Electromechanical coupling in a biopolymer gel*

T. J. Lewis†, J. P. Llewellyn and M. J. van der Sluijs
Institute of Molecular and Biomolecular Electronics, University of Wales Bangor, Dean Street, Bangor, Gwynedd LL57 1UT, UK

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Experiments on electromechanical coupling in aqueous gels of the polysaccharide agarose are described. The gels are prepared as free self-supporting discs between 1 and 4.5 mm thick immersed in water and in contact with a stainless-steel electrode system on a solid substrate. Mechanical vibrations, excited in the gel when a small (<1 V r.m.s.) alternating voltage is applied to the electrodes, are determined in magnitude and phase using an interferometer capable of measuring displacements down to 1 pm. The typical gel response exhibits two major and two minor resonances having frequencies that vary with gel thickness and concentration in a range below 2 kHz. The major resonances are interpreted in terms of a bulk transverse shear wave and a related surface wave, while the minor resonances give evidence of combination wave interactions. There is excellent agreement between the rigidity modulus deduced from the velocity of the surface wave and that obtained earlier by conventional rheological methods. Evidence is given to suggest that the gel vibrations are generated by voltage-induced oscillatory motion of ions within the double layers at the gel–electrode contacts. The magnitude and phase of the vibrations in the gel can be changed by applying bias voltages at the electrodes and by adding KCl to the water surrounding the gel.

(Keywords: agarose gels; electromechanical coupling; bulk and surface waves; combination waves; gel–electrode double layers)

INTRODUCTION

In the presence of water, certain biopolymers will form gels, the stability and viscoelasticity of which are determined by a balance of forces arising from the elasticity of the polymer strands, the polymer–polymer affinity moderated by the degree of hydrophobicity, and the electrostatic interactions between charges on the polymer strands and in the water phase. The presence of water and the polyelectrolyte nature of most biopolymers make the biogels different from gels formed by the crosslinking or condensation of synthetic polymer precursors. In spite of the fact that most biogels of interest have water contents that could be as much as 99% by weight, they behave rheologically as homogeneous solids but with low rigidity moduli[1]. In living systems the gel phase is ubiquitous, having both passive and active roles in intra- and extracellular situations[2].

Amiya and Tanaka[3] have demonstrated how discontinuous volume changes may be induced in gelatin, agarose and DNA gels when water conditions such as ionic content or pH are altered and the balance of forces changed. In view of the polyelectrolyte nature of these gels, with localized charge on the polymer strands and counter-charge in the water, we may also expect that they will respond to electric fields.

In fact, the presence of steady electric fields between electrodes applied to synthetic polyelectrolyte gels has been shown[4,5] to cause the negatively charged polymer networks to shrink towards the anode while mobile counter-ions (hydrated H^+) in the water phase move towards the cathode, where excess water is exuded as the gels shrink. Most of these measurements have been made under application of steady electric fields when the volume changes occur slowly over relatively long periods of time. This long response time appears to be confirmed by the experiments of Osada and Hasebe[6], who have reported that there was no response to alternating fields in the frequency range 6 Hz to 5 kHz. This result is surprising, bearing in mind the polyelectrolyte nature of the gel and the fact that interfacial polarization can be readily induced in electrolytic solutions in low-frequency alternating fields.

We should also note that any gel–metal electrode contact in equilibrium will have an associated electrical double layer with an inner (Stern) layer of adsorbed ions on the electrode surface and an outer more diffuse (Gouy–Chapman) layer in the gel proper in which an excess of ions of one sign will exist. One contribution to these charged layers will be ions in the aqueous phase of the gel and therefore similar to a regular electrolyte. The other contribution will be the bound charges on the polymer strands in both the adsorbed Stern layer and the outer Gouy–Chapman layer. In biological situations biogels are likely to exist in only weak average electric fields but they will be contained by interfacial membrane regions where much greater net double-layer charges and electric fields will be present. This is a situation that is not unlike that of the double layer of a gel–metal electrode contact.

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† To whom correspondence should be addressed

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In the present study we have investigated the electromechanical properties of aqueous gels of agarose, which is a polymeric disaccharide with alternating residues of β-D-galactopyranose and 3,6-anhydro-α-L-galactopyranose. It is understood that the polymer network of this gel forms as a result of random coil–helix transformations in sections of the single primary polymer chains, and these, being less hydrophilic than the random flexible conformation, associate to form junction zones of double and then aggregated double helices[7,8]. The junction zones are likely to be less well defined and static than the nodes of a chemically crosslinked network. This feature, together with the flexible random-coil nature of the rest of the network and the low weight fraction of polymer in the gel, will lead to a low modulus of rigidity.

We have sought to obtain conditions in the gel more akin to those in the biological situation with electric fields established only at one interface of a sample and the rest of the gel field-free. This is different from the bulk uniform field condition used by Tanaka *et al.*[4] and by Osada[5]. We shall show that disturbances generated by the local electric fields can cause mechanical strains that are propagated throughout the rest of the gel and, in appropriate circumstances, set up mechanical resonances.

EXPERIMENTAL

The gels were made from commercially available agarose (Pharmacia Type C as used for electrophoretic studies) without further purification. It is a natural product with a small amount of residual sulphation of the order of 0.3%, which will result in some charged SO_3^- groups on the polymer chains.

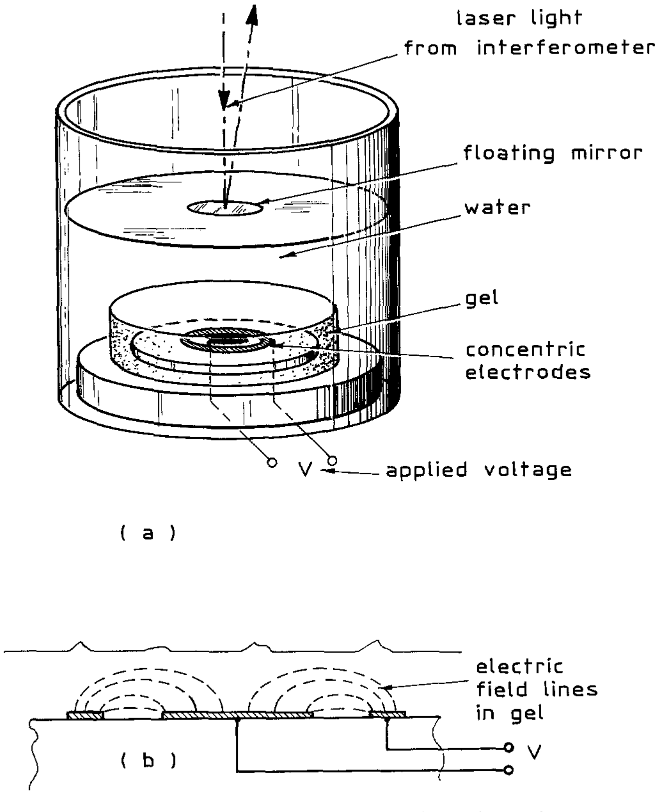

Figure 1 (a) The general arrangement for determining the response of a cylindrical disc of gel to an alternating electric field. The distance between gel and mirror is normally about 1 mm, but in the diagram this has been increased for clarity. (b) The electric field pattern generated in the gel between the electrodes

The experimental arrangement is shown in *Figure 1a*. Known weights of agarose were dissolved in deionized water at 90°C and poured into a mould placed over planar metal electrodes mounted on an electrically insulating base. In this way cylindrical discs of gel of about 32 mm diameter were formed, in intimate contact with the electrodes on the lower face but otherwise unconstrained. The electrodes consisted of a central disc, 11 mm diameter, surrounded by a concentric annulus 2 mm wide and with a spacing of 3.5 mm.

For most of the experiments stainless-steel foil (0.125 mm thick) was used to form the electrodes, but experiments with platinum electrodes produced similar results. The electrodes were polished with 0.075 μm alumina powder and then cleaned ultrasonically with a detergent and finally with deionized water. As indicated in *Figure 1b* the electric fields are confined to one face of the gel and will be most intense in the inter-electrode space. The alternating applied voltage was, in most measurements, kept below 1 V r.m.s. in order to avoid heating the gel. Since the electrodes were buried in the gel and care was taken to insulate the connections to the electrodes, it was possible to surround the gel samples with deionized water, so maintaining an equilibrium environment for the gel without an electrical short-circuit.

To measure the gel response with minimum disturbance, a small mirror (10 mm diameter) was floated centrally on the water surface above the gel (*Figure 1a*), being held there by surface tension forces. Vertical movement of the mirror was monitored by arranging it to be in one arm of a Michelson optical interferometer, which employed a feedback loop to minimize drift of the optical fringes due to temperature change, mechanical vibration or acoustic noise[9]. The cell and optical system were mounted on an optical vibration-proof table in a ground-floor laboratory where the temperature could be maintained at about 20°C. Any contraction or expansion of the gel was communicated to the mirror through the intervening water space. Provided this was about 1 mm (a spacing that was maintained constant throughout the experiments), the measured displacements of the mirror appear to coincide with displacements of the gel surface to within a few per cent.

Cyclic movement of the gel in response to an alternating voltage from a variable-frequency generator applied to the electrodes produced a corresponding cyclic movement of the mirror. The consequent optical response from the interferometer was fed via a photodiode to a phase-sensitive detector. In this way the in-phase (X) and out-of-phase (Y) components of the movement of the gel with respect to the applied voltage could be measured down to 1 pm. Using this highly sensitive method it is possible to detect electrokinetic responses of the gel to local electric fields as low as $10\ \mathrm{V\ m^{-1}}$.

RESULTS

A typical in-phase (X) response of the mirror following electrical stimulation of a 2% w/w agarose gel, 4.5 mm thick, is shown in *Figure 2*. The frequency characteristics of the feedback loop in the interferometer system prevent measurements at frequencies below 200 Hz, but we believe that the features of major interest are above this value. The displacements, strongly dependent on frequency, are below 1 nm in magnitude and are found to vary linearly with the applied alternating voltage up

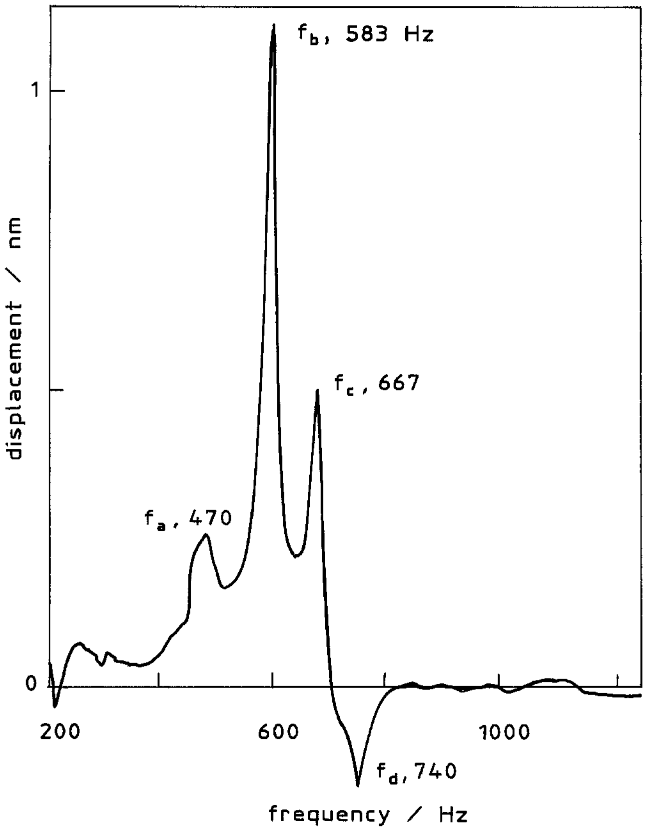

Figure 2 In-phase component of the frequency response of a 4.5 mm thick, 2% agarose gel sample. Two major (f_b,f_c) resonances and two minor (f_a,f_d) resonances occur

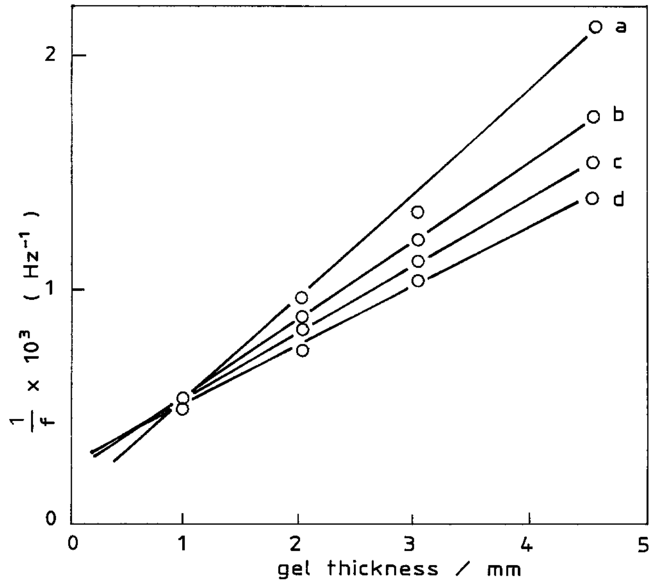

Figure 3 Resonant frequency–thickness relationships for the four resonances (f_a,f_b,f_b,f_d in *Figure 2*) of 2% agarose gel samples

to 1.5 V r.m.s. Four major resonances (f_a to f_d) are found and can be confirmed by the typical complementary resonance behaviour of the out-of-phase (Y) component (not shown). In addition to the resonances there is a background response that decays with increasing frequency.

The general features shown in *Figure 2* appear for all the large number of agarose samples investigated and are reproducible to within a few per cent. Resonance f_a is broad and less well defined than the rest and f_d is of opposite phase. Resonances f_b and f_c appear to be the dominant processes. The resonant frequencies vary with gel thickness as shown in *Figure 3* and with gel concentration as shown in *Figure 4*.

When a steady negative bias voltage is applied to the inner central electrode, it is possible to invert the phase of the response. The speed of the inversion depends on the bias voltage, and there appears to be an onset bias voltage below which inversion does not occur. The ability to effect an inversion also appears to depend on electrode conditions and is encouraged when the electrodes have been recently cleaned. A typical sequence following application of a 1.5 V negative bias is shown in *Figure 5*. Ultimately all four resonances decrease in amplitude and f_b and f_c invert in sign, but the initial response is an increase in the signal particularly in the case of the f_c resonance. If, after the inversion has reached a steady state, the bias voltage is removed, the resonances gradually revert back to their original phases. However, there is an initial increase in the inverted signal before reversion begins, a phenomenon similar to that occurring when bias is first applied (*Figure 5*). If positive rather than negative bias is applied, the normal resonances are unchanged but inverted ones revert more rapidly. In all these changes of magnitude and phase there is little, if any, change in the resonant frequencies.

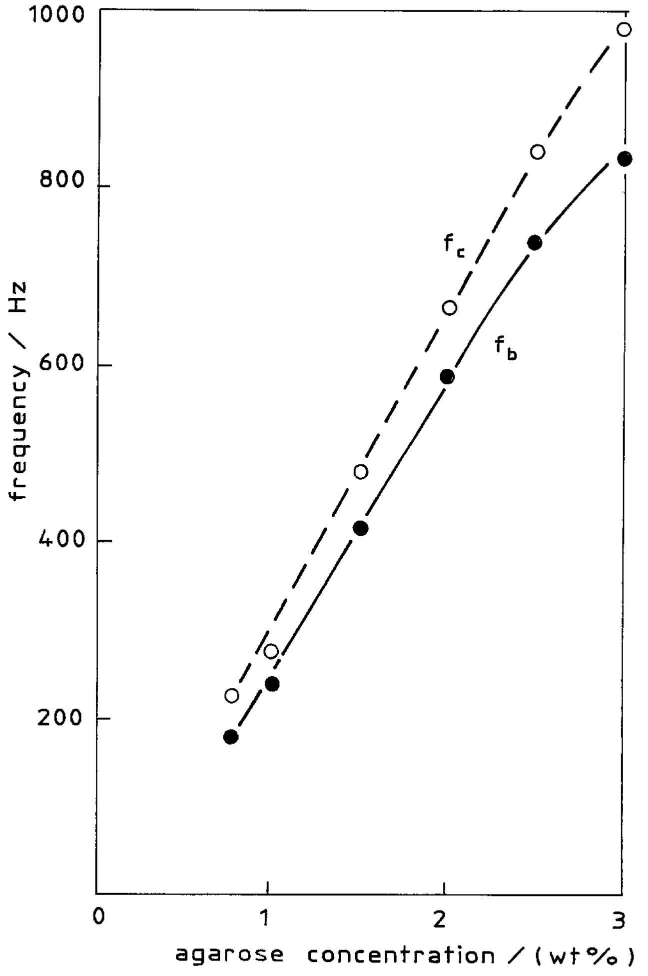

Figure 4 Dependence of the f_b and f_c resonant frequencies on agarose concentration for 4.5 mm thick samples

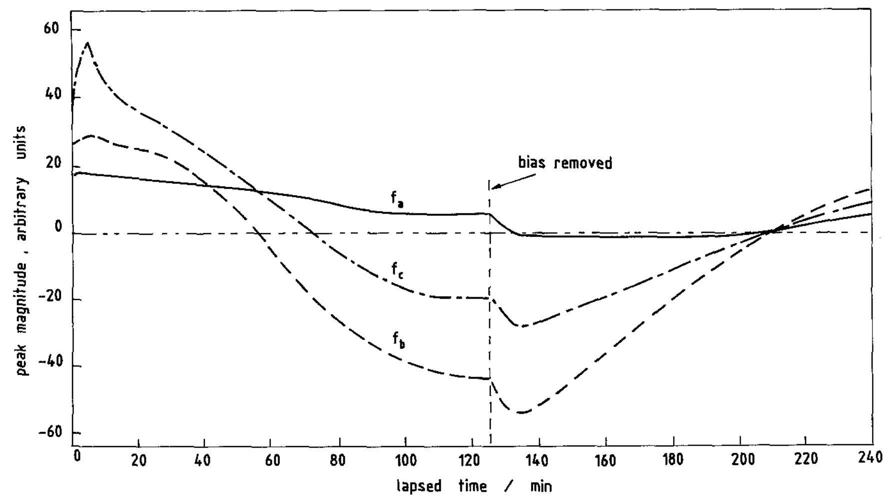

Figure 5 The time dependence of the magnitudes of the f_a, f_b, f_c resonances for a 4.5 mm thick, 2% agarose gel following application of a -1.5 V bias to the central electrode at zero time and removal after 125 min

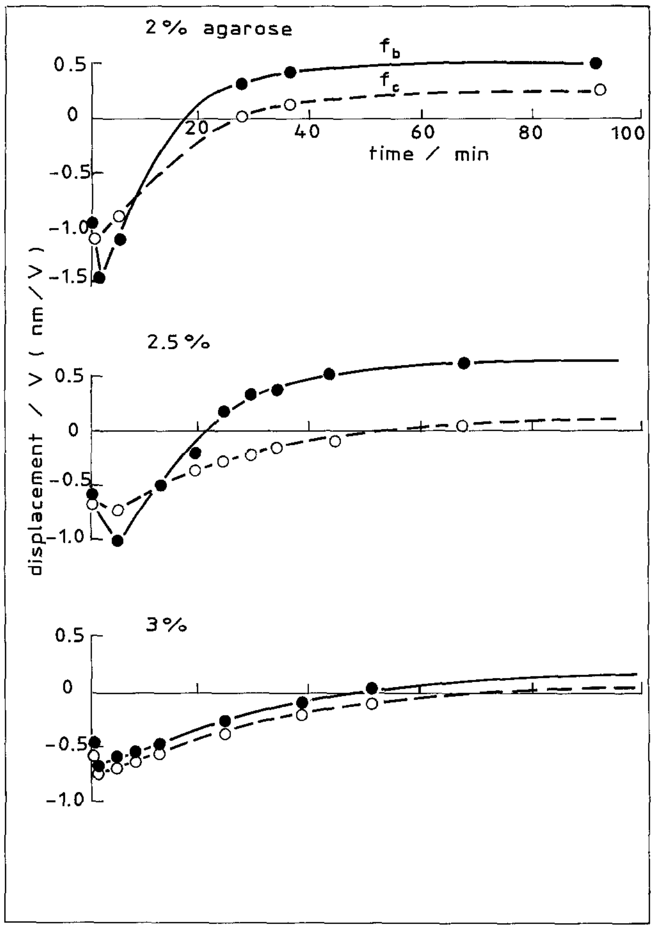

Figure 6 Time-dependent reversion of the inverted phases of f_b (●) and f_c (○) resonances for 4.5 mm thick gels after replacing the surrounding water by a 10^{-2} N KCl solution

The magnitude and sign of the response are also influenced by the ambient conditions of the gel. As shown in *Figure 6*, the substitution of a 10^{-2} N KCl solution for deionized water surrounding the gel causes inverted resonances (generated by appropriate negative bias of the central electrode) to revert back in a much shorter time than the natural reversion. The reversion is most rapid for the least concentrated gel. There is again an initial increase in signal before reversion begins, which is greatest for the less concentrated gel. The existence of a significant time interval before the changeover is completed, which is least for the 2% gel, suggests that it is necessary for ion diffusion through the gel to occur for a change to take place. (The pore size of the gel will vary inversely with agarose concentration.) Again, as found under bias conditions, the resonant frequencies do not appear to be changed.

The inverse effect

With the electrode and gel geometry employed here it is also possible to demonstrate an inverse effect in which direct mechanical stimulation of vibrations in the gel generates an electrical signal between the electrodes. For this an arrangement similar to that shown in *Figure 1a* was employed but with the mirror removed and a circular disc immersed in the water above and parallel to the gel. The disc was vibrated mechanically, producing waves that were transmitted through the water into the gel. As a result electrical signals were induced between the electrodes, which could be monitored using phase-sensitive detection. The in-phase electrical signal for a 4.5 mm thick, 2% agarose gel is shown in *Figure 7*. Three resonances are found in the frequency range in which the electrically excited resonances occurred (*Figure 2*). It is not surprising that the resonant frequencies are different in the two cases because the mechanically induced resonance will involve the vibrating disc and its assembly as well as the gel on its base in the water. The substitution of 10^{-2} N KCl solution for deionized water surrounding the gel caused a gradual collapse of the induced signal (*Figure 7*) on a timescale comparable with that found for the electrically induced resonances (*Figure 6*).

DISCUSSION

Resonances

The linear variation of the inverse of the resonant frequency with gel thickness (*Figure 3*) can be interpreted in terms of standing waves in the gel. There will be some degree of acoustic mismatch at the top and bottom

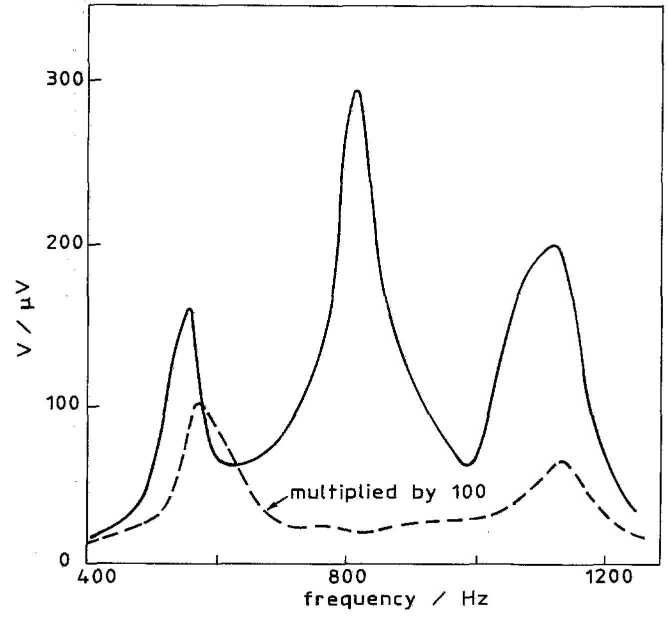

Figure 7 The inverse effect. The in-phase electrical signal generated as a result of mechanical vibration of a 4.5 mm thick, 2% agarose gel: (——) gel in water; (– – –) collapse of signal after immersion in 10^{-2} N KCl solution

faces of the gel where it makes contact with the water and with the solid base. Consequently, if l is the length of the gel and Δl is an equivalent additional acoustic length allowing for these mismatches, then a resonance at the fundamental frequency will occur when the wavelength of the standing wave is $2(l + \Delta l)$. The fundamental resonant frequency is then equal to $v/2(l + \Delta l)$, where v is the wave velocity. If Δl is assumed to be frequency-independent, the slope of the characteristics in *Figure 3* will be $2/v$. For the dominant resonances f_b and f_c the velocities v_b and v_c are 6.0 and 7.1 m s^{-1} and Δl_b and Δl_c are 0.6 and 0.8 mm. Interpreting the velocities in terms of a modulus G so that $v = (G/\rho)^{1/2}$, where ρ is the gel density, it is possible, using *Figure 4*, to determine G as a function of gel concentration for both of the resonances f_b and f_c. The result is shown in *Figure 8* together with the values of the storage shear modulus for an agar gel obtained by Clark and Ross-Murphy[1] using a conventional rheological spectrometer operating at 10 rad s^{-1}. The agreement between the moduli derived from f_b and their values[1] is very good especially in view of the considerable difference between the two derivations. On the other hand, the moduli derived from f_c are a factor of 1.3 times greater than the Clark and Ross-Murphy values.

If we conclude that shear waves are being generated in the gel by the electrical forces and are responsible for the f_b resonance, then the nature of the other resonances is less obvious, especially as they do not form a harmonic series. In a very large number of experiments under a variety of conditions of gel sample size and concentration, we have found that the ratio f_b/f_c of the dominant resonances is remarkably close to 0.87. This would be the expected ratio if f_c and f_b corresponded to a transverse shear wave in the bulk and a Rayleigh surface wave respectively in a system for which the Poisson ratio was zero[10]. Now, Tanaka *et al.*[11] have suggested that, while the Poisson ratio of the gel is expected to be close to 0.5 because of the incompressibility of the water phase, the Poisson ratio of the polymer *network* is likely to be zero. This is because the polymer network under uniaxial compression will, like a sponge, shrink along the direction of the compression but not spread out in transverse directions.

The Rayleigh surface wave is a mode that decreases in amplitude with distance from the surface but extends over several wavelengths. In the present case, therefore, it might be a mode involving a large part, if not the whole, of the sample volume.

If indeed f_c and f_b correspond respectively to bulk transverse and Rayleigh surface waves of the gel, then the very good agreement between the moduli deduced from f_b and from the measurements made by Clark and Ross-Murphy[1] suggests that their rheological measurements might involve the surface forces, a situation that could arise if the gel were undergoing torsional shear. More work is required to clarify this point.

There are also interesting relationships concerning the other resonances f_a and f_d. Under a variety of gel conditions the relationships $f_b - f_a = f_c - f_b$ and $f_d - f_c = f_c - f_b$ hold with only a few per cent error, so that f_a, f_b, f_c and f_d are equally spaced in frequency. These frequency conditions are well known to arise when non-linear combination wave interactions are occurring. It suggests that resonances f_a and f_d arise as a result of combination wave interactions of the dominant resonances f_b and f_c in the gel. If this is a correct interpretation, then there is the possibility of stimulated or wave pumping actions at low frequency within a gel matrix.

Wave generation

The tentative model we have for generating shear waves in the gel involves the double layer at the gel–metal

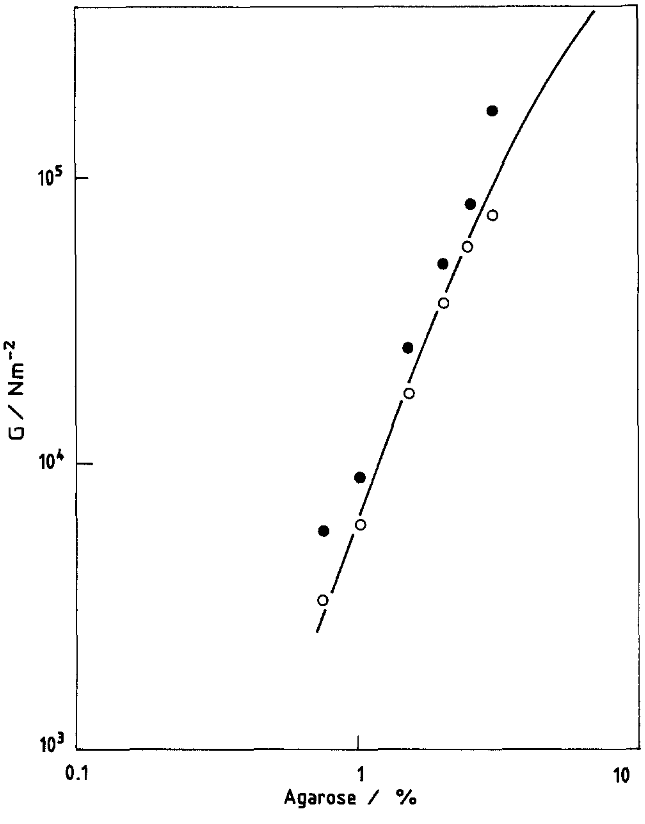

Figure 8 Modulus G obtained from the wave velocities of f_b (○) and f_c (●) resonances as a function of gel concentration. The full curve shows the shear modulus obtained by conventional rheological measurement (ref. 1)

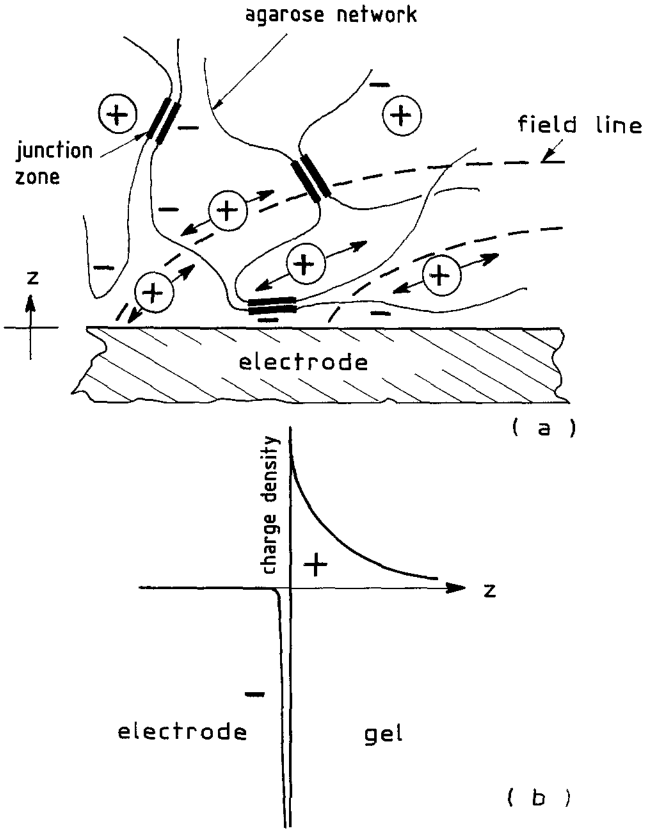

Figure 9 A double-layer model for the normal gel–stainless-steel electrode interface. (a) An excess of mobile positive ions (H^+) over localized negative centres (SO_3^-) on the agarose network is assumed to exist, creating (b) a double layer with net positive charge in the gel. The mobile positive ions will oscillate back and forth along field lines created by the alternating potential difference between the electrodes

electrode interface as shown in *Figure 9*. When the gel is first made on the electrode system, the negative bound charges of the sulphate groups on the agarose network together with mobile counter-ions in the water phase will be brought into contact with the metal electrodes. To attain equilibrium, charge transfer will be necessary to establish a double-layer potential difference with a net space charge on the gel side of the interface. We have been able to measure the formation potential difference when contact is made to stainless-steel electrodes and found it to be of the order of 100 mV. This is likely to consist of solvated protons (H^+) in the water phase extending out as a diffuse (Gouy–Chapman) layer a distance of perhaps 10^{-8} to 10^{-7} m into the gel. The double layer in the gel also has an associated capacitance, which we have found from a.c. impedance measurements to be of the order of 10^{-6} F m^{-2} at 1 kHz.

Under the action of the electric field, the mobile ions will tend to oscillate back and forth along field lines connecting the inner and outer electrodes (*Figure 9*), the excursions they make decreasing as the frequency increases. Because of the coupling between the ions and the gel arising from electrostatic interactions and from viscous forces between the ions, the water phase and the gel network, part of the ion momentum will be transferred to the gel. Because the double layer has an excess of one sign of charge (positive in this case), there will be a net momentum transfer, the phase of which with respect to the applied voltage will depend on the sign of the double layer. For the electrode configuration employed, a radial transverse momentum may be expected to be imparted across the gel face in the neighbourhood of the electrodes. The component of momentum normal to the interface will be weak partly because field lines with a significant normal component will pass through the weaker regions of the double layer and also because the direction of such normal components will be in opposition at the two electrodes. The double layer may be expected therefore to be a shear wave generator when an alternating voltage is applied to the electrodes. Increasing the voltage applied will increase the ion velocities and their oscillatory excursions in the field may be expected to depend linearly on voltage except at very high fields or sufficiently low frequencies when the build-up of polarization at the electrodes will ultimately limit the process.

Application of a negative bias voltage to the central electrode will have two effects. First, it will immediately tend to draw positive ions from the gel into the double-layer area, and this extra concentration of positive charge will increase the shear wave generation. The enhanced signal found at the beginning of negative biasing (*Figure 5*) is indicative of this process. Secondly, when the field resulting from the bias is sufficiently strong, electron injection can be expected at the electrode, first neutralizing the positive ions ($H^+ + e \rightarrow H \rightarrow \frac{1}{2}H_2$) and then gradually creating a net negative (OH^-) charge. The double-layer strength declines, goes through zero, and then grows to a steady-state value of opposite sign. The shear wave generation will follow suit, decreasing in magnitude and finally reversing in phase (*Figure 5*). When the bias is reduced to zero, electron injection ceases but, at the same time, positive-ion drift into the double-layer region will also be reduced. Shear wave generation is temporally enhanced but, as the negative space charge of the double layer in the gel declines and is replaced by the equilibrium net positive charge, so the generation will decline to zero, reverse in phase and recover the original value.

The diffusion of a KCl solution into the gel after negative bias has been applied and a negative double-layer space charge established in the gel will have several effects. First, K^+ ions will be available to neutralize the negative charge and restore the equilibrium positive space charge. The presence of the electrolyte will also tend to reduce the extent of the double layer, confining it more closely to the electrodes, and at the same time will provide increased Debye screening of the fixed negative charges on the polymer strands and the counter-charges in the water, so decoupling the two systems. This latter effect might be expected to change the overall network forces and therefore the modulus of the gel, but, since the resonant frequencies appear not to be altered when KCl is present, the effect cannot be large.

The double-layer concepts described above also provide a reasonable explanation of the inverse effect in which electrical signals are generated by gel vibrations. These vibrations will modulate the double layer, moving charges relative to the electrodes and thereby inducing currents in the electrode circuit. Transverse shear vibrations will be particularly effective in inducing currents.

The steady decay of the background response of the gels and the absence of significant resonances at frequencies above the four shown in *Figure 2* are a characteristic of all experiments. This may be due to increased gel absorption at the higher frequencies, but a more plausible explanation is that the efficiency of generation at the interface decreases with increasing frequency because of reducing ion movement. The a.c. impedance measurements have shown that the electrode capacitance falls rapidly as the frequency increases, but unfortunately the corresponding capacity current is a measure of total ion movement independent of sign and so cannot be used to determine the net ion movement that would be responsible for wave generation.

CONCLUSIONS

Using an interferometric technique it has been possible to monitor the small (<1 nm) unrestrained mechanical responses of agarose gel samples when weak electrical stimulation is applied across one face of the gel. Two dominant low-frequency resonances are found. One can be interpreted in terms of a transverse shear wave velocity and the other is likely to be a Rayleigh wave involving the gel surface with a somewhat lower effective modulus, which is in very good agreement with that determined by conventional rheological measurements. Two other weaker resonances are also found, which appear to be related to the dominant resonances in such a way as to suggest that wave combination interactions take place in the gel. If that is so, then there is the possibility of having stimulated wave mixing and pumping processes in gel matrices under appropriate conditions.

The electrokinetic effect demonstrated here is unlike that investigated by Tanaka *et al.*[4], Osada[5] and others in that the bulk of the gel is not subjected to an electric field and the induced mechanical processes occur on a very much shorter timescale. The transverse motion appears to be induced by local transverse ion currents at the electrodes and closely associated with the interfacial double layer. Since it is possible to produce the inverse effect, gel networks are potential vehicles for a variety of electrical–mechanical interconversions. At present it seems likely that the frequency response of the ionic source limits the operating frequencies to below 2000 Hz, but there is no reason to suppose that the gel network would not respond at higher frequencies.

We have found similar processes to occur in gelatin gels and we believe that the processes considered here might occur universally in all biogels and indeed in synthetic polymer gels where a polyelectrolyte network exists and a significant double-layer contact would be established at a metal electrode.

ACKNOWLEDGEMENTS

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