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DRUG DIFFUSION: A FIELD GRADIENT ELECTRON PARAMAGNETIC RESONANCE STUDY

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ABSTRACT

In order to gain information on the bioavailability of dermotherapeutics, field gradient electron paramagnetic resonance (FG EPR) spectroscopy was used. The characterisation of diffusion processes and distribution functions of different spin labeled molecules (Tempol, spin-labeled Lidocaine) within the ointment bases or skin is obtained by computer reconstruction of the spectra considering the diffusion - concentration profiles derived from the model and comparison with the experimental FG EPR spectra.

Results on the diffusion in some ingredients of ointment bases, emulsion gels and skin showed, that this method furnishes reliable data on molecular transport in these systems. The translational diffusion coefficients are larger for hydrophilic media than for hydrophobic ones, where a good correlation with the dynamic viscosity was found.

INTRODUCTION

The physicochemical properties of drugs, ointment bases, and skin have considerable influence on topical therapy. Diffusion parameters characterising the release of the drug from the vehicle and its penetration through the skin barriers are valuable in gaining information on the bioavailability of dermotherapeutics. Measurements which are most frequently made to assess topical bioavailability involve determination of how much and how fast the applied drug penetrates the skin. It must be stated at the outset, that no single, generally accepted technique to determine percutaneous absorption is yet available[1]. Modern investigations of drug diffusion in ointments and skin are based on measurements of drug release from a topical vehicle, and on measurements of the percutaneous absorption. Drug release studies invariably involve simple in vitro methods. In vitro release and skin absorption experiments are performed using glass diffusion cells which are divided into donor

and receptor compartments, separated by the excised human skin or other membranes (animal skin, synthetic membranes). The rate of drug transport across the membrane is also influenced by the interface conditions[2]. The variety of techniques used to study drug transport do always not allow a direct comparison of the experimental data. In our work a method is presented where small size samples can furnish data on the diffusion of spin-labeled molecules within ointment bases and skin.

MATERIALS AND METHODS

Preparation of Samples

Representative ingredients (according to Ph.Jug.IV) for topical vehicles were chosen and included: purified water, glycerine, polyethylene glycol 200, Miglyol 812^R, olive oil, liquid paraffin. Hydrogels of PMMA-Li were prepared by mixing the polymer (copolymer of methacrylic acid and its methylesters - Eudispert^R, Röhm Pharma) with Li_2CO_3 solution (0.276 g Li_2CO_3/g of polymer) and water to 100 g. As a hydrophilic emulsion gel (O/W cream), Unguentum hydrophilicum nonionicum DAB 9 was used, and as a lypophilic emulsion gel (W/O cream), Lanae alcoholum unguentum aquosum DAB 9.

For "in vitro" percutaneous penetration measurements pig ear skin was used. The skin was soaked in 0.2 mol/l solution of N-ethyl-maleimide (24 hours) to prevent the reduction of nitroxides.

Spin-labeled Molecules

The spin probe Tempol (1-oxyl-2,2,6,6-tetramethyl-piperidine-4-ol) and spin labeled Lidocaine (sl-Lidoc) analogue (α -[N-methyl-(1'oxyl-2',2',6',6'-tetramethyl-4'-piperidinyl)]-amino-N'-2,6-dimethyl-phenylacetamide were chosen. The structures of the spin probes are shown in Figs. 2 and 3.

Preparation of Spin-labeled Lidocaine Analogue

A mixture of 1.1 mmol (216 mg) chloroacetyl-2,6-dimethylxylide, 1 mmol (185 mg) 1-oxyl-2,2,6,6-tetramethyl-4-methylaminopiperidine, 1.1 mmol (165 mg) sodium iodide and 6 ml dry acetone was heated under reflux for six hours. After acetone evaporation, the residue was washed with ether (2 x 5 ml) and then dissolved in 10% potassium carbonate water solution. The aqueous phase was extracted with ether (3 x 5 ml) and the product was isolated by the usual procedure. Pink crystals [single spot on TLC plate, Rf (chloroform/methanol 9/1) = 0.68, melting point 163 - 164^{o}C] were characterised by mass and IR spectroscopy.

Measurements

EPR measurements were carried out on a Varian E-109 spectrometer. Helmholtz field gradient coils were mounted in the spectrometer parallel with the modulation coils. In the first approximation, the gradient was linear within the sample measured. Field gradients up to

0.485 T/m were used, as no excessive heating of the coils occurs. The liquid vehicles were taken in glass capillaries, semisolids and skin in tissue cells. The sample was oriented with the normal to the large face perpendicular to the field gradient and laboratory magnetic field directions. The spectra were measured with the following spectrometer settings: 20 mW microwave power at 9.3 GHz, modulation frequency 100 kHz, modulation amplitude 0.1 mT. The scan time, time constant and receiver gain were optimized for the best signal-to-noise ratio. All experiments were performed at room temperature.

Rheological Measurements

The viscosity was measured in an S/N cylindrical system on a rotational viscosimeter of Rheotest 2 (VEB MLW Pruffgeräte, Medingen, GDR) at 20°C, at a rate of shear $D_r = 81\ s^{-1}$.

RESULTS

In order to gain information on the penetration of drug molecules into the skin, the ointment basis has to be considered, as it is used to bring the drug in to close contact with the skin surface.

Our idea is the following: experimentally the diffusion coefficient (D) of spin-labeled molecules in ointment bases were measured. The penetration of the drug

into the skin was also measured for the situation where the drug is applied in the ointment bases. The spatially resolved FG EPR spectra allow us to evaluate the concentration profiles within the ointment bases, as well as within the skin. To evaluate these data, a model was constructed and the transport parameters were calculated. The model is described in the Appendix.

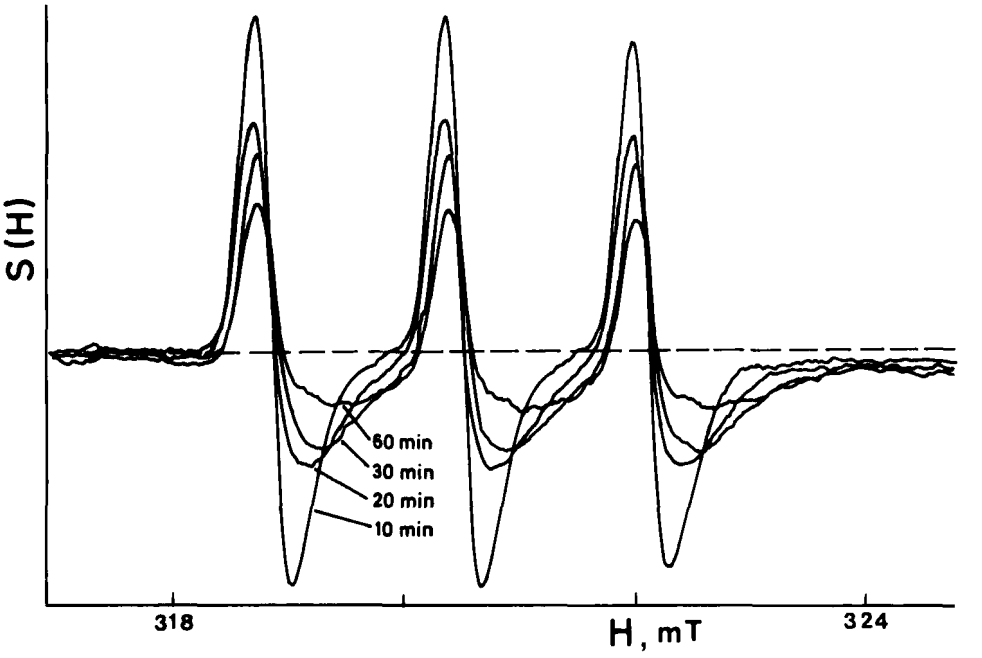

FIGURE 1

EPR spectra of Tempol distributed within a sample of hydrogel and a thread soaked with hydrogel which was initially supplemented with a 10^{-2} mol/l Tempol solution. The Tempol concentration profile evolving with time within the thread and the hydrogel sample furnishes the observed spectra measured with the spatial resolution provided by an imposed 4.8 mT/cm magnetic field gradient parallel to the laboratory magnetic field (H). The corresponding time of measurement after the contact of the thread and sample are indicated for each spectrum.

FG EPR spectra taken at different times to follow the concentration profile evolution within the sample and the spin probe source carrier are given in Fig.1. The line shapes are changing with time as the molecules gradually penetrate into the sample. The evaluated data on rotational and translational diffusion, as well as on hyperfine splitting, are shown in Tables 1,2.

The evaluated data show a correlation between the measured transport parameter for the spin probe molecules and the viscosity of the media.

The rotational as well as translational diffusion parameters can be separated into two groups with respect

TABLE 1

EPR data obtained for Tempol in different media

Medium	a_N mT	$\tau, 10^{-10}$ s	$D, 10^{-7}$ cm^2/s	η mPa.s
purified water	1.64	0.29	90.0	10
glycerine (Glyc)	1.63	3.80	4.5	120
polyethylene (PEG) glycol 200	1.56	1.47	5.1	50
Miglyol 812® (Migl)	1.50	0.82	3.9	30
olive oil (Ol.o)	1.51	1.38	0.5	60
liquid paraffin (Paraf.)	1.47	1.00	1.5	120

The isotropic hyperfine splitting a_N and the rotational correlation time τ were evaluated from the EPR spectra, and the traslational diffusion coefficient D from the FG EPR spectra. The viscosity data η obtained on the rotational viscosimeter were added to characterize the media.

TABLE 2

EPR data obtained for spin-labeled Lidocaine in different media.

Medium	a_N mT	τ, 10^{-10} s	D, 10^{-7} cm^2/s
purified water	1.65	1.2	80.0
glycerine	1.57	18.8	8.3
polyethlene glycol 200	1.55	8.8	8.1
Miglyol 812^R	1.50	6.4	4.9
olive oil	1.48	7.7	0.9
liquid paraffin	1.45	4.1	1.9

The corresponding abbreviations and viscosity of the media are given in Table 1.

to the properties of the media (Figs. 2,3,4,5). There is a linear increase of the rotational correlation time (τ) with the viscosity of the media, and an approximately inverse relation for translational diffusion. The rotational diffusion is slightly slowed down in hydrophilic media as compared to hydrophobic ones. On the other hand, the translational diffusion coefficients are larger for hydrophilic media than for hydrophobic ones. The liquid paraffin dissolved spin probes show shifts to larger values of D and to smaller values of τ, which means that in both situations the mobility is fast as compared to what is expected from the value of the viscosity and the data on D obtained for other media.

The penetration of Tempol within the hydrogel at

different polymer concentrations was measured. There is a linear relation between the diffusion coefficient and the polymer concentration (Fig.6).

DISCUSSION

The applied FG EPR method[3] furnished the translational diffusion coefficient (D), in addition to the rotational diffusion data and the polarity properties. The best correlation was found between the dynamic viscosity and the rotational correlation time, except for liquid paraffin, which shows deviations as detected by both spin probes (Figs. 2, 3). The relation between τ and viscosity (η) is linear, as expected from the known expression[4,5]:

$$\tau = 4\pi r^3 \eta / 3kT \qquad (1)$$

where r is the radius of a spherically symmetric molecule, k the Boltzmann's constant and T the absolute temperature. The inclination $\delta\tau/\delta\eta$ is 5 times larger for sl-Lidoc. than for Tempol (Figs. 2, 3). The ratio of the average molecule radii of sl-Lidoc. and Tempol evaluated from the experimental τ values and Eq.(1) is 1.7, as compared to the value estimated from the space filling CPK models which is about 1.4. It might be that rotation of sl-Lidoc. is too slow with respect to the fast change of the piperidine ring conformation (chair-bath) which brings the π orbital symmetry axes close to parallel with the long axis of the molecule. Therefore

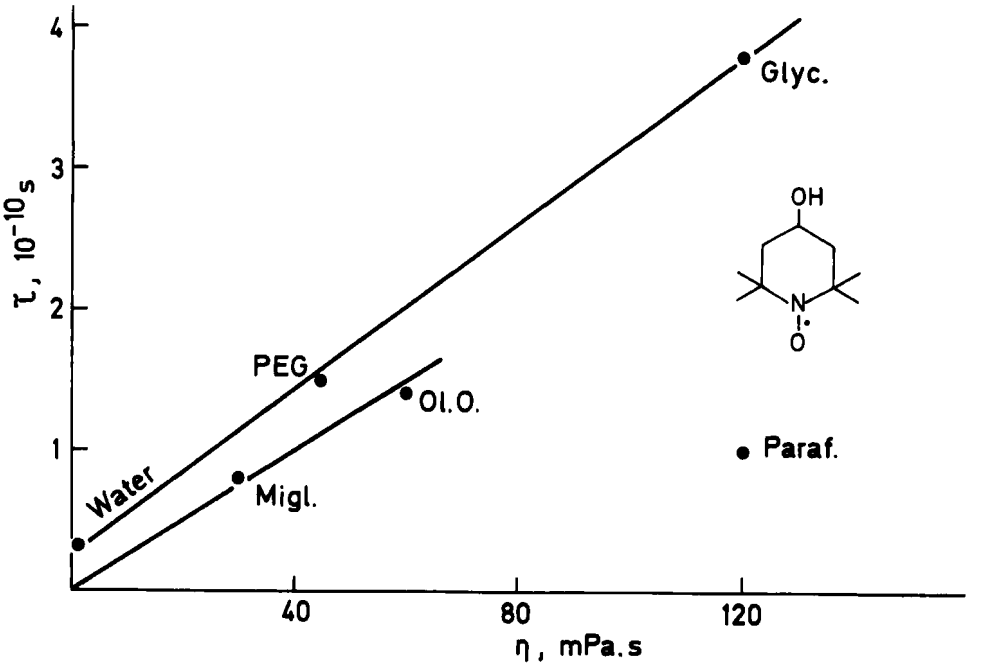

FIGURE 2

The rotational correlation time for Tempol molecules dissolved in different media.

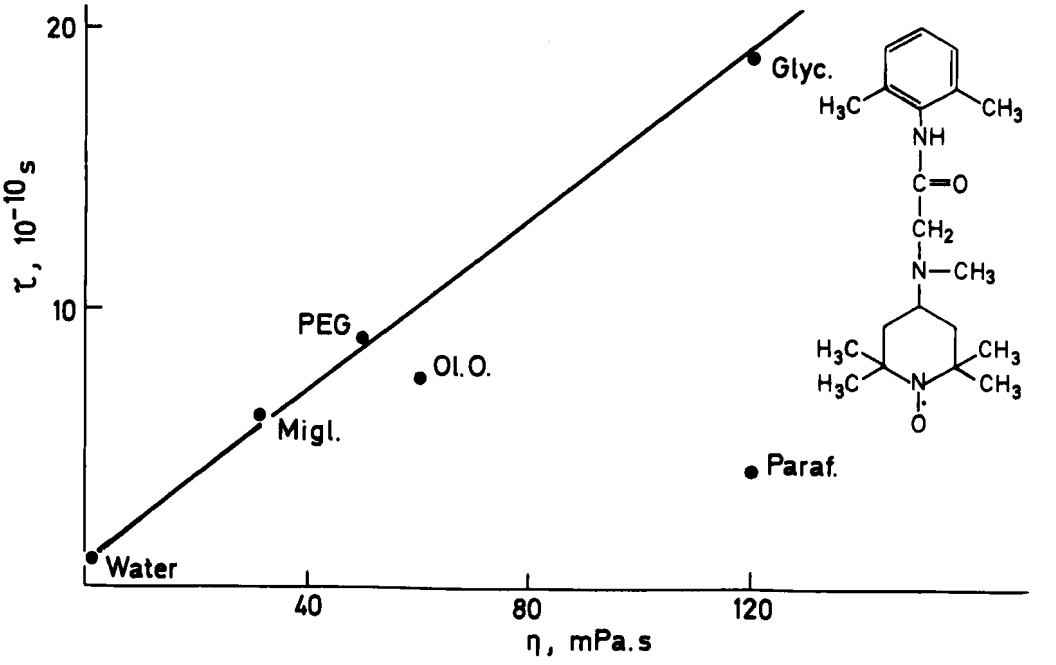

FIGURE 3

The rotational correlation times for spin-labeled Lidocaine molecules dissolved in different media.

only rotation about the axis perpendicular to the direction of the elongated molecule are operative in the relaxation process, leading to line broadening from which our data on τ were derived.

There is a slight decrease in τ for the lipophilic bases, but liquid paraffin is certainly an exception. Its dynamic viscosity is relatively high, D is higher than expected from the value of dynamic viscosity (Figs. 4,5). Hydrophilic media furnish larger translational

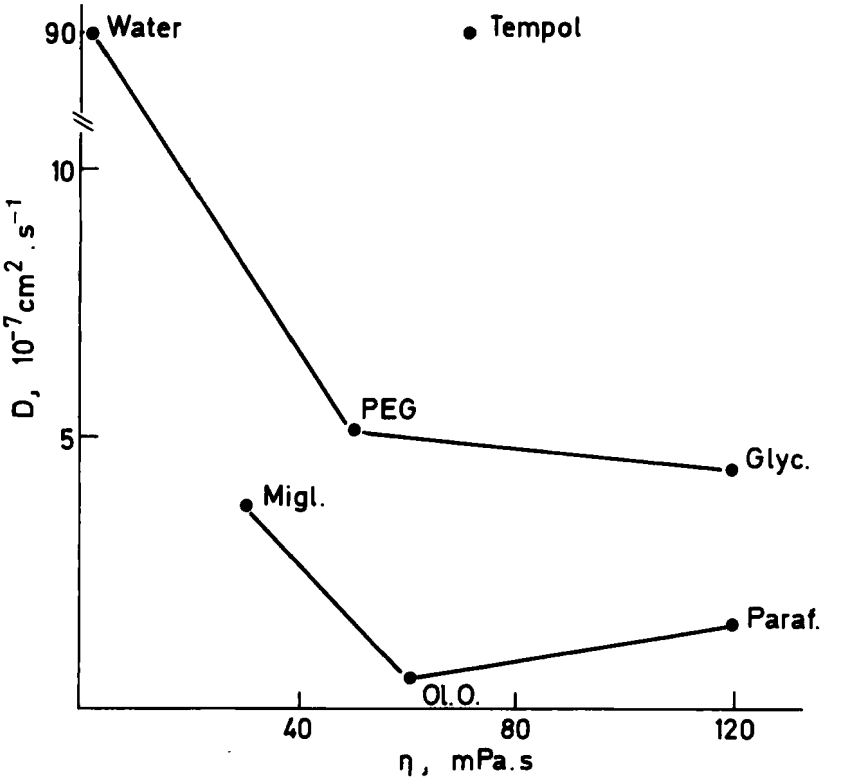

FIGURE 4

The traslational diffusion data for Tempol in different media plotted against the viscosity of the media. (The abbreviations for the media are identified in Table 1).

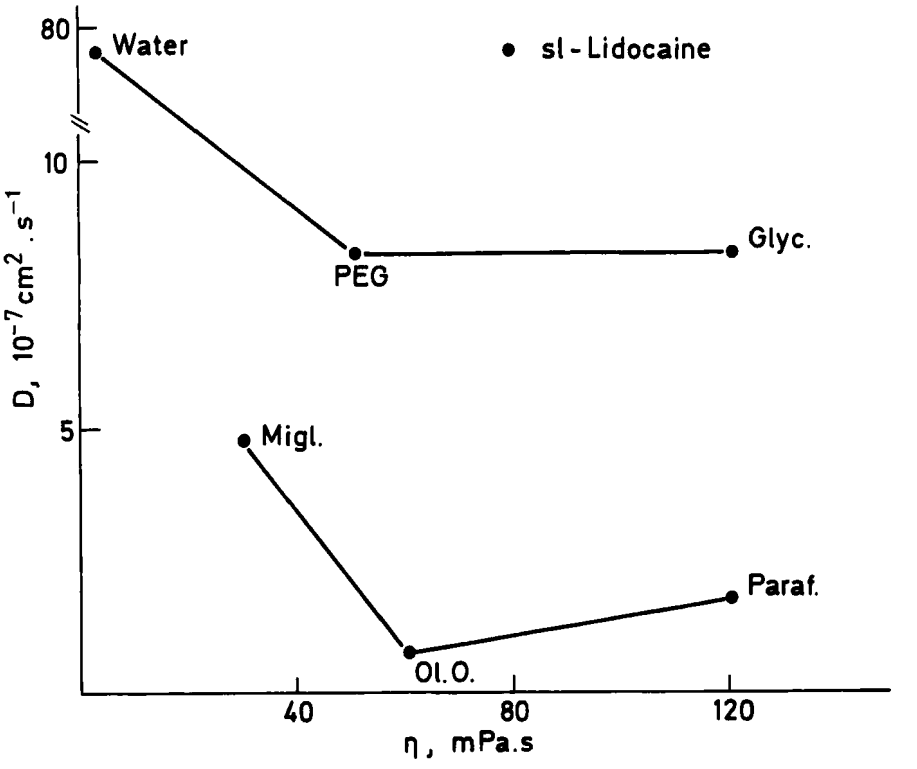

FIGURE 5

The traslational diffusion data for spin-labeled Lidocaine against the viscosity of different media.

diffusion coefficients than hydrophobic ones, which is quite the opposite, but less pronounced, for rotational diffusion where the hydrophobic media show larger diffusion rates (smaller τ values) than hydrophilic media.

Interactions of the diffusing molecules with the media certainly depend on the structural properties of both[6]. The inclination $\delta\tau/\delta(1/\eta)$ is larger for sl-Lidoc. than for Tempol. Correlation between the polarity of the medium and the diffusion coefficient can be

qualitatively described by a rapid increase of D when the polarity approaches the value of pure water. Glycerine is certainly an exception. It should be stressed that Tempol does not dissolve completely in glycerine. This might explain the anomalously small value of D at a relatively large hyperfine splitting a_N (Tables 1,2).

An interesting insight into the transport parameter and the structure of the solvent is represented by the diffusion data of spin probe in a hydrogel. A linear decrease of D with increasing concentration of the polymer was found (Fig.6). Diffusion is hindered both by

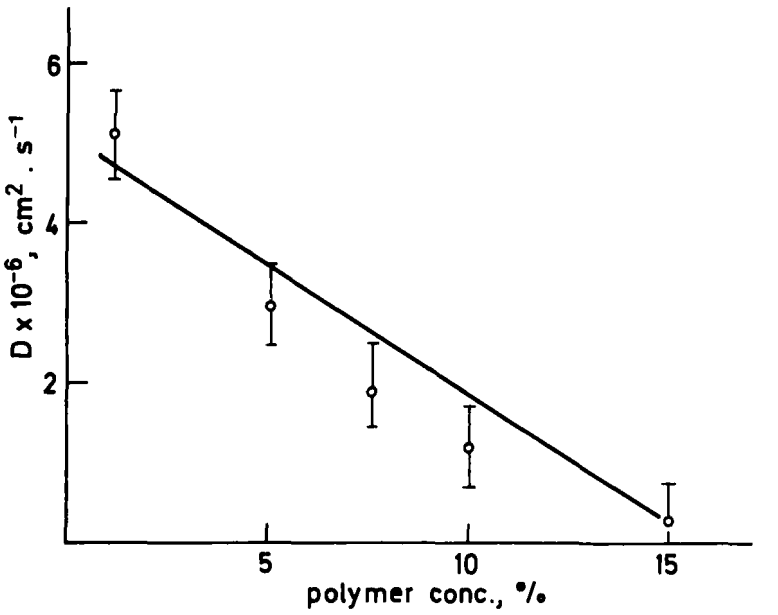

FIGURE 6

Translational diffusion coefficients of Tempol molecules in hydrogels with various polymer concentrations.

obstructions imposed by the polymer chains in the hydrogel network and by the immobilization of the water molecules[7,8].

We also determined the diffusion coefficients of Tempol in emulsion systems. For Unguentum hydrophilicum nonionicum $D = 2x10^{-7}$ cm^2/s , and for Lanae alcoholum unguentum aquosum $D = 1.5x10^{-7}$ cm^2/s , respectively.

In our experiment the diffusion of Tempol was determined in a segment of pig skin. The tissue is not homogeneous and consists of structurally different layers. The diffusion coefficient was determined by assuming the skin to be homogeneous. Again using the same technique as before, the differences in the surface acceptance as well as surface contact were avoided. The everage diffusion coefficient determined in pig skin is $2x10^{-6}cm^2/s$. This value is close to $D=1.8x10^{-6}cm^2/s$ found for the same molecule diffusing in rat liver tissue[9]. The problem of skin nonhomogeneity i.e. the layer structure, might also influence the value of the diffusion coefficient.

CONCLUSION

The diffusion results presented on lipophilic and hydrophilic systems - ointment bases and skin have - shown that FG EPR is a versatile technique for application to heterogeneous systems irrespective of the physical state, chemical properties, presence or absence

of water, and surfactants. The FG EPR technique was developed as a specific, accurate and sensitive method for characterizing the diffusion processes of spin-labeled drugs in ointment bases and in skin.

APPENDIX

The translational diffusion coefficient was evaluated from the EPR spectra measured in the magnetic field gradient which provides the spatial resolution of the spin probe concentration profiles within the source and the sample. The source was initially loaded and brought in to contact with the sample. Both the source and sample are measured and this distribution is reproduced in the spectrum. The source of thickness h and the supposition of a "semi-infinite sample", with the initial concentrations in the isolated system

$$c = c_o \ , \ t = 0, \ 0 < x < h$$

$$c = 0, \ t = 0, \ h < x$$

are leading to the solution of the differential equation

$$\delta c/\ \delta t = D\ \delta^2\ c/\delta x^2 \quad , \qquad (2)$$

when both the source and the sample have the same diffusion coefficient[10]

$$\frac{c(x_i,t)}{c_o} = \frac{1}{2}\left\{\text{erf}\left(\frac{h - x}{2\sqrt{Dt}}\right) + \text{erf}\left(\frac{h + x}{2\sqrt{Dt}}\right)\right\} \quad . \qquad (3)$$

In our model the FG EPR spectrum was simulated by reproducing the spectrum for a series of experimentally

relevant values of the diffusion constant and the corresponding independent variables x and t, which indicate the distance and time. Distance goes from one side of the source x = 0 to the other at x = h and proceeds within the sample. The time is counted from the instant the source and sample have been joined. The spectrum is reproduced by convolution of the distribution function $f(x,t) = c(x,t)/c_0$ with the original line shape of the spectrum in the absence of the field gradient, which was approximated by the first derivative of a

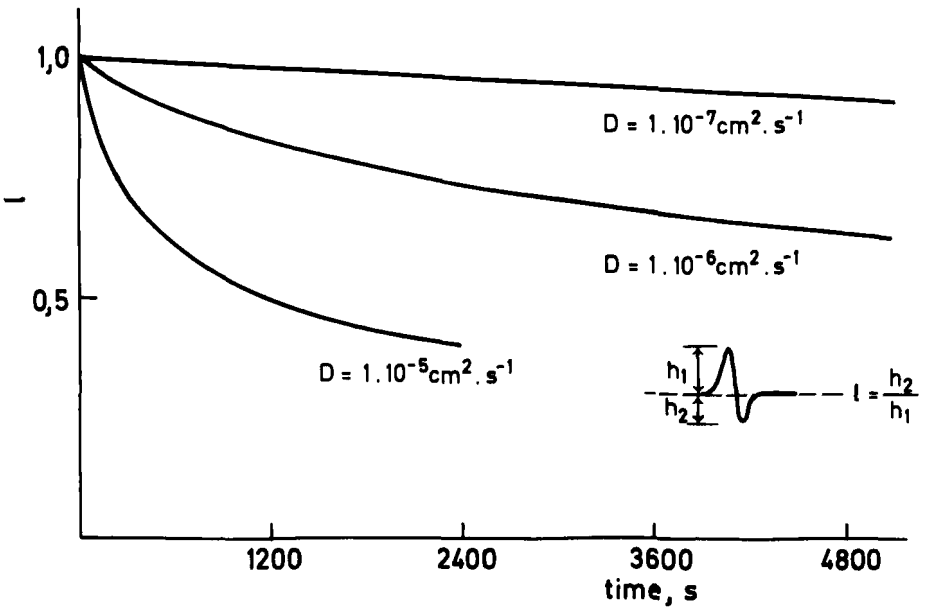

FIGURE 7

The time dependence of the line shape parameter "l" for Tempol determined from the calculated spectra (Appendix) for different values of diffusion coefficients. Other parameters taken to fit the spectra are (line width of spectra in the absence of a field gradient 0.2 mT, and the field gradient used in the experiment 4.8 mT/cm).

Lorentzian line L with the line width $\gamma = 1/T_2$:

$$L'(x-x',\gamma) = \frac{1}{\pi}\left\{\frac{2\gamma\,(x - x')}{[\,\gamma^2 + (x - x')^2\,]^2}\right\} \qquad (4)$$

so that the spectrum S is

$$S(x - x') = \int_{-\infty}^{\infty} f(x',t)\; L'(x - x', \gamma)\,dx. \qquad (5)$$

The parameters l evaluated from the calculated spectra were plotted (Fig.7) and used to determine the diffusion coefficients from the experimental spectra.

ACKNOWLEDGEMENT

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