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Recent Physical Methods in Transdermal Drug Delivery Research

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This paper gives an insight into the new hi-tech analytical probes that are used for the biophysical measurements of the human stratum corneum, its barrier functions and lipid composition. Electron Microscopy is used for quantitative ultrastructural studies in the skin, and to detect many changes in the various layers and organelles of the skin after treatment with a penetration enhancer. ATR-FTIR is a powerful *in vivo* technique for studying the biophysics of skin functions, in particular, the intercellular lipid domains and the degree of hydration of the stratum corneum. EPR is a spectroscopic method of detecting unpaired electrons (paramagnetic species) and requires the presence of a paramagnetic nitric acid moiety. Nitroxide skin probes are used for measuring membrane fluidity and polarity, lipid - protein interactions, and to study the skin penetration of spin - labelled drugs non - invasively. Thermal analysis techniques such as DTA and DSC are used to investigate the physical properties of stratum corneum and the measurement of lipid and protein thermodynamic behaviour in model and biological membranes.

TRANSDERMAL permeation studies have become the foremost choice with the researchers interested in controlled drug delivery systems because of their non invasiveness and beneficial effects over many other delivery systems. Most of the studies which have been undertaken in this area have emphasized mainly on the drug kinetics or barrier studies of the skin. It has been widely recognised that the barrier properties of the skin reside mainly in the stratum corneum. Of now, passive and triggered transdermal drug delivery research is aimed specifically to overcome/bypass the barrier function of the stratum corneum. The recent trend is to study and find out the details of the various mechanisms explaining the passage of drugs through skin, or retention/metabolism of drug(s) in the skin.

The effect of various external stimuli such as heat, chemicals, magnetism, electricity and ultrasound, are gen-

studied by conventional methods of drug diffusion, both *in vitro* and *in vivo*. But now hi-tech analytical probes, for example, Electron Microscopy (SEM, TEM with superior tracer stains), Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR - FTIR) and Calorimetric methods (DTA, DSC) are being utilised by researchers to get a better insight into the structural changes and mechanisms involved. Some of these methods have been discussed in the following sections.

Electron Microscopy : It is high resolution microscopy. The resolving power (resolution) which is the limit where two closely spaced points can be distinguished as two distinct identities, is much higher in an electron microscope as compared with that achieved by light microscope. Now it is possible to achieve a resolution of 2.5 A by Transmission Electron Microscope. As the resolution depends on various physical properties of electrons such as wavelength, accelerating voltage and scattering power, different kinds of electron microscopes have been developed, for example, Transmission Electron Microscope (TEM), High Voltage Electron Microscope (HV - EM) and Scanning Electron Microscope (SEM). Electron

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microscopy has made significant contributions in the field of biological and biomedical sciences. With the advancements in instrumentation technology, they are also being used for quantitative ultrastructural studies. These studies help in finding out many changes in the various layers and organelles of the skin after treatment with any type of penetration enhancer. The process of tissue preparation and staining are important and skillful steps in electron microscopy. After tissue separation, the thin sections for electron microscopy are prepared in the following way. Samples consisting of thin sections are fixed overnight in 2 % gluteraldehyde with 0.06 % $CaCl_2$ in 0.1 M sodium cacodylate buffer, pH 7.4. After washing with the buffer the sample is fixed for 90 minutes in 2 % osmium tetraoxide containing 0.5 % potassium ferrocyanide. The sample is then dehydrated in a graded ethanol series and propylene oxide and further embedded in a low viscosity epoxy resin. Thin sections are obtained by using a microtome. These are further melted on a water bath to get wax free sections. Staining can be done either with uranyl acetate or lead citrate. These sections are then ready to be photographed by an electron microscope.

Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR)

This technique has recently been identified as a powerful *in vivo* technique, for studying the biophysics of skin barrier functions. Naruhito Higo *et al.*[1] have indicated that this technique has the potential to accurately quantify the distribution of transdermally delivered drugs in the stratum corneum. The application of ATR-FTIR to study the biophysical properties of the stratum corneum has been reported by Mak *et al*[2]., Bommannen *et al*[3]., Potts *et al*[4]. also. Yoshikazu et al[5]. have used this technique to examine the effect of fatty acids, fatty amines and propylene glycol on the molecular mobility of rat stratum corneum lipids and proteins[6].

In contrast to transmission spectroscopy, where the sample intercepts the path of the IR beam, in ATR, the sample is placed on an IR crystal, the geometry of which permits total internal reflection. Thus the IR beam is directed to the crystal (the internal reflection element, IRE) from which an evanescent wave exists and penetrates into the sample that is in contact with the IRE. The symmetric trapezoidal geometry of the optics then guides the beam back to the detector of the spectrophotometer. In this way a spectrum of human stratum corneum *in vivo* can be obtained[7]. FTIR spectra are reported to be collected in the frequency range 4400-400 cm^{-1}. IR spectroscopy can be used to identify biomembrane lipids on observing the C-H stretching absorbances from the methylene groups of the lipid acyl chains. For example, as the degree of disorder of the lipid acyl chain increases, the stretching absorbance undergoes a blue shift (shift towards a shorter wavelength). The shift in the peak of this band has been used thereof to monitor lipid freedom. The integrated intensity (area under the curve) of an infrared absorbance is directly proportional to the amount of the absorbing species[8].

Many authors (farinas *et al*[9]., Wurster *et al*[10]. Harrick[11]) have described the application of ATR-FTIR spectroscopy to diffusion studies in polymers and semisolids. Pellett *et al*[12], used diffusion cells and ATR-FTIR spectroscopy to monitor the permeation of a model compound, 4 - cyanophenol, across silicone membranes. The authors found a close correlation between permeability coefficients measured by the use of regular diffusion cells and ATR-FTIR spectroscopy. They suggested that the deconvolution of diffusioning and partitioning effects is more reliable when performed using ATR-FTIR spectroscopic technique.

Electron Paramagnetic Resonance (EPR) Imaging : EPR is a spectroscopic method of detecting unpaired electrons (paramagnetic species), for example, free radicals in heterogeneous biological materials with high specificity and sensitivity. The EPR technique is also used for measuring biophysical and biochemical parameters employing spin labels. The basic requirement for the spin labelling technique is the presence of a paramagnetic nitric acid moiety. The nitroxides are sensitive to motion, polarity, structural order and fluidity, oxygen tension, redox processes, bioenergetic factors and chemical reactivity of their biophysical surroundings. Therefore nitroxide spin probes are extensively used in molecular and cell biology for measuring membrane fluidity and polarity, lipid-protein interactions, pH, electrochemical gradients, surface potential and volumes. Nitroxides are also employed for spin labelling of drugs, thus allowing detection and biological interactions of the labelled compounds by means of EPR spectroscopy. Potential applications of EPR skin imaging include *in vitro* and *in vivo* studies on skin membrane fluidity and polarity, electron redox processes, reaction of specifically targetted

nitroxides with reactive chemical groups and so on. Furthermore, liberation, penetration and distribution of labelled drugs can be analyzed noninvasively in animal and human skin. EPR skin imaging is novel approach in experimental dermatological research and will considerably stimulate progress in skin pharmacology. Fuch et al[13]. investigated skin penetration of spin labelled drugs such as estradiol and procaine, by EPR imaging. They used a skin-permeable and skin-impermeable drug to validate the penetration measurements.

Thermal Analysis : In the last few years there have been several reports in which thermal analysis techniques have been used to investigate the physical properties of stratum corneum[14]. Bulgin and Vinson[15] detected two different types of water in the stratum corneum, using "Differential Thermal Analysis (DTA)". Walkely[16] used nonfreezable water in neonatal rat stratum corneum. Wilkies et al.[17] used DTA in conjunction with X-ray diffraction techniques to study the structure of the crystalline lipid component of the human stratum corneum. Van Duzee[18] developed a thermal analysis technique to detect the melting of lipids and denaturation of proteins in the stratum corneum. Transitions were observed at 40° and 75°, 85° and 107°. The transitions at 40° and 75° were attributed to the melting of the lipids. The transitions at 85° was identified as due to denaturation of a nonfibrous protein which changed the state of water absorbed and that water contributed to the ordering of the lipid and protein. The thermal analysis technique may prove to be of value in studying the interaction of materials with the skin.

In DSC, the sample and the reference containers are heated separately by individual coils that are heated (or cooled) at the same rate. Platinum resistance thermometers monitor the temperature of the sample and the reference holders and electronically maintain the temperature of two holders constant. If a thermodynamic event occurs, which is either endothermic or exothermic, the power requirements for the coils maintaining a constant temperature will differ. This power difference (P) is plotted as a function of the temperature recorded by the programming device. The parameter measured in DSC is the heat flow, dH/dt or the differential or excess specific heat as a function of sample temperature. If the lipid-protein complex recorder pen should trace a straight horizontal baseline it indicates a zero differential heat output. If a thermal event occurs, the recorder pen is deflected from the baseline. the direction of the deflection depending on whether the event is endothermic or exothermic and the magnitude of deflection depending on the amount of excess specific heat absorbed or liberated.

Using a new, high sensitivity differential scanning calorimeter capable of very slow scanning rates and large sample volumes, Selwyn et al[19]. examined the thermal transitions in neonatal mouse stratum corneum.

When properly applied, DSC is probably the single most technique for the measurement of lipid and protein thermodynamic behaviour in model and biological membranes. The instrumentation required is comparatively inexpensive, the measurements can normally be made relatively quickly and the interpretation of the data obtained is fairly straight forward. Also the currently available high sensitivity DSC instruments require only relatively dilute suspensions of the material, permitting accurate control of pH and ionic composition of the aqueous phase. Unlike some other techniques which have been utilised to study lipid and protein thermotropic behaviour, DSC accurately reports the entire course of broad phase transition. Moreover unlike a number of spectroscopic techniques, DSC does not require the introduction of foreign probe molecules into the system under study. This technique is unique in providing a direct and accurate measurement of the thermodynamic parameters of the phase transitions under investigation. However, this technique does not provide direct information about molecular structure and dynamics. This can be done by a combination of DSC with a direct structural technique such as Nuclear Magnetic Resonance (NMR), Infrared or Raman Spectroscopy which provides a very powerful approach to understand thermal events in membrane systems at the molecular level.

The skin permeability modifications can also be determined by other noninvasive techniques such as the transepidermal water loss determination method. Noninvasive blood flow measurements can be done by Laser Doppler Velocimetery which allows to evaluate the skin pharmacokinetic modifications by measuring the lag time before vasodilatation and after the application of vasoactive agents.

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