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POLYMER MATRIX CONSIDERATIONS FOR TRANSDERMAL DEVICES

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Introduction

The transdermal delivery of drugs has taken a sudden surge of popularity during the past two years probable due to the successful introduction of a transdermal scopolamine for motion sickness by CIBA and a transdermal nitroglycerin by Key Pharmaceuticals. CIBA and Searle are also introducing transdermal forms of nitroglycerin.

Literature showing the feasibility of these and other drugs for transdermal delivery have existed in the pharmaceutical literature for more than a decade. Many studies carrying out permeability measurements on cadaver skin and animal skin have been published and these have been written in varying degrees of analytical sophistication. Some are very useful and convey a fundamental understanding of the basic problems and limitations involved (1-4).

The early literature has reasonably established that the stratum corneum layer of the epidermis is rate limiting to transdermal penetration of molecules. The stratum corneum is composed of multilayers of epithelial cells that have become rich in fibrous proteins. The some 10-20 layers of

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stratum corneal cells also are bounded by largely intact cell membranes. Therefore the process of stratum corneum permation is limited by the molecular environment provided by the stratum corneum. Since stratum corneum is primarily a two structural component system the permeability problem reduces to the interactions between the permeating solute molecule, the structural proteins, cell membranes of the stratum corneum and the aqueous medium in which the structural components are bathed.

It has also been well demonstrated that permeability of the stratum corneum is proportionate to the so called partition coefficient, implying that a lipid layer is limiting to permeability. Scheuplein (2) demonstrated the colinearity between partition coefficient and cadaver skin permeability. Such observations imply membrane limited permeability.

For a variety of molecules having the capability of surface binding to protein implies that the fibrous proteins of the stratum corneum may also drastically impede permeation.

Two important events must occur before molecules can successfully permeate membranes in general or stratum corneum in particular. First the molecules of interest must cross biological membranes. Ionic materials having exposed charged sites and therefore sizable hydration spheres have considerable difficulty in crossing even a single biological membrane. The energy considerations require that some force compel a hydrated polar molecule to pass through a hydrophobic lipid layer. This, of course, will occur statistically as a rare event. Some molecules such as many steroids have positive interactions with membrane phospholipids, orient in biomembranes and flip from one side of a bilayer to the other side with a many hour half-life. Therefore, even through these molecules are hydrophobic in character they may still be expected to have a low stratum

corneum permeability. Other classes of chemical have other problems and could be commented upon class by class. Many drugs are organic nitrogen bases and consequently have pH dependent permeability. For the most part each chemical of interest to use transdermally must be analyzed and studied separately.

The use of a class of materials known as permeability enhancers has been employed in attempts to increase stratum corneum permeability. In order for these enhancers to be effective they must modify the stratum corneum environment in some way. Increasing the solubility in the stratum corneum, increasing solute diffusivity, increasing local stratum corneum membrane permeability, decreasing protein binding or modifying some other limiting effect are some potential ways in which enhancers may mechanistically work.

The present chapter treats some of the diffusional events that are of interest in constructing an effective transdermal device that will release drugs to the skin and also treats some of the diffusional events that take place in skin during permeation. It is important to have some understanding of the physical behavior of the diffusion process and molecular motion in general. What is to follow attempts to treat some of the useful aspects relevant to transdermal devices and skin permeability.

Regardless of the amount of diffusion theory, spectroscopy or other special knowledge the final determining factor as to the efficacy of a transdermal device relates only to how well it works on the patient.

Molecular Motion

The motion of molecules is intimately connected to the fundamental nature of matter. Molecules are capable of several

types of motion. In a general sense these are limited to vibrational, rotational, translational, and flexing movements the latter of which comes about primarily by bond rotation. Of these, the present treatment is most concerned with translational diffusion and secondarily with rotational motion. Flexing motions which give rise to conformational states of micro and intermediate size molecules are extremely important in intermolecular close encounters and such considerations as receptor site binding. Although important they will not be dealt with in the present treatment.

The Einstein-Stokes' equation describes the general condition for rotational motion of a quasisperhical molecule,

$$Tc = \frac{4\pi r^3 \eta}{3\kappa T} \qquad (1)$$

where Tc is the average isotropic rotational correlation time, κ is Boltzman's constant, η is viscosity in poise and T is temperature in °K. The equation is confusing because it describes the time required for a given particle to rotate a segment of an arc in a fraction of a unit time. An improvement or an alternate form to deal with this problem stated simply uses the reciprocal form and changes Tc to a term we will call isotropic rotation (R_i) and the expression is given as,

$$R_i = \frac{3\kappa T}{4\pi\eta r^3}. \qquad (2)$$

The overall units can be expressed as a rate, number of segment rotations per unit time. For example, the water molecule has a Tc of close to 10^{-12} second; therefore, 10^{12} rotational events per second. This term is easier to handle and is more comparable and more compatible with the familiar Stokes' equation for diffusion,

$$D = \frac{3\kappa T}{6\pi r\eta}. \qquad (3)$$

This latter equation describes the diffusion coefficient (D) in isotropic media.

Equations (2) and (3) have relatively comparable units in that both are expressed in rates. One striking difference exists between the two. R_i depends on $(1/r^3)$ for a given rotational state which means that rotational motion is tightly coupled to molecular size. For translational diffusion using measurements of the diffusion coefficient (D), D depends only on (1/r); therefore, translational motion is not very dependent on molecular size. General methods for measurement of D in bulk phase assume isotropic conditions.

More detailed measurements or measurements carried out over micro dimensions for both R_i and D require measuring both of these parameters under conditions and in preparations that are difficult or impossible for generally used methods. For example, molecules having electron paramagnetic resonance (EPR) properties dissolved in a "solid" agar slab have about the same rotational mobility as the same probe dissolved in water. This observation is striking and means that molecular behavior over angstrom dimensions and over laboratory dimensions may appear very differently.

The agar slab is clearly a solid for purposes of laboratory manipulation; consequently, might be expected to confer restricted rotational motion for a small solute due to solute polymer interaction. In the case of agar, the average polymer spacing is very large compared to molecular size and does not detectively slow molecular rotation. Translational motion as quantified with the diffusion coefficient has somewhat different constraints in heterogeneous media. As a result diffusion will not be a constant over all dimensions. This comes about due to caging effects. Since the polymer lattice has an average polymer spacing and occupies a minority of total space the solute will

have a greater diffusivity over short dimensions than over long dimensions. For completely homogeneous media the diffusion coefficient is a constant over all dimensions. For a very regular polymer lattice having vacancies or polymer-free spaces (cage) all of about the same size then the measured diffusion coefficient by the electron paramagnetic resonance (EPR) method using collisional frequency (D_c) of a single solute species as the measurement method for D_c will be greater for high solute concentrations than for low solute concentrations. The obvious reason is because diffusion within a polymer cage is restricted only by the solvent while diffusion between cages is also restricted by the polymer molecules themselves. Conventional methods of measuring diffusion measure only the long range type of diffusion and average all the various limitations. In attempting to understand a given diffusion matrix it may; however, be important to know how the D_c for small dimensions compares to the D_c for large dimensions.

There is a straightforward relationship between R_i and D in isotropic media. This relation only holds for isotropic media where the forces that limit molecular movement within dimensions of the order of the molecular size itself and for many multiples of the molecular size are the same. For heterogeneous media such as polymer gels the two terms R_i and D will not conform to colinearity over large concentrations and over lare ranges of molecular spaces. Algebraic combination of equations (2) and (3) yields a composite where D can be calculated by measurements of R_i, and

$$D = 0.67r^2 R_i \tag{4}$$

where R_i can be calculated by EPR measurements from the equation,

$$R_i = 1.59 \times 10^9 W_o^{-1}\left[\left(\frac{h_o}{h_{-1}}\right)^{\frac{1}{2}} - 1\right]^{-1} \tag{5}$$

where W_o is the midline width and h_o and h_{-1} are the mid and high field line first derivative heights. Combined R_i and D_c values can be obtained from a variety of probe molecule structures and can be used to give important information on characterizing diffusional environments.

The term D_c introduced above gives a measurement of D over controlled dimensions. The concentration of the solute probe may vary from about 0.1M to 10^{-5}M. The on-center average cubic lattice spacing for solute molecules is given by:

$$S = \frac{(10^{27}A^3N)^{1/3}}{M^{1/3}} = \frac{11.84}{M^{1/3}} \qquad (6)$$

where N is Avagodro's number, M is molarity and S is linear dimension in Å. The cubic lattice spacing value for a 0.1M solution is 25.5Å and for a 10^{-5}M solution is 549.6Å. For a polymer gel having an average lattice spacing of 100Å the D_c values will be concentration dependent and will show a greater D_c at high solute concentrations where most collisional events between solute molecules are within cages than at lower solute concentrations where most solute collisions require travel between cages. For this reason the terms D and D_c may be different in heterogeneous media but are almost identical for all solute concentrations in homogeneous media.

However, such low concentration gels as agar may approach colinearity for small molecules where R_i/D_c equals a constant for all solute probe concentrations because the polymer lattice spacing is large. The heterogeneity provided by polymer gels results in barriers to the long range diffusion of molecules but are essentially not affected by the short range forces that limit rotational motion.

Appropriate methods are now available to analyze systems having extensive diffusion and are capable of making appropriate

characterization of these systems. The spin-label method employing electron paramagnetic resonance is used as an example of how to obtain such information from pharmaceutical systems that have important diffusional functions.

Spin labels have been used extensively in the past to obtain useful information about the motional state of the spin label probe molecule or spin label solute itself and how motion is allowed for by the local molecular environment. A preponderance of these papers have dealt with the freedom of rotational motion (5,6). These data and interpretations have been used to obtain information about biological membranes, about binding of small molecules to larger molecules and in general, about ligand (probe) interactions with larger surfaces. Another set of spin label literature deals with the measurement of translational diffusion (7-9). These data deal with lateral and perpendicular motion of molecules within and across biological membranes. The method of measuring translational diffusion depends on near encounters between spin label molecules. The diffusional problem can also be approximated by measuring spin label near encounter frequency in isotropic or near isotropic media. This approach is useful to study diffusion matrix problems.

Fig. 1 shows the spin label molecules used in the present report. Fig. 2 shows an example of a spin label I taken through a viscosity series. Fig. 2G shows relatively unrestricted rotational motion. Fig 2F shows slightly restricted motion and Fig. 2E shows still more restricted motion. Fig. 2A shows a rigid glass spectrum characteristic of essentially no rotational motion. Spectra in between show varying degrees of rotational motion. By use of equation (5) the appropriate spectral measurements can be used so that R_i values can be calculated.

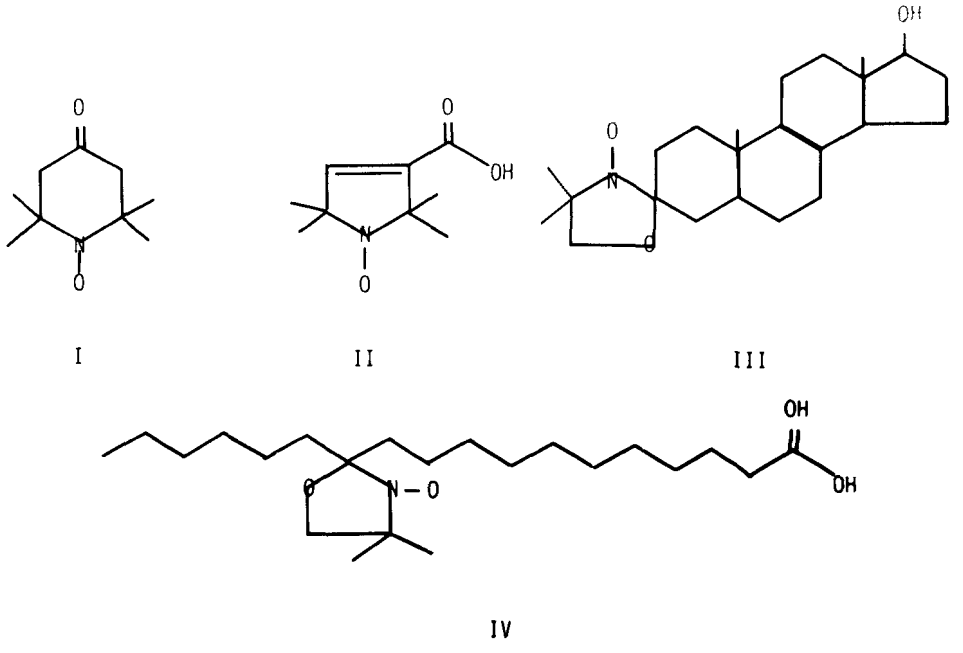

Fig. 1. Structure of Representative Spin Labels

I, the structure of a commonly used small water soluble spin label frequently referred to as TEMPONE. The chemical notation for this spin label may be written as 2,2,6,6-tetramethylpiperidone-N-oxyl. II, another commonly used water soluble spin label, 2,2,5,5-tetramethyl-3-carboxylpyrrolidinyl-N-oxyl. III, a spin labelled steroid representative where the spin label is a 2,2,5,5-N-oxyl-oxazolidine on ring position A3. IV, this is a derivative of stearic acid where the spin label has been placed on carbon 12 as a 2,2,5,5-N-oxyl-oxazolidine. More exact and other trivial names for III and IV are not necessary and can be found under varying names in the literature.

Fig. 3 shows a special series where the same spin label is increased in concentration from 3a to 3e. The spectrum showing 3e is 5mM. Fig. 3d is a 10^{-2} molar concentration, Fig. 3c at 2 x 10^{-2} molar, Fig. 3b at 4 x 10^{-2} molar, and

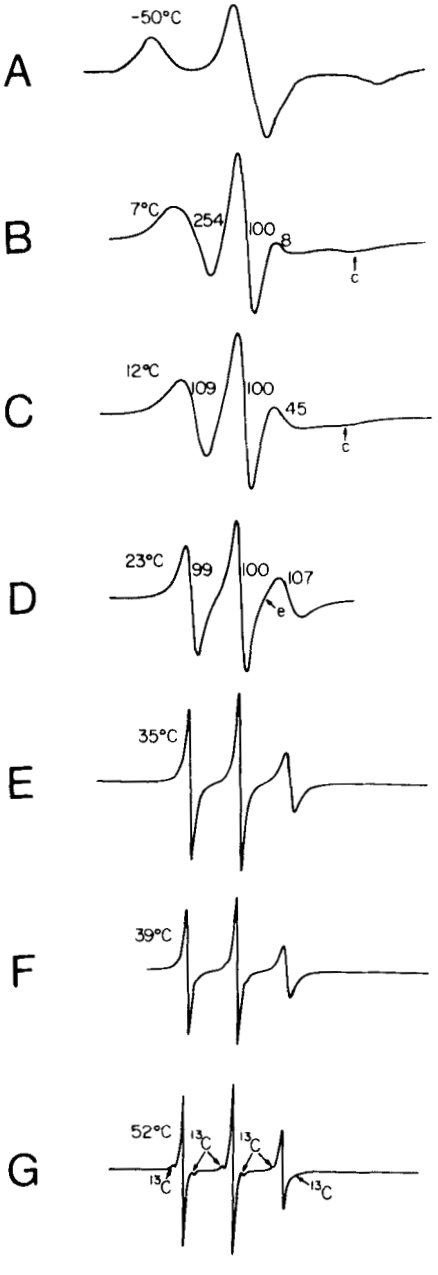

Fig. 2. A Motion Series for the Spin Label Probe I

This motion series was done in glycerol and was carried through the temperature series from -50°C to 52°C. At -50° the glycerol is in a glass state where very little if any crystalline structure is shown yet the bulk material is inflexible and will fracture if bent. A, is typical of spin label motion that is in the range where no rotational motion can be detected. It is essentially identical as that of a crystal containing a spin label which has been ground into a powder and randomized and then had its spectrum taken. B, warming the sample to 7°C substantially increases the molecular motion and that is revealed in the accompanying spectra of spin label probe I. By increasing the temperature from 7 to 12° it can be seen in C that the motion has further increased. The numbers that are shown by each line B and C are integrated numerical values for each of the first derivative spectral lines and for purposes of quantifying rotational motion only have relevancy when all three lines are approx. 100. D, by the time the temperature has been elevated to 23°C the motion of all three lines reveals a spectrum with an integrated intensity for each line that is approx. 100. The small (e) is meant to indicate that there is essentially no inflection point between the mid and high-field lines in this spectrum. E, by the time the spectrum has reached 35°C each of the lines has continued to narrow and allows for quite accurate measurements to be taken for purposes of R_i values. F, at 39° the spectrum continues to narrow and at G, the spectral lines have now narrowed to the point where the natural abundance of carbon 13 hyperfine splittings can be seen and are shown in the spectrum. That is only an indication that the line widths are still narrowing and the solution viscosity is continuing to decrease with continued temperature elevation.

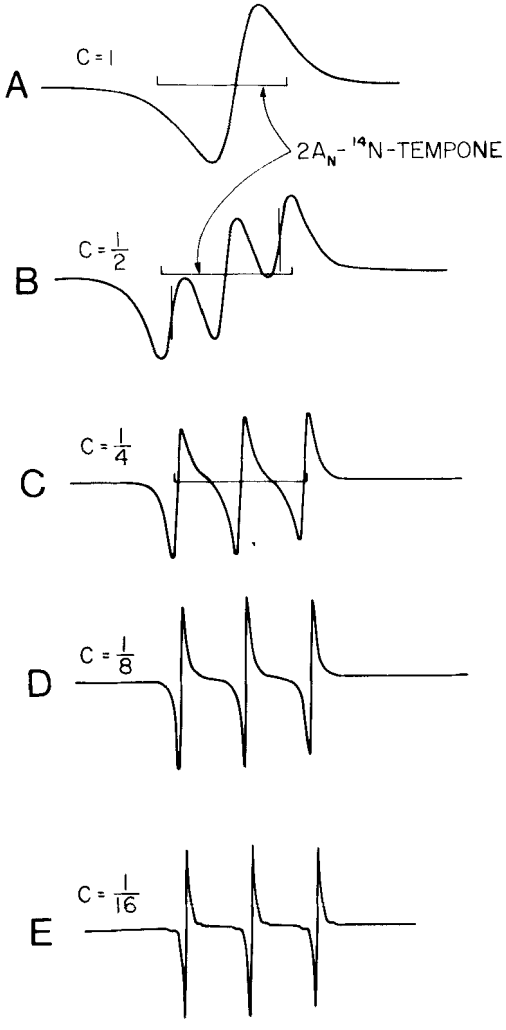

Fig. 3. Spin Label Probe I is Used to Reveal the Effects of a Range of Concentrations to Illustrate Difference in Collisional Frequency Between Spin Label Molecules.

Spin label probe I was dissolved in a mixture 1:1 glycerol:water at an initial concentration of 0.1M. Spectrum A was taken at 0.1M and reveals a near saturated spin label concentration where the collision frequency is adequately high that all three spectral lines have fused into a single contribution. Spectrum B, has a concentration to 0.05M and shows that now each of the three overlapping spectral lines can be seen. Spectrum C reduces the spin label concentration to 25mM and shows that now each of the three spectral lines are fairly well separated. Spectrum

Fig. 3a at 0.1 molar. Fig. 3a is used to show a case where spin label concentration has reached a limiting case for low viscosity conditions where the three lines have now fused into one single spectral contribution and the saturable concentration is near 0.1M. The near encounter or collision frequency of spin label molecules is dependent upon solvent viscosity, spin label concentration, spin label size, temperature and structural features imposed by the solvent or matrix environment. The effect of D_c coefficient known as collision-determined diffusion coefficient described in this way is both proportionate to near encounter frequency (ω_{ex}) and inverse to molarity (M). ω_{ex}/M normalizes for probe concentration and in isotropic media is independent of probe concentration.

$$D_c \propto \frac{\omega_{ex}}{M} \tag{7}$$

The term ω_{ex} is directly measurable from other line shape measurements. The usual measurement taken is the first derivative peak to peak line width. The line width (ΔH) at a known concentration is subtracted from the minimal line width (ΔH_m) for that preparation and the difference is the line broadening caused by concentration-dependent collisional effects,

$$\Delta H_c = \Delta H - \Delta H_M. \tag{8}$$

Since only half of the actual collisions are reflected in the line width the relation,

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Figure 3 (cont.):

D further reduces the spin label concentration to 12mM and shows that the lines have now become considerably more narrow. Spectrum E reduces the spin label concentration to 6mM and shows that the lines are now quite narrow. The text deals in appropriate spin label measurements that can be taken from such a series of spectra as this to determine D_c values.

$$C_f = 2\Delta H_c, \tag{9}$$

holds where ΔH_c is in collisions per unit time, usually in the megahertz range. This allows for a useful equation to be constructed,

$$D_c = \frac{K\omega_{ex}}{M} \tag{10}$$

where K is a proportionally constant and D_c is expressed in cm^2/sec. The diffusion coefficient calculated in this manner has a concentration dependency because of the relation between solute cubic lattice spacing and the polymer matrix barriers to long range diffusion. Equation (10) can be used to calculate D_c values and polymer diffusion matrices for average probe cubic lattice spacings ranging from about 25Å to 250Å. Spectra taken at the more dilute probe concentrations can be used to measure R_i values. Both sets of values, D_c and R_i, help to evaluate the potential usefulness of a given polymer matrix environment.

Fig. 1 shows four examples of probe molecules. Others can be synthesized to closely mimic most drugs. Use of such a probe drug-analogs in the proper manner immediately tells the investigator if the probe is free to rotate and move about in a given matrix environment. Such data acquisition allows the investigator to modify and improve matrices without laborous conventional experiments.

Polymer Matrix Conditions

Two principal terms that limit bulk diffusion of molecules trapped in a heterogeneous polymer containing diffusion matrix are solubility of the solute in the fluid phase and weak molecular interactions that may occur between the solute and the stationary phase. These interactions give rise to mass action equilibria conditions which can be regarded as the

basis for controlled rate release from the surface of a diffusion matrix.

For the consideration of a solute (D, or drug or probe) under such conditions where there is competition between the solute being dissolved in the fluid phase and being bound to a stationary phase (S_b, binding site) a mass action equation describes the equilibrium,

$$D{:}S_b \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} D + S_b \qquad (11)$$

having a forward (k_1) and reverse rate (k_{-1}). The equation is shown in this form because the complex $D{:}S_b$ takes place before polymer matrix construction takes place.

For Key Pharmaceutical nitroglycerin diffusion matrix (Nitro-Dur^{TM}) the reverse rate constant (k_{-1}) is negligible. This condition keeps nitroglycerin in a saturated state in the fluid phase until $D{:}S_b$ becomes limiting. For the case of Nitro-Dur zero-order release occurs until about 65-75% of the nitroglycerin has been depleted.

The method and approaches discussed so far do not require a concentration gradient of solute species to take diffusional measurements. In fact, the translational motion characterized in this analysis as a diffusion coefficient occurs equally whether the diffusing species has come to spacial randomness or not. The general diffusion condition for D is expressed in cm^2/second and states constant unit surface area per unit time. With a limited quantity of diffusing molecules from a point source this condition also states a declining close encounter frequency as the diffusing molecules get further apart. In three dimensional isotropic media the relation C_f/M is a constant. The mean free path (L) also increases with concentration lowering.

The general treatment of permeability through membranes has been developed by the use of concentration gradients. Fick's laws describe these conditions and allow energy and flux terms to be combined with diffusion terms.

In the consideration of a point diffusion source, $\partial M/\partial t$, where M declines with time, the solute invades new space at some rate D (usually a -D to show that the concentration at the point source declines). Fick's equations were not meant to describe space in general but space that contains a membrane where diffusion becomes a smaller value in the vicinity of the membrane. So a second term, $\partial C/\partial X$ is imposed to describe a concentration gradient where (C) is concentration and (X) is the thickness of the membrane, all over a defined surface area, (A). A common equation used to describe flux (J) is stated in a similar manner; however, allows values to be obtained from experimental conditions.

$$J = \frac{\Delta C_M D_S K_{S/M}}{\delta} \qquad (12)$$

(J) is always described in units of quantity/surface area/unit time and for transdermal studies usually in $\mu g/cm^2/hr$. Conditions may be set up where (J) can be directly measured but certain of the other terms may still be unknown or extremely difficult to quantify.

For a transdermal delivery system there are two sets of diffusional events, those that occur in the matrix and those that occur in skin. The most important terms for this consideration are diffusion (D) and concentration (C). Diffusion is a rate term and concentration is a quantity per unit volume term. Differential concentration (ΔC, concentration gradient shows how concentration changes from one zone to another). The product D times C limits J and increasing either D or C (preferably both) increases J ideally to a limiting value. In

equation (12) the term, $K_{S/M}$ is partition coefficient between the solute dissolved in the skin and the solute dissolved in the matrix, $K_{S/M} = C_S/D_M$ and is the effective concentration term for the above consideration of the product of D times C. The important D term is the diffusion coefficient in skin, D_S; therefore, D_S times C_S limits the real transdermal flux rate.

Conditions for zero order delivery of a drug transdermally has some additional requirements. Since the solute must transfer out of the matrix at a nonchanging rate the system is better defined if the solute is maintained at a constant (ideally saturated) concentration in the fluid phase of the matrix. For these conditions to be met D_m must be much greater than D_s and as a result the relative concentration of solute between skin and matrix is unimportant; that is, $D_m >> D_s$ as long as other requirements are met.

Nitro-Dur

The Key Pharmaceuticals diffusion matrix for nitroglycerin is composed of a simple mixture of solvents and polymers. Polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) form the lattice structure. While both PVA and PVP are highly water soluble the water solubility of the mixture is reduced due to cooperativity in hydrogen bonding between the PVA hydrogen donor and the PVA hydrogen acceptor. A lactose triturate of nitroglycerin is directly mixed with the solvent polymer mixture while in the liquid state. Upon cooling the nitroglycerin is partitioned between binding sites on lactose, the two polymers and the fluid phase and is described by the simple mass action equation shown earlier, where

$$S_b{:}D \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S_b + D \qquad (11)$$

S_b is the sum of all solid state binding sites, D is nitrogly-

cerin (a drug), k_1 is the dissociation rate constant and k_{-1} is the association rate constant. For nitroglycerin the dissociation rate keeps the liquid phase saturated until the undissociated reservoir becomes limiting.

Fig. 4 shows average data taken from cadaver skin permeability studies over some 48 hours. The skin permeability rate (J) remains constant and is described as zero-order kinetics.

Similar data to the in vitro data has been obtained from volunteers and patients. These data are obtained by taking blood samples from subjects wearing Nitro-Dur. The blood levels remain constant for a 24 hour period. It is somewhat difficult to relate these blood levels to in vitro flux rates since not all the pharmacodynamic parameters and, extraction of nitroglycerin from skin parameters, are well defined. The order of kinetics, however, seems to be zero-order just as the in vitro data shows.

Placing an active Nitro-Dur on the surface of a Nitro-Dur placebo matrix reveals a flux rate of 40-45 $\mu g/cm^2/hr$. That flux rate appears to be the diffusion limiting maximum obtainable from Nitro-Dur.

One characterization of Nitro-Dur as a diffusion matrix employed the EPR techniques described earlier. Table 1 shows data obtained by using probe I in Nitro-Dur at varying concentrations. Probe I has a molecular weight of 170 and a free nitroxide group. It is chemically about the same size as nitroglycerin and is similar in that both have a high thermodynamic activity coefficient.

Table 1 shows the R_i and D_c values for probe I in water and indicates that the calculated D_c value using equation (4) (D_c*) from R_i measurements are similar. Probe I in Nitro-Dur has slightly restricted rotational motion compared to water

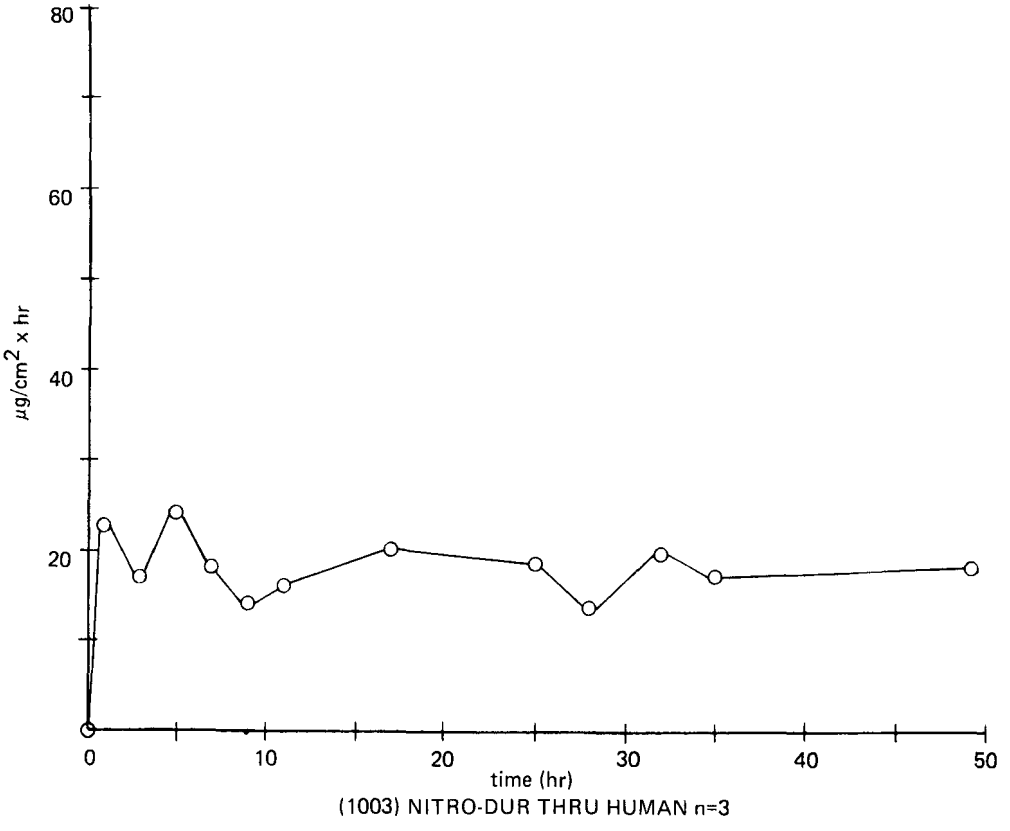

Fig. 4. In Vitro Data Taken from the Use of Nitro-Dur on Human Cadaver Skin to Measure the Flux of Nitroglycerin Passing Through Cadaver Skin.

The ordinate of Fig. 4 reveals the rate measurement as $\mu g/cm^2/hr$ and the abscissa reveals the time sequence over which the measurements took place. This figure reveals a relatively zero order release of nitroglycerin for an average of three samples. This is a typical type measurement which is frequently taken in the Key Pharmaceuticals laboratories on an in vitro preparation.

and a somewhat more restricted D_c than is expected from the use of equation (4). The added restriction to translational motion at a probe concentration of 0.01M is undoubtedly due to polymer interference with diffusional processes. As

Table 1. Probe I Comparative Motion.

Table 1 shows comparative R_i values for spin label probe I in water and in Nitro-Dur. It also shows comparative D_c values measured in water and Nitro-Dur. The D_c* values are calculated balues to give perfect agreement with the R_i values. It can be seen in the isotropic aqueous medium that the two values are the same while in a polymer matrix system they are different by a factor of 2.7.

Medium	R_i	D_c	D_c*	Probe
H_2O	5×10^{10}	6×10^{-5}	6×10^{-5}	I
Nitro-Dur	2×10^{10}	1×10^{-5}	2.7×10^{-5}	I

expected in the polymer lattice and heterogeneous environment of Nitro-Dur translational diffusion is somewhat more hindered than rotational motion. Other more regular or tighter polymer gels may have a considerably greater interference with long range molecular movement.

References

1. G.L. Flynn, S.H. Yalkowsky and T.J. Roseman, J. Pharm. Sci., 63, 479(1974).
2. R.J. Scheuplein, J. Invest. Dermatol., 45, 334(1965).
3. R.J. Scheuplein, J. Invest. Dermatol., 67, 31(1976).
4. R.J. Scheuplain, J. Invest. Dermatol., 52, 63(1969).
5. O.H. Griffith, D.W. Cornell, and H.M. McConnell, J. Chem. Phys., 43, 2909(1965).
6. A.D. Keith, M. Sharnoff and G.E. Cohn, Biochim. Biophys. Acta, 300, 379(1973).
7. P. Devaux, C.J. Scandella and H.M. McConnell, J. Magn. Reson., 9, 474(1973).

8. E. Sackmann and H. Träuble, J. Am. Chem. Soc., 94, 4482(1972).

9. E. Sackmann and H. Träuble, Am. J. Chem. Soc., 94, 4492(1972).