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Short communication

Surface enhanced Raman scattering (SERS) study of membrane transport processes

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Due to the excellent barrier properties of the stratum corneum, the outerlayer of the skin, it is usually important to enhance drug penetration to ensure that therapeutic tissue levels are achieved for local, regional or transdermal topical therapy. At a fundamental level, for skin and other membranes, understanding of how physical properties influence transport is essential to predict and enhance penetration. Structure-penetration relationships and mechanism of action of chemical enhancers (formulation excipients which increase drug penetration) have been developed based on the principals of Fickian diffusion of partitioning into and diffusion through the membrane. For example, Kasting et al. (1987) showed that skin penetration could broadly be predicted from lipid solubility (partition coefficient) and molecular size. More recently, (Abraham et al., 1995; Roberts et al., 1996) hydrogen bonding has been identified as a major determinant of diffusion across stratum corneum.

Investigation of stratum corneum membrane permeability is usually carried out in vitro by determination of diffusion profiles using Franz-type diffusion cells (Barry et al., 1995). This technique allows the determination of permeability coefficients from which partition and diffusion coefficients can be derived from the slope or the steady state flux and the lag time (Barry et al., 1995). Measurement of lag times of less than 1 h are not normally feasible with this system and the time required to obtain sufficient data can range from 24 h to 1 week. In order to measure the amount and rate of transport of diffused material the method requires removal of small samples of a receiving solution, thus causing disruption to the system. Also, because of its nature, transport across fully-hydrated stratum corneum is determined which does not represent the in vivo condition.

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Attenuated total reflectance fourier transfer infrared spectroscopy (ATR-FTIR) has recently been used to examine diffusion through synthetic membranes (Watkinson et al., 1995) and has had some success in allowing the deconvolution of diffusional and partioning phenomena where the diffusion path length is known. The technique allows more rapid sampling (typically 3 min between scans) which increases accuracy of determining time-lag. In order to improve understanding of the penetration process, further techniques to measure and deconvolve partioning and diffusion phenomena are required.

Surface enhanced Raman scattering (SERS) has previously been used to measure diffusion through thin films deposited from the gas phase or from solution onto bulk silver substrates, (Blue et al., 1989; Hong et al., 1991) but here we report a method suitable for measurements on preformed membranes, which uses colloidal dispersions of silver particles as the SERS substrate. For SERS-active molecules the SERS technique enables discrimination between different compounds and measurement of their respective interfacial arrival times and concentration growth rates in a receiving solution through a chosen membrane. Although molecules vary considerably in their ability to participate in the SERS effect, molecules which possess unsaturation, especially delocalised π systems associated with benzenenoid or unsaturated heterocyclic structures, and which adsorb at silver or gold surfaces, appear in general to show high SERS intensity. A large fraction of molecules of pharmaceutical interest contain such unsaturated cyclic systems, and we believe therefore that this method will have wide applicability in both fundamental and industrial membrane (e.g. skin) transport studies.

This first set of experiments demonstrates that SERS can be used to observe interfacial arrival times and that the derived data are reproducible under the given experimental conditions.

In this preliminary report the diffusion of the model compound pyridine through a Silastic (polydimethylsiloxane) membrane of thickness 0.02″ (supplied by SmithKline Beecham, Consumer Healthcare, Weybridge, Surrey) was studied with a silver colloid used as the SERS substrate (Creighton et al., 1979). For the colloid to be SERS active the particles have to be aggregated and it has been shown that aggregates which absorb light in the red region of the spectrum support the strongest SERS (Blatchford et al., 1982).

The membrane was supported on a holder which was placed in the path of a helium-neon 632.8 nm laser (power 9.4 mW). Silver colloids were prepared by the reduction of silver nitrate solution by sodium borohydride (Creighton et al., 1979) and were aggregated by the addition of magnesium chloride solution. A 10 μl volume of aggregated colloid suspension was placed on the underside of the membrane and the laser focused close to the interface using 244 cm^{-1} SERS band due to adsorbed chloride ions as an internal reference. The SERS spectrum was acquired with a liquid nitrogen-cooled CCD multichannel detection system with an integration time of 8 s and slit width of 200 μ. This gave a time difference between scans of 10 s. After scan 1, 1 μl of pure pyridine was applied to the top side of the membrane. Fig. 1 shows typical sequential SERS spectra of pyridine. A representative diffusion profile obtained for these systems is shown in Fig. 2. In Fig. 2, t_A is the time of the first detectable signal of a molecule at the interface. The intensity of the signal remains relatively constant before an increase in intensity/concentration is observed at t_G. In eight experimental runs t_G was consistently determined as 180 ± 20 s showing that, for this experimental procedure, the results are repro-

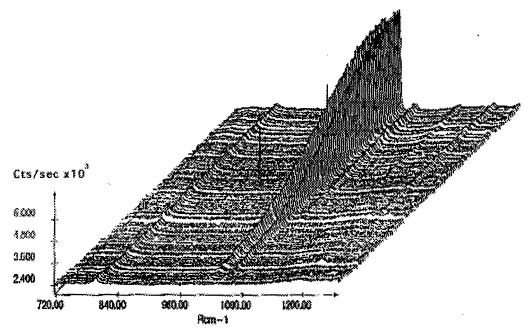

Fig. 1. Sequential spectra showing the arrival and growth in concentration of pyridine following the movement through a 0.20″ Silastic membrane.

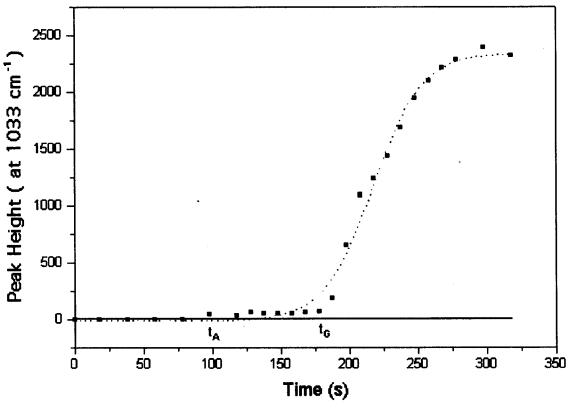

Fig. 2. Plot of intensity against time for the experiment described in the text. The figure shows the first detectable signal at t_A and rapid increase of intensity at t_G (from the back extrapolation of the slope of the graph).

ducible. The growth in concentration of pyridine in the receiving solution is measured and from these measurements it is possible to obtain the concentration, rate and diffusion coefficient of the penetrant.

It appears, from these observations, that two mechanisms of diffusion are observed, a rapid diffusion perhaps through channels or pores within the membrane structure (t_A) and a diffusion through the bulk of the membrane giving rise to a slower onset of growth of penetrant (t_G) but responsible for the greater part of the mass transport. Franz cell studies only measure an overall concentration growth in a receiving solution, from which an estimate of an arrival time may be calculated via back extrapolation but from which interfacial arrival times are not accessible.

Calculation of the diffusion coefficient through the 0.02 membrane is in the order of 10^{-9} cm^2 s^{-1}; the same order as would be expected for diffusion of small molecules in water. A mixed system of 2 M pyridine and 0.1 M diphenyldisulphide (DPDS) in ethanol solution applied to the top of the silastic membrane in place of pyridine was also investigated. This produced spectra in which the individual interfacial arrival times of both pyridine and DPDS could be distinguished. (Fig. 3). This result shows that using SERS it is possible to discriminate between the movement of different molecules across a membrane and to observe different interfacial arrival times (t_A values) and concentration growth rates in the receiving solution (from t_G onwards).

Thus and importantly, SERS offers a rapid non-invasive method for study of interfacial arrival times of molecules at the receiving interface of a membrane i.e. it can record the time taken for a molecule to travel through the membrane i.e. a true rate. The system is not dependent upon the removal of quantities of receiving solution as in Franz cell-based studies, nor on the long lag times which are typical of that technique. Neither does it have the restriction of sufficient close contact (approximately one infrared wavelength) between crystal and membrane required in ATR-FTIR nor is it affected by a change in the refractive index of the membrane due to the solvation of penetrant in the membrane as can occur in ATR-FTIR. In the technique described here the only constraint is that the diffusing molecule be SERS active. For transdermal diffusion studies the most important component that is required to be SERS active is the drug itself. If an enhancer is not SERS active the effect it has on the transport of the drug through the membrane can still be measured by a decrease or increase in t_A and/or t_G.

This technique has many applications in studies of the diffusion of molecules through both biological and synthetic membranes, such as: the transport of drugs through membranes from topical formulations; the release of drugs from synthetic

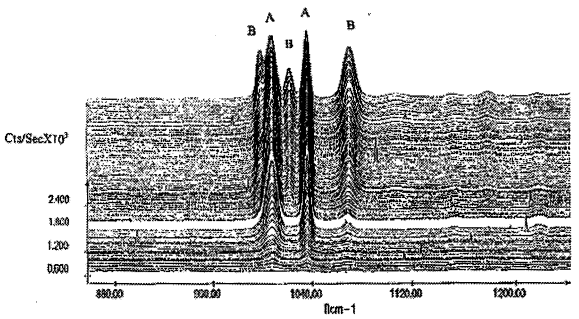

Fig. 3. Sequential spectra of the arrival and growth of the mixed solution of pyridine and DPDS. It can be clearly seen that pyridine (A) arrives before DPDS (B) and also has a faster intensity growth rate.

delivery systems such as patches and polymer implants; the diffusion of agrochemicals from packaging material; the effectiveness of barrier protection; the transfer of plasticisers, contaminants from/through food packaging. All of these areas require an understanding of the effectiveness of the barriers, either synthetic or biological, that have been constructed to afford separation of different chemical systems.

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