stored at 15 °C or higher temperatures (room temperature or 32 °C), a significant decrease in assay values was observed. When stored at 5 °C or −20 °C, stable assay results were received. While these results gave us information on how to appropriately store the analytical samples for HPLC analysis, it also indicated special precautions for transdermal experiments conducted at 32 °C according to OECD. Adding sodium azide (NaN_3) to the human skin extract prevented effectively the decrease in assay values (Fig.). The same observations were obtained, although to a lesser extent, with BA dissolved in a phosphate buffered saline (PBS) solution and in water.

We conclude that adding a preservative such as NaN_3 is required to perform reliable long-term *in vitro* skin permeation experiments with BA.

Experimental

Human skin extract was obtained by placing a full-thickness skin piece of approximately 6.5 cm in 50 mL of a 0.1 M phosphate buffered saline (PBS) solution pH 7.4 (Sigma, St Louis, CA, USA) [skin PBS], to which 0.1% m/V sodium azide (Mallinckrodt Baker, Phillipsburg, NJ, USA) was added [skin PBS + NaN_3]. The solutions containing the skin pieces were incubated for 24 h at 37 °C and used immediately.

This gave the same skin exposure/solution ratio as in our experimental settings for *in vitro* transdermal penetration experiments with Franz diffusion cells. The skin used was obtained from the abdominal region of a 45 years old healthy male patient (plastic surgery); the subcutaneous fat was removed and the full-thickness skin was kept in a refrigerator at −20 ± 3 °C until it was used.

BA solutions at three different target concentrations were prepared (i.e. 80 μg/mL, 20 μg/mL and 2 μg/mL) in six different solvents. The chosen solvents were: water (HPLC grade, Fisher Scientific, Leicestershire, UK) [water], 0.1% m/V formic acid in water/acetonitrile 70/30 V/V (i.e. mobile phase of the used HPLC system) [MF], 0.1 M PBS solution at pH 7.4 [PBS], 0.1% m/V sodium azide in the same PBS solution [PBS + NaN_3], skin extract in PBS solution [Skin PBS] and skin extract in 0.1% m/V sodium azide in PBS solution [Skin PBS + NaN_3].

Solutions were incubated for up to 24 h at five different temperatures: −20 ± 3 °C, 5 ± 3 °C, 15 ± 3 °C (i.e. the temperature of the autosampler of the HPLC), room temperature (21 ± 3 °C) and at 32 ± 3 °C (i.e. the most commonly used working temperature for *in vitro* transdermal penetration experiments and recommended by OECD).

Samples taken at different times were assayed by an in-house developed and validated HPLC method using a Waters Alliance 2695 equipped with a Waters 2996 PDA detector and thermostatted autoinjector (all Waters Corporation, Milford, Massachusetts, USA). Analytes were separated on a LiChrospher 100 RP (125 × 4 mm, 5 μm, Merck, Darmstadt) column kept at 30 °C. The mobile phase was a mixture of acetonitrile/water (70/30 V/V) buffered with 0.1% m/V formic acid at a flow rate of 1 ml/min. UV detection was at 229 nm. Injection volume was 25 μL. Under these conditions, BA eluted at approximately 4.5 min.

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Studies of the skin permeation of lipophilic drugs: paclitaxel

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The objective of this investigation was to develop and validate skin permeation methodology for a highly lipophilic drug with respect to sink conditions and intactness of barrier during the course of experiment, using water permeation and trans epidermal water loss as tools.

Skin, being a lipophilic and highly tortuous barrier, severely limits the molecular transport of drugs into the body. However, permeation of lipophilic molecules is less restricted than that of hydrophilic drugs. The ideal characteristics required for efficient transdermal delivery of a drug include (Naik et al. 2000) a) molecular weight < 500 Da; b) log P: 1 to 3 and c) daily dose <10 mg. A practical problem in transdermal delivery research is the development of a suitable permeation methodology for poorly water-soluble molecules. This problem is even more severe during the evaluation of transdermal escape of topical drugs. In such a situation, higher amounts of drug will be localized in the skin without sink conditions, leading to false positive results.

Aqueous solubility of paclitaxel (PCL) is as low as 0.7 μg/ml, but the compound is appreciably soluble in ethanol (EtOH, 36.4 mg/ml, Straubinger 1995; Panchagnula et al. 2004). Due to its poor aqueous solubility, physiological buffers cannot provide necessary sink conditions. Therefore, EtOH was incorporated into phosphate buffered saline (PBS) (pH: 7.4). However, EtOH itself, being a penetration enhancer (Williams and Barry 1992) can alter the skin barrier if it diffuses back into the stratum corneum (SC) from the receptor compartment. In the current study, to evaluate the effect of ethanolic receptor phase on skin barrier function, a series of binary combinations of EtOH and PBS were prepared and their influence on skin barrier function was evaluated, using ^3H_2O permeation and transdermal epidermal weight loss (TEWL) as markers.

Hydro alcoholic solutions have often been used as receptor phase in skin permeation studies of poorly water soluble drugs (Liu et al. 1991; Dayan and Touitou 2000; Ben-Shabat et al. 2005). However, this methodology was not systematically evaluated yet. In the current investigation, skin barrier integrity was evaluated using two complimentary methodologies. Water permeation and TEWL were earlier employed to assess skin barrier alteration upon the

application of permeation enhancers (Bronaugh and Stewart 1986; Nangia et al. 1998). These two techniques are based on water transport across water impervious, skin barrier in opposite directions. Water being the simplest endogenous molecule, gives an exact idea of skin barrier alteration. Although it is polar in nature, still is expected to be sensitive to the alterations in lipid portions of skin, in light of the fact that, an alteration in lipid bilayer by penetration enhancers will have a global impact on alteration of aqueous pathways as well (Barry 1991).

The water permeation reflects the barrier function of the skin only at lower fractions of water absorption, while as it approaches unity, skin permeation is not reflected by fraction of water absorbed (Nangia et al. 1998). Hence, water permeation was studied up to 48 h only. The water permeation estimated from the skin in contact with receptor fluids containing increasing proportions of EtOH revealed that, skin barrier had remained intact at least for 48 h when EtOH : PBS 1 : 3 was used as receptor phase. Only 5% of water applied has permeated during 48 h, essentially indicating the intactness of the skin barrier function. However, barrier was compromised within 9 h when EtOH proportion was increased to 50%. A slight alteration in barrier function was also observed in case of PBS alone without preservative. The intactness of barrier as measured with respect to water permeation was in the order of EtOH : PBS 1 : 3 > EtOH : PBS 1 : 2 > PBS > EtOH : PBS 1 : 1, suggesting that at higher EtOH proportions, skin barrier has compromised (Fig. 1A).

Although, water permeation has been used for long in transdermal delivery research for the quality control of skin used in ex vivo permeation (Bronaugh and Stewart 1986; Scott et al. 1986), its application is having several limitations such as (Nangia et al. 1998):

- It cannot be used when the permeant is ^{3}H-labelled, and ^3H_2O probe itself can alter skin barrier, by altering the hydration state of skin, thus affecting the permeation of solutes which will be applied subsequently
- Since diffusion is a slow process, this technique requires several hours for assessment of barrier function

In order to overcome these problems, another biophysical tool, TEWL was developed based on the similar principle of water permeation across the skin. It is used to assess macromolecular changes in barrier properties of skin as a function of water permeation across the barrier. TEWL results with the same set of receptor phases supported the water permeation findings (Fig. 1B). The TEWL increased drastically after 48 h when PBS alone was used as receptor phase, indicating severe alteration in skin barrier, if preservative is not incorporated. Further, it also altered within 10 h when higher EtOH proportions (>33%) were present in the receptor fluid. Both methodologies indicated the intactness of skin barrier at 25% EtOH in PBS as receptor fluid. Hence, EtOH : PBS 1 : 3 was used as receptor phase in subsequent investigations. Effect of vehicles on skin barrier function was evaluated by pretreatment of the skin with all vehicles in neat for 4 h. The results indicate that water permeation has increased several times (Fig. 2) when compared to control. Water permeation from intact skin without any pretreatment was taken as control. For choosing a vehicle to enhance drug delivery across the skin, this inherent barrier alteration is a prerequisite, apart from solubility and/or partitioning enhancement of drug into skin. EtOH : PBS 1 : 3 was optimized as receptor phase to study PCL permeation through skin ex vivo. Skin barrier integrity was maintained up to 84 h in this receptor phase, suggesting its suitability to study drug permeation

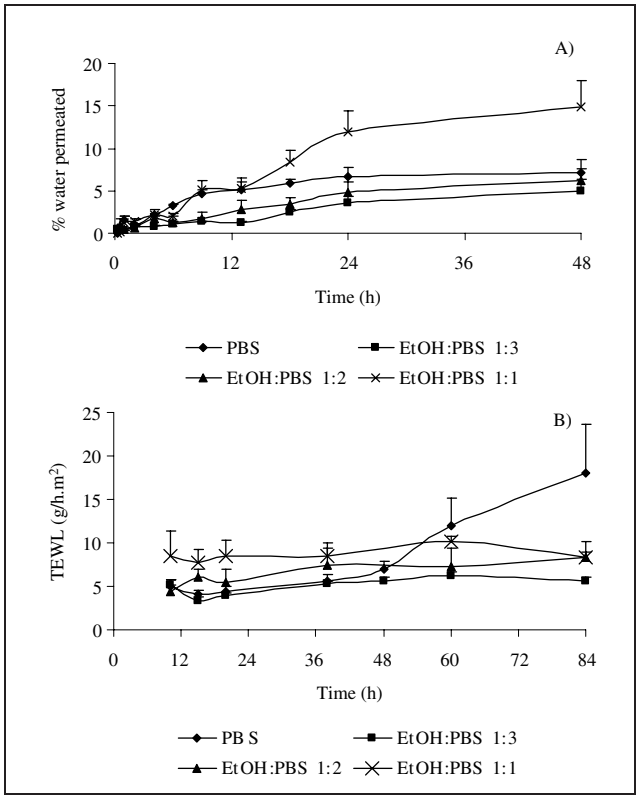

Fig. 1: Validation of receptor phase containing binary combinations of EtOH and PBS for skin permeation studies. (A) Using water permeation (B) With TEWL as probe
Key: EtOH-Ethanol; PBS-Phosphate buffered saline (pH 7.4). The ratios in the legend indicates the volumetric ratio of the components

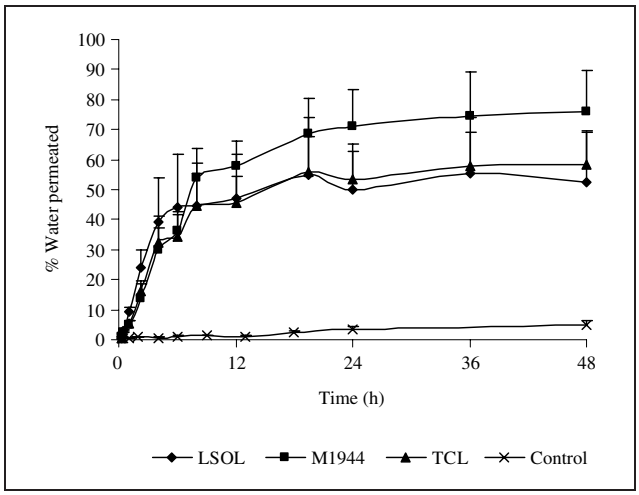

Fig. 2: Effect of vehicle pretreatment on water permeation through skin
LSOL-Labrasol; M1944-Labrafil M1944; TCL-Transcutol

even up to three days including 12 h initial skin acclimatization period. All vehicles tested were found to alter skin barrier, a prerequisite for vehicle selection to enhance drug penetration and/or permeation.

This study clearly demonstrates the significance validation of receptor medium in ex vivo transdermal permeation studies.

Experimental

The protocols for all the ex vivo and in vivo experiments performed on SD rats were approved by IAEC, NIPER. The radioactive material was handled in the premises approved for the purpose in accordance with the

statutory requirements of the AERB, GOI and the biological waste was disposed according to the IAEC, NIPER and AERB, GOI protocols. Skin penetration and permeation studies were performed according to protocols which were previously reported (Panchagnula et al. 2004). Ex vivo skin permeation studies were performed ($n = 4$) with unjacketed vertical type Franz diffusion cells with a diffusion surface area of 0.785 cm^2 and 5.2 ml of receptor cell volume, stirred at 800 rpm using a dry block heating and stirring module. Receptor compartments were filled with fluids containing binary combinations of EtOH and PBS (1 : 1, 1 : 2, and 1 : 3). In order to attain ~32 °C at skin surface, receptor phase was maintained at 37 ± 0.5 °C (Qvist et al. 2000). Frozen skin pieces were thawed to room temperature and mounted over diffusion cells with dermal-side in contact with receptor phase, equilibrated for 3 h, and then air bubbles were removed. Subsequently, donor compartments were filled with 300 l of ^3H_2O, and covered with laboratory film to prevent evaporation of donor phase. Samples (200 µl) were withdrawn until 72 h, at specified intervals from receptor compartment, followed by replacement with fresh receptor solution. At the end of the study drug was quantified in receptor samples by radiochemical method.

Transdermal epidermal weight loss (TEWL) was determined after equilibrating skin for 10 h. TEWL was measured up to 84 h with various receptor phases containing increasing concentration of EtOH in PBS, by placing the collared probe on the receptor compartment. Always the standard deviation of measurement was 2% of mean values collected over the previous 20 s. All measurements were performed in a single ventilated room having controlled temperature between 28 and 30 °C, and relative humidity of 38–40%. Vehicle influence on skin barrier was studied after pre-treatment with neat vehicle for 4 h. For this purpose, skin was first equilibrated with optimized receptor phase containing EtOH : PBS 1 : 3 for 12 h. After that, vehicles (500 µl) were applied for 4 h and then wiped carefully with tissue paper. Subsequently, ^3H_2O was applied (300 µl) and its permeation was studied for 48 h using the procedure mentioned above. Flux values (µg cm^{-2} h^{-1}) were calculated from slopes of steady state portion of the Cartesian plots constructed between cumulative amounts of water permeated (µg cm^{-2}) as ordinate and time (h) as abscissa. Lag times (h) were calculated by extrapolation of the steady state portion to abscissa. Flux and lag time were compared between various treatments using unpaired t-test at $P = 0.05$ (Sigmastat 2.03, SPSS Inc., USA).

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Inhibitory effects on the digestive enzyme α-amylase of three *Salsola* species (Chenopodiaceae) *in vitro*

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Hypoglycaemic effects of *Salsola kali*, *S. soda*, and *S. oppositifolia* (Chenopodiaceae) aerial parts were examined using *in vitro* assay based on the inhibition of α-amylase. The *S. kali* ethyl acetate fraction was the most active with a IC_{50} value 0.022 mg/ml. Through bioassay-guided fractionation processes two flavonol glycosides, isorhamnetin-3-*O*-glucoside and isorhamnetin-3-*O*-rutinoside, were isolated by silica gel column chromatography and characterized by spectroscopic methods. Isorhamnetin-3-*O*-rutinoside showed an interesting activity (IC_{50} 0.129 mM).

The plant kingdom is a wide field to search for natural effective oral hypoglycaemic agents that have slight or no side effects. More than 1200 plant species have been recorded to be used empirically for their alleged hypoglycaemic activity (Marles and Farnsworth 1995).

One therapeutic approach for treating diabetes is to decrease post-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise. A number of naturally occurring reversible glycosidase inhibitors are known such as nojirimycin, castanospermine, swainsonine, and acarbose, and these have been subjected to intensive study including the synthesis and testing of a number of analogues. *Salsola* species are well known in traditional medicine (Woldu and Abegaz 1990). In Russia *S. collina* was one component of the biologically active food additive "Heparon" recommended as a hepatoprotector when the hepatic cells are exposed to alcohol, medications and various toxins. It has also an anti-inflammatory and mild cholagogue effect. Aqueous extracts of *S. tuberculatiformis* have been used by bushmen women as an oral contraceptive (Swart et al. 2003). *S. soda* has been used as a food plant; the main edible parts are the buds. Chemical constituents of members of the genus have been studied to some extent. *S. kali* are reported to contain tetrahydroisoquinoline alkaloids such as salsoline and salsolidine, fatty acids, and flavonoids (List and Hörhammer 1969–1979; Tomàs