Spectral broadening of light scattered from polysaccharide gels

W. Mackie, D. B. Sellen and J. Sutcliffe*
Astbury Department of Biophysics, University of Leeds, Leeds LS2 9JT, UK
(Received 7 April 1977; revised 1 August 1977)

Light scattering Rayleigh linewidth measurements have been made on 0.5% calcium alginate gels using the autocorrelation method. The degree of spectral broadening has been found to be small (~2%) although two relaxation times (~7 and ~50 msec) within the broadened component have been measured. The variations in the degree of spectral broadening and the relaxation times with angle of scatter were found to be much less than predicted by any existing theory, and no systematic variation in any of the parameters with temperature was detected within the range 3° to 88°C. By scanning through the gel with the focused laser beam, however, it has been shown that long range (tenths of millimetres), long term (tens of minutes), fluctuations in alginate density occur. It is possible that the observed relaxation times represent the continual rearrangements of polymer chains which, assuming a junction zone type of structure, would be necessary for these fluctuations to take place. Oscillations were generally found to be superimposed upon the autocorrelation functions and these have been positively identified with bulk oscillations of the specimen. A theory is advanced explaining why bulk oscillations manifest themselves in the autocorrelation function and predicting the marked increase in amplitude with angle of scatter observed experimentally. Experiments are also described in which the diffusion coefficients of dextran fractions and globular proteins within 0.5% gels were measured by investigating the heterodyne beat spectrum. It was found that the movement of macromolecules with $1/D_{20w}$ less than 3×10^4 sec/mm^2 was virtually unimpeded by the gel. Diffusion constants fell to about one third of those in aqueous solution for values of $1/D_{20w} \sim 9 \times 10^4$ sec/mm^2. Thus there are large interstitial spaces within the gel and if a junction zone type of structure is correct, each junction zone must consist of an association of some hundreds of chains. Some preliminary results are presented of experiments with agarose and bovine vitreous humour.

INTRODUCTION

In recent years measurement of the Rayleigh linewidth of scattered light has been extended to include the light scattered from gels[1-9]. This paper describes extensive measurements with calcium alginate gels together with some preliminary measurements with the gels of other polysaccharides, and suggests a theoretical interpretation of the results. The degree of spectral broadening has been found to be small, and this has made possible measurement of the diffusion coefficients of small compact macromolecules within gels by investigating the heterodyne beat spectrum. Some measurements of this type using dextran fractions and globular proteins are described.

EXPERIMENTAL

Calcium alginate gels were prepared by dialysing 0.5% solutions of sodium alginate in 0.1 M NaCl against 0.1 M $CaCl_2$ using dialysis tubing 6.5 mm in diameter. Each rigid cylinder of gel so formed was removed from the tubing, and then supported with its axis vertical in a cylindrical light scattering cell in which it was surrounded by water. The light scattering cell was a small weighing bottle, 20 mm in diameter, with its rear side blackened. Gels for the experiments with dextran fractions and the globular protein, bovine plasma albumin, were prepared by mixing these at concentrations sufficient to give a suitable level of scatter, with the sodium alginate prior to dialysis. Each resultant gel was then placed in the light scattering cell and surrounded by dextran or globular protein solution of the same concentration. Experiments were also made using the globular protein, chymotrypsinogen. Because of the presence of calcium in the preparation used, it was not possible to mix this with the sodium alginate prior to dialysis, and in this case the chymotrypsinogen was allowed to diffuse in after the gel had been formed. As dextran is a good bacterial growth medium, a bactericide (sodium azide) was added in these experiments. The sodium alginate consisted of 70% guluronate and was obtained from the seaweed *Laminaria hyperborea*. It had a weight-average molecular weight of 700 kg/mol and due to the high solution viscosity, 0.5% represented the highest concentration of solution which could be conveniently handled. Prior to making the gels the sodium alginate solutions were centrifuged for 1 h at 100 000 g and then made to pass through a Millipore filter of pore size 1.2 μm.

Light scattering Rayleigh linewidth measurements were made by obtaining the autocorrelation function of the homodyne beat spectrum using the apparatus previously described[10-11]. The light scattering cell was surrounded by a water jacket so that the temperature of the gel could be held constant at values between 3° and 88°C. Measurements were commenced 48 h after preparing the gel.

* Present address: Physics Department, Royal Devon and Exeter Hospital, Exeter, Devon, UK.

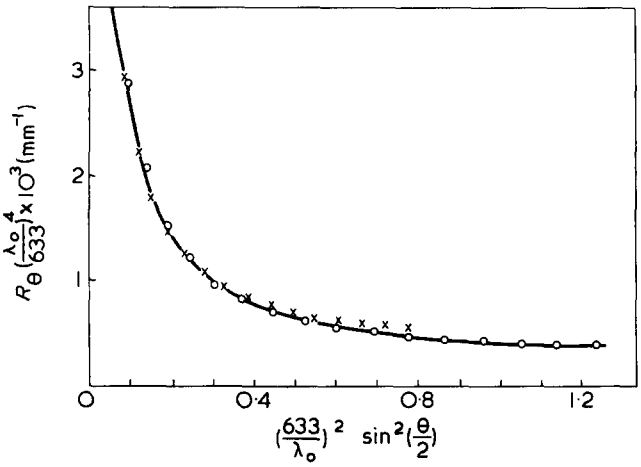


Figure 1 Rayleigh ratio of light scattered from a 0.5% calcium alginate gel: X, 546nm; O, 436nm

RESULTS AND DISCUSSION

Conventional light scattering measurements

The intensity of light scattered from the alginate gels was found to be some two orders of magnitude greater than that from macromolecular solutions at concentrations commonly used in light scattering experiments. In order to verify that the scattering arose from density fluctuations at a molecular level, and not from dust or other macroscopic impurities, conventional light scattering measurements were made at $\lambda_0 = 436$ and 546 nm using the Aminco apparatus (American Instrument Co. Inc., Silver Spring, Maryland, USA). According to the Debye theory[12], if $\lambda_0^4 R_\theta$ is plotted against $\sin^2(\theta/2)/\lambda_0^2$ a single plot should be obtained and *Figure 1* shows this to be the case. For convenience the plot has been converted to that expected at the laser wavelength (636 nm). The geometrical optics of the system presented calibration difficulties and the absolute values of the Rayleigh ratio, R_θ, are only approximate. As the optical path length within the gel was shorter than the length observed by the detecting system of the Aminco apparatus the normal $(\sin\theta)^{-1}$ scattering volume correction was not applied. *Figure 1* shows data for a freshly prepared gel. However measurements on a gel some four months old (stored surrounded by water) yielded a plot of identical shape.

Rees[13] has proposed a rigid structure for alginate gel in which micelle-like junction zones of the polyguluronate are formed with the calcium ions in an 'egg box' configuration, the interzonal chains being mainly polymannuronate. Light scattered from this type of structure would arise mainly from the junction zones, the interconnecting chains scattering much less. As far as the overall scattered intensity is concerned the system could be regarded as a random spatial distribution of junction zones and classical solution theory[14] applied – although of course the dynamics of the system would be quite different. A Guinier plot however does not yield a straight line but a curve bending sharply upwards with decreasing angle. It must be assumed, therefore, that the spatial distribution of junction zones does not obey Gaussian statistics, there being fluctuations with distance in mean concentration of junction zones which increases the scatter at low angles. A simple picture is one of small clustered regions in which the junction zone concentration is higher than in their surroundings, the clusters being randomly positioned with respect to each other. It would be expected however that single junction zones would give rise to most of the scattered intensity at high angles. Assuming this to be so a very approximate molecular weight of 7000 kg/mol may be calculated by taking the concentration and dn/dC to be that of the original sodium alginate solution. This would indicate that there is on average one junction zone per $(100\ \text{nm})^3$ in a 0.5% gel, and assuming that 100 nm therefore represents the maximum length, that each junction zone consists of an assembly of some hundreds of chains. These conclusions are of course meaningful only on the assumption that alginate gel does in fact have a junction zone type of structure.

Autocorrelation functions

Figure 2 shows a typical normalized autocorrelation function of intensity fluctuations of light scattered from an alginate gel. Typically 10 min of integration time was required. This was achieved by making repeated measurements with a 20 sec time constant, so that any large short term systematic variations (such as in the oscillations) could be detected. S here is the value of ϕ expected at $\tau = 0$ if the spectral broadening were complete; this was found from the coherence factor and the d.c. component of the photoelectric signal (see Appendix). The degree of spectral broadening is small and therefore given by $\phi_0/2S$. The autocorrelation function in this case represents the Fourier transform of the spectral distribution.

Apart from the oscillations, which will be discussed later, *Figure 2* may be represented as the sum of two exponentials, and this was found generally to be the case. *Figures 3a* and *3b* show the two relaxation times and *Figures 3c* and *3d* show the degree of spectral broadening and the percentage of spectral broadening attributable to the shorter decay, as a function of angle of scatter and temperature, for one gel sample. Four gels were investigated with similar results. Surprisingly, no systematic variation with temperature was detected in the range 3° to 88°C and the error bars each represent the standard error in the mean value of all the results obtained at a given angle of scatter. There was however a correlation between the degree of spectral broadening and the order in which the experiments were carried out, the degree of spectral broadening increasing with time. No such variation was detected in the other parameters.

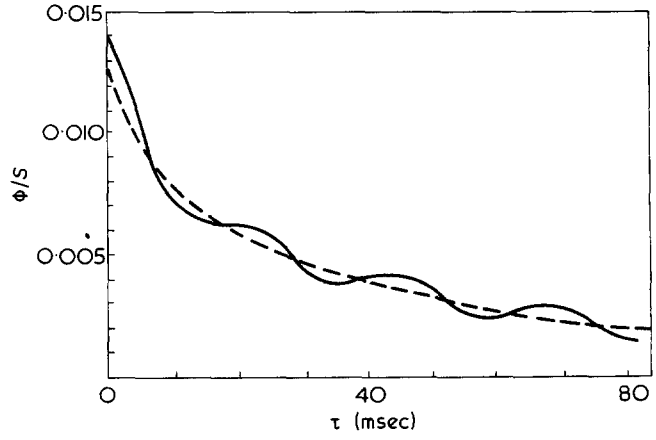
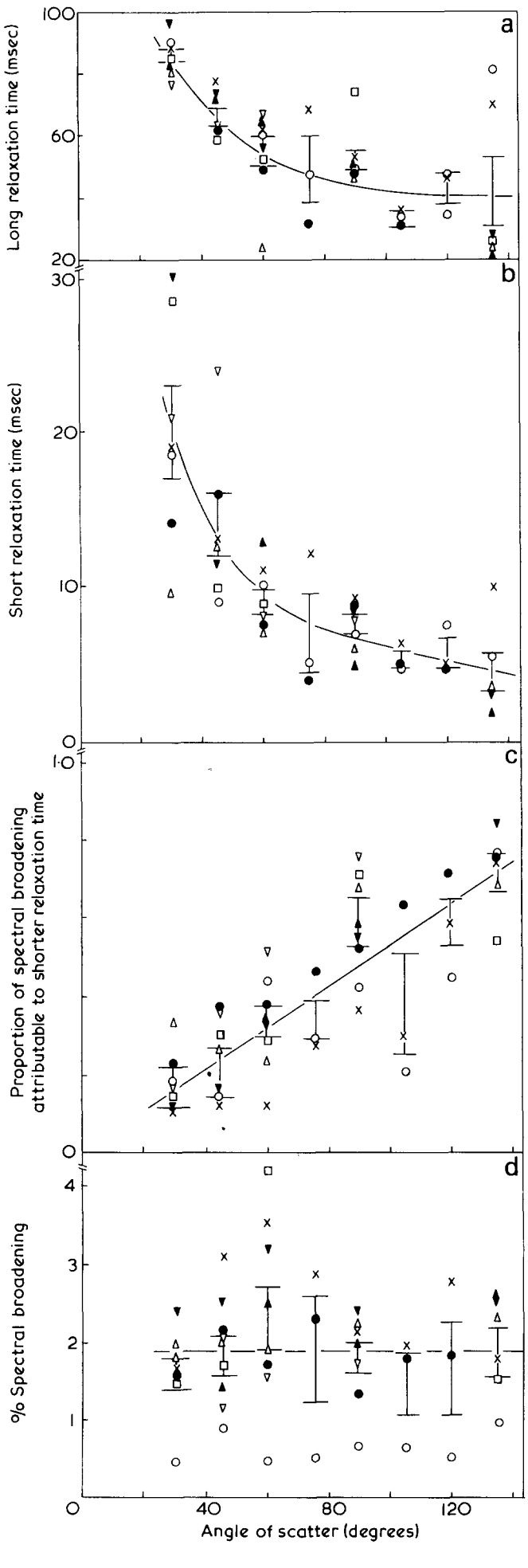


Figure 2 Normalized autocorrelaton function of intensity fluctuations of light scattered at 90° from a 0.5% calcium alginate gel at 20°C. The specimen is a cylinder 20 mm long and 6 mm in diameter supported with its axis vertical and surrounded by water



A theory for the spectral broadening of light scattered from gels has been formulated by Tanaka *et al.*[1]. The broadened component of the scatter is assumed to arise from freely diffusing fluctuations in polymer segment density, the effective diffusion coefficient being given by the ratio of the longitudinal elastic modulus to the force per unit volume required to maintain unit relative velocity between the polymer network and solvent. The spectral linewidth then varies as $\sin^2\theta/2$, and this has been verified experimentally for polyacrylamide[1], polystyrene[2,3] and poly(dimethyl siloxane)[4] gels. In these experiments the degree of spectral broadening was found to be small and increase with angle of scatter, the unbroadened component being attributed to long range stationary fluctuations in polymer density, occurring over distances not small compared with the wavelength. *Figure 3* however shows that in the present work neither relaxation varies inversely as $\sin^2\theta/2$, much less variation being observed, and that there is no detectable variation in the degree of spectral broadening with angle of scatter. An entirely different theoretical approach has been taken by assuming that scattering centres diffuse independently under the influence of restoring forces proportional to their displacements from mean positions[7–9]. The spatial density fluctuations of this model contain a time invariant component associated with the fixed mean positions, so that partial spectral broadening is predicted, as well as non-exponential autocorrelation functions. The theory predicts that when the degree of spectral broadening is small (in the case of highly restricted diffusion), it varies as $\sin^2\theta/2$, which is clearly not the case in the present work. Even if additional, long range, stationary fluctuations are postulated a satisfactory agreement with the data cannot be obtained. Thus the variations in the relaxation times and degree of spectral broadening with angle of scatter, observed in the present work, are not consistent with either theory and an alternative explanation will now be given.

Alginate chains have been shown to be very stiff in solution[15]. If the gel structure is assumed to be of the junction zone type then it would be expected to be very rigid due to the long persistence length of interzonal chains. Thus only small temporal fluctuations would be expected, and hence a small degree of spectral broadening as actually observed. However, by measuring the overall scattered intensity as the sample was moved short distances relative to the focused laser beam (*Figure 4a*), and by observing changes with time in a given position (*Figure 4b*), it was shown that large fluctuations in alginate density occur, but these these are very long range (tenths of millimetres) and long term (tens of minutes)*. It is unlikely that these fluctuations could occur with a junction zone type of structure without continual rearrangements of polymer chains. Thus in the

* Fluctuations of this sort are not observed in conventional light scattering measurements (*Figure 1*) where the illuminiated volume is some four orders of magnitude larger. They enhance the overall scattered light intensity only by randomly refracting the incident light. As the maximum refractive index difference involved is of the order of 0.001, this effect is negligible within the range of angles investigated.

Figure 3 Relaxation times (a) and (b) and the associated degrees of spectral broadening (c) and (d) of light scattered from a 0.5% calcium alginate gel as a function of angle of scatter and temperature. The bars each represent the standard error in the mean value of all the data at a given angle. X, 3°C; ○, 21°C; ●, 38°C; ▽, 49°C; △, 53°C; ▼, 67°C; ▲, 78°C; □, 88°C

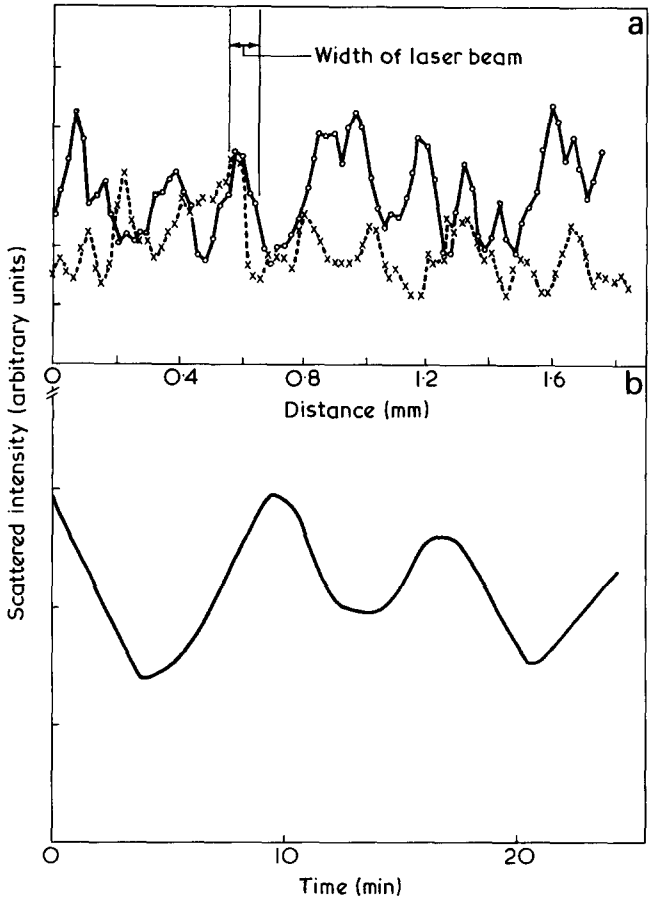


Figure 4 (a) Fluctuations of scattered intensity with distance observed by moving a 0.5% calcium alginate gel relative to the focused laser beam. ○, ——, 30°; X, – – –, 135° (scattered intensity x 10) (b) Fluctuations of scattered intensity with time at a fixed position

short term† the structure could be regarded as stationary except for the migration of peripheral polymer chain segments between junction zones, causing the latter to fluctuate in mass. Assuming the junction zones to be polydisperse this situation would be consistent with the experimental results as regards variation with angle of scatter. The angular variation in the relative contributions of the shorter and longer relaxation times to the spectral broadening suggests that they are associated with fluctuations in mass of single junction zones and clusters, respectively. The temperature dependence of the relaxation times, however, would be affected by a complexity of factors, e.g. the proportion of chain segments free of junction zones at a given time would be expected to increase with temperature. It would in any case be necessary to postulate the exact mechanism by which these molecular events occur in order to discuss their temperature dependence.

In all light beating experiments it is standard practice to use a focused laser beam in order to maximize the coherence factor. However the question arises as to whether the observed phenomena are natural or arise from a local heating effect, although it must be emphasized that the explanation in terms of molecular events given above could apply in either case. Determination of the temperature of the gel within the laser beam is intractable. However the turbidity of the original alginate solution was $\sim 10^{-4}$mm^{-1} and its wavelength dependence indicated the absorption coefficient to be much smaller ($<10^{-6}$mm^{-1}). The focused beam from the 50 mW laser was ~0.1 mm wide over the entire path length within the specimen due to the diffraction limitation and the long focal length of the lens. Hence assuming that no additional absorption arises on gelation, a local heating rate of <0.1°C/min may be calculated. It is unlikely that this would upset the thermal balance within the specimen and the phenomena observed are thought to be natural. It would in fact be difficult to envisage a junction zone type of structure in which local rearrangements of polymer chains did not take place, as reverse osmosis, which requires the structure to be partly in solution, is necessary for the stability of the gel.

Autocorrelation function oscillations

The superimposed oscillations on the measured correlation functions were found to be undamped, (over the time range of the autocorrelation function), and for a given gel the frequency to be independent of both time and angle of scatter. The amplitude tended to vary at random with time, although sometimes it varied periodically with a period of several minutes and sometimes oscillations were not observed at all. The maximum amplitude relative to the zero time value of the autocorrelation function, however, increased some hundred fold with angle of scatter between 30° and 135°. It was found that on shortening the length of the specimen, (*Figure 2* represents a specimen 20 mm in length), the frequency of oscillation increased and the amplitude decreased. The amplitude of the oscillations could be temporarily increased by gently tapping the cell table. Thus these oscillations can be positively identified with bulk oscillations of the specimen which are possibly thermally maintained and represent cooperative movement between alginate and solvent. It is still necessary to explain how they manifest themselves as oscillations in the autocorrelation function. It is possible that the long range, long term fluctuations in alginate density described above, coupled with bulk oscillations of the specimen, lead to oscillations in scattered intensity as the gel moves across the beam. Whereas this effect may make some contribution to the observed oscillations, it cannot be the major cause as the fluctuations shown in *Figure 4a* are independent of angle of scatter. The oscillations in the autocorrelation function must arise mainly from the oscillatory relative movements within a single coherence volume‡ resulting from the oscillatory strain. A simple analysis of this situation will now be given.

It is proposed that within a single coherence volume oscillatory relative movement resulting from oscillatory strain leads to optical interference, the phase differences oscillating with time and varying linearly with distance. Mathematically this situation is analogous to that in which light is scattered from a rod-like molecule in a fixed orientation of oscillating length but fixed mass. The modulation in scattered light intensity is given by:

† Spectral broadening arising from the *long* term fluctuations would be due to heterogeneity of motion within a single coherence volume resulting in a randomly varying distribution of Doppler shifts, and over the time scale of the observed relaxation times (e.g. *Figure 2*) would result in long term random positive fluctuations of the base line. However in the present work the bandwidth of the electronic system (lower 3db point ~0.2 Hz) was not sufficient for this effect to be observed.

‡ This is the volume over which light may be regarded as scattered in a single phase in the absence of any relative motions within it, and within which optical interference occurs as a result of motion. It is that volume which is just resolved (in an image forming sense) by the scattered light received system.

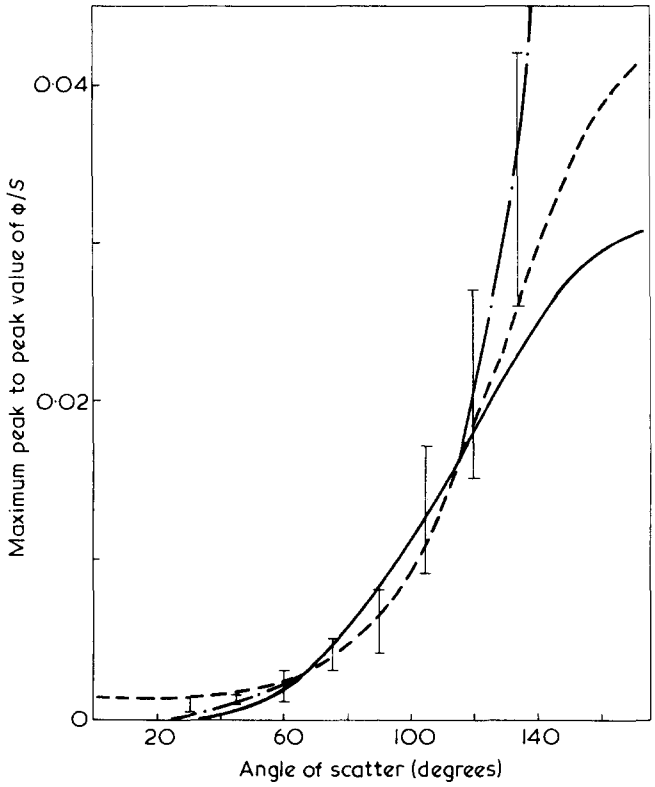


Figure 5 Oscillations in the autocorrelation function (see for example *Figure 2*) as a function of angle of scatter. The error bars represent the standard errors in the mean values of ten sets of data. ——, $\sin^4\theta/2$ variation; — — — —, $(1/4 + \sin^4\theta/2)^2$ variation; — · — · —, $\tan^2\theta/2$ variation (see text)

$$\gamma I\left(\frac{\sin(\mathbf{K}\cdot\mathbf{a}/2\,\sin\omega t)}{\mathbf{K}\cdot\mathbf{a}/2\,\sin\omega t}\right)^2 \qquad (1)$$

where **K** is the scattering vector, **a** the *maximum* relative displacement within the coherence volume and ω the frequency of oscillation of the strain. I is the scattered light intensity and γ the coherence factor. If it is assumed that relative displacements are small compared with the wavelength a second order approximation may be made which yields an alternating component:

$$\frac{\gamma I}{24}(\mathbf{K}\cdot\mathbf{a})^2\cos 2\omega t \qquad (2)$$

Thus there is an intensity modulation of twice the frequency of the strain oscillation. This gives rise to an oscillation of the same frequency in the autocorrelation function with a peak to peak value of ϕ/S equal to:

$$\frac{2(\mathbf{K}\cdot\mathbf{a})^4}{24^2} \qquad (3)$$

Expression (3) predicts that the magnitude of the oscillation in the autocorrelation function depends upon the direction of the strain oscillation and is a maximum when the strain oscillation is in the direction of the scattering vector. Thus the random nature of this phenomenon is explained. The peak to peak value of ϕ/S should vary from zero to:

$$\frac{8}{9}\pi^4\left(\frac{a}{\lambda}\right)^4\sin^4\theta/2 \qquad (4)$$

where λ is the wavelength *in the gel*. *Figure 5* shows a statistical analysis of ten complete sets of data. The bars represent standard errors in the mean values. It can be seen that the maximum value of ϕ/S does not follow a $\sin^4\theta/2$ law, the oscillations being larger at both high and low angles when the best fit is made. However, it has been assumed in the above theory that the coherence volume is independent of angle of scatter. In fact it has been shown experimentally[10,11] that the coherence factor, γ, is independent of angle of scatter so that the coherence volume must vary as cosec θ, i.e. in proportion to the volume from which scattered light is received. In order to take account of this variation it is necessary to know how the shape of the coherence volume varies with angle of scatter. If the simplifying assumption is made that the coherence volume is a prolate spheroid with major axis in the direction of scatter and ratio of major to minor axis equal to cosec θ, then the magnitude of **a** is now a function of direction. It can be shown that the autocorrelation function oscillations are now a maximum when the strain oscillation is at an angle ψ to the scattering direction where:

$$\tan\psi = \sin^2\theta\,\tan\theta/2 \qquad (5)$$

(cf. the scattering vector is at an angle $\theta/2$ to the scattering direction) and that $\sin^4(\theta/2)$ in expression (4) should be replaced by $[1/4 + \sin^4(\theta/2)]^2$, **a** now representing the value for $\theta = 90°$. This gives a much better fit to the data although an equally good if not better fit may be obtained by simply assuming that the oscillations in intensity are modified by a factor cosec θ, in which case $\sin^4\theta/2$ is replaced by $\tan^2(\theta/2)/4$. However in view of the statistical nature of the data and the simplifying assumption made in taking account of variation in coherence volume, agreement between theory and experiment is as good as can be expected. The magnitudes of the autocorrelation function oscillations indicate values of a/λ of the order of 10^{-1} corresponding to oscillating strains of the order of 5×10^{-4}.

It must be emphasized that the predictions of the above theory would not necessarily be expected to apply in cases other than that of a rigid gel supported in the manner described in this paper, as it is necessary for elastic oscillation in the appropriate direction to be possible. Oscillations in the autocorrelation function do not appear to occur in experiments on synthetic polymers[1–4,7,9]. It is likely therefore that bulk oscillations of the specimen are triggered by the long range, long term fluctuations in alginate density, and occur only for rigid gels having a comparable structure (in a purely physical sense) to alginate gel.

Diffusion of compact macromolecules within alginate gel

The diffusion coefficients of chymotrypsinogen, bovine plasma albumin and seven different dextran fractions within alginate gel were determined and compared with their diffusion coefficients in aqueous solution (*Figure 6*). The effect of changing the concentration of the diffusing macromolecule was not investigated although it had already been shown that in each case the diffusion coefficient in aqueous solution was independent of concentration[11,16], and the concentrations used in the present work were within the ranges previously investigated§. They ranged from 5 to 0.3% with in-

§ The globular proteins were previously investigated[11] in unbuffered salt solution at pH~5, and no buffers were added in the present work. A more recent investigation[17] covering a wide range of pH and using various types of buffer did not show any significant variation in diffusion coefficient within the range of concentrations of globular protein used in the present work (below 3%).

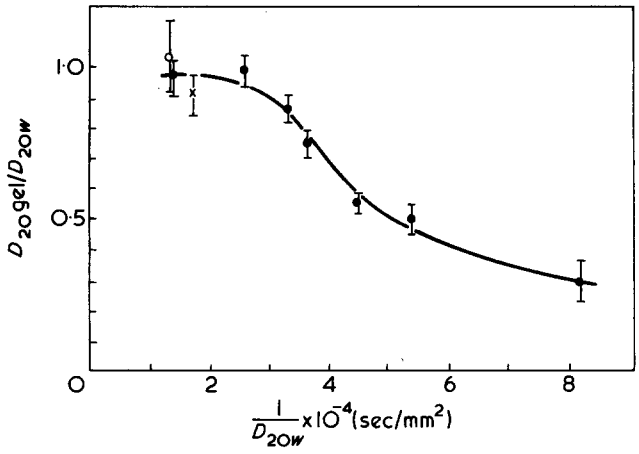


Figure 6 Diffusion coefficients of compact macromolecules within 0.5% calcium alginate gels, D_{20} gel, compared with those in aqueous solution, D_{20w}. ●, Dextran fractions; ○, bovine plasma albumin; X, chymotrypsinogen

creasing molecular weight.

The autocorrelation function in these experiments consisted essentially of an exponential decay corresponding to the heterodyne beat spectrum between the light scattered from the solution and the unbroadened component of the light scattered from the gel, superimposed upon the autocorrelation function due to the light scattered from the gel. These could easily be separated as the relaxation times differed by two orders of magnitude. The homodyne beat spectrum due to the light scattered from the solution was assumed to be negligible, as in all cases the intensity of light scattered from the gel was some two orders of magnitude greater than that scattered from the solution. It was necessary to use thin discs of gel only 2 mm thick for these experiments so as to reduce the superimposed oscillations resulting from bulk oscillations of the specimen to a minimum. So far as the effects of dust are concerned these experiments were somewhat easier than those with the dextrans and globular proteins in solution, as the dust particles were immobilized within the gel.

The exponential decay corresponding to the heterodyne beat spectrum is given by $\exp(-K^2D\tau)$ where $K = 4\pi\sin(\theta/2)/\lambda$. In each case measurements were made at five angles of scatter between 30° and 135° and the decay times found to vary inversely as $\sin^2\theta/2$ as predicted theoretically. The error bars shown in *Figure 6* correspond to the standard error in the mean value of D determined in this way, combined with the corresponding error in the value of D determined in aqueous solution. *Figure 6* shows that movement of compact macromolecules with values of $1/D_{20w}$ less than 3×10^4 sec/mm^2 (corresponding to an equivalent hydrodynamic diameter of 10 nm on the basis of a hard sphere model) is virtually unimpeded within the gel and that macromolecules three times larger are far from immobilized. This is consistent with a junction zone type of structure with large interstitial spaces. It is also consistent with the molecular weight of the junction zones derived from the conventional light scattering experiments (7000 kg/mol), suggesting that for a 0.5% gel there is on average one junction zone per (100 nm)^3. In considering the shape of the curve shown in *Figure 6* it must be remembered that in general the hydrodynamic interaction between diffusing macromolecules and gel will be partially compensated by an increase in osmotic pressure due to the fact that the macromolecules are diffusing within a smaller volume than in solution. This compensating effect would be enhanced by a distribution of size of interstitial spaces.

PRELIMINARY INVESTIGATIONS OF SOME OTHER POLYSACCHARIDE GELS

Agarose

An agarose gel was formed by dissolving commercial agarose at a concentration of 0.5% in hot water (>80°C) and allowing to cool to 20°C. Initially very large oscillations were observed which remained constant in frequency but whose amplitude varied at random with time. After about a week however the oscillations disappeared completely. These observations have been reported previously by Prins *et al.*[6]. Whereas the oscillations are undoubtedly associated with long range thermal gradients persisting within the gel it is likely that the actual phenomenon observed is due to bulk oscillations which are triggered off by the diffusion currents rather than the diffusion currents themselves. The oscillations observed were much too large for the theory given in this paper to be quantitatively applicable. The fact that persisting oscillations of the type observed with alginate did not occur may be a consequence of the way the gel was supported. In the case of agarose the gel filled the light scattering cell so that bulk elastic oscillations in the horizontal direction were constrained after the gel had equilibriated.

After the oscillations had disappeared the autocorrelation function was found to consist of a single exponential with a relaxation time of 30 msec and associated degree of spectral broadening ~1%. Both of these parameters were found to be independent of angle of scatter between 30° and 135°. As with alginate however there was a tendency for the degree of spectral broadening to increase with time. Thus the results for agarose and alginate are qualitatively similar and it may be speculated that similar explanations apply.

Bovine vitreous humour

Bovine vitreous humour consists mainly of hyaluronic acid. It is not a true gel in the accepted sense, in that it has no quasi-static modulus and readily adopts the shape of the light scattering cell. Measurements were made directly at room temperature on material freshly extracted from bull's eyes. Not surprisingly, in view of the physical properties of the material, the autocorrelation function exhibited no oscillations but could be represented by the sum of two expontials whose relaxation times varied continuously from 33 to 16 msec and from 3 to 2 msec in the angular range 30° to 135°. In this respect the behaviour of bovine vitreous humour is similar to an alginate gel and very different from that of a solution. The degree of spectral broadening was however much higher, ~7% at the time of measurement, approximately one quarter of which was attributable to the shorter relaxation time. Here again however, the spectral broadening increased with time, in this case to such an extent that it was not possible to determine whether or not there was any systematic variation with angle. Material which had been kept deep frozen for several days exhibited complete spectral broadening. Although the autocorrelation functions in this case were not exponential, it was found that functions determined at different angles of scatter could be superimposed when plotted against $\tau\sin^2\theta/2$. This is indicative of a diffusion process and the data could be represented by two diffusion coefficients 1.7×10^{-7} and 3×10^{-6} mm^2/sec. Thus bovine vitreous

humour quickly becomes unstable after extraction and the structure appears to break up on freezing. It is possible that *in vivo* the integrity of the system is maintained by short-lived junction zones and that these are represented by the shorter observed relaxation times. The longer relaxation times possibly represent the dynamics of clusters similar to those suggested for alginate gel. However investigations of complex systems such as bovine vitreous humour is probably best approached through the study of model systems, e.g. a study of pure hyaluronic acid solutions as a function of concentration and temperature.

CONCLUSIONS

The degree of spectral broadening of light scattered from calcium alginate gel is small and the variation in the parameters associated with the broadened component with angle of scatter does not conform to any existing theory. The existence of very long range, long term fluctuations in alginate density may, however, be demonstrated, and it is possible that the observed relaxation times represent the continual rearrangements of polymer chains which, assuming a junction zone type of structure, would be necessary for these fluctuations to take place. Bulk oscillations of the specimen manifest themselves in the autocorrelation function. This is due to optical interference within a single coherence volume resulting from the oscillatory strain.

The diffusion coefficients of compact macromolecules through a 0.5% calcium alginate gel indicate large interstitial spaces having dimensions consistent with a very approximate molecular weight for the junction zones of 7000 kg/mol derived from the scattered intensity at high angles. If a junction zone type of structure is correct, then each junction zone must consist of an association of some hundreds of chains.

ACKNOWLEDGEMENT

This research was supported by Science Research Council Grant B/RG/6957/6.

REFERENCES

1 Tanaka, T., Hocker, O. and Benedeck, G. B. *J. Chem. Phys.* 1973, **59**, 5151
2 Munch, J. P., Candau, S., Duplessix, R., Picot, C., Herz, J. and Benoit, H. *J. Polym. Sci. (Polym. Phys. Edn)* 1976, **14**, 1097
3 Munch, J. P., Candau, S. and Hild, G. *J. Polym. Sci. (Polym. Phys. Edn)* 1977, **15**, 11
4 Candau, C., Munch, J. P. and Herz, J. *IUPAC 16th Microsymp. Macromolecules Prague* 1976
5 Prins, W., Rimai, L. and Chompff, A. J. *Macromolecules* 1973, **5**, 104
6 Wun, K. L., Feke, G. T. and Prins, W. *Discuss. Faraday Soc.* 1974, **57**, 146
7 McAdam, J. D. G., King, T. A. and Knox, A. *Chem. Phys. Lett.* 1974, **26**, 64
8 Carlson, F. D. and Fraser, A. B. *J. Mol. Biol.* 1974, **89**, 273
9 Wun, K. L. and Carlson, F. D. *Macromolecules* 1975, **8**, 190
10 Sellen, D. B. *Polymer* 1970, **11**, 374
11 Sellen, D. B. *Polymer* 1973, **14**, 359
12 Debye, P. *Ann. Phys.* 1915, **46**, 809
13 Bryce, T. A., McKinnon, A. A., Morris, E. R., Rees, D. A. and Thom. D. *Discuss. Faraday Soc.* 1974, **57**, 221
14 Debye, P. *J. Appl. Phys.* 1944, **15**, 338; *J. Phys. Colloid Chem.* 1947, **57**, 221
15 Smidsrød, O. and Haug, A. *Acta Chem. Scand.* 1968, **22**, 793; *Biopolymers* 1971, **10**, 1213; Smidsrød, O. *Carbohydr. Res.* 1970, **13**, 359; Smidsrød, O., Glover, R. M. and Whittington, S. G. *Carbohydr. Res.* 1973, **27**, 107; Noy, R. *PhD Thesis* Leeds (1976)
16 Sellen, D. B. *Polymer* 1975, **16**, 561
17 Phillies, G. D. G., Benedeck, G. B. and Mazer, N. A. *J. Chem. Phys.* 1976, **65**, 1883

APPENDIX

Analysis of the autocorrelation function in the general case of partial spectral broadening

Figure A1 shows a spectral distribution for an arbitrary case of partial spectral broadening, together with the corresponding phase correlation function, one being the Fourier transform of the other. The phase correlation function $F(\tau)$ may be written:

$$F(\tau) = A + Bf(\tau) \tag{A1}$$

At zero correlation delay time $F(\tau) = A + B = I$, the overall scattered intensity. The parameters most likely to be interpretable in terms of any molecular model are the normalized Fourier transform of the broadened component of the spectral distribution $f(\tau)$, and the degree of spectral broadening $B/(A + B)$. The problem is to find these from the autocorrelation function of the photoelectric signal. In general this autocorrelation function, $\phi(\tau)$ is given by:

$$\phi(\tau) = k\{F(\tau)\}^2 \tag{A2}$$

where $k = (c^2\gamma^2)/2$, c is the overall sensitivity of the photoelectric system and γ a coherence factor. Substitution of (A1) in (A2) yields a term kA^2. This is the autocorrelation function of the homodyne beat spectrum resulting from the unbroadened component and would only be observable after infinite integration time. Thus in practice:

$$\phi(\tau) = k(\{F(\tau)\}^2 - A^2) \tag{A3}$$

Experimentally γ may be found by making measurements upon any system for which the spectral broadening is complete, i.e. for which $A = 0$, and any macromolecular solution is suitable for this purpose. In this case $\phi_0(\tau) = kI^2 = (c^2\gamma^2I^2)/2 = (s^2\gamma^2)/2$, where s is the d.c. signal resulting from the scattered light. Thus γ may be found from the zero time value of the autocorrelation function $\phi_0(\tau)$, and the d.c. signal. In the present work a 0.5% solution of Ludox was used for this purpose and γ was found to be 0.31 independent of angle of scatter.

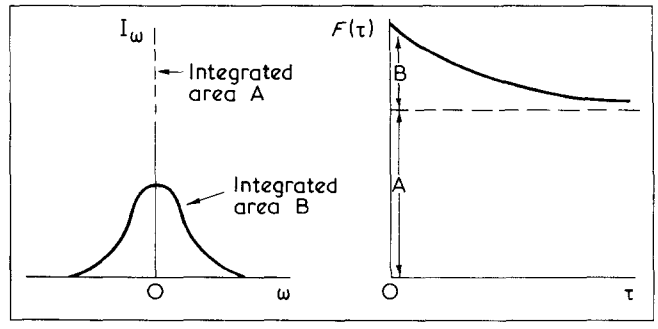


Figure A1 An arbitrary case of partial spectral broadening showing the intensity distribution function I_ω as a function of the displaced frequency ω, and the phase correlation function $F(\tau)$ as a function of the correlation delay time τ

In the general case it is useful to define S as the value of $\phi_0(\tau)$ expected if the spectral broadening were complete. Thus experimentally $S = s^2\gamma^2/2$ and in terms of the phase correlation function:

$$S = k(A + B)^2 \tag{A4}$$

Also from equations (A1) and (A3):

$$\phi(\tau) = k\{2ABf(\tau) + B^2[f(\tau)]^2\} \tag{A5}$$

$$\phi_0(\tau) = k(2AB + B^2) \tag{A6}$$

Equations (A4)–(A6) yield:

$$f(\tau) = \frac{[\phi(\tau) - \phi_0(\tau) + S]^{1/2} - [S - \phi_0(\tau)]^{1/2}}{(S)^{1/2} - [S - \phi_0(\tau)]^{1/2}} \tag{A7}$$

and

$$\frac{B}{A + B} = 1 - \left[1 - \frac{\phi_0(\tau)}{S}\right]^{1/2} \tag{A8}$$

In the case of complete spectral broadening A = 0 and equation (A7) becomes:

$$f(\tau) = \left[\frac{\phi(\tau)}{\phi_0(\tau)}\right]^{1/2} \tag{A9}$$

In the case where the degree of spectral broadening is small, i.e. $A \gg B$ equations (A7) and (A8) becomes:

$$f(\tau) = \frac{\phi(\tau)}{\phi_0(\tau)} \tag{A10}$$

$$\frac{B}{A + B} = \frac{\phi_0(\tau)}{2S} \tag{A11}$$

This is the heterodyne case where the broadened component beats with the unbroadened component and the homodyne beat spectrum of the broadened component may be neglected. By making a second order approximation it may be shown that equation (A10) is valid within a fractional error of p of the zero time value of $f(\tau)$ for values of $\phi_0(\tau)/S$ less than $16p$.