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The use of second-order derivative UV spectroscopy to monitor the percutaneous absorption of naphazoline and oxprenolol

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Summary

Derivative spectroscopy was used to measure the transfer of the cationic drugs, oxprenolol and naphazoline, across full-thickness human skin in vitro. Flow-through diffusion cells were employed. These allowed the receptor fluid to be pumped through a microprocessor-controlled UV spectrophotometer. Spectra were then recorded at preprogrammed intervals. From the second-order derivative curves it was possible to evaluate the concentration of each diffusant within the receptor phase. Confirmation of the technique was established using HPLC. The steady-state fluxes of both drugs were measured. The diffusion of naphazoline and to a lesser extent oxprenolol were enhanced in the presence of lauric acid. The use of derivative spectroscopy in this manner provides a rapid, labour-saving technique for the continuous monitoring of the absorption of various drugs across excised skin.

Introduction

The measurement of in vitro permeation rates for various drug molecules across skin is becoming important both in the testing of potential candidates for transdermal drug delivery and for the evaluation of cutaneous toxicity.

The rate of drug absorption across skin has in the past been studied using high-performance liquid chromatography (HPLC) and radiotracer studies. Unfortunately, both methods suffer from drawbacks. Firstly, HPLC analysis is time-consuming and often too insensitive. In the second technique radiolabelled permeants are expensive and not always readily available. In addition, skin metabolites cannot be easily detected and labels can be exchanged within the skin. Both methods also require regular manual sampling and analysis.

Conventional ultraviolet spectroscopy is unsuitable for drug analysis in a skin diffusion cell. Water-soluble proteins and amino acids cause a continuous increase in absorbance towards shorter wavelengths (Fig. 1). Thus, the UV absorbance of a permeating drug molecule becomes a shoulder on the broad absorption band (Fig. 2) (Green et al., 1987).

Derivative spectroscopy has been used to eliminate broad absorption bands resulting from turbidity and matrix interference; Fell et al. (1981) used second-order derivative spectroscopy to assay paraquat in serum, plasma and dialysis fluid.

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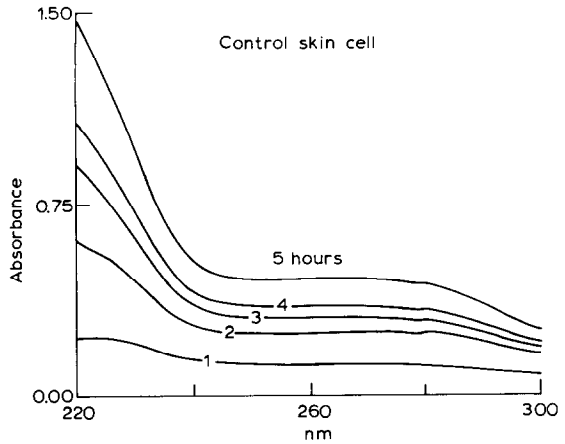

Fig. 1. The hourly increase in absorbance of the receptor phase of a skin diffusion cell.

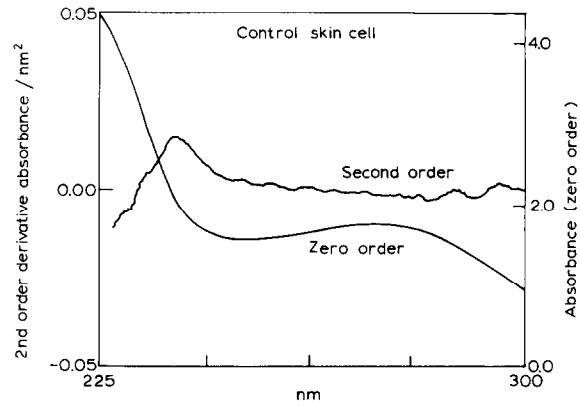

Fig. 3. Zero and second-order derivative spectra of the receptor phase of a control skin diffusion cell after 48 h. The lower curve shows the zero-order derivative.

In second-order derivative spectroscopy the spectra is differentiated twice with respect to wavelength, with the result that broad bands are suppressed relative to sharper absorption bands. Consequently, background interference is greatly reduced (Fig. 3). In this paper the use of derivative spectroscopy as a continuous automated technique for the measurement of the percutaneous absorption of the cationic drugs oxprenolol and naphazoline is described. The two molecules were chosen since they possess sharp UV absorbance peaks. In addition the effect of lauric acid as a 'penetration enhancer' was evaluated. Lauric acid is known to increase the transdermal flux of naloxone hydrochloride across human skin in-vitro (Aungst et al., 1986). In an earlier study its effectiveness as an ion-pairing agent for various cationic molecules has been demonstrated (Green and Hadgraft, 1987).

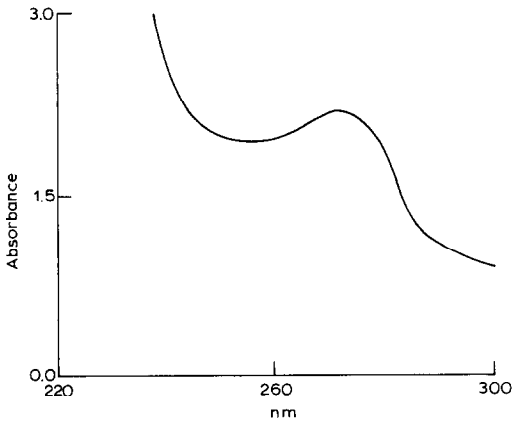

Fig. 2. Absorbance of oxprenolol hydrochloride in the receptor phase of a skin diffusion cell after 45 h.

Theory

If the Beer–Lambert law (Eqn. 1) is obeyed over a specified concentration range, then Eqn. (2) can be used to quantify a drug in the nth derivative order.

$$A = \epsilon.C.L \tag{1}$$

$$\frac{d^n A}{d\lambda^n} = \frac{d^n \epsilon.C.L}{d\lambda^n} \tag{2}$$

where A is the absorbance of the drug at wavelength λ, ϵ is the molar extinction coefficient and L the cell path length.

Fig. 4 shows the zero and second-order derivative curves for oxprenolol hydrochloride. The upper curve shows the second-order derivative. The minima of this curve at A and C corresponds to maxima in the lower zero-order curve.

Oxprenolol concentration can be determined from the second-order curve by measuring the displacement of each minimum from their adjacent maximum at B and D (the spectrophotometer

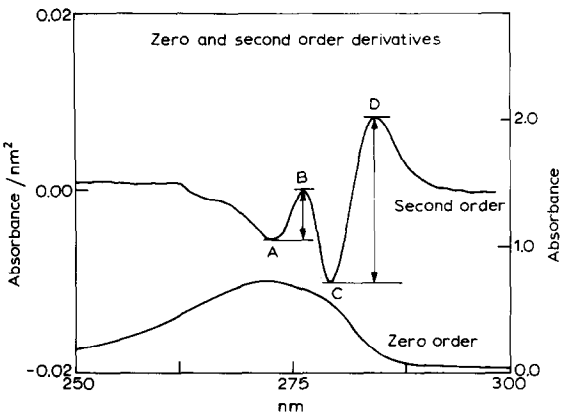

Fig. 4. The zero and second order derivative spectra of oxprenolol hydrochloride (180 μM) in pH 7.4 phosphate-buffered saline.

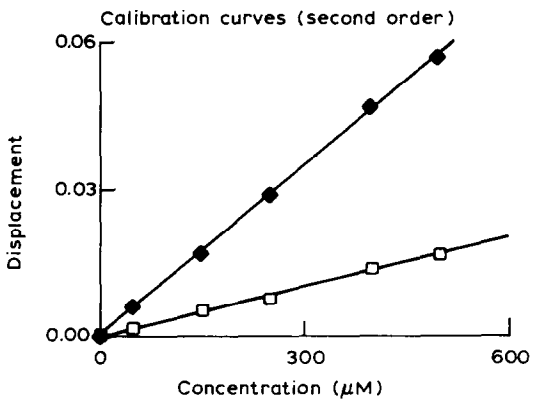

Fig. 5. Second order derivative calibration curves for oxprenolol hydrochloride for displacement AB (□) and CD (◆).

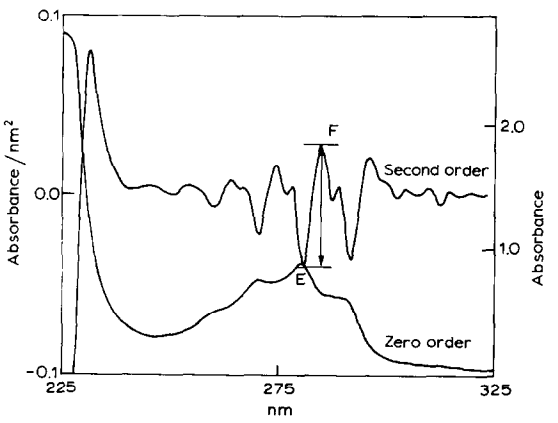

Fig. 6. The zero and second order derivatives of naphazoline hydrochloride (140 μM) in pH 7.4 phosphate-buffered saline.

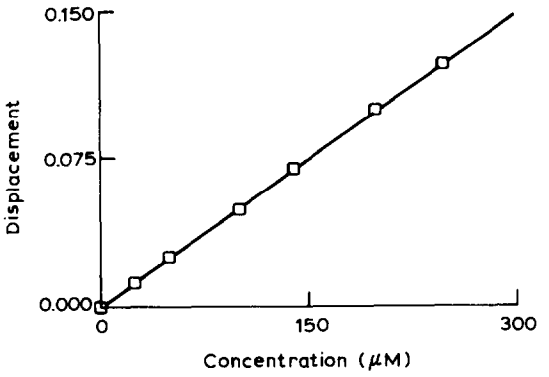

Fig. 7. Second order derivative calibration curve for naphazoline hydrochloride in pH 7.4 phosphate-buffered saline.

records the maximum and minimum values of the second-order curves in absorbance · nm^{-2} units). This method is used to suppress any background interference. If the displacements AB and CD are measured as a function of concentration then the Beer–Lambert plot can be shown to be linear (Fig. 5).

Naphazoline can be quantified in the same manner as oxprenolol by measuring the displacement EF (Figs. 6 and 7).

Materials and Methods

Materials

Naphazoline hydrochloride was obtained from Aldrich Chemical Company, Oxprenolol hydrochloride was a gift from Ciba-Geigy (Horsham, U.K.). All reagents were of GPR grade and supplied by BDH Chemicals, except for lauric acid which was biochemical grade, and absolute ethanol (James Burrough Ltd). Cellulose nitrate 0.45 μm pore size membrane filters were obtained from Whatman Ltd. Reagents used for HPLC were methanol (HPLC grade, Rathburn Chemicals Ltd.), heptane sulphonic acid (Fisons) and acetic acid (Aldrich Chemical Co.).

Preparation of skin membranes

Abdominal strips of caucasian cadaver human skin were obtained at autopsy, from a number of female and male donors (age 64–88). The subcutaneous fat was carefully trimmed away from the

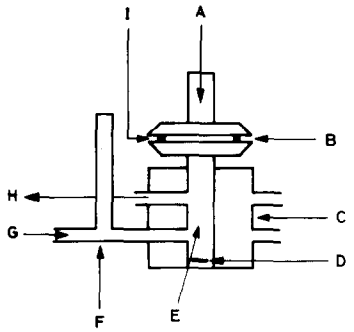

Fig. 8. Flow-through penetration cell. A, donor phase; B, skin membrane; C, water jacket; D, magnetic stir bar; E, receptor phase; F, T-joint bubble trap; G, receptor phase input; H, receptor phase exit to UV flow cell; I, O-ring.

dermis. Remaining fat was carefully removed by blunt dissection and swabbing with pH 7.4 isotonic phosphate-buffered saline. The stratum corneum was gently swabbed with the donor phase (pH 8.0 phosphate–citrate buffer) to remove any contaminants. The skin membranes were either used immediately or stored frozen for a maximum of 7 days.

In vitro skin diffusion cells

Skin membranes were placed between the stoppered donor and receptor compartments of a skin penetration cell (Fig. 8) (model LG-1083-PC, LGA skin permeation systems, CA, U.S.A.). An O-ring mount, situated around the outer edge of the membrane, was used to seal the skin to the penetration cell. The cells had a cross-section area of 0.8 cm^2. The water jacket of the penetration cell is connected, via a manifold, to a circulation water bath to maintain the receptor phase at 37 °C. The skin surface temperature was measured and found to be 32 °C. The receptor phase contained pH 7.4 phosphate-buffered saline (ca. 15 cm^3). A peristaltic pump (Watson-Marlow 502s multichannel cassette pumphead) was used to allow the receptor phase to flow continuously, through a flow cell, situated in a Uvikon 860 microprocessor controlled UV spectrophotometer (Kontron Instruments).

The receptor phase was boiled then filtered under vacuum, prior to use, using a cellulose nitrate 0.45 μM pore size membrane filter. T-piece bubble traps were placed before the fluid intake port to prevent air bubbles from entering the receptor compartments. This minimised problems of air bubble formation under the skin surface.

Skin permeation

The penetration cells were allowed to equilibrate with the receptor phase, at 37 °C, for 1 h, prior to the addition of 50 μl of an ethanolic solution of lauric acid (0.1 M) to the skin surface. After a 2-h period the alcohol had evaporated to leave a thin film of the fatty acid deposited in the outer layers of the skin. A solution containing the permeant (2 ml) was then introduced, into the stoppered donor phase (0.05 M solution in pH 8.0 phosphate–citrate buffer).

Control cells were prepared where the skin surface was treated with 50 μl of ethanol without lauric acid.

Spectra were recorded automatically, between 225 and 325 nm, at preprogrammed 3-h intervals for 48 h. The spectrophotometer was allowed to scan at a speed of 100 nm min sampling at 0.25 nm intervals. These parameters were found to be optimal for the analysis of oxprenolol and naphazoline. At higher sampling intervals the resolution decreased, whereas at lower intervals the available curve memory within the microprocessor was reduced.

At the completion of each experiment, the concentration of the drug in the receptor phase was determined by direct comparison with the appropriate calibration curves. The concentration was plotted as a function of time and the slope of the steady state region was determined by linear regression analysis. Lag times were evaluated by extrapolation of this linear portion back to the time axis.

Permeability coefficients, K_p, (cm/h) were evaluated from Eqn. 3:

$$J = K_p A C_d \quad (3)$$

Where J (mol/h) is the steady-state flux of the solute traversing the skin membrane of cross-sectional area A (cm^2) and C_d, the initial concentration of the drug in the donor phase.

TABLE 1

Reverse phase chromatographic conditions required to analyse skin permeants

Drug molecule	Mobile phase	Detector wavelength (nm)	Flow rate (ml/min)	Retention time (min)
Oxprenolol hydrochloride	0.005 M heptane sulphonic acid in 750 ml methanol, 5 ml acetic acid and 245 ml distilled water	273	1.5	4.8
Naphazoline hydrochloride	0.005 M heptane sulphonic acid in 950 ml methanol 5 ml, acetic acid and 45 ml distilled water	280	2.5	3.7

Each experiment was performed in triplicate using skin samples from the same donor.

High performance liquid chromatograpy (HPLC)

Drug analysis using the second-order derivative techniques was validated using HPLC. The system comprised of a UV variable wavelength detector (LDC Spectromonitor III, Milton Roy), a dual piston pump (model III G constametric, LDC) and an Apex, 5 μm particle size octadecyl silica reverse-phase chromatographic column, 250 mm $\times$ 4.6 mm i.d. (Jones Chromatography). Table 1 lists the other chromatographic parameters.

Samples were removed from the receptor phase and injected directly into a fixed volume Rheodyne sample injector with a 100 μl loop. The peak areas were evaluated using an LDC computing integrator. The amounts of oxprenolol and naphazoline were quantified by direct comparison with a standard calibration curve. The assay value of each sample was obtained as a mean from two separate injections.

Results and Discussion

Figs. 9 and 10 show the effect of lauric acid on the in vitro percutaneous absorption of oxprenolol and naphazoline, respectively, as determined by second-order derivative spectroscopy. The transdermal flux of naphazoline, and to a lesser extent oxprenolol, was enhanced in the presence of lauric acid. The lag times of both of the drug molecules were not affected by the fatty acid (Table 2). The precise mechanism in which lauric acid enhances drug permeation across skin is currently under study. The mean assay values determined by second-order derivative spectroscopy were found to

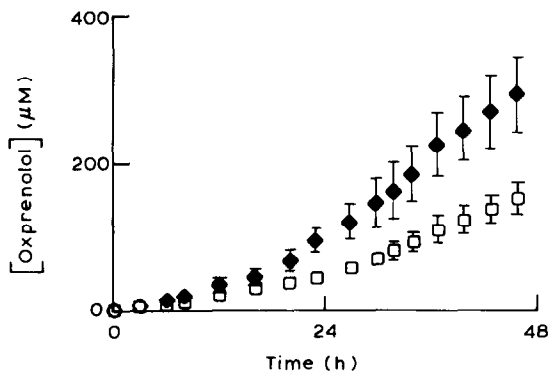

Fig. 9. In-vitro percutaneous absorption of oxprenolol (±S.D.) following skin pretreatment with 100 mM lauric acid (◆) and ethanol as control (□).

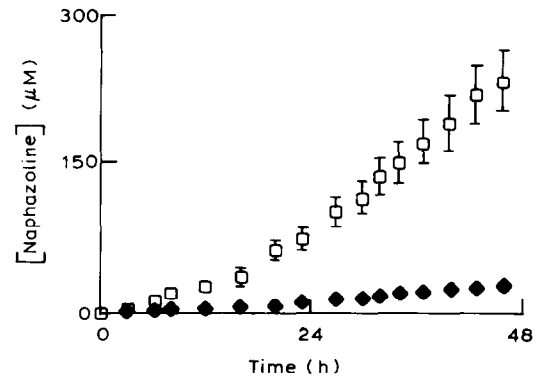

Fig. 10. In-vitro percutaneous absorption of naphazoline (±S.D.) following skin pretreatment with 100 mM lauric acid (□) and ethanol as control (◆).

TABLE 2

Effect of lauric acid on the transfer of oxprenolol and naphazoline across excised human skin (n = 3)

Permeant	Skin pretreatment	Lag time (±S.D.) (h)	Permeability coefficient (K_p) (±S.D.) ($cm/h \times 10^{-4}$)
Oxprenolol hydrochloride	Control	13.6±1.8	17.8±3.5
	0.1 M lauric acid	12.9±1.7	33.9±6.3
Naphazoline hydrochloride	Control	10.6±2.0	3.1±0.7
	0.1 M lauric acid	12.3±1.9	26.6±5.1

correlate well with values obtained by HPLC. Figs. 11 and 12 show the correlation for naphazoline ($r = 0.994$, slope = 1.037, intercept = −5.59, $n = 10$) and oxprenolol ($r = 0.996$, slope = 0.980, intercept = −1.20, $n = 10$), respectively.

Apart from the advantages of using flow-through diffusion cells, as opposed to the traditional static Franz cell (Bronaugh and Stewart, 1985), this method of analysis also has the ability to sample the receptor fluid at frequent regular intervals. This allows for a more accurate evaluation of permeability coefficients and lag times from the linear steady state region of the flux profiles.

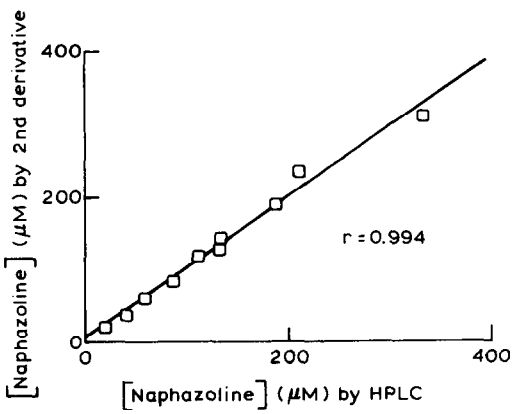

Fig. 11. Correlation of HPLC and 2nd derivative assay results for naphazoline in the receptor phase of a skin diffusion cell.

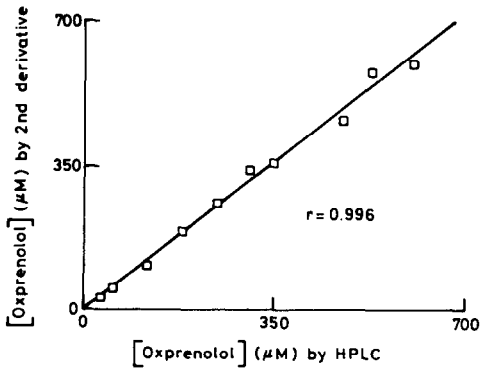

Fig. 12. Correlation of HPLC and 2nd derivative assay results for oxprenolol in the receptor phase of a skin diffusion cell.

The findings of this paper confirm that derivative spectroscopy can be conveniently and accurately used to monitor automatically the in vitro percutaneous absorption of oxprenolol and naphazoline across full-thickness human skin.

Acknowledgements

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