Use of ATR-FTIR Spectroscopy to Study the Diffusion of Ethanol Through Glycerogelatin Films

A. Margarida L. Tralhão,[1] Adam C. Watkinson,[2,3] Keith R. Brain,[1,2] Jonathan Hadgraft,[1] and N. Anthony Armstrong[1]

Received July 4, 1994; accepted November 22, 1994

The use of ATR-FTIR spectroscopy to study the permeability of a glycerogelatin film is described. Measurement of the diffusion coefficient of ethyl alcohol-d in the film showed excellent reproducibility. Comparison of results from this technique with those previously obtained using an air-flow receptor phase diffusion cell show good agreement in terms of lag time assessed diffusion coefficients. ATR-FTIR spectroscopy revealed time-dependent changes in the composition of the glycerogelatin film during the diffusional process. It was also demonstrated that the concurrent assessment of both diffusant penetration and film composition is feasible.

KEY WORDS: ATR-FTIR; deuteration; diffusion coefficient; ethanol; glycerogelatin films; soft gelatin capsules.

INTRODUCTION

The soft gelatin capsule consists of a shell containing gelatin, water and a plasticizer (usually glycerol), and a fill which may be oily or hydrophilic. The range of substances which can be used as fills is limited, since molecules may diffuse from the fill into the shell and vice-versa. It is important to assess the extent of this diffusion so that the viability of new formulations and the stability of the final pharmaceutical dosage form can be predicted. Ethanol is an important solvent which is currently used in the formulation of soft gelatin capsule fills, but its use is hindered by its high hydrophilicity and small molecular size, both of which lead to rapid diffusion through the shell. Techniques which have previously been described to monitor the diffusion of molecules through gelatin columns and/or films (1–5) include the use of diffusion cell assemblies with air-flow receptor phases and spectrophotometric methods. This paper describes the use of attenuated total reflectance Fourier transform infra-red (ATR-FTIR) spectroscopy to monitor the diffusion of ethyl alcohol-d through glycerogelatin films.

The method of application of the ATR-FTIR system was similar to that described by various authors in diffusion studies on polymers (6–10) and semisolids (11). The film sample is sandwiched between the impermeable ATR crystal, and the reservoir of either pure or formulated diffusant which provides an essentially constant concentration, C_0, of diffusant in the upper surface of the film. The film is initially devoid of diffusant. As diffusion into the film occurs, there will be a build up of diffusant concentration at the film/crystal interface. An analytical solution describing the build up of diffusant concentration at the film/crystal interface with time can be obtained, using Fick's second law and the relevant initial and boundary conditions (12), and is given by Equation 1.

$$\frac{C}{C_0} = 1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\exp\left(\frac{-D(2n+1)^2\pi^2 t}{4h^2}\right) \quad (1)$$

where C is the diffusant concentration at the interface, t is the time, D is the diffusion coefficient of the diffusant and h is the film thickness. For large values of time, the $n = 0$ term in Equation 1 predominates and the long-time approximation of Equation 1 is given by Equation 2.

$$\ln\left[\frac{\pi}{4}\left(1 - \frac{C}{C_0}\right)\right] = -\left(\frac{\pi^2 D}{4h^2}\right)t \quad (2)$$

Equation 2 may be fitted to experimental data in a plot of

$$\ln\left[\frac{\pi}{4}\left(1 - \frac{C}{C_0}\right)\right]$$

against time. The diffusion coefficient, D, may then be obtained from the gradient of this plot and the film thickness, h.

The principle of operation of the ATR-FTIR system is as follows. The ATR crystal is mounted on the FTIR spectrometer, and the IR beam is internally reflected along the length of the ATR crystal. With the film sample placed on the upper surface of the ATR crystal, the total internal reflection is modified, in that the IR beam enters the sample to a small depth which is determined by the optical parameters of the beam, crystal and film. The IR beam is then attenuated according to the IR absorption characteristics of molecules in the surface region of the film. Assessment of the diffusant concentration at the film/crystal interface may therefore be performed by monitoring changes in the IR spectrum. The equipment is programmed to obtain spectra at predetermined intervals.

A limitation of the technique is that the diffusant under investigation must have an absorption band in its IR spectrum which can be distinguished from the IR absorption spectrum of the test film. Because the main IR bands (the C – H and O – H stretches) of ethanol were found to overlap with those of the glycerogelatin film, a deuterated analogue of ethanol (C_2H_5OD, ethyl alcohol-d) was used. The O – D stretch (~2500 cm^{-1}) is at a lower frequency than the O – H stretch (~3300 cm^{-1}) (Fig. 1). The deuteration of ethanol has been shown to have an insignificant effect on the diffusion process in synthetic membranes (13). An additional benefit of this approach is that the concurrent measurement of ethyl alcohol-d at the film/crystal interface and of the O – H stretches associated with the film constituents becomes possible.

MATERIALS AND METHODS

Ethyl alcohol-d (99.5+ atom % D) was obtained from

[1] Welsh School of Pharmacy, UWCC, Cardiff CF1 3XF, United Kingdom.
[2] An-eX, Redwood Building, King Edward VII Avenue, Cardiff CF1 3XF, United Kingdom.
[3] To whom correspondence should be addressed.

0724-8741/95/0400-0572$07.50/0

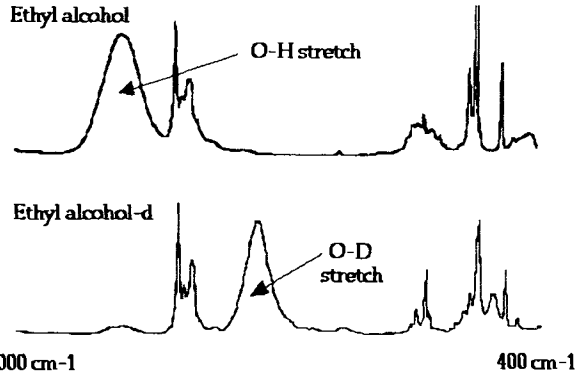

Fig. 1. Comparison of the IR spectra of ethyl alcohol and ethyl alcohol-d.

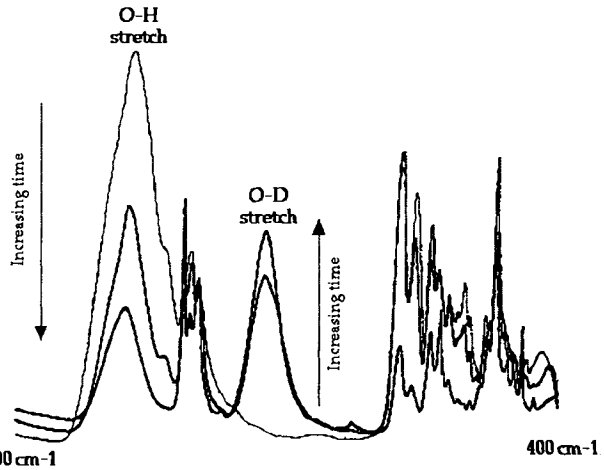

Fig. 2. Increase in O-D peak area (from penetrating ethyl alcohol-d) and decrease in O-H peak area (from film) with time.

Aldrich, UK and used as received. Absolute ethanol (99.7%) was obtained from James Burroughs, UK.

Films of approximately 0.3 mm thickness, were cast from a gel containing 39.7% water, 40% gelatin, 20% glycerol and 0.3% Carbopol 934P (14). After conditioning for at least five days at 33% RH, strips of the film were cut out and their thicknesses determined using a micrometer. The water content was measured as approximately 13%.

The film samples were placed in direct contact with the surface of a ZnSe attenuated total reflectance crystal (with a 45° angle of incidence to the IR beam) mounted on a Nicolet 710 FTIR spectrometer. Once the film was flat on the crystal the PVC trough was placed on top of it. The trough and film were sealed together with petroleum jelly, and the join was monitored for leaks throughout the experiment. The ethyl alcohol-d was then placed in the PVC trough and the trough covered with a plastic film held in place by a brass weight. The spectrometer was linked to a PC equipped with Nicolet Omnic software to allow the automated collection and subsequent manipulation of IR spectra. All non-linear curve fitting was carried out using UltraFit software (Biosoft®, Cambridge, UK) on an Apple Macintosh computer.

In order to assess whether there was any upward diffusion of film constituents into the donor phase, a sample of absolute ethanol was placed in contact with a film as described above, and left for 22 hours. The donor phase was then removed from the surface of the film and concentrated by evaporation of the majority of the ethanol. An ^{1}H-nmr spectrum of this donor phase sample was collected at 360 MHz on a Bruker WM360 spectrometer in D_2O solution, and compared with a spectrum of absolute ethanol.

RESULTS AND DISCUSSION

Typical examples of spectra over the time course of the experiment are shown in Figure 2. It can be seen that the area of the O−H peak reduces with time while that of the O−D peak increases. The area under the O−D peak was taken to be directly proportional to the concentration of ethyl alcohol-d at the interface. Normalised plots of areas of O−D and O−H peaks as a function of time are shown in Figure 3 for three replicate experiments. Even before normalisation, the spread of data is small showing the excellent reproducibility of the method. Normalisation of the data was carried out with respect to the final value of the area measured.

In Figure 3(a) it can be seen that there is an initial lag period of approximately 500 minutes, after which there is a rapid increase in concentration towards a plateau after about 900 minutes. Attempts at fitting Equation 1 to the full experimental data set were unsuccessful because the lag time for the data is anomalously long compared with the subsequent rate of rise in concentration. This implies either that the diffusion is non-Fickian or that the effective diffusion coefficient is not constant with time. The experimental data was then plotted in the form of Equation 2, as shown in Figure 4. It is apparent that a period of linearity exists after a lengthy lag time. The gradients of these plots yield a mean value for the diffusion coefficient of ethyl alcohol-d of $3.56 \pm 0.26 \times 10^{-4}$ mm^2 min^{-1}. The straight line part of the curve was extrapolated back to $y=0$ to give values of lag time. These were then used to obtain a second estimate of the diffusion coefficient using the expression $D = h^2/6t_{lag}$ (12). A value of $3.08 \pm 0.03 \times 10^{-5}$ mm^2 min^{-1} was obtained. This is in agreement with the value of $3.00 \pm 0.30 \times 10^{-5}$ mm^2 min^{-1} based on lag time measurements found previously (14). The fact that there is such good agreement between these measurements supports the validity of the assumption that the area under the O−D peak is directly proportional to the concentration of ethyl alcohol-d at the interface. There is an order of magnitude difference in the values of diffusion coefficient calculated using Equation 2 ($3.56 \pm 0.26 \times 10^{-4}$ mm^2 min^{-1}) and the lag time ($3.08 \pm 0.03 \times 10^{-5}$ mm^2 min^{-1}). This shows that the effective diffusion coefficient is not constant over the duration of the experiment. However, application of Equation 2 to the long-time data is valid, as it only assumes a constant diffusion coefficient from its point of application.

One hypothesis which may explain these results is that there may be a decrease in the barrier properties of the film with time, leading to a large increase in the value of the diffusion coefficient during the course of the experiment. If this is the case then there may be a threshold concentration of ethanol at which the film becomes significantly more permeable to the diffusant. If such a phenomenon occurs it will be more easily detected in the situation where there is an accumulation of ethanol in the film (allowing the build up of higher concentrations) rather than where sink conditions allow the solvent to exit from the distal side of the film.

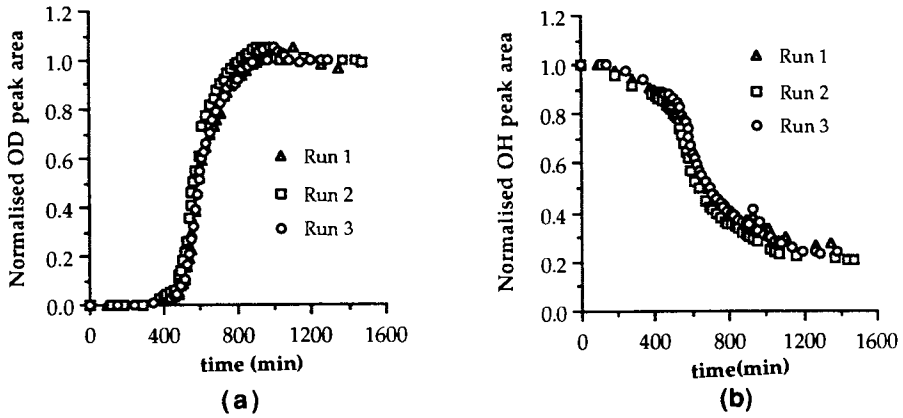

Fig. 3. (a) Normalised O-D peak areas as a function of time; (b) Normalised O-H peak areas as a function of time.

A second hypothesis involves the film 'soaking' up ethyl alcohol-d molecules, which perhaps displace bound water within the film structure. Once the binding sites are full, the experimentally seen lag phase will be complete and the free non-retarded diffusion of ethanol into the film would begin (15). This would be observed as a lengthy lag phase followed by rapid diffusion. It has been demonstrated previously (16,17) that there are at least two types of water in gelatin structures and that the 'free water population' can exchange with water that is bound to the gelatin. Although the possibility of an analogous process occurring with ethanol and bound water has been questioned (18) it has not been disproved.

Figure 3(b) shows that there is a decrease in the film associated O – H peak area with time. This reduction may be caused by the upward diffusion of water, glycerol and other low molecular weight hydrophilic molecules out of the interfacial region. In contrast to the arrival of ethyl alcohol-d at the interface there is no lag time associated with this process of diffusion. Association of rapid upward diffusion of hydrophilic molecules with the movement of ethanol into the film is suggested by the fact that both occur at the same time (Figure 3(a) and 3(b)).

The degree of upward diffusion of hydrophilic molecules was qualitatively assessed by sampling the ethyl alcohol-d donor phase before and after the experiment and running an IR spectrum of the samples. The spectrum of the ethyl alcohol-d donor phase at the start of the experiment

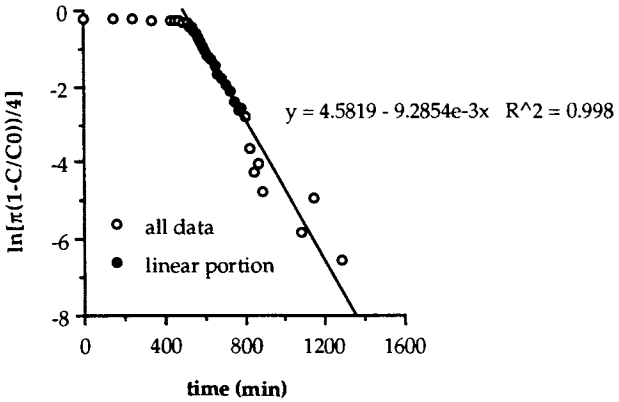

Fig. 4. Example of data plotted according to Equation 2 with a regression fit to the linear portion.

contained a strong absorption at ~2500 cm^{-1} (O – D stretch) and a very weak absorption at ~3300 cm^{-1}, presumably due to O – H contamination from absorbed atmospheric H_2O. Examination of the IR spectrum of the donor phase at the end of the experimental time frame again showed the presence of OD, but in addition, contained a strong absorption at ~3300 cm^{-1}. This was interpreted as being the result of upward diffusion of components of the film, containing O – H groups, into the donor phase. A control experiment was run concurrently with the glycerogelatin film absent to correct for any absorption of atmospheric water that might occur in the donor phase. Over the same time period and under the same conditions as the experiment the control donor phase showed no increase in the O – H peak area. This result suggests that the absorption increase seen in this spectral region in the presence of the membrane was due to diffusion of species out of the film. A further control experiment where no ethanol was placed on the film surface then showed no appreciable reduction in the O – H peak area associated with the film. This shows that the loss of O – H containing material from the film was dependent on the presence of ethanol.

Figure 5 compares the ^{1}H-nmr spectra between 0 and 4 ppm of ethanol and of the donor phase sample taken after 22 hours contact with the film. The ethanol spectrum shows a CH_3 triplet and CH_2 quartet. The ^{1}H-nmr taken after 22 hours contains additional peaks that can be explained by the presence of a quantity of glycerol in the donor phase. This implies that the O – H peak seen in the IR spectrum of the donor phase, as discussed above, is partly due to the presence of glycerol. The diffusion of glycerol and/or other plasticising components out of the film would be expected to produce an increase in crystallinity (19), and therefore in the tensile strength, which has been associated with a reduction in diffusion coefficients (20). However, the combination of long lag times and rapid subsequent diffusion which are observed experimentally suggest that such an effect is not dominant over the time scale of the experiment.

CONCLUSIONS

The work described in this paper shows that ATR-FTIR spectroscopy can be used to assess the diffusion of ethanol through glycerogelatin films. Changes in the composition of

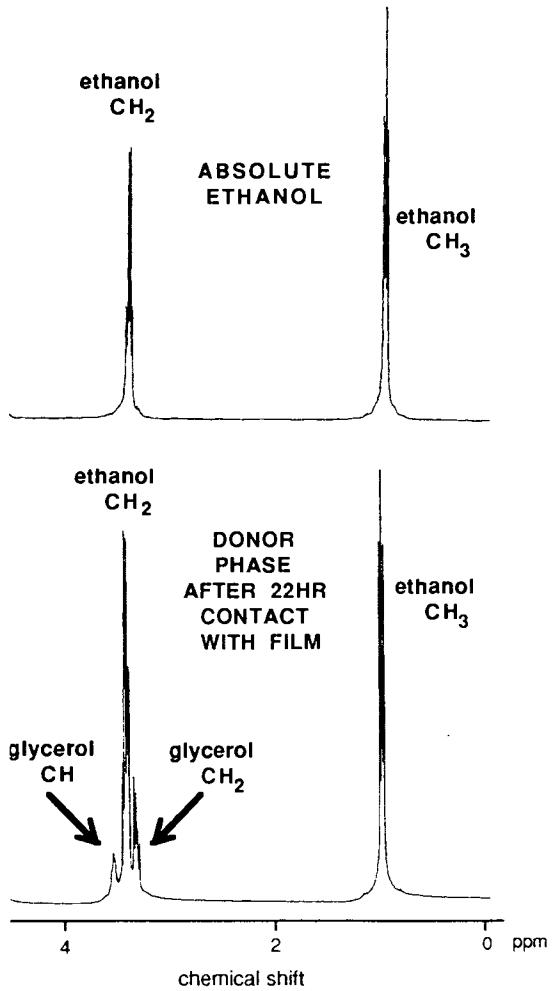

Fig. 5. ^{1}H-nmr of absolute ethanol and the donor phase after 22 hours.

glycerogelatin films during the diffusion of ethanol through them can be monitored. It has been demonstrated that hydrophilic constituents (including glycerol) diffuse out of the films when they are placed in contact with either ethanol or ethyl alcohol-d.

In principle ATR-FTIR spectroscopy could be used for evaluating the diffusion through glycerogelatin films of any substance with a characteristic absorption in the IR-transparent region of the film (~1800 cm^{-1} to ~2600 cm^{-1}). Deuterated analogues (e.g., OD or CD) can be used where appropriate. Changes in the film during the diffusion process due to interactions with the diffusant can be assessed. It is feasible that changes in the film structure due to factors such as relative humidity could also be monitored.

ACKNOWLEDGMENTS

The authors would like to thank JNICT for the award of a grant to AMLT. Thanks are also due to Dr. M. Hewlins and Mr. D. Jervis of the Department of Chemistry, UWCC for assistance in the ^{1}H-nmr studies.

REFERENCES

1. N. A. Armstrong, K. C. James, R. C. Moreton and F. S. S. Morton. Diffusion of volatile hydrophilic substances through glycerogelatin films. *J. Pharm. Pharmacol.* 42, Suppl. 5P (1990).
2. N. A. Armstrong, T. Gebre-Mariam and K. C. James. An apparatus for investigating drug migration into gelatin capsule shells. *Int. J. Pharm.* 34, 125-129 (1986).
3. J. R. Nixon, P. P. Georgakopoulos and J. E. Carless. Diffusion from gelatin-glycerine-water gels. *J. Pharm. Pharmacol.* 19, 246-252 (1967).
4. F. S. Hom, S. A. Veresh and W. R. Ebert. Soft gelatin capsules II: Oxygen permeability study of capsule shells. *J. Pharm. Sci.* 64, 851-857 (1975).
5. H. A. Smith. Sorption rates of water-soluble dyes on soft gelatin capsules. *J. Pharm. Sci.* 63, 639-641 (1974).
6. H. Brandt. Determination of diffusion specific parameters by means of IR-ATR spectroscopy. *Experimentelle Tech. Phys.* 33, 423-431 (1985).
7. K. Hemmelmann and H. Brandt. Investigation of diffusion and sorption properties of polyethylene films for different liquids by means of IR-ATR spectroscopy. *Experimentelle Tech. Phys.* 34, 439-446 (1986).
8. H. Brandt and K. Hemmelmann. On the evidence of non-linear sorption at the surface polyethylene/ATR-element for the diffusion of ethylacetate in polystyrene films. *Experimentelle Tech. Phys.* 35, 349-358 (1987).
9. K. C. Farinas, L. Doh, S. Venkatraman, and R. O. Potts. Characterisation of solute diffusion in a polymer using ATF-FTIR spectroscopy. *Macromolecules*, 27, 5220–5222 (1994).
10. A. C. Watkinson, K. R. Brain and J. Hadgraft. The deconvolution of diffusion and partition coefficients in permeability studies. Proceedings of the 21st International Symposium on Controlled Release of Bioactive Materials, p160–161, Nice, France (1994).
11. D. E. Wurster, V. Buraphacheep and J. M. Patel. The determination of diffusion coefficients in semisolids by Fourier transform infrared (FT-IR) spectroscopy. *Pharm. Res.* 10, 616-620 (1993).
12. J. Crank. *The Mathematics of Diffusion.* 2nd edition. Oxford University Press, New York, 1975.
13. M. A. Pellett, A. C. Watkinson, K. R. Brain, and J. Hadgraft. Diffusion of deuterated ethanol in synthetic membranes using ATR-FTIR spectroscopy. *Eur. J. Pharm. Sci. P.* 2, P147 (1994).
14. A. M. L. Tralhão and N. A. Armstrong. Ethanol diffusion in glycerogelatin films containing non-neutralised Carbopol 934P. Proceedings of the 3rd UKaps annual conference, Leicester, Hadgraft, J. Kellaway, I. W. and Parr, G. D. (Eds), p79 (1994).
15. G. L. Flynn, S. H. Yalkowsky, and T. J. Roseman. Mass transport phenomena and models: theoretical concepts. *J. Pharm. Sci.* 63, 479-510 (1974).
16. J. Maquet, H. Théveneau, M. Djabourov, J. Leblond and P. Papon. State of water in gelatin solutions and gels: An ^{1}H nmr investigation. *Polymer* 27, 1103-1110 (1986).
17. M. Djabourov, J. Leblond and P. Papon. Gelation of aqueous gelatin solutions. I. Structural investigation. *J. Phys. France* 49, 319-332 (1988).
18. R. C. Moreton. Migration of substances into and through soft capsule shells. PhD Thesis, University of Wales 1992.
19. L. A. Nielsen. *Mechanical Properties of Polymers and Composites.* Vol. I. Marcel Dekker, Inc., New York 1974.
20. A. M. L. Tralhão and N. A. Armstrong. Evaluation of some physical parameters of glycerogelatin gels and their relation with diffusion coefficients. *Pharm. Res.* 11, S-161 (1994).