depletion of this enhancer into skin as result of its high lipophilicity. Prior to transport experiments, the %DDP depleted in both chambers during equilibration with skin was measured as a function of time. Also, this depletion test was carried out in presence of 5% HP-β-CD. Then, the permeability experiments with hairless mouse skin were carried out with 3.75 x 10^{-4} M solution of DDP in 5% w/v HP-β-CD.

RESULTS AND DISCUSSION

A-Solubility experiments for corticosterone in absence and in presence of 5% HP-β-CD

Effect of polyvinylpyrrolidone

The effect of 5% HP-β-CD as solubilizing agents for CS had been proved in previous study.[7] In the present study, the effect of PVP alone or in presence of cyclodextrin HP-β-CD on the solubility of CS was determined and presented in Table (1). The data demonstrated that the addition 0.25 and 1 % w/v PVP did not affect the drug solubility at 37° (0.23 mg/ml solubility limit, Table 1). This was evidence that there was no complexation or interaction between PVP and CS at these two concentrations. On the other hand, it was found that autoclaving CS with 0.25 and 1 % w/v PVP increased the solubility of CS about 3 fold to be 0.78 and 0.79 mg/ml for both concentrations respectively. This increase in solubility was related to the effect of PVP on crystal growth inhibition that resulting in the super-saturation CS/PVP solution in which the drug thermodynamic activity was increased by 3 folds. The data also showed that 0.25 % PVP was the minimum crystal growth inhibitory concentration. 5% HP-β-CD at 37° showed a 10 fold increase in solubility compared to PBS (2.36 mg/ml). The addition of 5% HP-β-CD to 0.25 % PVP at 37° had no effect on the solubility of CS (2.4 mg/ml) compared to that solution free from PVP (2.36 mg/ml). While, autoclaving of this mixture at 121° revealed a marked inhibitory effect of PVP on crystal growth, leading to increase the solubility of CS about 3 fold (7.8 mg/ml).

Effect of HP-β-CD and chemical enhancers

Figure 1 (a and b) presented the phase-solubility diagrams of OP and DDP in PBS solution of different concentrations of HP-β-CD, respectively. At HP-β-CD concentration below 5% (W/V) the solubility diagrams were of Higuchi's A_L-type. 1:1 complex governed the solubility behavior of both OP and DDP in HP-β-CD solution. The complexation binding constants (K_C) between the two enhancers and HP-β-CD were estimated from the slope of the phase-solubility diagrams as previously described.[9] The complexation binding constants (Kc) determined was about 941.7 and 1346.8 $(M)^{-1}$ for OP and DDP, respectively. From the Kc values, a correction factors for the amount of free enhancer concentration in the donor chamber in transport experiments was calculated. It was found that this correction factor was 10 for OP (i.e., the free OP will be 1/10 of total OP conc.) added in donor chamber in presence of cyclodextrin, while it was about 25 for DDP. The results presented in Table 1 showed that octyl-pyrrolidone (OP) 2.3 x 10^{-3} M of OP as a chemical enhancers had no effect on the CS solubility (0.23mg/ml). However, mixing the chemical enhancer with 5% of HP-β-CD showed obvious effect on increasing the solubility of CS by 10 fold to be about 2.4 mg/ml with 2.3 x 10^{-3} M of OP 2.3 x 10^{-2} M of OP and 2.3 x 10^{-4} M of DDP. This result proved that HP-β-CD could be used as a solubilizing agent for highly lipophilic skin permeation enhancers and drug that accordingly increased the permeation enhancement of these low concentrations of enhancers.

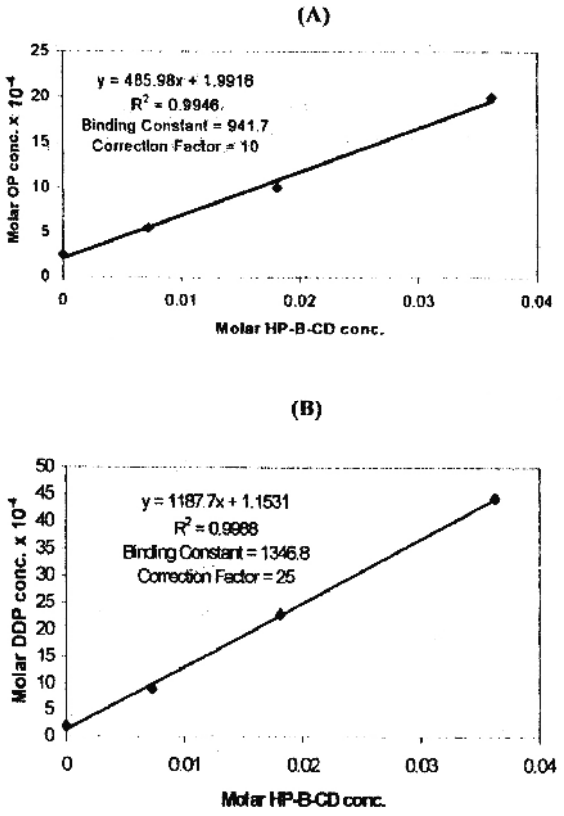

Fig. 1: Phase solubility diagram of: (A) Octyl-pyrrolidone (OP) in HP-β-CD, (B) Dodecyl-pyrrolidone (DDP) in HP-β-CD.

B- The effect of polyvinylpyrrolidone and enhancers on CS permeability through hairless mouse skin

Effect of polyvinylpyrrolidone

The results of transport experiments with trace level ^{3}H-CS with 0.25 and 1 % PVP in CS saturated solution with and without autoclave treatments through hairless mouse skin are presented in Table (1). The data revealed that the increase in solubility and thermodynamic activity by autoclaving in PVP solutions had no effect on the permeability coefficient (P nearly 2.8 cm/sec x 10^{-7}) and the flux values of ^{3}H-CS (around 3.8 x10^3 dpm/min.cm^2). This result was related to the fact that ^{3}H-CS donor concentration was the same in all experiments carried out in the present study. However, the flux of non-radioactive CS calculated from the ^{3}H-CS was increased about 3 to 4 fold with autoclaving in PVP (nearly 12.5x10^{-4} mg/min. cm^2).

The data also show that presence of 5% w/v HP-β-CD at 37° decreased the apparent ^{3}H-CS permeability coefficient and flux ^{3}H-CS by 10 fold compared to those solutions free from HP-β-CD. This result is attributed to complexation effect of HP-β-CD on decreasing the drug thermodynamic activity while, there was no change in CS flux at the same temperature. This result is in agreement with the study of Loftsson et.al.[10] who, reported that hydrocortisone flux from saturated solution through cellophane membrane was unaffected by cyclodextrin concentration. The result also show that the decrease in the apparent CS permeability coefficient due to CS/ HP-β-CD complexation was to the same extent in PVP/ HP-β-CD solution equilibrated at 37° without autoclaving and after autoclaving to be 0.23, 0.25 and 0.27 cm/sec x10^{-7} respectively. This result prove that there is no interactions between PVP and HP-β-CD. In the experiments that were conducted with CS solutions in different conditions without addition of the ^{3}H-CS, the flux of CS was directly measured by HPLC. There is no difference between CS flux from PVP/ HP-β-CD solution at 37° than that in PBS 3.76 and 3.8 mg/min.cm^2 x10^{-4} respectively. On the other hand, the increase in CS flux in PVP/ HP-β-CD about 3-fold after autoclaving (13.3mg/min.cm^2 x10^{-4}) compared to other condition of HP-β-CD without autoclaving could be related to excess CS free from complexation in the supersaturated solution. The results are in agreement with this explanation that PVP increased corticosterone flux only by autoclaving through inhibition of crystal growth and increasing the drug thermodynamic activity. This explanation is in agreement with the results of Simonelli *et al*.,[3] who found that PVP inhibited Sulfathiazole single crystal growth. Also the results in this investigation agreed with that of Corrigan *et al*.,[2] that co-precipitation of drugs with PVP increased the drug solubility due to the formation of a high-energy amorphous phase rather than complex formation. They also reported that the flux from the PVP containing solution, saturated with crystalline hydrocortisone was about 15% higher than from the aqueous solution. Their study revealed that fluxes obtained from co-precipitate-containing systems were higher and increased with increasing hydrocortisone content.

Recently, increasing thermodynamic activity beyond the saturation point has been explored with success. The use of such supersaturated states showed great potential as inexpensive technique that avoided altering the integrity of the stratum corneum.[11,12] Figures 2 and 3 show the histograms representation of the change in flux and permeability coefficient of CS through hairless mouse skin.

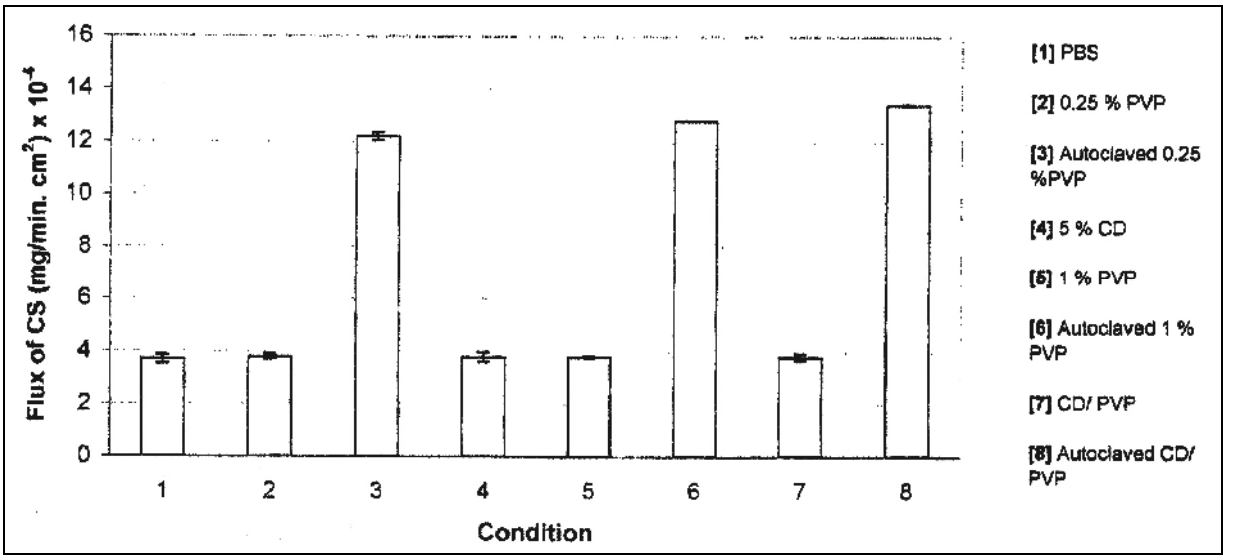

Fig. 2: Histogramic representation of the change in CS flux through hairless mouse skin, upon different treatment conditions.

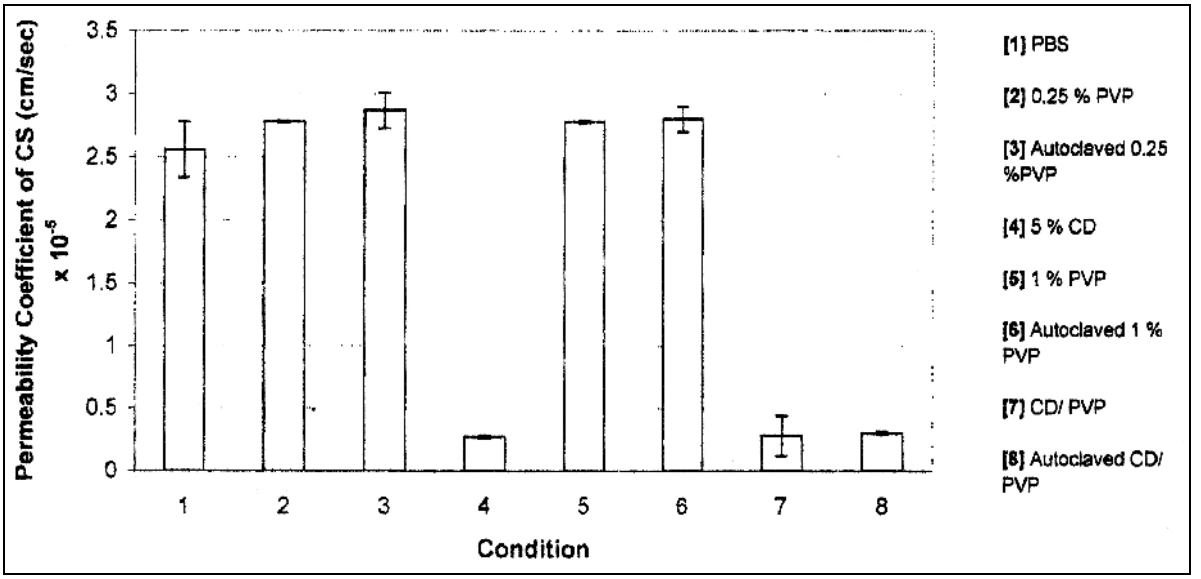

Fig. 3: Histogramic representation of the change in CS permeability coefficient through hairless mouse skin, upon different treatment conditions.

Effect of HP-β-CD and enhancers on permeation of the corticosterone

Octyl-pyrrolidone (OP)

The use of octyl-pyrrolidone (OP) as a chemical enhancer was dependent on previous results showed that 2.3 x 10^{-3} M concentration of OP increased the flux of drugs by 10 times.[13] The transport experiments were conducted with 2.3 x 10^{-3} M of OP solution in PBS and also10 times this concentration (2.3 x 10^{-2} M) with 5% HP-β-CD. The permeability coefficient and flux of ^{3}H- CS and CS are presented in Table (1).

The permeability experiments with hairless mouse skin were carried out after equilibration of skin with OP for 2 hr prior to addition of the drug. The data show that 2.3x10^{-3} M concentration of OP significantly increased the permeability coefficient of ^{3}H-CS through skin by 10 fold (23.0 cm/secx10^{-7}) compared to phosphate buffered saline (PBS) free from OP (2.6 cm/secx10^{-7}). This effect was also followed by increase with the same ratio on the ^{3}H- CS and CS flux. On the other hand, the addition of 5 % HP-β-CD to 2.3 x 10^{-3} M of OP solution in PBS reduce the drug flux 10-fold due to reduction of free enhancer concentration after complexation. The concentration 2.3 x 10^{-2} M OP with 5 % HP-β-CD was used to compensate the reducing effect of 5 % HP-β-CD.

Dodecylpyrrolidone (DDP)

The pervious results confirm that cyclodextrin could be used as a solubilizing agent for the highly lipophilic DDP and at the same time the free DDP concentration can be estimated. The permeability experiments with hairless mouse skin cannot be carried out with DDP, due to depletion of this enhancer into skin as result of its high lipophilicity. Prior to transport experiments, the % DDP depleted in both chambers during equilibration with skin was measured by HPLC as a function of time.

Also, this depletion test was carried out in presence of 5% HP-β-CD. Data in Table (2) show that percentage depletion of DDP after 90 min in both donor and receiver chamber is 26% and 70% respectively. However, addition of 5% HP-β-CD lead to complexation of DDP and reduced the percentage depletion in both chamber to be 0.8 and 1.15% in donor and receiver chamber respectively. Therefore, the permeability experiments with hairless mouse skin can be conducted with DDP in cyclodextrin solution. The permeability coefficient and flux of ^{3}H- CS were determined in 3.75 x 10^{-4} M solution of DDP in 5 % w/v HP-β-CD (Table 1). The data reveal that a 10-fold enhancement of CS flux could be obtained from the later solution.

Table 2: Effect of HP-β-CD on depletion of DDP in donor and receiver chambers during equilibration with hairless mouse skin.

Time (min) of equilibration with skin	Starting DDP conc. in both chambers 1.5 x 10^{-5}		Starting DDP conc. in both chambers 3.75 x 10^{-4} M/5% HP-β-CD	
	Remaining DDP conc. X 10^{-5} M in: Donor	Receiver	Remaining DDP conc. X 10^{-4} M in: Donor	Receiver
15	1.2	0.6	3.73	3.72
60	1.1	0.5	3.71	3.70
90	1.1	0.45	3.72	3.70
% Depletion	26.6%	70%	0.8%	1.15%

Conclusion

From the above results it could be concluded that autoclaving Unlabeled corticosterone (CS), with polyvinylpyrrolidone PVP maintained supersaturated solution through inhibition the crystal growth. This led to an increase CS flux across hairless mouse skin as a result of increasing the drug thermodynamic activity. Octyl-pyrrolidone (OP) and dodecylpyrrolidone (DDP) are effective as chemical enhancers especially in presence of hydroxypropyl–beta-cyclodextrin (HP-β-CD) that act as a solubilizing agent for the highly lipophilic skin permeation enhancers. It also prevents depletion of such lipophilic enhancers into skin and increasing their permeation enhancement effectiveness. So their effects on the *in-vitro* permeation of ^{3}H-CS from gels and transdermal therapeutic patches will be studied in part II.

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