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A comparative study of gelatin gelation using photon correlation spectroscopy and a conventional gelation timer

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Summary

Photon correlation spectroscopy was used to study aqueous gelatin solutions. Diffusion coefficients were strongly dependent on polymer concentration, sample time and method of data analysis. To study gelation, the light scattering behaviour of monodisperse probe particles was monitored within gelling systems. This reduced problems associated with the direct measurement of the diffusional characteristics of the gelatin molecule by producing a simple autocorrelation function and the means of a reproducible analysis. A linear relationship was obtained between this information and gelation times determined by a conventional method of gelation timing.

Introduction

Gelatin is a complex polypeptide that results from the mild degradation of collagen containing tissues. It possesses a similar chemical composition to its parent collagen and a range of intriguing properties. The gel and film forming abilities have been exploited in pharmaceutical, photographical and edible products industries without a full understanding of the mechanisms involved.

Technological development has provided information on the physicochemical properties of macromolecules. However, difficulties can arise in interpreting experi-

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mental data for a complex heterodispersed polymer such as gelatin. This is further complicated by the method of manufacture which determines the percentage of ionizable carboxyl groups. The flexibility of the polypeptide chains allow intra- and inter-molecular interactions, and the magnitude of these effects depends on the polymer–solvent relationship and the total composition of the system. Polydispersity also influences behaviour, but the different properties of gels arising from gelatins produced by different manufacturing processes and sources are too complicated to be explained by molecular weight differences alone.

The importance of the physical properties of gelatin gels and films has become recognized over the years and included in gelatin specifications. To date, gel strength remains the property on which commercial values of gelatin are based. In film formation the speed at which a gelatin solution sets is also extremely important, but there are no standard methods that adequately determine this property. Several methods for gelation timing are described in the literature, but result in very different values (Wainewright, 1977). Most apply to a particular gelatin use or product and have their own relevant conditions. There are no reports of correlation between the various empirical methods or whether the ranking order of a given series of gelatins depends on the method of test. For example, none of these methods adequately relate to film formation on mould pins during hard capsule manufacture. An approach to standardizing these methods would be to study a more fundamental property of the gelatin molecule. During gelation the freedom of motion of the chains are increasingly restricted. A measure of the changes in diffusive ability are a reflection of the gelling process, and can be determined most readily by photon correlation spectroscopy (pcs).

This work illustrates the dependence of diffusion coefficient (D) on gelatin concentration, sample time and method of data analysis. It indicates the complexities of studying a polydispersed system such as gelatin and describes a technique for studying gelation by means of monitoring a probe particle. The light scattering behaviour of the probe particle in different gelling systems was compared with gelation times determined by a conventional gelation timer.

Materials and Methods

The photon correlation spectrometer was supplied by Malvern Instruments, Malvern, Worcs. It comprises a He-Ne laser (35 mW) operating at 632.8 nm. Scattered light was collected at an angle of 90° by a photomultiplier assembly (RF313) and the signals transmitted to a 64-channel multibit correlator (K7025). The correlation data was analyzed using a Malvern Application (k7025-spec-22) programmed Commodore 32K Pet, or alternatively a "Weighted Nonlinear Least Squares Regression Analysis" (NONLIN-74) (Metzler et al., 1974).

To illustrate dependence on sample time and method of analysis, 2% alkaline ossein gelatin solutions (Croda 174L) were prepared using filtered pH 6 phosphate buffer (0.05 μm membrane filter). The solutions were held at 50°C for 2–3 h to destroy thermal history and equilibrated at 35°C before determining the diffusion coefficients.

To study concentration effects on diffusion coefficients, gelatin (Rousselot limed ossein 32272) solutions ranging from 1 to 14% were prepared using distilled filtered water, adjusted to pH 6 with potassium hydroxide or hydrochloric acid and held at 50°C for 2–3 h to destroy thermal history. The diffusion coefficients were determined at 35°C and expressed as a plot of log D vs log concentration (Fig. 3).

Experiments on latex/gelatin systems were performed using six gelatin samples:

Rousselot, limed ossein 32272. Croda, limed ossein 174.
Rousselot, limed ossein 32976. Croda acid ossein 163.
Rousselot, acid ossein 05759. Neinburger, limed ossein 06528.

1% solutions were prepared using pH 7 Sorenson's Buffer, incorporating latex particles (mean diameter 0.091 μm, S.D. $\pm$ 0.0058 μm, Dow Chemicals) to a concentration of 1 μl/ml gelatin solution. A 10% solids latex suspension therefore, results in gelatin solutions containing 0.01% latex spheres. These were filtered through a 0.45 μm filter and held at 50°C for 2–3 h prior to placing in the temperature-controlled vat of the instrument set at 14°C. Light scattering experiments were performed at sample time 100 μs and the results analyzed by a Malvern programme. Diffusion coefficients were determined every 3 min up to gelation and expressed graphically as log D vs time (min).

Gelation times of the six gelatins were determined by the Kodak bar method (Janus, 1958). 6% solutions were prepared using pH 7 phosphate buffer and stabilised at 40°C prior to the experiment. The apparatus consisted of a hollow chrome bar enclosed in a perspex box. A temperature of 20°C was maintained by pumping water from a thermostatically controlled water bath through the bar. Drops were placed at 2 s intervals along the bar using a hypodermic syringe (needle size no. 12). These were left for a predetermined time of "X + 2" s and then the bar rotated through 90° and left for a further 15 s. At the end of the time period the number of drops to the first undistorted drop were counted and the gelation time determined using the following formula:

$$(\text{No. of drops to } 1^{\text{st}} \text{ undistorted drop} \times 2) + (X + 2)$$

The time X s is the time that allows half the number of drops to set and may differ with gelatin source.

Results and Discussion

The principle of pcs is that macromolecules and small particles diffract light in a characteristic pattern when radiated by a coherent light source. In solution, random or Brownian Motion results in a temporal variation in the diffraction pattern which enables the velocity to be calculated. During a homodyne experiment, an autocorrelation function is generated from the scattered light intensity. When normalized this takes the form:

$$G^2(\tau) = B(1 + Ae^{-2\Gamma\tau})$$

TABLE 1
GELATION TIMES (min) DETERMINED AT 20°C BY THE KODAK BAR METHODS

Gelatin sample [a]	Gelation time (min)
Rousselot limed ossein 32272	57
Rousselot limed ossein 32976	44
Rousselot acid ossein 05759	40
Neinburger limed ossein 06528	81
Croda limed ossein 174	59
Croda acid ossein 163	40

[a] 6% solutions pH 7

where $\Gamma = DK^2$; D = diffusion constant; K = scattering vector; B = background; A = instrumental optical constant.

The Malvern programme plots the natural log of the correlation function versus

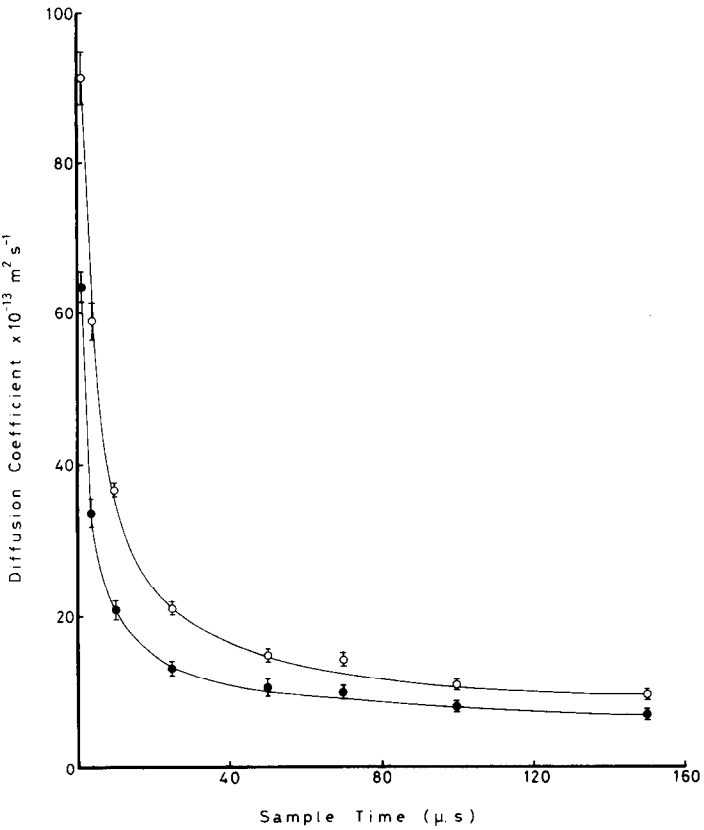

Fig. 1. The effect of sample time on diffusion coefficient at 35°C for a 2% gelatin solution pH 6 (Croda limed ossein 174). ○, cumulant expansion analysis; ●, linear fit analysis.

delay time and calculates a diffusion coefficient from the decay constant (Γ).

$$\ln\left[\left(G^2(\tau)/B\right) - 1\right]/A = -2\Gamma\tau$$

For many experiments the semi-log plot of the correlation function is rarely an ideal linear fit, thus indicating that the original function is not a simple exponential. In the case of polydispersed samples a sum of exponentials has to be fitted to the above expression. Therefore the curve-fitting procedure can be modified and represented by a polynomial as in the "Method of Cumulants" (Koppel, 1972). A diffusion coefficient can be calculated from the first cumulant and obtainable from the Malvern application programme.

The light-scattering phenomena of the gelatin molecule is principally displayed as two decays, a "fast" and a "slow" mode. The fast decay has been associated with a "mutual" or "cooperative" diffusion coefficient which is claimed to express the internal motions or flexing of segments of overlapping polymer chains. Conse-

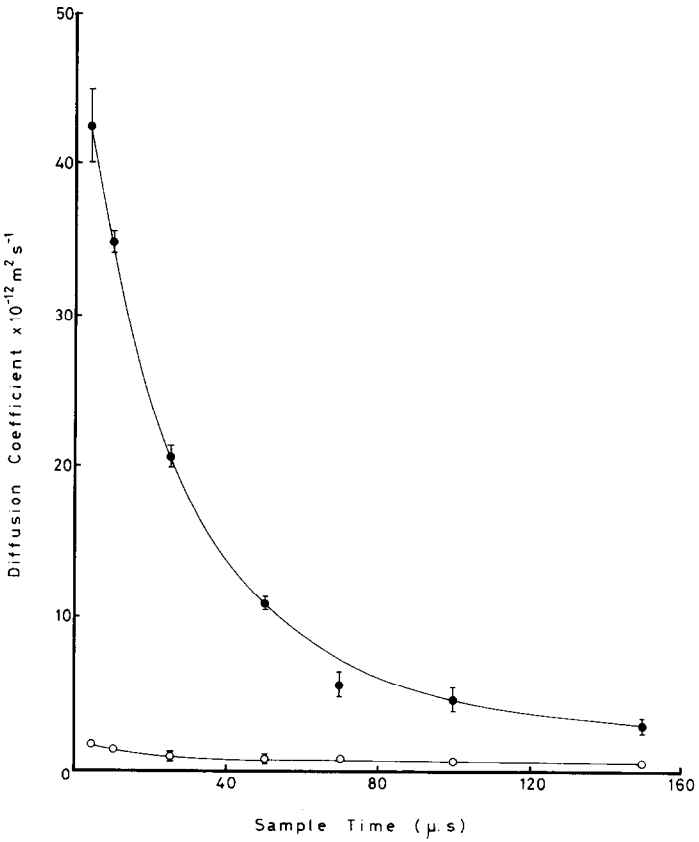

Fig. 2. The effect of sample time on diffusion coefficient at 35°C for a 2% gelatin solution pH 6 (Croda limed ossein 174). analyzed by NONLIN-74. ●, 'fast' decay; ○, 'slow' decay.

quently the value of the mutual diffusion coefficient increases with concentration. The slow mode has been attributed to the slow relaxation of self diffusion (Amis et al., 1981). This is concerned with the centre of mass motion of a polymer chain in its surroundings. However the values for self diffusion obtained by pcs are much smaller than values obtained by other methods, and it has been proposed that the slow mode is attributable to the translation of an entangled polymer (Brown et al., 1983; Chang and Yu, 1984). The concentration dependence of this relaxation parallels that of self-diffusion and corresponds to it at infinite dilution.

During a pcs experiment both "fast" and "slow" decays contribute to the linewidth. Their separation is not a simple procedure but this can be attempted by choosing sample times well removed in time scale. Fig. 1 illustrates the decrease in diffusion coefficient with sample time. Diffusion coefficients calculated from the first cumulant are slightly higher in value than those calculated from the simple linear fit. This difference is greater at short sample times due to the influence of the fast decay, indicating the importance of sample time selection. Over the range 1–40 μs values obtained for diffusion coefficient will be markedly different. To reduce errors of this nature, analysis of the slow decay is best performed using longer sample time where the diffusion coefficient has reached a fairly constant value. A two-component analysis attempts to separate the two decays more completely, and

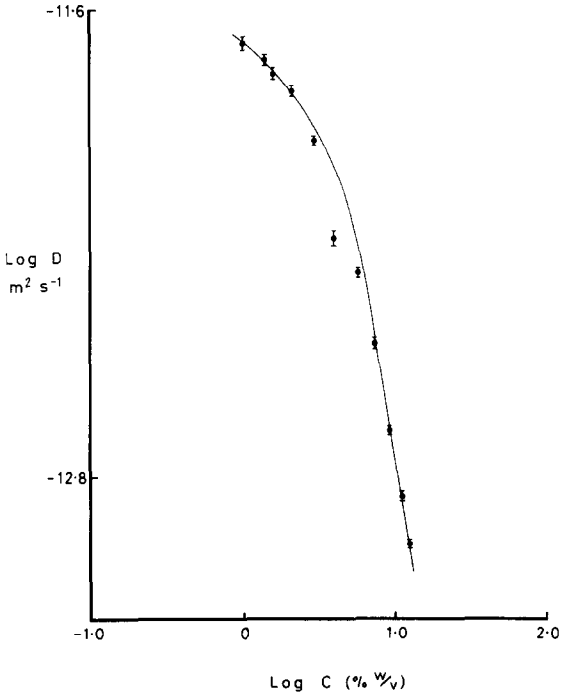

Fig. 3. Log diffusion coefficient vs log concentration at 35°C for 1–14% gelatin solutions pH 6 (Rousselot limed ossein 32272). data from Thomas et al. (1983).

the characteristic profiles are illustrated in Fig. 2. The value of the slow diffusion varies to a small degree only, regardless of sample time. The slow decay bears an average 75% contribution to the total intensity of the signal. This increases by approximately 5% when the gelatin concentration is increased from 2–6%.

Fig. 3 illustrates that above approximately 4% gelatin concentration there is a dramatic decrease in the diffusion coefficient and a linear relationship between log D and log concentration suggesting a reflection of polymer–polymer interactions. In dilute solutions at 35°C molecular domains remain fairly unperturbed, thus slight increases in polymer concentration have a limited effect on molecular diffusion in this range. As the overlap concentration is approached, entanglements can severely restrict chain mobility resulting in a concentration-dependent decrease in the diffusion coefficient.

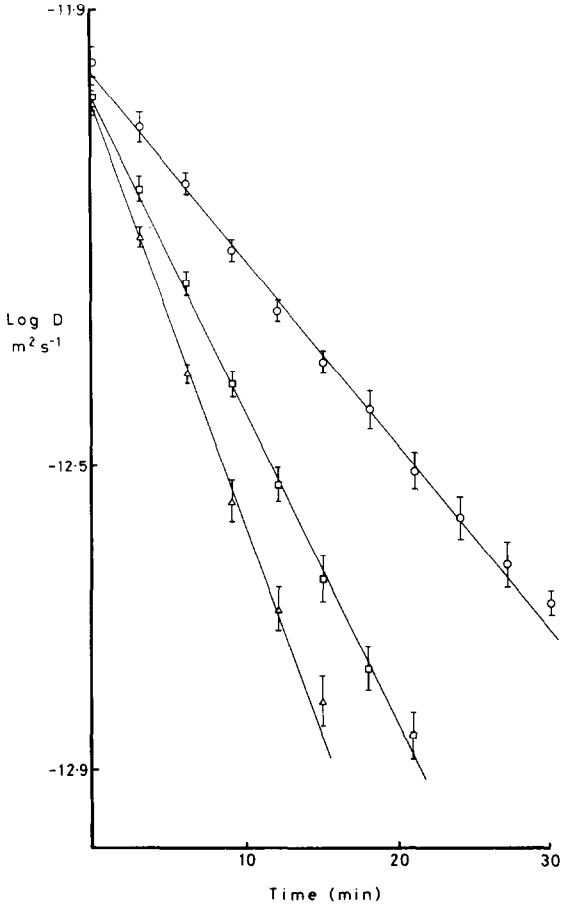

Fig. 4. Log diffusion coefficient vs time at 14°C for latex spheres in 1% gelatin solution pH 7. ○, Neinburger limed ossein 06528; □, Rousselot limed ossein 32272; △, Croda acid ossein 163.

As stated above, the autocorrelation function applies to a narrow distribution of non-interacting rigid spherical particles. If the distribution is wide or the particles are irregularly shaped then the autocorrelation function is no longer a single exponential but a sum of decay rates. The situation is further complicated by the intramolecular motions of a flexible, random coil polymer such as gelatin. The diffusive behaviour of polymer chains have been interpreted by various models (De Gennes, 1976a and b). However, the proposed mechanisms have not been verified and are complicated by concentration effects. To obviate problems associated with the gelatin molecule, the behaviour of monodispersed latex particles were studied within gelling systems. The ideal nature of the probe particle produces a simple autocorrelation function which lends itself more readily to reproducible analysis.

Figs. 4 and 5 illustrate the decrease in the slow diffusion of the latex particle

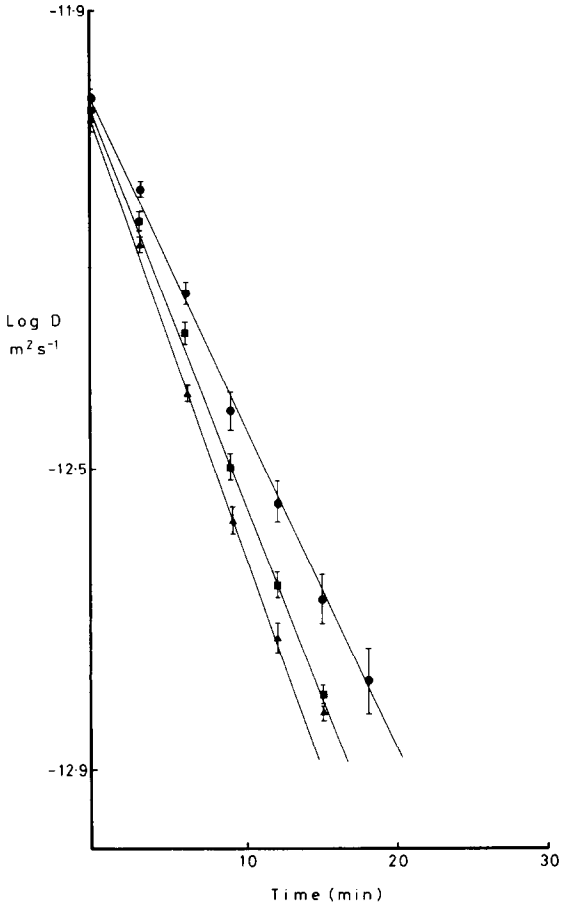

Fig. 5. Log diffusion coefficient vs time at 14°C for latex spheres in 1% gelatin solutions pH 7. ●, Croda limed ossein 174; ■, Rousselot limed ossein 32976; ▲, Rousselot acid ossein 05759.

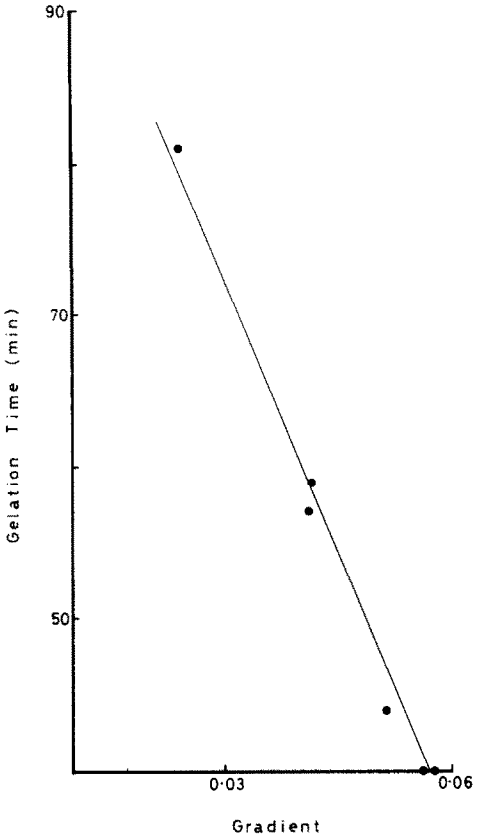

Fig. 6. Gradients (calculated from pcs studies of latex spheres in 1% gelatin solutions) vs gelation times (determined by the Kodak bar methods).

during the first stages of gelation. The values of the gradients between log D and time differ with gelatin source and bear a linear relationship (Fig. 6) to gelation times determined by the Kodak bar method, thus giving a correlation between two entirely different methods for studying gelation.

In solutions, gelatin molecules become strongly adsorbed by the attachment of certain groups along the peptide chains to the hydrophobic surface of the latex particle. At low temperatures molecular aggregation occurs and interchain linkages become more probable. This time-dependent structuring is reflected in the decreased diffusivity of the probe particle.

The different values of the gradients indicates that the motion of the latex particle is influenced to varying degrees by the kinetics of a particular gelatin, each of which has a characteristic rate of molecular aggregation and reversion to the "triple helix configuration".

The signal intensity obtained from the latex/gelatin system is principally due to the latex particle but bears a contribution from the gelatin molecule. This proportion

differed with gelatin sample but tended to lie between approximately 0.6% and 3% of the total intensity at the start of the experiment. This percentage contribution increases over the gelation period and as the motion of the particle decreases. This can be established by two component analysis or by directly comparing the correlograms obtained from gelatin/latex systems and gelatin systems alone.

A consequence of this is a departure from linearity towards the end of the time period. The diffusive ability of the latex particle has reached a minimum. However, the weak signal is influenced by other scattering effects and artificially higher values are obtained. The build-up of a network is likely to increase the amount of scattered light. In addition during gelation the signal from the "slow" decay diminishes but the "fast" decay remains relatively unaltered, thus gaining more significance in the curve fitting procedure.

The Kodak bar method for gelation timing is a fairly rapid procedure. It is reported as giving results of physical significance (Janus et al., 1965). Drop size, speed of rotation of the bar etc., can be varied considerably without affecting the results. Conversely it is arguable that subtle differences in gelatins affecting gelation rates will pass undetected. Gelation times rely on a subjective assessment of drop distortion. This is likely to vary with operator, but under the same conditions should yield a similar rank order of gelation times. A simple means of characterizing different gelatin sources on a kinetic basis is provided. The bar method gives limited information on the mechanism of gelation. To further a study on the molecular aspects of gelation techniques, using photon correlation spectroscopy may have more potential. Both methods reflect the increase in viscosity of the gelling solution. In the bar method it is the viscosity that resists drop distortion, whilst in pcs the increase in viscosity is reflected in the restricted motion of the probe particle. The above work has shown that it is possible to correlate the two different methods for studying gelation.

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References

Amis, E.J., Janmey. P.A., Ferry, J.D. and Yu, H., Quasielastic light scattering measurements of self-diffusion and mutual diffusion in gelatin solutions and gels. Polym. Bull., 6 (1981) 13–19.

Brown, W., Johnsen, R.M. and Stilbs, P., Quasielastic light scattering (QELS) in semi-dilute polymer solutions a comparison of "slow-mode" diffusion with self-diffusion from FT-pulsed field gradient NMR. Polym. Bull., 9 (1983) 305–312.

Chang, T. and Yu, H., Self-diffusion of gelatin by forced Raleigh scattering. Macromolecules, 17 (1984) 115–117.

De Gennes, P.G., Dynamics of entangled polymer solutions. 1. The Rouse model. Macromolecules, 9 (1976a) 587–593.

De Gennes, P.G., Dynamics of entangled polymer solutions. II. Inclusion of hydrodynamic interactions. Macromolecules, 9 (1976b) 594–598.

Janus, J.W., in Stainsby, G. (Ed.), Recent Advances in Gelatin and Glue Research, Pergamon, London-New York-Paris-Los Angeles, 1958, pp. 214–218.

Janus, J.W., Tabor, B.E. and Darlow, R.L.R., The setting of gelatin sols. Koll. Zeit., 205, 2 (1965) 134–139.

Koppel, D.E., Analysis of macromolecular polydispersity in intensity spectroscopy: the method of cumulants. J. Chem. Phys., 57, 11 (1972) 4814–4820.

Metzler, C.M., Elfring, G.L. and McEnen, A.J., A users manual for NONLIN and associated programs, revised edn.; Research Biostatistics, Upjohn Company, Kalamazoo, 1974.

Thomas, M., Kellaway, I.W. and Jones, B.E., A technique for studying gelatin gelation. J Pharm. Parmacol., 35 (1983) 6P.

Wainewright, F.W., In Ward, A.G. and Courts, A. (Eds.), Science and Technology of Gelatin, Academic Press, London-New York-San Francisco, 1977, pp. 531–533.