The effect of ageing on the rheological properties of gelatin gels

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The effect of ageing at 4° on the viscoelastic properties of 1·5 and 2·0% acid and alkaline processed gelatin gels has been investigated. Viscoelastic properties were not markedly affected by ageing time although evidence of a decrease in gel structure with increasing ageing time was apparent. This has been tentatively attributed to hydrolysis of amide links. 1·5% alkaline gels were shown to be more rigid than the corresponding acid gels and this has been discussed in terms of differences in molecular weight, polydispersity, charge and charge distribution.

The increase in the rigidity modulus (G) of gelatin gels which occurs during maturation is the result of a complex and undefined ageing process. A rapid rate of increase in rigidity is observed initially which subsequently falls although an equilibrium value is never obtained. Ferry (1948) has shown that by precooling the gel at 0° for 24 h and then raising the temperature to the experimental value, rigidity increases rapidly at first but reaches a value which is approximately constant within 3–5 h.

The viscoelastic properties of gelatin gels have been investigated by Miller, Ferry & others (1951) using a concentric cylinder apparatus to measure stress relaxation in 5·92% gels. These workers observed a distribution of relaxation times between 10^3 and 10^6 s. The distribution was shifted to shorter times by increasing the temperature and to longer times by increasing the time of ageing before stress application. In the present work non-destructive creep compliance tests have been used to monitor the structural changes which occur on ageing of both acid and alkaline processed gels at 4°.

MATERIALS AND CHARACTERIZATION

The gelatins used were commercial acid and lime processed ossein samples (Croda Ltd.) suitable for use in hard gelatin capsule manufacture. The manufacturer's specifications* indicated that the gelatins did not vary markedly in terms of physical characteristics. The samples were further characterized in terms of molecular weight, axial ratio and isoelectric point. Number average molecular weights (M_n) of the samples were determined at 30° in 2M potassium thiocyanate using a Melabs CSM-2 membrane osmometer.

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* Bloom, g at 6 2/3 % acid (AC) 249, alkaline (AL) 243; viscosity, mps at 12½ % AC 123, AL 190; viscosity degradation, % AC 16, AL 20; insoluble particles ACAL none; particle size, % passing 3/16″ BSI ACAL 100; % passing no. 32 BSI AC 6, AL 6; *CHEMICAL TESTS*: Moisture % AC 12·0, AL 10·5; pH AC 5·3, AL 5·6; ASH, % AC 0·1, AL 0·2; SO_2, ppm AC 40, AL 24; As, ppm AC none, AL 1; heavy metals, ppm AC, AL none.

Increasing thiocyanate ion concentration brought about a decrease in the number average molecular weight of the acid and alkaline samples: this was 45 500 and 57 000 respectively for the two gelatins at 1·0 M thiocyanate; 44 800 for acid gelatin at 1·5 M thiocyanate, and 40 300 and 52 800 respectively at 2·0 M thiocyanate. Also the interaction coefficient approached zero in 2 M KCNS in both cases, indicating a decreased tendency to aggregate, i.e. little or no solute-solute interaction.

Axial ratios were determined from limiting or reduced viscosity measurements using a "U" tube viscometer. 2 M Potassium thiocyanate was used as the solvent and viscosities were measured in the concentration range 0·1–0·6% w/v. The axial ratio of the acid sample was found to be 21·6 and that of the alkaline sample 25·4. Stainsby (1952) and Chernyak & Pasynskii (1953) found axial ratios of 25·0 and 24·0 for alkali processed gelatins.

Isoelectric points of the acid and alkaline gelatins have been determined indirectly by "U" tube viscometry and by direct determination of mobility. These mobilities were determined at 25° in Michaelis acetate-barbitone buffer in the pH range 4·0–7·0 in a microelectrophoresis apparatus, (Rank mark II, Rank Bros., Bottisham, Cambridge), using a capillary cell. The gelatin samples were adsorbed onto polyvinyl toluene (PVT) latex 1·87 μm diameter, 10^5 particles ml^{-1} or polystyrene latex 0·8 μm diameter, 10^8 particles ml^{-1} and the final gelatin concentration in buffer solution was 0·05% w/v.
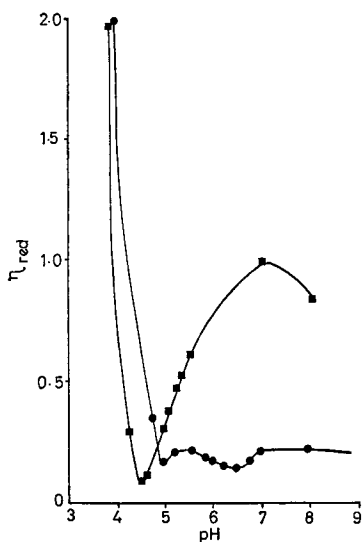

FIG. 1. Effect of pH on dilute solution viscosity of 2·0% w/v acid and alkaline gelatins at 25°. ■ Alkaline gelatin. ● Acid gelatin.

Fig. 1 shows the effect of pH on dilute solution viscosity. It is evident that a distinct isoelectric point exists at pH 4·6 for the alkaline sample whereas a single value for the acid gelatin is not apparent. With the acid gelatin the isoelectric point appears to be in the pH region 6·0–7·0. Similar behaviour in acid and alkaline processed gelatins has been observed by Stainsby (1952).
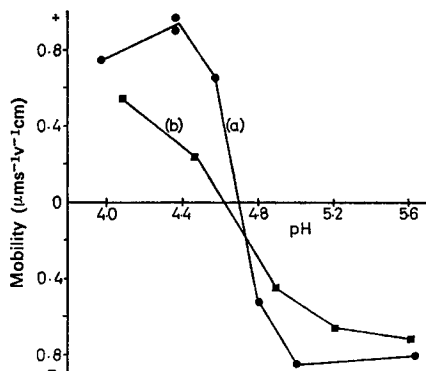
FIG. 2. Effect of pH on the electrophoretic mobility, at 25°, of alkaline gelatins adsorbed onto latex spheres. (a) PVT latex; (b) Polystyrene latex.

Fig. 2 demonstrates the effect of pH on the electrophoretic mobility of gelatin adsorbed on to either PVT or polystyrene latex. Mobilities presented are the mean of ten measurements, five in each direction. Determinations were carried out only in the upper stationary level since the method was used solely as a means of locating the isoelectric point. The isoelectric point of the alkaline gelatin occurred at pH 4·6 for gelatin adsorbed on to polystyrene latex and 4·7 for the PVT latex, the former value corresponding to the U-tube results. It was not possible to estimate the mobilities of the adsorbed acid samples since values were lower than the minimum measurable mobility (0·1 μm s^{-1} V^{-1} cm). However, the particle charge changes from positive to negative between pH 6·0 and 6·2 for both PVT and polystyrene latex. The isoelectric point for most acid gelatins is of the order of pH 9·0. The probable explanation of this discrepancy is that the acid sample used in this work was treated to produce a pH in solution close to that of the alkaline sample.

METHODS

1·5% w/v and 2·0% w/v gels were prepared by heating at 50° and maintained at this temperature for 1 h. The gels were then stored at 4° for the required ageing period. After equilibration at 25° for 2 h the semirigid gels were loaded between the cone and plate of an air turbine viscometer (Marriott, Irons & Harris, 1973) and equilibrated for a further 1 h at 25° in a saturated atmosphere. Creep compliance tests were carried out in the linear viscoelastic region for 90 min by which time the creep curve had been linear for approximately 25 min. Fig. 3 indicates the linear relation between stress
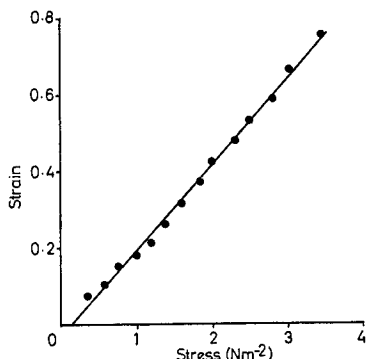

FIG. 3. Strain-stress relationship in a 1·5% w/v acid gelatin gel at 25°.

and strain in a 1·5% alkaline gel at 25°. A known stress was applied to the gel and the instantaneous shear compliance, J_0, was determined. The stress was removed and the gel allowed to relax for 5 min. This procedure was repeated at gradually increasing stresses. Linearity was monitored in the 1·5% w/v and 2·0% w/v gels by inspection of the creep recovery curve which was generated at the end of the actual creep test upon removal of stress. The maximum stress applied to any system was 3·87 Nm^{-2}. The system of optical levers was so arranged that full scale deflection of the pen (100 mm chart) resulted from an angular deflection of the cone of 0·0267 rad (1°30) which is within the theoretical limits of testing when using cone and plate geometry. Results were subjected to line spectral analysis as described by Warburton & Barry (1968) and a computer program was written to carry out the procedure.

RESULTS AND DISCUSSION

Fig. 4 shows the effect of ageing at 4° on the instantaneous shear compliance (J_0). The increase observed for both acid and alkaline 1·5% gels indicated a less rigid gel structure which may be due to the hydrolysis of amide links in the polypeptide chains or a decrease in the number of cross-links between chains.

Costello & Goyan (1964) investigated the viscoelastic properties of gelatin-glycerin mixtures as used in soft gelatin capsule manufacture. Stress relaxation was measured for thin films and the results analysed in terms of a mechanical model with four Maxwell units in parallel. The instantaneous elasticity, E_0, and the equilibrium elasticity, E_e, decreased with increasing ageing time up to 48 h indicating that the number of cross-links was decreasing or that polymer chains were contracting and becoming more amorphous. It was concluded that the decrease in E_0 and E_e in aged films up to 48 h was not due to hydrolysis effects and they proposed an orientation mechanism.

The scatter observed in the experimental data for the 1·5% acid precursor gels may be attributed to the effect of temperature (a 2% acid gel melted at 27·4° and the m.p.s of alkaline gels at 1·5 and 2·0% concentrations were 26·0 and 27·9), since tests were carried out at 25° which is close to the melting point of the gel (25·2°). The variance of the experimental points about the dotted regression line is 0·000036. The lower J_0 values observed for the 1·5% alkaline gelatins indicate the existence of a more rigid gel network. The 2% w/v acid gels showed a small decrease in J_0 with ageing time and hydrolysis effects were not evident.
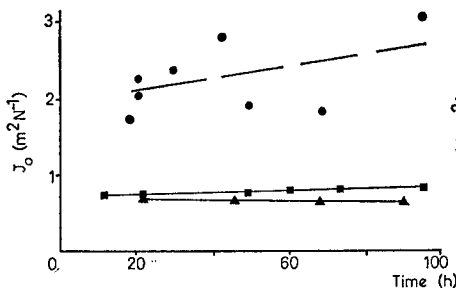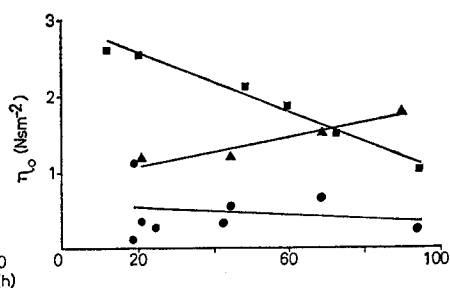

FIG. 4.   FIG. 5.

FIG. 4. The effect of ageing time on the instantaneous shear compliance (J_0) of acid and alkaline gelatin gels at 25°.   ● 1·5% w/v acid gel.   ■ 1·5 % w/v alkaline gel.   ▲ 2·0% w/v acid gel.
FIG. 5. Effect of ageing time on the steady state shear viscosity (η_0) of acid and alkaline gelatin gels at 25°.   ● 1·5% w/v acid gel.   ■ 1·5% w/v alkaline gel.   ▲ 2·0% w/v acid gel.

Fig. 5 illustrates the effect of ageing on steady state shear viscosity, η_0, which decreased with ageing time in the 1·5 % gels and increased with ageing time in the 2·0 % acid gels. The 1·5 % alkaline gels showed increased viscosities compared with the 1·5 % acid gels again indicating a more rigid gel network in the former.

The more rigid nature of the alkaline gel network may be due to (i) Differences in molecular weight and polydispersity. (ii) Differences in charge and charge distribution.

The alkaline gelatin possessed a higher molecular weight and axial ratio than the acid sample. The latter sample has been shown to be more polydisperse than the alkaline gelatin on the basis of gel filtration and polyacrylamide gel electrophoresis studies. It is also generally thought that acid gelatins contain more of the lower molecular weight material than do alkaline gelatins (Pouradier, 1958).

The net charge on the alkaline gelatin molecule in distilled water was higher than that on the acid molecule (Fig. 3). The alkaline gelatin solution (pH 5·6) exhibited a net negative mobility of $-0·815$ μm s^{-1} V^{-1} cm whilst a solution of the acid gelatin (pH 5·3) displayed a small net positive mobility of $+0·168$ μm s^{-1} V^{-1} cm. The alkaline gelatin would, therefore, appear to consist of a longer unit of higher molecular weight possessing a higher charge-weight distribution than the acid gelatin. Since the alkaline gelatin exhibited a preponderance of negative charges it might be expected that the polypeptide chains would be more expanded than those of the acid gelatin hence the rigidity and viscosity of the former would be greater .

The results for the line spectra analysis of the creep curves are shown in Tables 1 and 2. The curves have been fitted to a Burger's model so that J_0 is the instantaneous

Table 1. *The effect of ageing time on the viscoelasticity of 1·5 %w/v alkaline gelatin gels.*

Time of ageing (h)	No. of Voigt Units	J_0	J_1 (m² N⁻¹)	J_2	τ_1	τ_2 (s)	η_0	η_1 (Nsm⁻²) × 10⁻⁵	η_2
12	5	0·075	0·041	0·019	1096	166	2·62	2·68	0·88
21	3	0·073	0·057	0·019	1005	716	2·57	7·76	0·38
49	4	0·078	0·038	0·015	950	218	2·14	2·51	0·14
60	4	0·084	0·039	0·015	917	213	1·89	2·3	0·14
73	3	0·087	0·059	0·027	940	636	1·53	1·61	0·23
95	4	0·081	0·048	0·020	644	896	1·01	1·33	0·44

Table 2. *The effect of ageing time on the viscoelasticity of 2·0 %w/v acid gelatin gels.*

Time of ageing (h)	No. of Voigt Units	J_0	J_1 (m² N⁻¹)	J_2	τ_2	τ_2 (s)	η_0	η_1 (Nsm⁻²) × 10⁻⁵	η_2
21	4	0·0799	0·0221	0·00992	1121	89	12·7	5·0	0·18
45	3	0·0696	0·0208	0·00784	1148	299	11·8	5·4	0·37
69	3	0·0676	0·019	0·006	1152	240	15·6	5·5	0·4
90	3	0·0672	0·0179	0·00696	1249	254	18·6	6·9	0·36

Table 3. *Mean viscoelastic parameters of 1·5%w/v acid and alkaline gelatins aged at 4° for 21 h.*

	J_0	J_1	J_2 (m² N⁻¹)	J_3	$\tau 1$	$\tau 2$ (s)	$\tau 3$	η_0	η_1 (Nsm⁻² × 10⁻⁵)	η_2	η_3
Acid											
Mean (n = 4)	0·233	0·123	0·079		462	35·3		0·35	0·039	0·0044	
S.D.	0·061	0·0025	0·013		177	7·5		0·07	0·021	0·0078	
Std. Error	0·03	0·0013	0·006		89	3·8		0·035	0·011	0·00039	
Alkaline											
Mean (n = 4)	0·0793	0·051	0·00256	0·185	996	725	233	2·13	1·81	0·28	0·011
S.D.	0·00765	0·0047	0·000708	0·0742	18·5	6·32	182	0·41	0·44	0·09	0·0044
Std. Error	0·00383	0·0024	0·000354	0·0371	9·3	3·16	91	0·2	0·22	0·045	0·0022

shear compliance, J_1, η_i, and τ_i, the compliance, viscosity and retardation of the 'i'th Voigt unit and η_0 the residual shear viscosity. The root mean square error in all cases was less than 0·015. Although three, four and five Voigt units were fitted in some cases, only two are presented for each system. The results demonstrate the viscoelastic nature of 1·5% alkaline and 2·0% acid gels and indicate that under the conditions of the experiment these parameters do not vary greatly with ageing time. 1·5% acid gels consistently exhibit higher compliances, lower viscosities and shorter retardation times when compared with the 1·5% alkaline gels.

A statistical analysis of the results for 1·5% w/v acid and alkaline gels aged at 4° for 21 h is shown in Table 3. The figures provided are the mean of four determinations in each case and it is evident that even though the standard error is high in some cases, the differences in compliance and viscosity between the acid and alkaline samples are real and are not due to errors of measurement.

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