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Laser velocimetry for the non-invasive assessment of the percutaneous absorption of nicotinates

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Summary

The use of laser velocimetry for the non-invasive assessment of the effects of topically applied solvents and a model vasodilator, hexyl nicotinate, is reported. The importance of excluding solvent effects on the recorded response is emphasised and the value of the technique for predicting the effects of formulation changes is illustrated.

Introduction

The topical route of drug delivery has attracted much recent interest. In order to make best use of the opportunities which this route offers there is a need to understand both the physicochemical and the biochemical bases of transcutaneous transport. Examples of work carried out to these ends are those reported by Scheuplein and Blank (1973), Barry et al. (1985), Fox et al. (1979), Hadgraft (1983) and Cheung et al. (1985). Although much fundamental information can be abstracted from such studies involving the use of model systems there is still a need to study transdermal absorption in situ using non-invasive methods. The value of this alternative approach is shown by the success of the in situ skin blanching method for the assessment of steroid delivery and activity (McKenzie and Atkinson, 1964; Barry and Woodford 1974). A number of alternative methods including measurement of skin impedance (Kohli et al., 1985) and laser velocimetry (Guy et al., 1983) have been assessed with variable degrees of success. The latter appears to be highly promising for the assessment of the activity of drugs with a high degree of vasoactivity and is the subject of this report. In particular, aspects which are considered in this report are (a) identification of parameters which affect the response recorded on the laser Doppler meter given that an end objective is the use of the equipment for monitoring the performance of cosmetic and drug formulations and (b) evaluation of physicochemical parameters affecting transcutaneous transport of drugs and cosmetic agents using vasodilator nicotinate esters as model compounds.

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Theory

The theory of laser velocimetry or flowmetry has been well described by several authors (Nils-

son et al., 1980; Ware, 1981). When applied to monitoring cutaneous blood flow the coherent light impinging on the stationary structures are reflected back at the same frequency. Light reflected by moving blood cells, however, undergoes a frequency shift, a phenomenon known as the Doppler effect. The end result is a spectral broadening of the almost monochromatic laser light. Part of the back-scattered light is directed towards suitable photodetectors for subsequent electronic translation into electrical signals. The magnitude of the frequency shift can be related to the velocity of the moving particles thus providing the basis for relative quantification. Background noise from the environment, photodetector and the laser source itself decreases sensitivity and make measurements of low flow rates difficult. To reduce this problem some instruments, such as the one used in this study, gather light from two adjacent sites. Differential analysis of the two signals then leads to significant improvement in signal-to-noise ratio. The term flowmetry is often used instead of velocimetry for systems with this differential analyser but this appears to be confusing and has been used purely in a commercial context.

Materials and Methods

Measuring assembly

Laser-Doppler velocimetric (LDV) measurements were carried out using a Periflux PF 1d laser Doppler flowmeter (Perimed KB, Stockholm, Sweden). The instrument uses a 2-mW helium-neon laser operating at 632.8 nm. The light is brought to the skin surface via an optical probe. The probe head is held in a holder attached to the skin surface with double-sided adhesive tape. The approximate surface area illuminated by the laser is 28 mm^2.

The output signal from the flowmeter was recorded on paper using a pen recorder (LKB 2210 Recorder, Bromma, Sweden). The recorder speed was set at 0.1 mm/s. The recordings were made at instrumental settings of $\times 10$ gain, 4 kHz frequency limit and a 3-s time constant.

Procedure

The volunteer subjects chosen were of both sexes in the age range 20–26 years. They were fully informed of the nature of the study and the procedures involved. They were placed in a room in an adjustable reclining chair where measurements were carried out on the volar area of the forearm. The measurement room was air-conditioned and the relative humidity was closely monitored. The temperature of the room was maintained at $22 \pm 2°C$, while the humidity was measured to be 40–60% during the period of experimentation. Daily humidity changes were found to be negligible.

The various products tested were applied on the skin surface through the probe holder. Since the quantities applied were small (usually 5 μl) the products were applied using a microsyringe. After the application of a product the probe was inserted in its holder and LDV measurements were made. Due to the design of the probe holder the probe head remained 0.8 mm above the skin surface, thus ensuring that the product would not occlude and the risk of tissue compression was reduced.

High-performance liquid chromatography (HPLC)

The nicotinate esters were assayed by HPLC using reversed-phase chromatography with acetonitrile as the mobile phase and a 4.6 mm $\times$ 25 cm Whatman PXS-1025 ODS-2 column. Peak detection was by flow spectrophotometry at 265 nm. Calibration curves to enable interpolation of unknown concentrations were constructed using peak height as the concentration-dependent response.

Partition coefficients

The partition coefficients of the nicotinates were determined by shaking equal volumes of nicotinate containing solution in kerosine (grade of petroleum, with a distillation range 200–240 °C, used in cosmetic formulations) or isopropyl myristate and monopropylene glycol. All the solvents were presaturated with the partitioning solvent and vice versa, before use. The nicotinate content of both phases were measured by HPLC. The partition coefficient could then be calculated. Three initial concentrations (0.025, 0.0125 and 0.00625 M) were used to exclude the possibility of concentration-dependent partitioning.

Results and Discussion

Since pharmaceutical formulations are usually combinations of drug and excipients, the first part of the study was concerned with evaluation of any response produced by solvents which could be used as carrier for the drugs to be investigated. Water, polyethylene glycol, monoethylene glycol and kerosine were used as typical hydrophilic and hydrophobic solvents.

Kerosine, glycerine, monopropylene glycol and the polyethylene glycols did not exert any activity detectable by the LDV. Water however was found to produce an effect which was highly inconsistent. Some individuals indeed showed no response and on certain days even those previously responsive to application of water failed to do so. This positive response to water contradicts the results reported by Wahlberg (1984), possibly because in the latter study not enough test subjects were studied and therefore the inconsistent effect could easily have been missed. An alternative explanation is that in Wahlberg's study water was applied for 5 min and then wiped off before initiating the measurements. In the present study, 5 μl of water was applied and left on while the readings were taken. If a slow diffusion step is involved then the present experimental protocol increases the likelihood of seeing such delayed effects. An attempt to repeat Wahlberg's experiment however still led to contradictory results in that positive responses were observed in some cases.

The positive response to water is not easily explainable but it is possible that by hydrating the skin, the water induces localised temperature disturbance and therefore secondary vasodilation. To study this effect further, a series of linear aliphatic alcohols were applied to the skin and any induced response monitored by LDV. The alcohols also

TABLE 1

Variability in LDV response following application of a series of alcohols and water to the skin of the forearm

The symbols Δ, −, * and + refer to repeat experiments on the same subject on the same day. Values are times to onset of response (t_o)

Subject	Solvent									
	Ethanol	Propanol	Butanol	Pentanol	Hexanol	Heptanol	Octanol	Nonanol	Decanol	Deionised water
1 (LE)	540	1120		1270	500			> 1800	> 1800	300
										456
2 (MF)	230	820		600		320			> 3600	180
										780
3 (DG)		> 1800*		1250*		> 1800Δ	> 1800Δ	> 1800Δ		
		> 1800 +		> 1800 +		> 1800 +				
4 (DF)		> 1800*		> 1800*	370	350				
		> 1800 −		> 1800 −						
		> 1800Δ	> 1800Δ	> 1800Δ	1370 +	> 1800 +	> 1800 −			
5 (SL-S)	330		> 1800					> 1800		1320
6 (AZ)	600					200				350
										670
7 (RK)	430	> 1800*		570*						
	> 1800 +	> 1800 +	> 1800 +							> 1800
8 (NA-K)	540		> 1800		280					300
9 (SM)		1460		> 3600						
10 (CR-P)				> 1800*	740*					> 1800
11 (MR)					700*	> 1800*	1200			> 1800
12 (AC)							600			470
13 (JW-E)			> 1800				> 1800			
14 (CB)	640									

produced a highly inconsistent effect (Table 1). Again the results reported by Wahlberg contradict ours in that ethanol was said to be inactive in this respect.

The inconsistent response induced by the alcohols makes it difficult to detect any underlying change in the kinetics of the response as one progresses up the homologous series. The validity of the trend shown by the t_0 values, the time taken for the response to appear, would need to be confirmed by a much larger study. It should be noted that although 14 subjects were tested, because only a few of the individuals tested responsed, the variance in the t_0 values is unacceptably high for statistical comparison.

The effect of the solvents raises doubt about

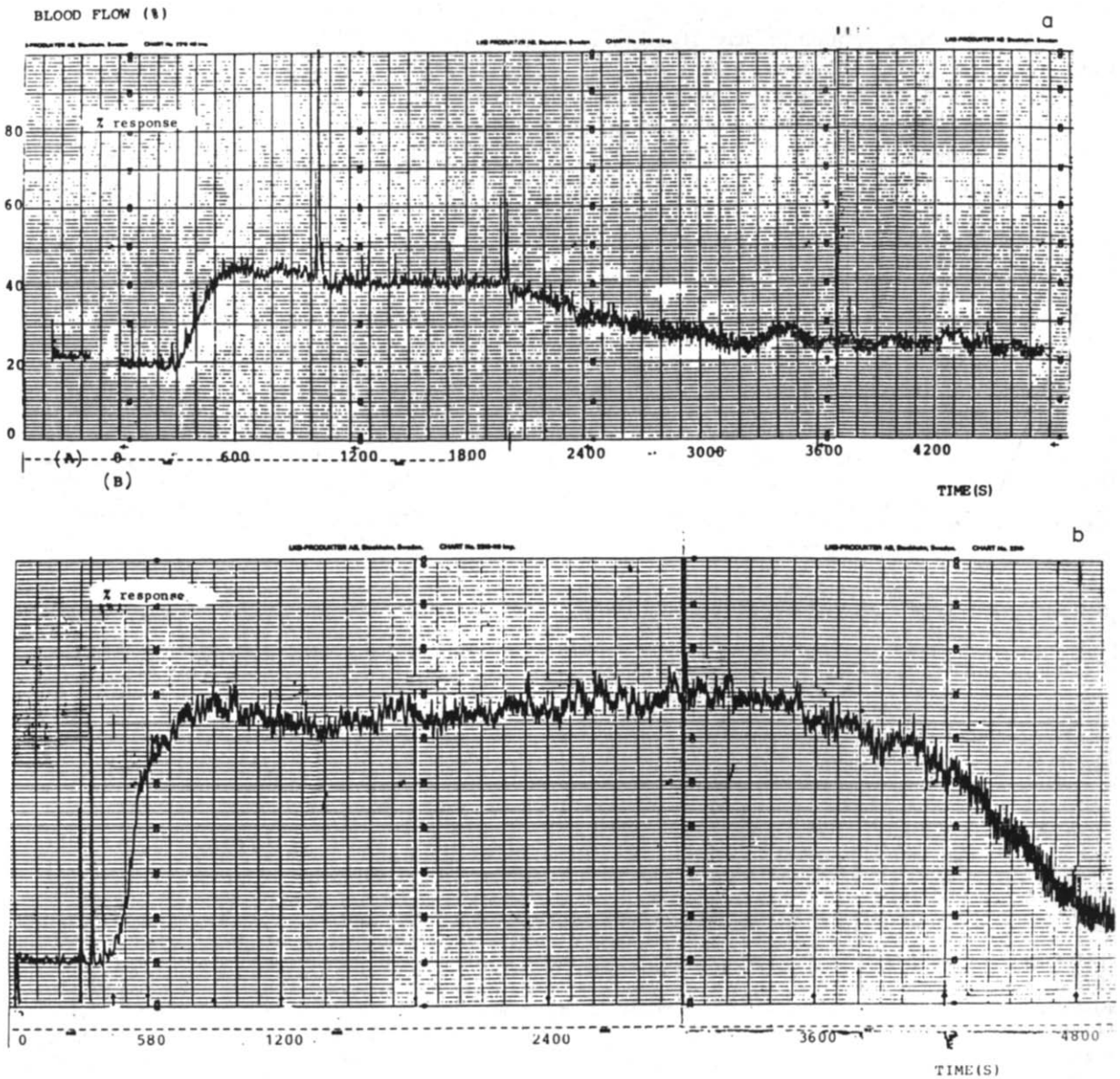

Fig. 1. a: typical response curve as measured by the LDV to 5 μl of 0.1 M hexyl nicotinate in kerosine. (A), pretreatment; (B), point when nicotinate solution was applied. b: LDV response to 5 μl of 0.01 M hexyl nicotinate in kerosine.

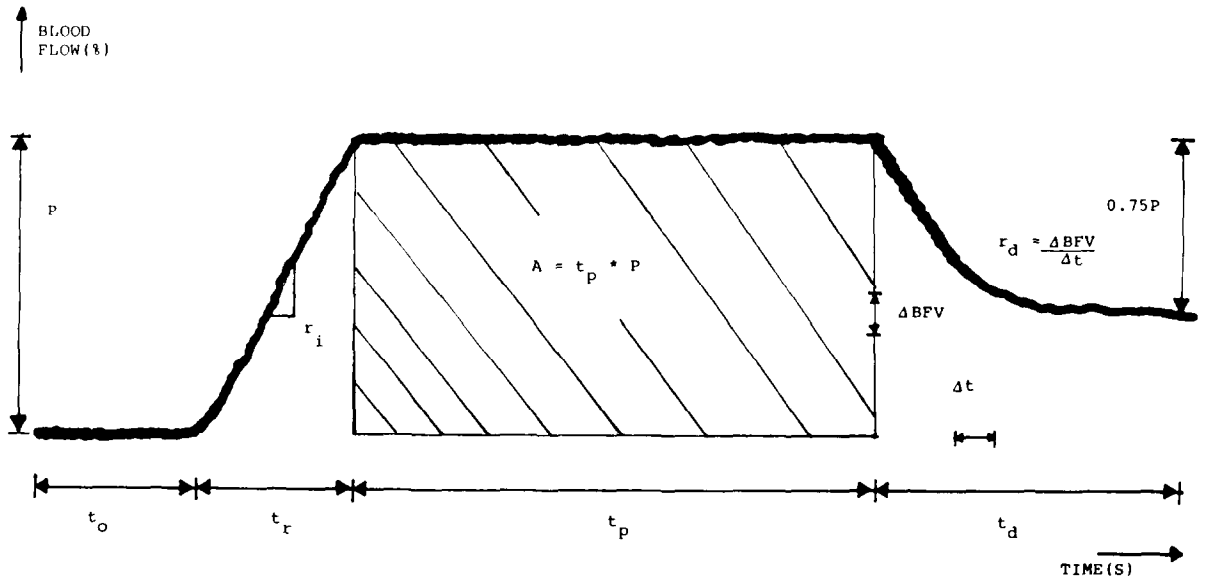

Fig. 2. Parameters used for LDV profile analysis.

the validity of studies in which the effect of carrier solvents is not taken into account. If both drug and solvent exert an effect then deconvolution of the data is required before meaningful conclusions can be drawn about the effect of each active species. It seems that this has not been done in any of the previously reported studies.

In all subsequent studies therefore only solvents which do not induce an effect within the timescale of study were used as carriers for the model alkyl nicotinate vasodilators. Monopropylene glycol and kerosine were used as model hydrophilic and lipophilic solvents, respectively.

Typical traces of the vasodilator response as monitored by the LDV are shown in Figure 1. Following a lag phase a sharp response which rapidly reaches a steady state is observed. This then decays inconsistently in that on occasions a seemingly exponential decay (Fig. 1a) profile is observed while on others a sigmoidal pattern is seen (Fig. 1b). The decay portion of the curves can therefore not be used for studying the effects of

TABLE 2

The parameters for LDV profiles of hexyl nicotinate in kerosine at different concentrations

Values are means ± S.D.; s = second; n = number of experiments. See Fig. 2.

Concentration (M)	$t_{o(s)}$	$t_{r(s)}$	$r_{i(s^{-1})}$	$P\%$	$t_{p(s)}$	$t_{o(s)}$	$r_{d(s^{-1})}$	$A_{(s)}$	n
0.2	318 ± 73	272 ± 88	0.238 ±0.185	41.0 ± 13.5					5
0.175	372 ± 86	242 ± 148	0.214 ±0.096	35 ±10					6
0.125	320 ± 100	246 ± 71	0.188 ±0.066	31 ±15.5					5
0.10	234 ± 86	214 ± 77	0.269 ±0.207	35 ±12	2178 ± 413	1836 ± 263	0.269 ±0.207	78454 ± 32814	6
0.05	638 ± 147	323 ± 144	0.0765 ± 0.05	44 ±16	2447 ± 572	1907 ± 298	0.0132 ± 0.00301	72971 ± 47828	5
0.025	696 ± 79	284 ± 71	0.101 ±0.036	32 ±13					5
0.01875	693 ± 242	323 ± 151	0.1495 ± 0.07	29.5 ± 17					5
0.00625	942 ± 404	430 ± 235	0.0684 ± 0.029	31 ± 9.5					5

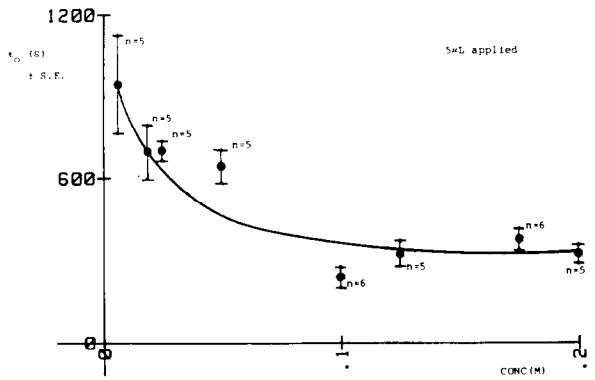

Fig. 3. Variation of LDV response (t_o) with concentration of hexyl nicotinate in kerosine.

different parameters on the response.

Although Guy et al. (1983) have reported on the successful use of maximal response ($P\%$) and time of maximal response to decay to 75% (t_d), to quantitatively study the effect of methyl nicotinate, in our hands the variability observed did not lead to satisfactory results. The variability in the data for the different parameters Fig. 2 is shown in Table 2. Only t_o, the lag times, were consistent enough to be used in a meaningful way. Part of the reason may be due to the existence of a variable concentration for saturation of erythemal response as reported by Guy et al. (1984). Below saturation, biexponential kinetics have been used successfully by the same authors to model the kinetics of the vasodilator response of methyl nicotinate.

In all subsequent studies comparing the different formulations only t_o was used.

The effect of concentration of hexyl nicotinate in kerosine on t_o is seen in Fig. 3. An increase in concentration initially leads to a decrease in t_o which must provide an index for the flux of the hexyl nicotinate through the epidermis.

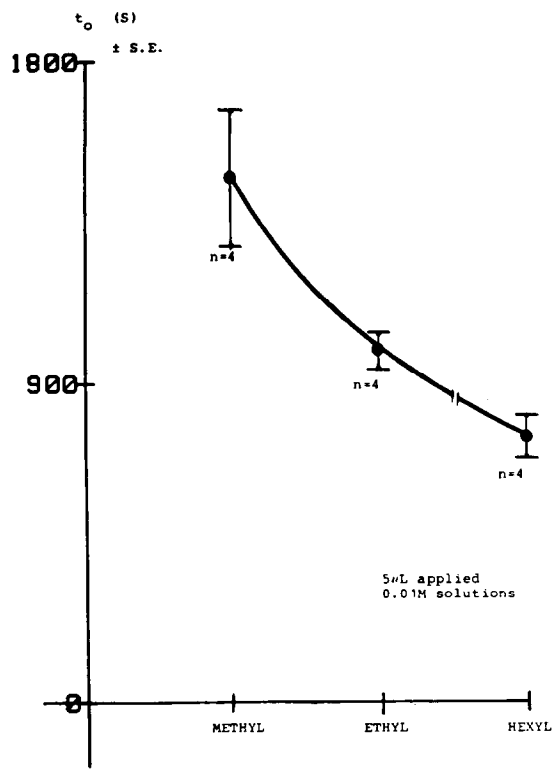

Fig. 4. Measurement of onset of LDV response for alternative nicotinates in MPG. y-Axis = t_o in s; values are means ± S.E.M.

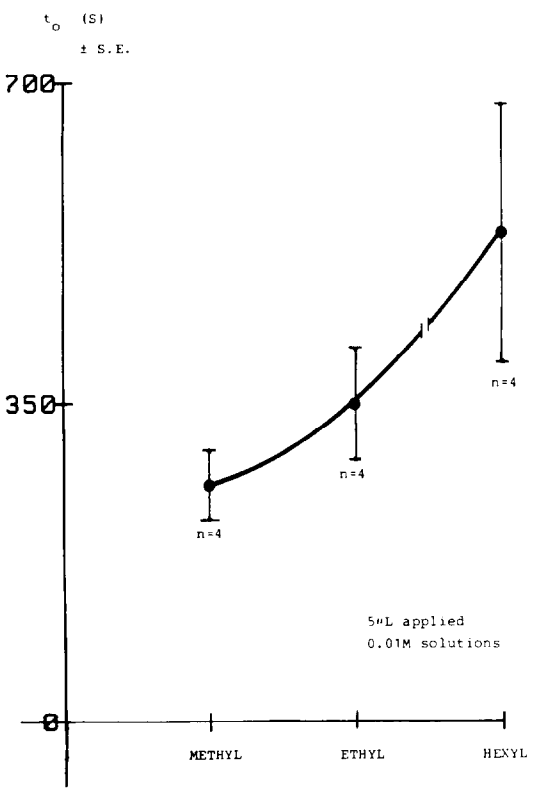

Fig. 5. Measurement of onset of LDV response for alternative nicotinates in kerosine. y-Axis = t_o in s; values are means ± S.E.M.

The t_o, as measured by the LDV, therefore appears to be a useful parameter for comparing the activity of different systems. The effect of different vasodilator nicotinate esters were therefore assessed using t_o. Fig. 4 shows that increasing chain length clearly decreased the t_o values when monopropylene glycol was used as the solvent. The reverse trend was observed when kerosine was used as the carrier (Fig. 5). These data can readily be rationalised by taking into account the physical chemistry of the systems. With the polar solvent, MPG, an increase in the alkyl chain length of the nicotinate increases its lipophilicity and therefore within the polar carrier, solubility of the ester is decreased and its thermodynamic activity is increased at any given fixed concentration (0.01 M in this instance). The converse is true with the lipophilic carrier. The combined data effectively demonstrate the value of further careful choice of base for specific applications. As shown, this choice can be made rationally by a consideration of the physical chemistry of the systems.

Conclusion

LDV thus appears a useful method for studying the effects of topically applied vasoactive drugs (Guy et al., 1983, 1984). This technique can be usefully expanded to investigate the effect of formulation on drug release as shown in this study. The dramatic effects of changing from a lipophilic to a hydrophilic base are illustrated in Figs. 4 and 5. These effects are of course well known. With LDV these effects and other effects such as those produced by alteration in the physicochemical properties of the drug molecule (Table 3) can be monitored non-invasively in vivo. The technique therefore usefully extends the range of methods available to the pharmaceutical formulator. Care must however be excercised in the use of the technique as a number of solvents, most notably water, produce a response (Table 1). Overlooking this may lead to totally erroneous conclusions. This sadly seems to have been the case in much of the earlier work with this technique.

TABLE 3

Partition coefficients of nicotinate esters in different solvent systems

Solvent System	Partition coefficient		
	Methyl nicotinate	Ethyl nicotinate	Hexyl nicotinate
Monopropylene glycol/isopropyl myristate	2.79	1.89	0.37
Monopropylene glycol/kerosine	11.67	6.33	1.01

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