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The Effect of Gelatin Grade and Concentration on the Migration of Solutes Into and Through Glycerogelatin Gels

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Abstract—The diffusion of 4-hydroxybenzoic acid and phenobarbitone through glycerogelatin gels was found to be independent of the type of gelatin used. Three types of gelatin, two acid-processed and one alkali-processed were studied, and the bulk viscosities of gels prepared from them was seen to vary considerably. However, the microviscosities of the gels, as measured by ESR, showed no significant differences. Thus microviscosity was the factor governing diffusion. Gelatin concentration in aqueous solutions without glycerol influenced microviscosity and hence diffusion. This is believed to be caused by dissolution of water-soluble fractions of the gelatin. Interstices in the gelatin matrix, though reduced in size when gelatin concentration is raised, are still too large to act as physical barriers to diffusing molecules. It is suggested that hydrated gelatin forms the matrix of glycerogelatin mixtures and that the interstitial fluid, through which migration occurs, consists almost entirely of glycerol and water.

It has been shown previously that the rate of migration through plasticized gelatin gels is dependent on the microviscosity of the gel, rather than its bulk viscosity. It was also shown that the composition of the plasticizer had an important influence on microviscosity and hence on migration (Armstrong et al 1987). In that work, the same sample of gelatin was used throughout. The present work investigates the effects of changing the grade and concentration of gelatin on the viscosities of the gels, and on the rates and extents of migration. The migrating species were 4-hydroxybenzoic acid and phenobarbitone, both of which have been used previously to study migration (Armstrong et al 1984). An HPLC technique for the determination of phenobarbitone in glycerogelatin gels was developed, and used in place of the previously employed spectrophotometric technique which was considered to be less accurate.

Materials and Methods

Materials

Gelatin I was acid-processed gelatin and of BP quality, obtained from BDH Ltd. Gelatin II was acid-processed and gelatin III was an alkali-processed gelatin. Both were obtained from R. P. Scherer Ltd, Swindon. Phenobarbitone and butobarbitone were obtained from May & Baker Ltd, Dagenham, and isopropyl myristate (IPM) from Fluka. Anhydrous sodium dihydrogen phosphate was reagent grade and diethylether and methanol were Analar. The remaining reagents and solvents were as described previously (Armstrong et al 1987). All were used without further purification.

Three glycerogelatin bases, A, B, and C, containing 38·5% w/w gelatin, 38·5% w/w water and 23·0% w/w glycerol were prepared from gelatins I, II and III, respectively. They were prepared by the method given in the British Pharmacopoeia for the production of Glycerol Suppository Base BP, except that the gel masses were degassed in a vacuum oven at 60°C until the excess water was lost and clear gels obtained.

Gelatin gels containing no plasticizer were prepared in the same way. Heating times and temperatures were the same for each gel, so that if thermal degradation of the gel occurred, it took place to the same extent in each case.

Photomicrographs of the gels were obtained using a scanning electron microscope (Cambridge Stereoscan 600, Cambridge Instruments, Cambridge, UK). A piece of aluminium foil was dipped into the molten gel and then plunged into liquid nitrogen. The gel was freeze dried overnight and then sputtered with gold before scanning.

Characterization of gelatins I, II and III

Electrophoretic mobilities were determined using a Malvern Zetasizer IIC, and the remaining data shown in Table 1 were provided by R. P. Scherer Ltd. The method for measuring diffusion rates has been described previously (Armstrong et al 1986), as also have methods for determining bulk and microviscosities, and use of the penetrometer (Armstrong et al 1987). Though no preservatives were used in the gels, no evidence of microbial spoilage was observed.

Analysis of glycerogelatin slices

4-Hydroxybenzoic acid was assayed using the method described previously (Armstrong et al 1986). A liquid chromatography method was developed for phenobarbitone using a multiwavelength detector (LDC-Milton Roy Spectromonitor III-wavelength, LDC Ltd, Stone, UK), a Rheodyne 7125 injection valve fitted with a 50 μL loop, a constant flow pump (Technicon Fast LC pump, Technicon Instruments, USA) and a strip chart recorder (CR 650S, J. J. Lloyd Instruments, Southampton, UK). A 25 cm × 4·6 mm internal diameter stainless steel C18 reversed plate column (Apex ODS, Jones Chromatography Ltd, Hengoed, UK) was employed.

The mobile phase, as used by Gill et al (1981) was a mixture of 600 mL 0·1 M sodium dihydrogen orthophosphate in distilled water and 400 mL methanol, adjusted to an

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apparent pH of 8·5 with 10 M NaOH. The solution was filtered through a 0·5 μm membrane filter and degassed by ultrasound sonication. Stock solutions containing 2·5 to 40 μg mL^{-1} of phenobarbitone and the internal standard, butobarbitone, in the mobile phase were pumped at a flow rate of 1 mL min^{-1}, and the detector was attenuated at either 0·1 or 0·05 aufs with the wavelength fixed at 240 nm. A rectilinear relationship was obtained between concentration and peak height responses. Well resolved chromatograms were obtained, and the retention times for phenobarbitone and butobarbitone were 5·0 and 8·5 min, respectively.

Glycerogelatin gels containing barbiturate were dissolved at 37°C in a mixture of 0·2 mL of 0·1% internal standard in mobile phase and 0·3 mL of 0·1M HCl, and adjusted to a pH of 1 with further HCl. 5 mL of ether was added, mixed for 15 min using a rotary mixer, centrifuged and the ether layer collected. The aqueous layer was extracted twice more with 5 mL ether, and the combined extracts evaporated to dryness in a stream of nitrogen. The residue was reconstituted with 5 mL of mobile phase and 50 μL injected into the column.

Results and Discussion

In previous migration studies (Armstrong et al 1984), phenobarbitone concentrations were followed spectrophotometrically. This method had the disadvantage that the irrelevant absorption of gelatin occurs in the same region as the absorption of phenobarbitone. The HPLC procedure was developed for this reason. The new process was tested by incorporating known quantities of barbiturate into glycerogelatin bases and comparing peak heights with their calibration results. Percentage recoveries of $100 \pm 0{\cdot}9$ and $100 \pm 0{\cdot}6$ were obtained for phenobarbitone and butobarbitone, respectively.

The three gelatin samples were found to vary considerably in their chemical and physical properties as shown in Table 1. These differences will account for differences in the rheological properties in the gels made from them as indicated by bulk viscosity and penetrometer data (Table 2).

Bulk viscosities were recorded at elevated temperatures since it was impossible to measure this property at room temperature. However, it has been shown (Gebre-Mariam 1988) that viscosity is related to temperature in a manner analogous to that expressed by the Arrhenius equation. Bulk viscosities at all temperatures and penetrometer readings at ambient temperatures followed the same rank order, and plots of log viscosity against reciprocal of temperature were rectilinear. Estimating the bulk viscosity at 23°C by this means gives values of 26·0, 47·0 and 56·8 Pas for gels A, B and C, respectively.

Thus there is a more than two-fold difference in bulk viscosity, presumably accounted for by the different properties of the three gelatins. Yet the microviscosities of the three gels show negligible differences. Thus, the matrix which is formed in the gels is dependent on the gelatin type, but the properties of the fluid in the interstices of the gel are not. These data also suggest that the interstices in the gel are large enough not to have any retarding effect on TEMPOL, the ESR probe.

Diffusion data for 4-hydroxybenzoic acid and phenobarbitone are given in Table 3, using both isopropyl myristate and octanol as the solvents applied to the top of the glycerogelatin columns. Though the diffusion coefficients and fractions vary with diffusant and solvent, for any given

Table 1. Properties of gelatin samples.

Property	Gelatin I	Gelatin II	Gelatin III
Mean molecular weight*			
Number average	34676	40024	45687
Weight average	89632	101929	112557
Bloom strength (g)**	145	203	155
Viscosity (cP)**	2·65	3·12	3·76
Loss on drying (%)**	10·5	11·5	8·81
pH**	4·70	5·10	5·75
Ash (%)**	0·33	0·46	0·56
Iso-ionic pH***	8·54	7·49	4·89
Electrophoretic mobility ($\mu m\ s^{-1}\ v^{-1}\ cm^{-1}$)	+0·67	+0·83	−1·10

* By gel permeation chromatography.
** British Standard 757.1975.
*** By ion exchange.

Table 2. Bulk and microviscosities of the glycerogelatin gels.

Base	A	B	C
Bulk viscosity (Pas)			
at 45°C	5·53	8·36	10·30
50°C	3·70	4·60	5·80
55°C	2·62	3·00	4·48
60°C	2·02	2·40	3·30
Penetrometer reading (mm)	1·3	0·9	0·6
Microviscosity ($Pas \times 10^{-3}$)	7·15	7·49	7·35

Table 3. Migration data for bases A, B and C.

	Apparent diffusion coefficient ($mm\ h^{-1}$)					
	Isopropyl myristate			Octanol		
	A	B	C	A	B	C
4-Hydroxybenzoic acid	0·051 (0·003)	0·050 (0·002)	0·050 (0·003)	0·041 (0·002)	0·041 (0·003)	0·042 (0·002)
Phenobarbitone	0·031 (0·001)	0·032 (0·001)	0·033 (0·001)	0·030 (0·001)	0·030 (0·001)	0·030 (0·001)
	Fraction migrated after 360 h					
4-Hydroxybenzoic acid	0·89	0·90	0·89	0·22	0·23	0·21
Phenobarbitone	0·36	0·37	0·37	0·062	0·063	0·064

The figures in parentheses represent the confidence limits of the means ($P' = 0{\cdot}01$). Each result is the mean of 16 replicates.

combination of diffusant and solvent, there are no significant differences for diffusion data obtained with the three gelatins. From this it must be concluded that though gelatin type will be of importance in the properties of capsules, it has very little influence on migration into the shell.

A possible explanation is that since gelatin forms a matrix, it makes a negligible contribution to the interstitial fluid, which is a mixture of water and glycerol. However, in the absence of glycerol, gelatin has a marked effect on microviscosity.

The effect of a change in gelatin concentration on microviscosity is shown in Fig. 1. The gels used in this case are mixtures of gelatin I and water. There is a considerable increase in microviscosity as the gelatin concentration is raised. It must be borne in mind however that some of the gelatin concentrations reported here could not be used for soft shell capsules. Diffusion coefficients of 4-hydroxybenzoic acid from isopropyl myristate were determined, and these, when plotted against microviscosity, are shown in Fig. 2. Here again, diffusion is linearly related to microviscosity.

The reason why the microviscosities of gelatin/water mixtures increase with increasing gelatin concentration may be that the gelatin matrix becomes more dense. Hence the interstices are reduced in size and the ESR probe encounters hindrance to its rotation.

Migun & Prokhorenko (1987) have studied the viscosities of polar fluids in microcapillaries, and have shown that significant changes in viscosity are apparent when the radius of the capillary is less than 0·5 μm. A scanning electron micrograph of a 20% solution of gelatin III in water (Fig. 3) shows a porous structure with apertures of about this size, though it is accepted that the method of sample preparation may have some effect on the apparent pore size. Thus some increase in viscosity would be anticipated for this reason. The other gelatins gave similar pictures. Also smaller interstices would have the effect of providing a more tortuous pathway through which diffusing molecules must pass. However the pore sizes indicated by electron microscopy are still much bigger than the molecular sizes of both diffusants and the ESR spin probe.

A second possibility is that in gelatin-water gels, soluble components are released from the gelatin. Hence the medium through which both spin probe and diffusant must pass consists not of water but an aqueous solution of the soluble fractions of the gelatin. Microviscosity data suggest that glycerol suppresses this process. Table 4 gives microviscosities of mixtures of components of the shell. The microviscosity of water is increased by a factor of nearly 4 with the addition of 38·5% gelatin. However, addition of a similar proportion of gelatin to a mixture of glycerol and water has a

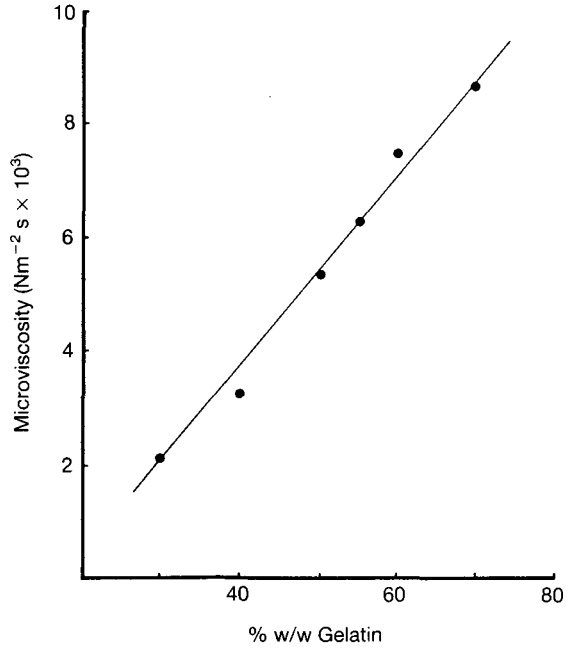

FIG. 1. The effect of gelatin concentration on microviscosities of aqueous solutions.

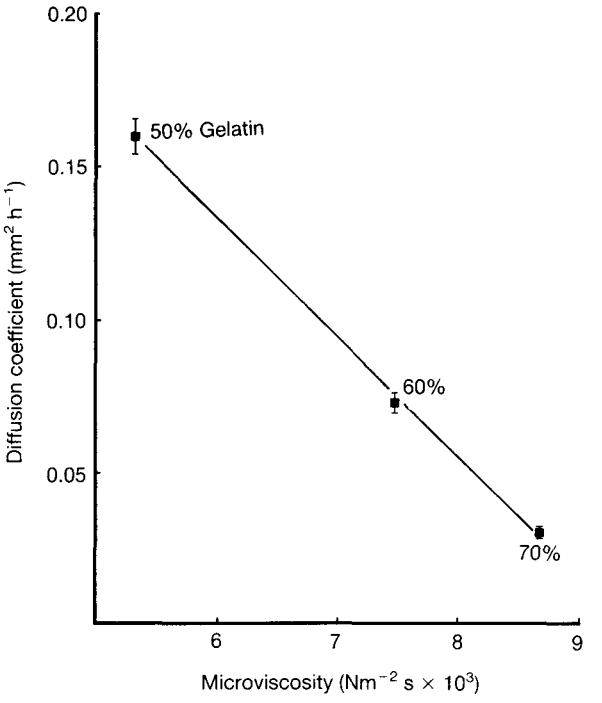

FIG. 2. The relationship between diffusion coefficient and microviscosity at three gelatin concentrations in aqueous solution.

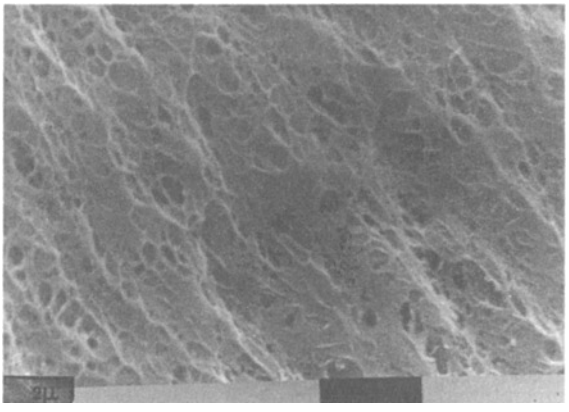

FIG. 3. Scanning electron micrograph of an aqueous gel containing 20% gelatin III.

Table 4. Microviscosities at 23°C of components of the glycerogelatin mass.

Component	Microviscosity (Pas × 10^{-3})
Water	0·93
Gelatin I: water (38·5:61·5)	3·00
Glycerol: water (23:38·5)	7·20
Gelatin: glycerol: water (38·5:23:38·5)	7·15

negligible effect. It can be assumed that the water-soluble components of gelatin do not dissolve so readily in the presence of glycerol. Experimental observations indicated that the solubility of gelatin in glycerol was less than 1%, compared with its higher solubility in water. Also it is known that gelatin hydrates in the presence of water. If a fraction of the water becomes associated with the gelatin molecules, it is unlikely that it will form part of the medium through which diffusion occurs. Thus the interstitial fluid would become richer in glycerol, and this too would depress dissolution of water soluble components of the gelatin.

The suggested constant composition of the fluid through which the solute migrates is supported by the quantities of solute which had migrated into the glycerogelatin gel after 360 h. After this time, the systems were close to equilibrium, so that the results given in Table 3 are a measure of the partition coefficients between the non-aqueous solutions and the glycerogelatin bases. For each combination of solute and non-aqueous solvent, the fraction which had migrated was constant, indicating that the aqueous partitioning phase was the same for each type of gelatin.

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