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# The influence of viscosity on the migration of chloramphenicol and 4-hydroxybenzoic acid through glycerogelatin gels

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Migration of chloramphenicol and 4-hydroxybenzoic acid from solutions in 1-octanol into Glycerol Suppository Base, BP and soft gelatin capsule shells is reported. Rates of migration through the gels, quantified in terms of diffusion coefficients, are given. An electron spin resonance probing technique was used to determine the microscopic viscosity. The latter, rather than the bulk viscosity, was shown to be the major rheological influence on the rate of diffusion.

The soft gelatin capsule is widely used especially for drugs dissolved or dispersed in an oily or polyethylene glycol base. The shell is made from an aqueous dispersion of gelatin and plasticizer, usually glycerol, or a mixture of glycerol and sorbitol. Since the shell contains a significant amount of water and there is a dynamic relationship existing between capsule contents and capsule shell during the drying stage, the possibility arises for migration of ingredients from the fill material into the gelatin shell, and/or water from the shell into the fill material. This may affect the formulation. Recently, Serajuddin et al (1986) reported that a water insoluble drug, α-pentyl-3-(2-quinolinylmethoxy)benzenemethanol, dissolved in non-aqueous hydrophilic bases, crystallized out and the solubility of the drug decreased by 45%, as a result of water migration from the containing shell into the fill material. Conversely, considerable migration of solute from oily vehicle to capsule shell can occur and the extent of the migration is dependent upon the aqueous solubility of the encapsulated solute and its partition coefficient between the encapsulated solution and the capsule shell (Armstrong et al 1984, 1985, 1986).

In the present work, diffusion rate was investigated in relation to macro- and microscopic viscosity. A number of methods have been employed to study microscopic viscosity (Neilson et al 1977; Malinski & Zagorski 1979; Donner et al 1981). Morse (1985) described an electron spin resonance (ESR) technique for measuring the microviscosity within human erythrocytes, and this technique has been used to characterize the microenvironment of a glycoprotein gel (Kearney et al 1984). In the work herein reported, ESR spectroscopy was used to measure the microscopic viscosities of glycerogelatin gels.

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## MATERIALS AND METHODS

### *Materials*

4-Hydroxybenzoic acid (99%), glycerol (Analar) and gelatin BP were obtained from BDH Ltd, crystalline chloramphenicol was from Sigma Chemical Co., 1-octanol was from Fluka and 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy (TEMPOL) from Aldrich Chemical Co. All were used as received. The composition of the gel bases used and their viscosities at 45–60 °C are given in Table 1. All bases were prepared by the method given in the British Pharmacopoeia for the production of Glycerol Suppository Base BP.

### *Experimental procedure*

An apparatus recently described by Armstrong et al (1986) was used for measuring migration of the substrates. In this, a solution of solute in octanol was applied to the top of a glycerogelatin column contained in a truncated disposable 5 mL plastic syringe. This was housed in a boiling tube, the whole covered with cling film and a metal cap and clamped vertically in a constant temperature water bath at 22·5 °C. After a preselected period of time, the oily solution was poured off and the film of solution adhering to the inside of the syringe removed with a tissue. Slices were cut off as the column was extruded, weighed immediately and dissolved in 0·1 M HCl in the case of 4-hydroxybenzoic acid and in freshly distilled water for chloramphenicol. Both were assayed spectrophotometrically. Columns containing the oil but no diffusant were used in a similar manner as simultaneous controls.

*Calculation of diffusion coefficients*

The diffusion coefficients were obtained from the slopes of the plots of the natural logarithm of the concentration against distance squared. It has been shown that the process follows Equation 1 (Armstrong et al 1985, 1986), in which Co is the concentration at the proximal end of the column and C is the concentration x mm away. D is the diffusion coefficient and t is time.

$$\ln C = \ln Co - \frac{x^2}{4Dt} \quad (1)$$

The root mean square distance, namely

$$x = \left(\frac{x_1^2 + x_2^2}{2}\right)^{\frac{1}{2}} \quad (2)$$

was calculated and used to represent the distance corresponding to the concentration of each slice in a column, where $x_1$ and $x_2$ are the distances of the extremities of the slice from the interface.

*Measurement of viscosities*

*(a) Bulk viscosity.* A rotational viscometer, (Rheomat 30, Contraves, Zurich), was used for measuring the bulk viscosities of the gel masses at elevated temperatures. Rates of shear were plotted against shear stress, and viscosities calculated from the slopes obtained. All gels showed Newtonian behaviour.

An indication of viscosity at 22·5 °C was obtained by applying a penetrometer (Gallenkamp, London) to a sample of each gel. These were poured into truncated syringes to give a column about 4 cm in height and allowed to set for 12 h. The penetrometer needle assembly (weight 51 g) was applied to the surface of the gel for periods of 5 seconds. All bulk rheological data are given in Table 1.

Table 1. Composition of gels (% w/w), and their bulk viscosities.

| Base | A | B | C |
|---|---|---|---|
| Gelatin | 14 | 38·5 | 38·5 |
| Water | 16 | 38·5 | 38·5 |
| Glycerol | 70 | 23·0 | – |
| Sorbitol | – | – | 23·0 |
| Viscosity (P)* | | | |
| at 45 °C | 10·3 | 55·3 | 82·9 |
| 50 °C | 4·4 | 37·0 | 42·8 |
| 55 °C | 2·9 | 26·2 | 32·9 |
| 60 °C | 2·2 | 20·2 | 26·8 |
| Penetrometer (mm) | 8·28 | 1·33 | 1·04 |

* 1 P = $10^{-1}$ Pas.

*(b) Microscopic viscosity.* Microscopic viscosities were measured by an ESR probing technique, using TEMPOL as a probe. To each 20 g of molten base, 2 mL of TEMPOL solution (17·2 mg/100 mL) was added and stirred. The bases were adjusted to the desired weight in a vacuum oven at 60 °C to provide a final concentration of $1 \times 10^{-4}$ M TEMPOL in each mixture. ESR spectra were recorded with a Varian E3 spectrometer at ambient temperature, with a power level of 5 mW and a modulation amplitude of 4G.

## RESULTS AND DISCUSSION

4-Hydroxybenzoic acid was used in this study as it had been used extensively in the past to study migration in glycerogelatin (Armstrong et al 1984, 1985, 1986). Chloramphenicol was chosen because it has a reasonable solubility in both octanol and water, and so could be expected to migrate from an octanol solution into the glycerogelatin base. The same concentration (0·062 M) was used for both substances.

Three bases were selected; Base A was Glycerol Suppository Base, BP, and Base B a mixture of glycerol, gelatin and water which is commonly used as a shell for soft gelatin capsules. The higher gelatin content of B gave a higher bulk viscosity. The glycerol in Base B was replaced with an equal proportion of sorbitol (Base C) to study the effect of a change in plasticizer. An intended similar substitution in Base A was not carried out as Base A contains insufficient water to dissolve the sorbitol. Hence the liquid phase would consist of a suspension of sorbitol particles in water rather than a solution, and it was considered that a valid comparison of diffusion data could thus not be made.

Plots of the natural logarithm of the concentration against $x^2$ were rectilinear and diffusion coefficients were calculated. A typical example is shown in Fig. 1 for the diffusion of chloramphenicol in Base B. Table 2 shows the mean diffusion coefficients of chloramphenicol and 4-hydroxybenzoic acid in the three bases at 22·5 ± 0·1 °C.

Due to the high viscosities of the gels at 22·5 °C, their viscosities could only be measured by a rotational viscometer at elevated temperatures. These data are given in Table 1, as are penetrometer readings at 22·5 °C. It can be seen that the same rank order applies at 45, 50, 55 and 60 °C, it is therefore reasonable to assume that this order applied at 22·5 °C, particularly as the same order is obtained with the penetrometer at that temperature.

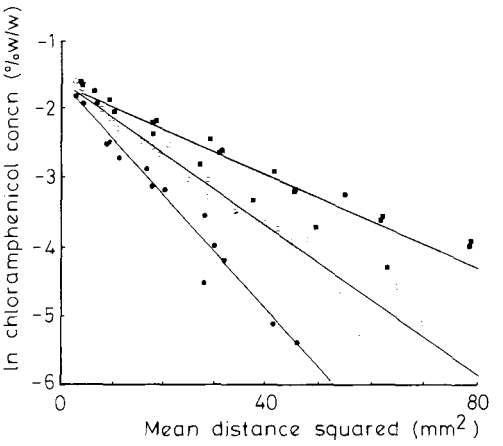


FIG. 1. The diffusion of chloramphenicol through base B. (●, 144 h; ○, 216 h; ■, 288 h).

Since movement of the solute through the column is diffusion controlled, it might be expected from the Stokes-Einstein equation that the process would be slower in the more viscous medium, but this is not so. Such behaviour has been observed in other gel systems (Farng & Nelson 1973; Kearney & Marriott 1986).

The anomaly arises because the bulk viscosity is not necessarily indicative of the diffusive resistance experienced at the solute molecular level. In polymer solutions and gels, diffusion of small molecules occurs via the spaces between the polymer segments which are filled by free solvent. Hence the viscous drag on the diffusant will be determined by the flow properties of this microenvironment, the so-called 'microviscosity'. The polymer interactions (cross-linking etc.) which determine the bulk rheological characteristics of gels will not significantly affect the diffusion of small molecules through the solvated matrix. Indeed, Taft & Malm (1939) showed that the diffusion rate of salts through aqueous gelatin solutions before and after setting was unchanged. If the crosslinking density is sufficiently high however, the dimensions of the interstices may approach the size of the diffusing molecules and in such instances selective diffusion (gel filtration) may occur.

The major hindrance afforded by the presence of the polymer itself would be through an obstruction effect. The extent of this effect has been derived theoretically for macromolecules in general (Wang 1954) and was shown to be linearly dependent on the volume fraction. As diffusing molecules have to diffuse around the polymer segments, the effect can be considered as arising from an increase in the path length. Hence the net rate of transfer of diffusant in any given direction will be reduced accordingly.

In the gelatin systems used in this work, the effect of the microviscosity is illustrated by the data obtained for Base B in which sorbitol was substituted for glycerol. The mean diffusion coefficient of 4-hydroxybenzoic acid, for example, was lowered from $0{\cdot}040 \pm 0{\cdot}002\ mm^2\ h^{-1}$ to $0{\cdot}027 \pm 0{\cdot}0018\ mm^2\ h^{-1}$. It thus becomes essential to measure the microscopic viscosity, and for this the technique of ESR probing was chosen.

When a small nitroxide molecule, e.g. TEMPOL is moving rapidly and isotropically in a non-viscous medium, the ESR signal consists of three narrow, symmetrically spaced peaks of equal height (Fig. 2a). If the spin probe is not tumbling freely, this leads to a differential line broadening in the spectrum (Fig. 2b, c). The mobility of TEMPOL, the spin probe, is assessed in terms of a measured rotational correlation time ($\tau$) which is determined from the relative peak heights of the ESR triplet.

The rotational correlation time of the probe was calculated from Equation 3 which was initially employed by Keith et al (1970) and later used by Morse et al (1975).

$$\tau = 6{\cdot}5 \times 10^{-10} \Delta H_O \left( \sqrt{\frac{h_o}{h_{(-1)}}} - 1 \right) \qquad (3)$$

$h_o$ and $h_{(-1)}$ are the amplitudes of the central and high-field lines, respectively, and $H_O$ is the line width

Table 2. Mean diffusion coefficients of chloramphenicol and 4-hydroxybenzoic acid through glycerol suppository base (Base A) and soft gelatin capsule shell base containing glycerol (Base B) and sorbitol (Base C). (n = 4, $P' = 0{\cdot}01$).

| Running time (h) | Diffusion coefficients ($mm^2h^{-1}$) Chloramphenicol | | | 4-Hydroxybenzoic acid | | |
|---|---|---|---|---|---|---|
| | Base A | Base B | Base C | Base A | Base B | Base C |
| 144 | 0·0090 (0·0003) | 0·020 (0·002) | 0·0155 (0·0017) | 0·021 (0·001) | 0·039 (0·0018) | 0·0275 (0·0007) |
| 216 | 0·0087 (0·0003) | 0·022 (0·003) | 0·0140 (0·0012) | 0·021 (0·002) | 0·042 (0·002) | 0·0270 (0·003) |
| 288 | 0·0087 (0·0004) | 0·023 (0·005) | 0·0110 (0·0017) | 0·021 (0·001) | 0·040 (0·0015) | 0·0260 (0·0017) |

The figures in parentheses represent standard errors.

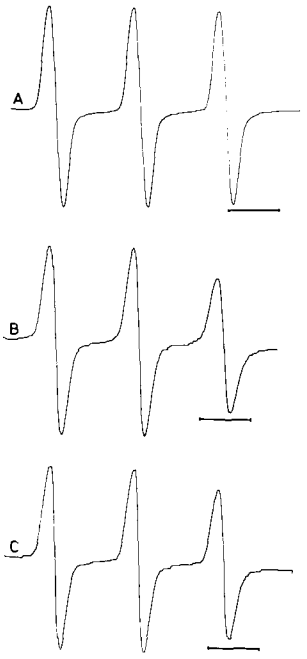


FIG. 2. ESR spectra of $1{\cdot}0 \times 10^{-4}$ M solution of TEMPOL at 25 °C in (a) distilled water, (b) Base A, (c) Base B.

of the central line in gauss. This form of the equation gives $\tau$ in nanoseconds.

The viscosity ($\eta$) of water at ambient temperature (0·8904 cP; $8{\cdot}904 \times 10^{-4}$ Pas) and the rotational correlation time of free TEMPOL in water were taken as standards and the microscopic viscosities of the different gels were calculated from Equation 4.

$$\tau_{gel}/\tau_{H_2O} = \eta_{gel}/\eta_{H_2O} \quad (4)$$

The results of the rotational correlation time of TEMPOL in different media and the corresponding microscopic viscosities are shown in Table 3.

Table 3. Rotational correlation times ($\tau$) for TEMPOL in water and gels, and the corresponding microviscosities.

| Solvent | $\tau$ (ns) | Microscopic viscosity (cP)* |
|---|---|---|
| Water | 0·027 | 0·89 |
| Base A | 0·408 | 13·30 |
| Base B | 0·199 | 6·52 |
| Base C | 0·332 | 10·86 |

* 1 P = $10^{-1}$ Pas.

Fig. 3 shows the relationship between the diffusion coefficient of 4-hydroxybenzoic acid and the microscopic viscosity. An approximately rectilinear plot is obtained showing that the microscopic viscosity plays a much more important role in determinating diffusion through the gel than does the bulk viscosity.

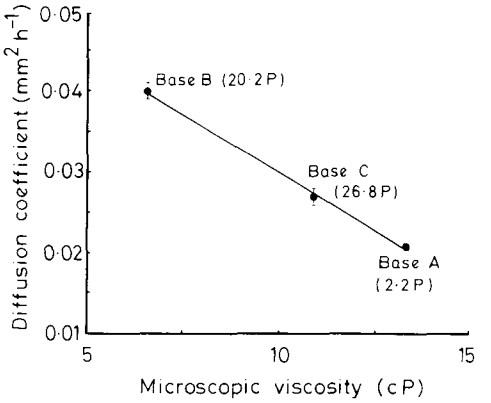


FIG. 3. The relationship between the microscopic viscosity of the three bases and the diffusion coefficient of 4-hydroxy benzoic acid. The bulk viscosity of each base at 60 °C is shown.

Thus diffusion can be controlled by varying the composition of the liquid filling the interstices of the gel and, to a lesser extent, by changing the volume fraction of the polymer used to make the gel.

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