Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Polymers 54 (2003) 83–95
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Effect of sucrose on agarose gels mechanical behaviour

Valery Normand[a,*], Pierre Aymard[b], Didier L. Lootens[a], Eleonora Amici[a], Kevin P. Plucknett[a], William J. Frith[a]

[a]*Unilever Research Colworth, Colworth House, Sharnbrook Bedfordshire MK44 1LQ, UK*
[b]*Danone Vitapole, 15 Avenue Galilée, 92350 Le Plessis Robinson, France*

Received 24 April 2003; accepted 9 May 2003

Abstract

The use of sucrose solutions as a binary solvent for the agarose biopolymer is investigated over a broad range of concentration. Three different molecular weight agarose samples have been studied in different sucrose/water ratios using small and large deformation mechanical measurements, DSC measurements, turbidity measurements and TEM microscopy. It is shown that the addition of sucrose results in a less heterogeneous gel network though the aggregation of helices into fibres is still present. The small deformation oscillatory measurements indicate that addition of sucrose induces a modification of the kinetics of gelation, i.e. a modification of the apparent gelation temperature and long-time modulus. Large deformation results indicate that increasing the sucrose/water ratio results in a large increase in the strain at failure, which can extend from 40% to more than 150%. This phenomenon can be explained by melting (under stress) and reforming of cross-links within a more homogeneous physical network (character exhibited by gelatin/water gels). Strain and stress at failure have been fitted empirically using an exponential function whose parameters can be interpreted in terms of agarose molecular weight and concentration. An inflexion point is always found at 20% w/w sucrose concentration when strain and stress at failure and also the Young's modulus are plotted as functions of sucrose concentration at a given agarose concentration.

In addition it is concluded that the effect of agarose average molecular weight on gelation and gel properties is reduced when sucrose is added.

Keywords: Average molecular weight; agarose; Gels

1. Introduction

The mechanical behaviour of agarose aqueous gels have been extensively studied (Clark & Ross-Murphy, 1987; Marvin, 1954; Normand, Lootens, Amici, Plucknett, & Aymard, 2000). It has also been shown that changing the solvent quality (for instance by addition of sucrose, glucose syrup…) changes the gel network's structure (Nishinari et al., 1992; Nishinari, Watase, Miyoshi, Takaya, & Oakenfull, 1995; Watase, Nishinari, Williams, & Phillips, 1990). Both experimental (small deformation rheology) and theoretical (Zipper model) results suggested that the number of cross-links increased and the size of the junction zones decreased in the presence of sucrose molecules (Nishinari et al., 1992, 1995). More recently, the fracture properties of agarose gels in very high sugar environment has been found to be unusual for an agarose gel network but has not been investigated in depth (Tsoga, Kasapis, & Richardson, 1999). The results of this study demonstrated an increase of the strain at failure of around 70% in compression for a sample containing a total of 85% w/w of sugar molecules. This phenomenon is largely explained by a reduction of intermolecular associative interactions, leading to a reduction in the average number of chains forming the agarose fibres, thus leaving more freedom to the bundles, to stretch and relax, as also seen for de-acylated gellan gels (Sworn & Kasapis, 1998). While this study was performed with only one type of commercial agarose (type I-A) in the present work, the influence of the molecular weight of the agarose is tested (Normand et al., 2000), particularly in relation to the effect of the sugar content on the large deformation mechanical behaviour.

The present study focuses on the effect of sucrose concentration on the failure properties of agarose gels but

* Corresponding author. Address: Firmenich SA, 7 Rue de la Bergère, CH-1217 Meyrin 2, Geneva, Switzerland.
E-mail address: valery.normand@firmenich.com (V. Normand).

0144-8617/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0144-8617(03)00153-X

could provide information which is relevant to other polysaccharide sucrose systems.

2. Materials and methods

Two different types of purified agarose (supplied by Sigma) have been used in the context of this study: a high viscosity agarose (Type I-A, low EEO, A-0169, *H*, lot 56H1046) and a low viscosity agarose obtained after enzymatic degradation, which was provided in two different lots (Type XII, A-7299, *L*1, lot 17H0207 and *L*2, lot 88H0070). The molecular weight distribution of these biopolymers have been estimated at 60 °C using Size Exclusion Chromatography with Multi-Angle Laser Light Scattering (HPSEC-MALLS). The concentration of the agarose solution used for this determination was 0.3% w/w in 100 mM LiCl solution. This solution was first heated to 95 °C for 30 min before being cooled down to 60 °C. The refractive index provides information on the amount of agarose being eluted, while the light scattered is proportional to the product of the concentration and the apparent mass, at a given angle ($c.M_{app}$). Measurement at several angles and several concentrations allows M_w and the radius of gyration R_g to be obtained by extrapolation (Zimm plot). Typical chromatograms for the three agarose variants are reported elsewhere (Normand et al., 2000), and corresponding molecular weight data (weight average molecular weight and polydispersity) for these samples are summarised in Table 1. Distributions of molar mass for the different samples clearly overlap, due to polydispersity index of about 1.8, and mean values differ by no more than 50%. However, as shown earlier, significant differences in rheological behaviour have been observed and discussed with regard to molecular weight distribution differences (Normand et al., 2000).

The gels were prepared by dissolving the biopolymer powder in sugar solution at 98 °C under strong stirring for 30 min, till a transparent solution formed. Sometimes, at high concentrations, it was necessary to leave the solution at rest for several hours, and at 98 °C, to remove the air bubbles suspended in the solution through its viscosity. The solutions were then cast in moulds and cooled down to 10 °C.

Commercial sucrose from Sigma (UK) (saccharose, $C_{12}H_{22}O_{11}$, batch 9914439249) was used in this study.

Table 1
Weight average molecular weight ($\bar{M}_w$) and polydispersity for the three batches of agarose investigated (Normand et al., 2000)

Sample	($\bar{M}_w$) (g mol^{-1})	$\bar{M}_w/\bar{M}_n$ (polydispersity)
56H1046 type 1-A (*H*)	162,000	1.731
88H0070 type XII (*L*2)	132,000	1.806
17H0207 type XII (*L*1)	116,000	1.837

The mechanical properties of the different agarose gel samples were measured using small deformation testing in oscillatory mode using a Carrimed CSL 500 with double concentric cylinders at large gap, adapted for the measurements of three dimensional gel networks (gap = 3 mm) (Normand & Ravey, 1997). The sample gels were supported between lower layers of perfluoro-methyl-decaline (density = 1.98), and mineral oil (density = 0.7) above, thus removing end effects and preventing evaporation. All the measurements were performed within the linear viscoelastic domain of deformation of the gel using the strain-controlled option. A constant strain of 0.2% amplitude was chosen to avoid disturbing the gel network during the measurement.

The large deformation and failure behaviour of the gels were assessed in both tension and compression using an Instron tension/compression testing machine (Instron 4501, High Wycombe, UK) at a constant 10 °C temperature. At least 8 samples of each composition were tested to assess the experimental error.

For the compression tests, hot solutions of agarose were poured into cylindrical moulds. The sample dimensions were: initial diameter 13 mm, and initial height 13 mm. Vaseline cream was used to lubricate the plastic moulds. The samples were kept for 24 h in the fridge after rapid quench cooling, prior to measurement. The tests were performed under lubricated conditions (dodecane) in order to avoid shear deformation due to surface interactions. All the samples were deformed at the same rate, which was 50 mm min^{-1}.

For the tension tests, the hot solutions were poured between two glass plates separated by 1.4 mm thick spacers (*e*) and cooled down quickly to 10 °C using two water baths, one set at 60 °C during filling of the hot solution, and the other one at 10 °C to gel the sample. After storage (24 h at 5 °C), samples were cut from the gel sheets using 'dog-bone' shaped cutters (60 mm gauge length and 6 mm width). Samples were then gripped using double-sided tape and polymeric glue, and stuck to cardboard tabs, in order to minimise handling damage. Tension tests were performed at a displacement rate of 100 mm min^{-1}. In both cases, true stress and true strain were considered (Normand et al., 2000). In fact, using such different displacement rate for tension and compression tests allows the system to deform at a similar strain rate in both tension (tensile test) and extension (compression tests) up to 50% strain at least.

Differential Scanning Calorimetry experiments were conducted using a Setaram MicroDSC III (F) batch and flow calorimeter. 800 ± 10 mg of hot solutions were poured into the sampling device at 25 °C. The reference used was the binary solvent present in the type of sample studied (i.e. water, 40, 60 and 70% w/w sucrose in water) (Nishinari et al., 1995; Watase et al., 1990). The temperature profile was identical for all the experiments: heating from 25 to 100 °C at 1 °C min^{-1}, then cooling from 100 to 10 °C at

1 °C min^{-1}, then heating from 10 to 100 °C at 1 °C min^{-1}. Agarose ordering enthalpy was evaluated from the area of the peak shown by heat flow versus the temperature.

The microstructure was visualised using Transmission Electron Microscopy (TEM). For this, samples were cut into small 1 mm cubes, and fixed in 0.05% aqueous ruthenium tetroxide for 4 h, followed by rinsing in two changes of distilled water for 15 min each. Samples were then dehydrated through 70, 90 and 100% (three times) alcohol, and twice in ethanol, for 30 min. Samples were then left in a London Resin White/GMA (7:3) resin for 96 h, this being changed every 24 h. Samples were finally embedded in 100% resin at 55 °C for 24 h. Sections were cut using an Ultracut E Microtome and counter stained using 1% uranyl acetate for 20 min and Reynold's Pb-citrate for 30 s. Sections were examined on JEOL 100CXII Transmission Electron Microscope at 80 kV.

The optical measurements were performed using a Perkin–Elmer Lambda 40 UV–vis scanning spectrophotometer in the range 400–800 nm and for a path-length of 1 cm. The correlation length of agarose gels, providing an indication of the 'mesh-size' of the networks, has been determined from the wavelength dependence of the turbidity, according to the method described elsewhere (Aymard et al., 2001). The principles of the method are here briefly recalled. The turbidity represents the sum of the scattered intensity over a range of angles. Provided interactions between the scattering of different particles can be neglected, the turbidity is essentially related to the so-called 'intra-particle dissipation factor', usually noted Q in the literature (Doty & Steiner, 1950):

$$Q = \frac{3}{8}\int_{\alpha}^{\pi} P(\theta)\cdot(1+\cos^2\theta)\cdot\sin\theta\cdot d\theta \qquad (1)$$

where α is the acceptance angle of the spectrophotometer, θ is the scattering angle and $P(\theta)$ is the form factor, whose angular-dependence is related to the shape of the scattering object. The form factor expression used here was that of Gaussian chains in entangled solutions ($c > c^*$), an expression formerly applied to gelatin sols and gels (Pezron, Herning, Djabourov, & Leblond, 1990):

$$P(q) = \frac{1}{1+q^2\cdot\xi^2} \qquad \text{for } q\xi < 1 \qquad (2)$$

with $q = 4\pi n_0 \sin(\theta/2)\lambda^{-1}$, n_0 the refractive index of the solvent and λ the wavelength of the radiation. ξ is the screening (or correlation) length, i.e. the average distance between entanglements. The correlation length can then give an indication of the mean 'pore-size' of the network, although the precise relation depends on the shape of the pores.

As shown previously, the wavelength dependence of turbidity, can be written as (Camerini-Otero & Day, 1978):

$$\frac{d\log\tau(\lambda)}{d\log\lambda} = -4 + \alpha_1 + \alpha_2 + \frac{d\log Q(\lambda)}{d\log\lambda} \qquad (3)$$

with

$$\alpha_1 = 2\cdot\frac{d\log(n_0)}{d\log(\lambda)}; \qquad \alpha_2 = 2\cdot\frac{d\log(dn/dc)}{d\log(\lambda)} \qquad (4)$$

α_1 and α_2 are usually referred to as 'dispersion coefficients' and arise from the wavelength dependence of the refractive index of the solvent (n_0) and of the refractive increment of the polymer (dn/dc), respectively. For wavelengths between 700 and 800 nm, $\alpha_1 = 0.0248$ and $\alpha_2 = 0.0922$ can be calculated for agarose in water (Podesva, Prochazka, & Medin, 1995; Thormaehlen, Straub, & Grigull, 1985). The derivative of $\log Q$ with regard to $\log\lambda$ was calculated for wavelengths between 600 and 700 nm, using the expressions given in Eqs. (1)–(4) and an acceptance angle of 3°, characteristic of the optical set-up used (Aymard et al., 2001). The wavelength exponent was then converted into an average correlation length.

Hot agarose solutions (>95 °C) were cooled in preheated spectrophotometer cuvettes and allowed to cool spontaneously to room temperature. Samples were then kept for 1 day at low temperature (5 °C) to ensure complete gelation had occurred and were then re-warmed to room temperature a few hours before the measurement. Turbidity spectra were recorded between 400 and 800 nm every 2 nm. The wavelength exponent was extracted by linear regression from a double logarithmic plot of the turbidity vs. the wavelength between 600 and 700 nm, with regression coefficients well above 0.99, and converted into a correlation length.

3. Effect of sucrose on gels of constant agarose concentration

3.1. Calorimetric measurements

Although calorimetric measurement only refers to the coil-helix transconformation of the biopolymer, it provides useful thermodynamic information on the influence of sucrose on helix formation that is necessary for the gel to exist.

Thermograms obtained for the low viscosity agarose L1 are shown in Fig. 1. The addition of sucrose produces a rather limited effect on the ordering process. The temperatures of onset and peak of the ordering shift toward higher values up to 60% sucrose and then decrease again. However, the melting is more strongly affected by sucrose addition, showing a constant increase with sucrose concentration over the concentration range investigated. The onset temperature of melting, and even the peak temperature of melting, slightly increase with sucrose content. This suggests an increased thermal stability of the helices, which will be commented on in the discussion.

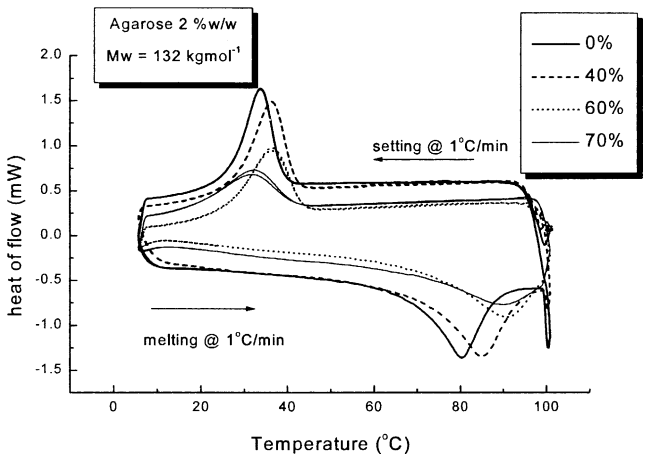

Fig. 1. Thermograms of agarose gels, for low viscosity agarose (*L*1), at different solvent composition.

Enthalpies of setting have been calculated using Eq. (5) and are reported in Table 2

$$\Delta H = \left[\int_{T_1}^{T_2} Q \cdot \mathrm{d}T \cdot \left(\frac{\mathrm{d}T}{\mathrm{d}t}\right)^{-1}\right] \frac{M_R}{mC} \tag{5}$$

where Q is the heat of flow, T the temperature, t the time, M_R the molecular weight of a residue (Normand et al., 2000), m the mass of the sample, and C the concentration in % w/w.

The enthalpy of the conformational transition for the setting of the gel is not dramatically affected by sucrose addition up to 60%, and the values extracted from these experiments are in good agreement with the ordering enthalpy of transition normally found for agar gels (Norton, Jarvis, & Foster, 1999). However, as the sucrose concentration is increased beyond this limit, the enthalpy of setting drops significantly, which either indicates that the coil to helix transition liberates less heat or that less ordering occurs when sucrose is added up to this extent.

3.2. Small deformation results

In this part, the gelation properties of agarose are discussed in sugar solution conditions and on cooling at 1 °C/min. This includes the effect of sugar on the gelation temperature, and the evolution of the elastic (G') and loss (G'') moduli with time and at constant frequency (1 Hz) and strain (0.2%).

Kinetic measurements using low and high viscosity agarose were made for different sucrose concentrations. The evolution of G' with time for both samples is shown in Fig. 1.

On a phenomenological point of view, the addition of sucrose leads to three consequences:

1. a shift in the apparent gelation temperature
2. a change in the rate of gel formation (that can be estimated from the time-course of the elastic modulus after the gel point)
3. a change in the final elastic modulus obtained at the end of the cure curve.

Furthermore, two domains of sucrose concentrations can be distinguished with regards to the three criteria mentioned: up to 60% sucrose, increasing sucrose concentration in the solvent leads to a decrease in the apparent gelation temperature, an increase in the rate of gel formation and in the elastic modulus obtained at long times. Within this range of concentrations, sucrose seems to accelerate and boost the gelation process. However, when sucrose concentration exceeds 60%, the reverse trends are observed and the gelation process seems dramatically slowed down. Similar, but not identical trends are observed for the low (Fig. 2a) and the high viscosity (Fig. 2b) samples.

The amplitude of the loss modulus reveals the viscosity of the network. The time dependence of the loss properties is shown in Fig. 3 for the same conditions. Unlike what was observed for the elastic modulus, no singularity around 60% sucrose is here shown: a monotonic increase in the loss modulus at long times is observed with increasing sucrose content in the binary solvent. This indicates that the network formed dissipates a higher fraction of the mechanical energy at high sucrose concentrations.

The measured gel temperatures, defined as the temperature of the cross-over between the elastic modulus and the loss modulus (Winter & Chambon, 1986), are reported in Fig. 4, together with the temperatures of onset and of maximal ordering extracted from the thermograms. Fig. 4 clearly illustrates previous comments on the existence of two sucrose concentrations domains: from 0 to 60% sucrose in the binary solvent, the ordering process shifts towards higher temperature, and as a result, the apparent gelation temperature increases. Above 60% sucrose, however, a dramatic decrease in all temperatures mentioned (onset of

Table 2
Enthalpy of setting of a 2% low viscosity agarose (*L*1) and its temperatures of onset and peak for setting and melting

[Sucrose] (% w/w)	$\Delta H_{setting}$ (kJ mol of residue^{-1})	Setting T_{onset} (°C)	Setting T_{peak} (°C)	Melting T_{onset} (°C)	Melting T_{peak} (°C)
0	10.73 ± 0.8	42.04	33.92	66.15	80.40
40	11.31 ± 1.1	45.56	36.50	70.50	85.10
60	10.57 ± 1.3	47.07	36.42	71.80	91.00
70	8.03 ± 0.9	41.6	31.30	69.50	90.15

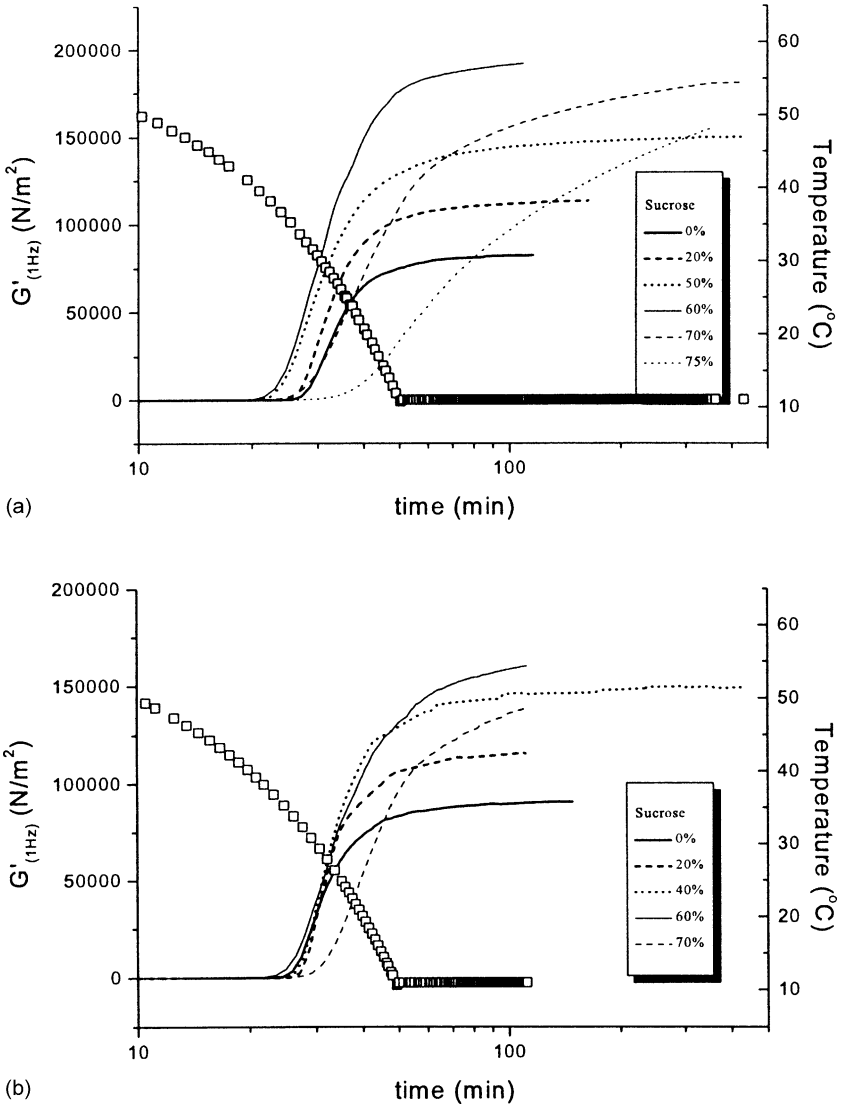

Fig. 2. Evolution of the elastic modulus with time for different conditions of sucrose concentration. (a) Agarose low viscosity (*L*1) 2%w/w; (b) Agarose high viscosity (*H*) 2% w/w.

ordering, peak temperature and gelation temperature) is seen.

It is worth noting that the gelation temperature of the low viscosity agarose is always higher than the gelation temperature of the high viscosity sample (data not shown), for all the sucrose concentrations tested.

3.3. Optical measurements

The addition of sucrose also leads to major changes in the optical aspect of the gels. At low sucrose content, agarose gels show, as usual, a marked turbidity while at high sucrose content, the turbidity virtually disappears and clear gels are obtained. Values of turbidity measured at 700 nm are shown in Table 3 for agarose gels with sucrose contents ranging from 0 to 60%. Higher sucrose contents (>60%) led to highly viscous samples, containing small air bubbles whose scattering dominates that of the gel network.

Hot agarose solutions are transparent because both the correlation length and the refractive index increment of single agarose chains are small. Upon gelation, turbidity appears due to the aggregation of helices into bundles. This provokes an increase in both the correlation length and the cross-sectional radius. In pure water, values of correlation length close to 80–90 nm have been reported for the same agarose gels at the same concentration (Aymard et al., 2001).

The decrease in the turbidity of the gels with increasing sucrose content could hence be explained by a reduction in the degree of helix aggregation, leading to finer, i.e. with smaller cross-section radii, and more numerous bundles, thus giving a smaller correlation length compared to pure water agarose gels. However, these changes could also simply reflect the changes in refractive index contrast between the agarose bundles and the mixed sucrose:water solvent. In other words, increasing sucrose content could

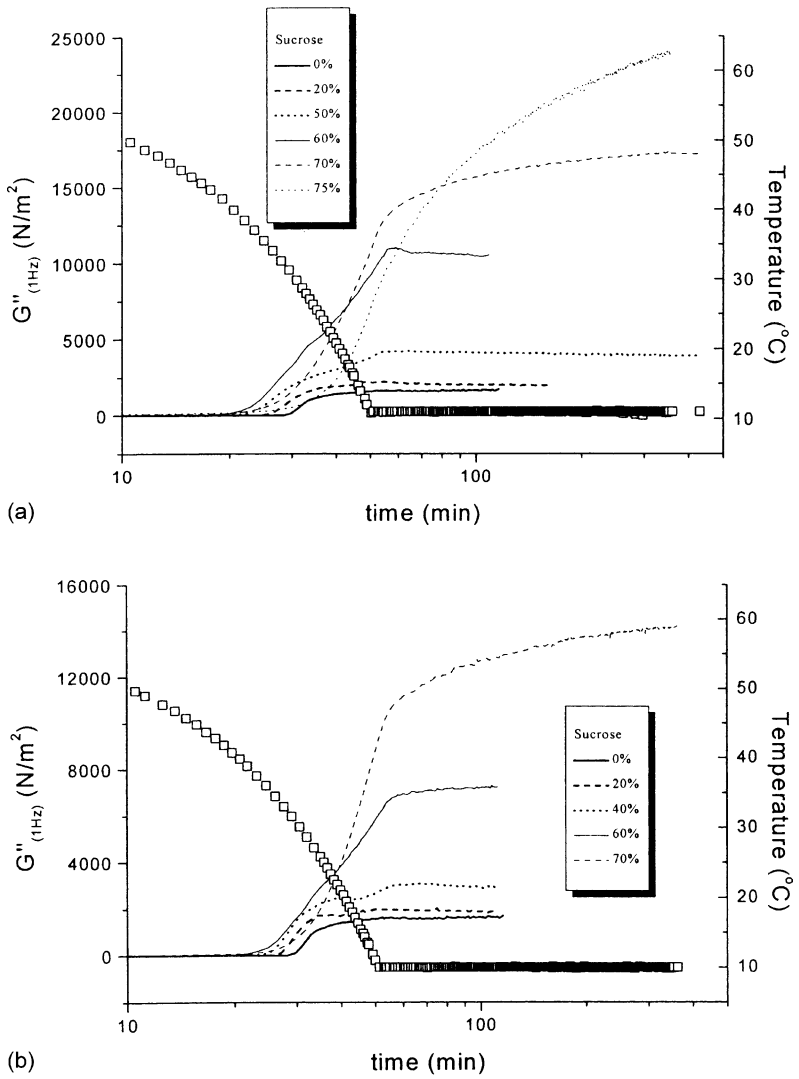

Fig. 3. Evolution of the loss modulus with time for different conditions of sucrose concentration. (a). Agarose low viscosity (*L*1) 2% w/w; (b) Agarose high viscosity (*H*) 2% w/w.

lead to a matching of the refractive indices, thus making the agarose bundles in sucrose:water mixtures 'invisible' to light, while the structure of the gels would remain unchanged. The turbidity at a given wavelength depends on the square of the refractive increment d*n*/d*c*, so that the changes in the contrast term are expected to provoke severe modifications of the turbidity value. However, the wavelength dependence of the turbidity shows less dependence to the contrast terms since the influence of the dispersion coefficients (wavelength dependence of the refractive index of the solvent and the refractive increment) are minor corrections that can be neglected. Thus, considering the wavelength dependence of the turbidity rather than the absolute value at a given wavelength (700 nm here) may allow distinction between the two possible effects (reduction in the extent of aggregation or refractive index matching). The evolution of the average correlation length with increasing sucrose contents is shown in Table 3 and Fig. 5. It is clear that the correlation length continuously

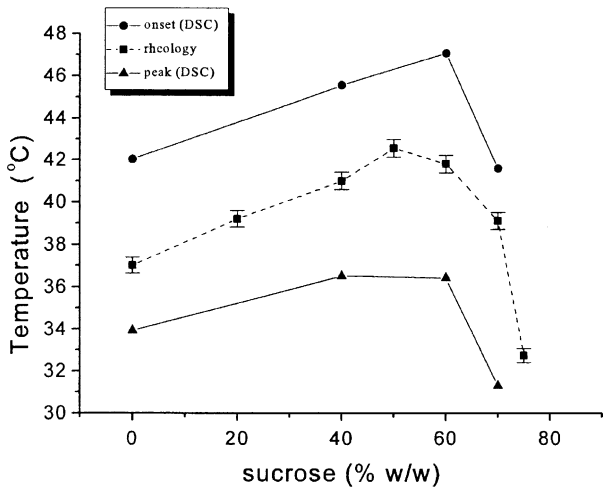

Fig. 4. Temperature of gelation for various sucrose concentration. Agarose concentration has been kept at 2% w/w for low viscosity agarose (*L*1). Temperature of onset and peak of ordering (calorimetry measurements) are also presented.

Table 3
Optical data for a 2% w/w agarose gel in different sucrose:water ratios

% Sucrose in the mixed solvent	Turbidity at 700 nm (cm^{-1})	Wavelength exponent (600–700 nm)	Correlation length ξ (nm)
0	0.406	−3.11	83
10	0.311	−3.16	75
20	0.232	−3.24	64
40	0.087	−3.69	30
60	0.029	−3.60	35

decreases with increasing sucrose content, hence indicating a progressive reduction in the degree of aggregation of helices. Agarose networks progressively evolve from a highly aggregated structure, with large pores of water surrounding thick bundles of helices to a more homogeneous fine-stranded gel structure. For 40 and 60% sucrose, the correlation length is only about 3–4 times that of the entangled solution and agarose networks behave in such conditions like fine-stranded gels such as gelatin (Pezron et al., 1990).

The fact that the reduction in turbidity is associated with the decrease in the correlation length clearly indicates that the microstructure of agarose gel changes with sucrose content and that possible refractive index matching effects are not sufficient to explain the dramatic reduction in turbidity.

According to TEM micrographs, the agarose gel network appears more homogenous (more uniform with smaller pore size) with increasing sucrose content, as shown in Fig. 6. The images there are for a low viscosity agarose (*L*1) 2% w/w network in various sucrose conditions.

Undoubtedly, the microstructure changes when sucrose is added. One can observe the porosity of a pure agarose in water network, which demonstrates the aggregation of the chains to form fibres leading to an inhomogeneous microstructure with polymer-poor and polymer-rich regions. When sucrose is added, the heterogeneity diminishes progressively and a more homogeneous structure appears. This structure is far finer, with the pore size having decreased extensively.

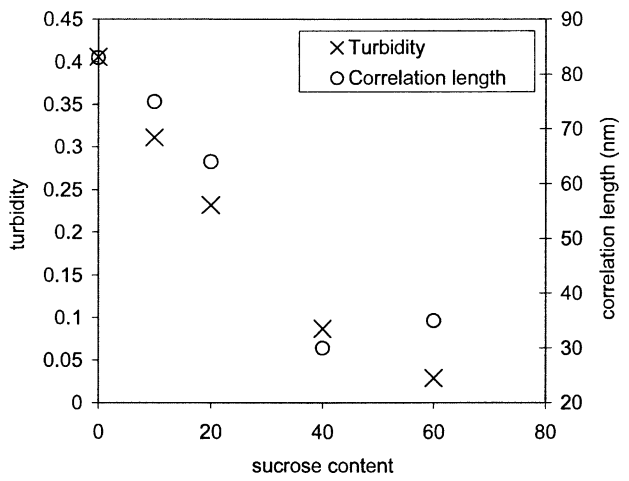

Fig. 5. Turbidity at 700 nm and correlation length measured for a 2% w/w agarose gel in different sucrose concentration.

3.4. Large deformation results and ultimate properties

Samples with different sucrose concentrations have been tested at large deformation. The same protocol as for pure agarose systems (Normand et al., 2000) has been used to determine the effect of sucrose on the stress/strain relationships for agarose-sucrose gels.

To test the influence of the sucrose concentration on agarose gel systems, constant concentrations of agarose were chosen (2, 2 and 5% w/w for high viscosity (*H*), low viscosity (*L*2) and low viscosity (*L*1) agarose respectively). The range of sucrose concentration investigated was from 0 to 80% w/w. Typical curves of compression for both agarose types are shown in Fig. 7 for the *H* and *L*1 agarose samples. The Young's modulus, the true stress and strain at failure were extracted from the figures and are commented here-after.

As far as Young's modulus is concerned, it has been previously shown in the small deformation rheological measurement section that the dependence of the shear elastic modulus is not monotonic when the sucrose concentration increases. Below 80% w/w sucrose the Young's modulus of a 2% w/w agarose gel increases, whilst above this sucrose concentration, the modulus falls. This could indicate, to a first approximation, that at 80% of sucrose, a 2% w/w agarose gel has a different network structure. The dependence of the Young's modulus as a function of the sucrose concentration, measured after 24 h of cure at 10 °C, is shown in Fig. 8.

As with the results obtained with the shear elastic modulus, the variation of the Young's modulus is not monotonic. However, the position of the maximum of the modulus varies also with agarose concentration. It thus appears that the higher the agarose concentration the lower the sucrose concentration for which the maximum modulus is reached. An empirical equation can describe the dependence of the Young's modulus on the sucrose concentration used in the binary solvent.

$$E = E_0 + a(b - [S])\left(\exp\left(\frac{[S]}{[S]_0}\right) - 1\right) \quad (6)$$

Here E is the Young's modulus, E_0 is the Young's modulus when water is used as a solvent, a and b are model's parameters and $[S]_0$ is a critical sucrose concentration. The values of these parameters are summarised in Table 4.

Regarding true stress and true strain at failure, Fig. 7 shows that they both increase with sucrose concentration at constant agarose concentration.

Fig. 9 shows the change in the true stain at failure when sucrose concentration increases for the three different

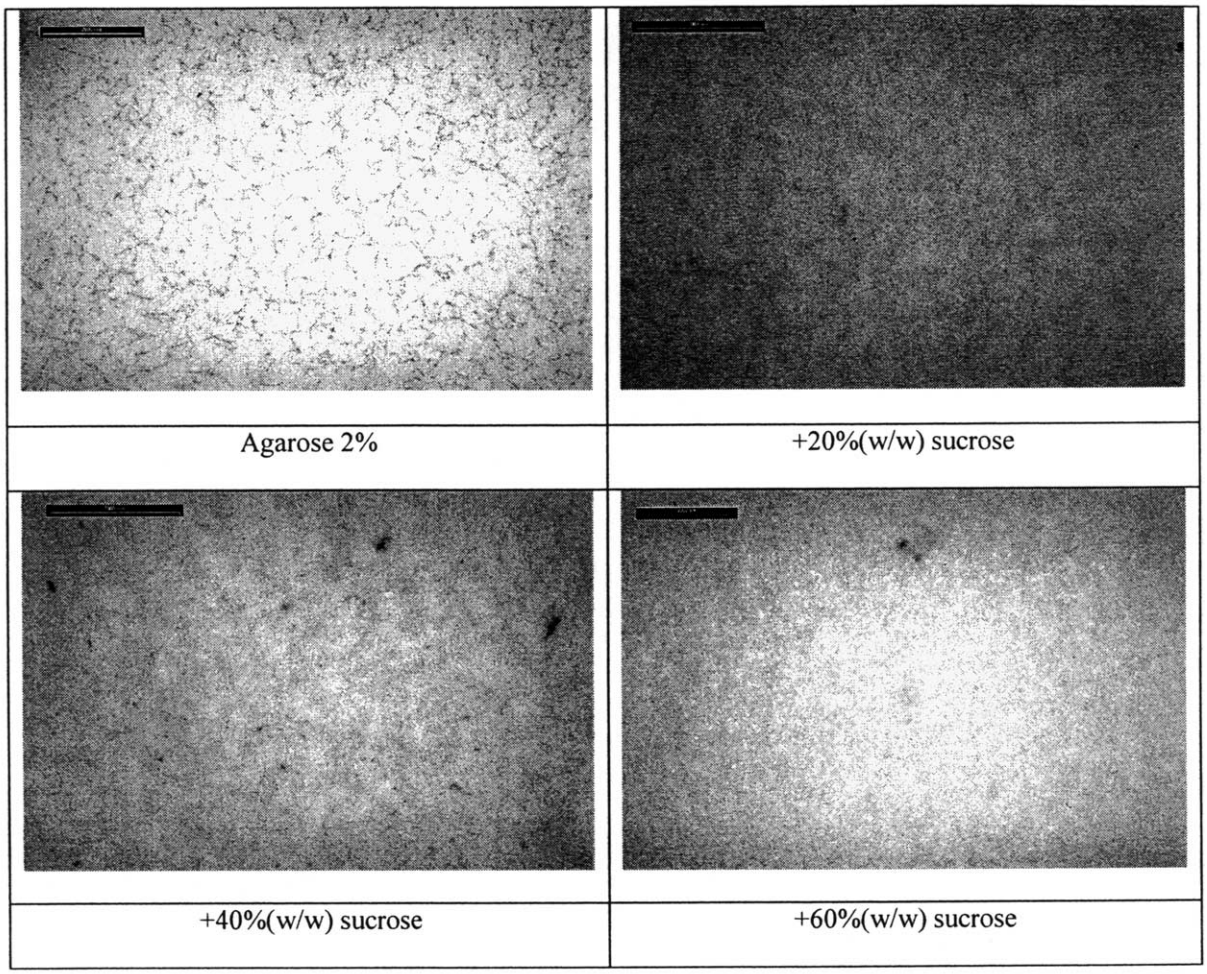

Fig. 6. Dependence of the microstructure of an agarose 2% w/w low viscosity (*L*1) on the sucrose concentration. Magnification × 20,000, scale bars represent 2000 nm for all images.

molecular weights of agarose. The effect of the sucrose on the strain at failure is very important: increasing the sucrose concentration leads to a transition from brittle gels (about 40% strain at rupture) to highly deformable (about 200%). This behaviour is rather unusual for polysaccharide gels. As the sucrose concentration increases, the strain at failure increases as well, and in an exponential manner. This dependence has been modelled with an empirical equation as a function of the sucrose concentration ([*S*]), using the measured data, i.e.

$$\varepsilon_f = \varepsilon_{f_0} + c\left(\exp\left(\frac{[S]}{[S]_0}\right) - 1\right) \quad (7)$$

Here ε_{f0} represents the strain at failure in the no-sucrose condition and for the agarose type considered, $[S]_0$ represents a critical sucrose concentration, adjusted to 20 ± 1%, and c is a front factor. The values of $[S]_0$ and c are shown in Table 4.

Similarly, the dependence of the stress at failure on sucrose concentration, for a constant agarose concentration, is plotted in Fig. 10. The stress at failure increases as a function of the sucrose concentration, and like the strain at failure (see Fig. 9), in an exponential manner. As for the dependence of the strain at failure on sucrose concentration, a similar empirical equation can be used to describe the evolution of the stress at failure.

$$\sigma_f = \sigma_0 + d\left(\exp\left(\frac{[S]}{[S]_0}\right) - 1\right) \quad (8)$$

The critical sucrose concentration $[S]_0$ used to fit the stress at failure data, is the same as that appearing in Eq. (6) and (7) ($[S]_0 = 20 \pm 1\%$ w/w). The front parameter d is reported in Table 4.

As shown in Table 4, all the parameters used to fit the data for agarose types *L*2 and *H* have similar values. This may be due to the similar agarose concentrations used in both cases, which would overcome the differences in molar mass. L1 gels, measured for a higher concentration (5% instead of 2%) give different fit parameters, which suggest that they depend on agarose concentration. When the agarose concentration is higher, the dependence of the sucrose appears different. Looking at the properties of the sucrose solutions, 22% w/w represents the limiting concentration at which sucrose-sucrose interactions appear

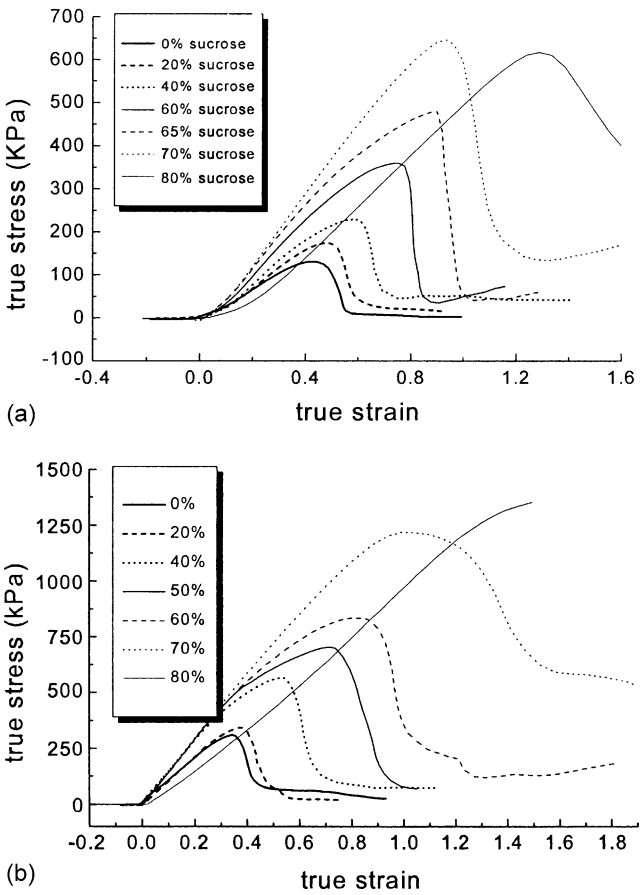

Fig. 7. Influence of sucrose concentration on the large deformation and fracture properties for constant agarose concentration systems. (a) 2% w/w high viscosity agarose (*H*) and (b) 5% w/w low viscosity agarose (*L*1).

(Mathlouthi, Cholli, & Koenig, 1986). However, the fact that this value, and the inflexion point on the dependence of the strain a failure as a function of the sucrose concentration are close, is difficult to explain. As a conclusion, it is encouraging to be able to fit the three sets of data with similar empirical relationships and with parameters of the same order of magnitude.

Although any detailed discussion of the values of these parameter is highly speculative, some comments will be given in the discussion.

In order to clarify this effect, the same experiment must be conducted for a different agarose concentration, which is reported in Section 6.1.

Table 4
Parameters of the fit used in Eqs. (6)–(8)

Type of gel	$[S]_0$	*a*	*b*	*c*	*d*
*L*1 5% w/w	21	2.2	79	3×10^{-2}	35
*L*2 2% w/w	19	0.4	95	1.7×10^{-2}	14
H 2% w/w	20	0.4	95	2.0×10^{-2}	14

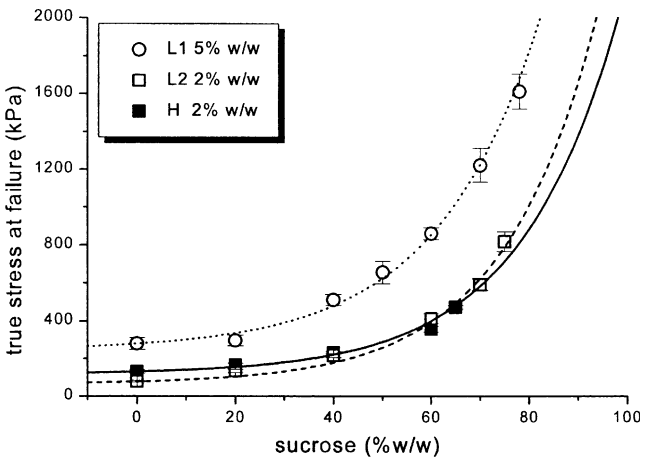

Fig. 9. Dependence of the failure strain on the sucrose concentration for the three different types of agarose. Lines are best empirical fits to the data using Eq. (7).

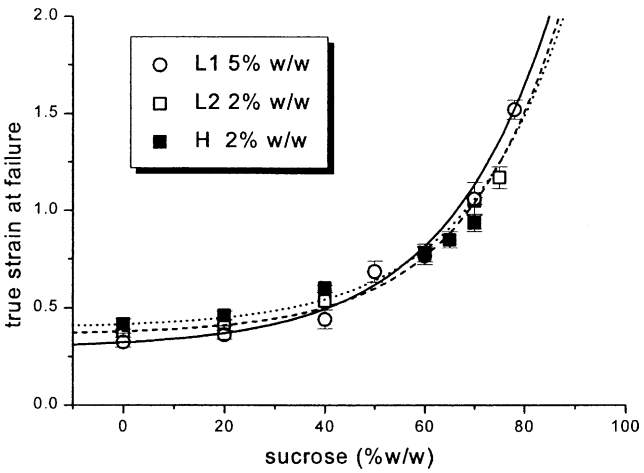

Fig. 8. Dependence of the Young's modulus on the sucrose concentration for three different type of agarose cured 24 h at 10 °C. Lines are best empirical fits to the data using Eq. (6).

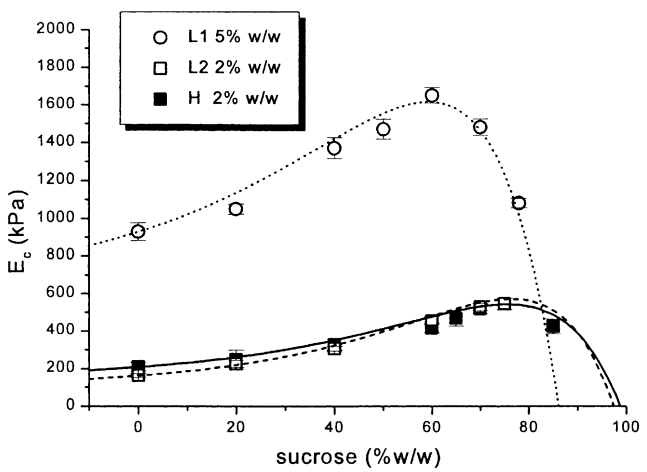

Fig. 10. Dependence of the failure stress on the sucrose concentration for three different type of agarose. Lines are best empirical fits to the using Eq. (8).

4. Effect of agarose concentration on gels at constant sucrose content

The present study of the effect of agarose concentration on gels at constant sucrose content has focused on the large deformation mechanical properties. To test the influence of the agarose concentration on low water content gel systems, a constant 60% w/w level of sucrose was chosen. Typical compression curves for the H and the L agarose samples are shown in Fig. 11a and b, respectively).

True stress and true strain at failure and Young's modulus, all increase with agarose concentration, at constant 60% w/w sucrose concentration. All of these quantities have been compared to those of pure agarose and are plotted in Figs. 12 (true strain at failure), 13 (true stress at failure) and 14 (Young's modulus) as a function of the agarose concentration.

As it has been demonstrated previously (Normand et al., 2000), the true strain at failure for pure agarose gels does not depend on agarose concentration. When 60% w/w sucrose solution is used as a solvent, the true strain increases with agarose concentration. By increasing agarose concentration at a constant sucrose concentration, it is possible to reach significantly high fracture strains.

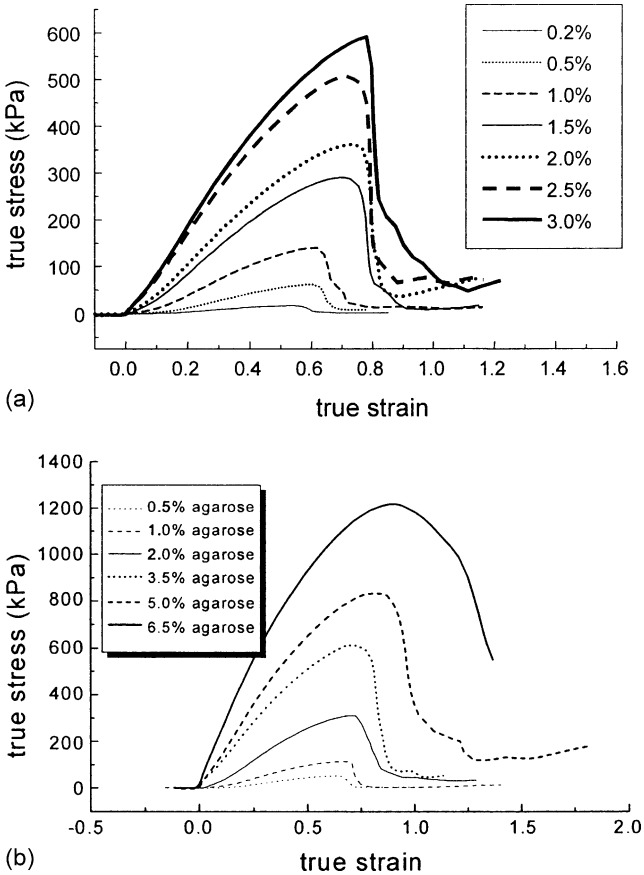

Fig. 11. Influence of agarose concentration on large deformation and fracture properties for agarose/60% w/w sucrose concentration systems. (a) High viscosity agarose (*H*) and (b) low viscosity agarose (*L*1).

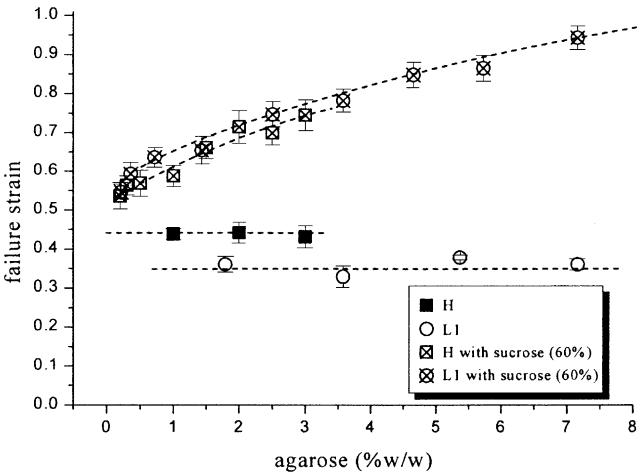

Fig. 12. Dependence of the strain at failure on agarose concentration for agarose/60% w/w sucrose concentration system. Comparison with pure agarose data from Normand et al. (2000).

In the same way, the true failure stress of an 'agarose/constant sucrose' system has been compared to the pure agarose systems. Fig. 13 shows the data concerning the two different agarose species. The stress at failure increases with increasing agarose concentration for all the different systems investigated whatever the solvent composition. Furthermore, power laws can be deduced from the data, there being a constant exponent (1.3 ± 0.02). Having the same exponent for the power laws suggests a similarity in structure as the agarose concentration increases. However, the difference seen for agarose in water, as a function of the average molecular weight, decreases when sucrose is added. This also indicates that the fracture behaviour of the gel, in presence of sucrose, is less dependent on the average molecular weight of the agarose. This has also been seen in Fig. 12 for the failure strain.

The same exercise was attempted for the concentration dependence of the Young's modulus. The latter was calculated at the very beginning of the stress–strain curve,

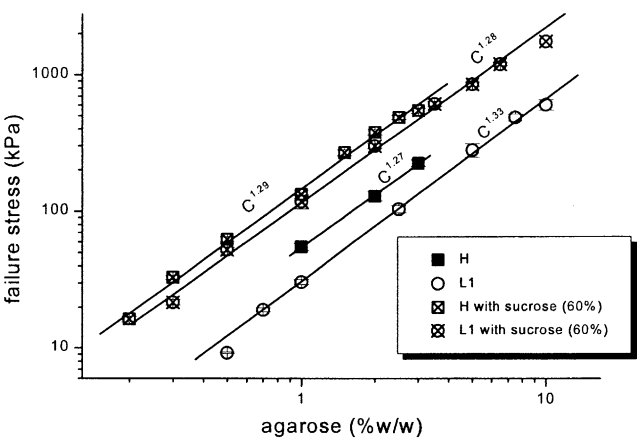

Fig. 13. Dependence of the stress at failure on agarose concentration for agarose/60% w/w sucrose concentration system. Comparison with pure agarose data.

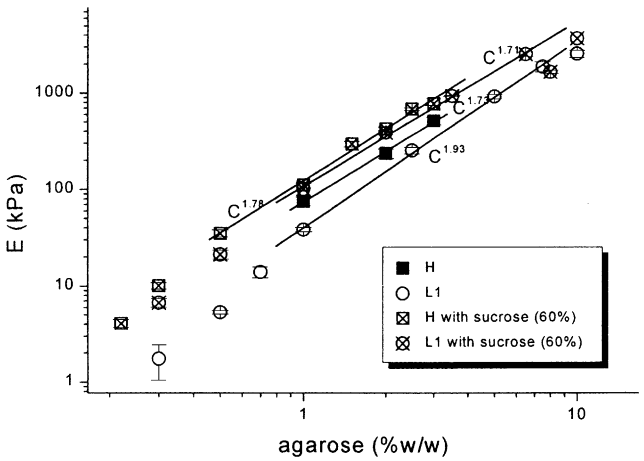

Fig. 14. Dependence of the Young's modulus after 24 h of cure at 5 °C and measured at 10 °C, on agarose concentration for agarose/60% w/w sucrose concentration system. Comparison with pure agarose data.

where the stress–strain profile was linear ($\varepsilon < 5\%$). The data are reported in Fig. 14.

In previous studies (Amici, Clark, Normand, & Johnson, 2000; Mc Evoy, Ross-Murphy, & Clark, 1985; Normand et al., 2000), the concentration dependence of the shear elastic modulus has usually been fitted with a power law. The exponents found by past authors lay between 2.0 and 2.5 for agarose in the range of concentration investigated. In the present study, the fitting by such a power-law for the concentration dependence of the Young's modulus (which can be related to the shear modulus if the Poisson's ratio is constant) yields exponents that are consistent with the values previously found. In fact, the range of concentration that has been investigated here is broader than in any other previous studies and significant deviations from the power-law are observed (at low and high concentrations). However, the global concentration dependence of the Young modulus is fairly similar, irrespective of differences in sucrose content or differences in molar mass. It can also be noted that the sucrose addition reduces the discrepancy between the two average molecular weights investigated, as it is the case for the stress at failure.

5. Discussion and conclusions

The results shown in this study consistently indicate that addition of sucrose markedly affect agarose gelation in a non-monotonic manner, which suggests that several processes may interplay. The different observations are briefly summarised with regards to sucrose content.

The addition of sucrose up to 60% has little effect on the coil-to-helix transconformation. Small deformation rheology measurements carried out in this range show that increasing sucrose concentration is associated with a decrease in the apparent gelation temperature, an increase in the rate of gel formation as well as in the elastic modulus obtained at long times. Addition of sucrose up to 60% hence seems to accelerate the gelation process and increase the elastic modulus of the networks. Large deformation measurements show an increase in Young's modulus, as well as a marked increase in the stress and strain at failure. With increasing sucrose contents, agarose networks evolve from a brittle (strain at failure about 40%) to a highly deformable (200%) behaviour. It should be noted that the loss modulus increases monotonically when sucrose is progressively incorporated in the system. This result show that more energy can be dissipated in the network, and indicates that the gel is more deformable and less keen to recover its original form when submitted to large deformations. The scattering properties of the gels are also modified by addition of sucrose. The turbidity and the wavelength exponent decrease. The latter indicates a reduction in the correlation length of the system, thus a more homogeneous network. This suggests an evolution from a coarse structure, characterised by a large correlation length and the presence of thick bundles of aggregates of helices at low sucrose concentrations, to a fine-stranded gel structure with a much smaller correlation length. These results have been confirmed by TEM observations.

Just above 60% sucrose, however, dramatic changes are observed. At 70% sucrose, for instance, the change in enthalpy upon setting decreases by 20% compared to 60% sucrose, suggesting that less helices form or that they are less energetic. Hence, gelation is delayed (lower apparent gelation temperature) and the growth rate of the gels is lower, yielding lower moduli values at long curing times. A similar downwards trend is observed on the Young's modulus obtained by large deformations measurements, while the failure strain and stress continue to increase with sucrose content.

The effect of sucrose on agarose gelation (and possibly on other helix-forming biopolymers) could be explained by several contributions. First, increasing the sucrose content in the binary solvent provokes an increase in viscosity. This increase is far from linear and becomes dramatic above about 55–60% as shown in Fig. 15. At 35 °C, temperature close to the gelation temperatures, the ratio of the viscosity at a given sucrose content to the viscosity of pure water increases by a factor 34 between 0 and 60% sucrose, while raising the sucrose content from 60% to only 64% doubles this viscosity ratio. Fig. 15, that shows the superimposition of viscosity of the sucrose:water mixture and the temperatures of gelation and melting, clearly indicates that the dramatic increase in viscosity of the water:sucrose mixture phenomenologically coincide with the slowing down of the gelation process. This could be explained by a reduction in polymer chain mobility. In terms of a nucleation and growth process, such a reduction in mobility would favour helix nucleation and inhibit the growth process, which would here be the packing of the helices into thick bundles. Such an effect is consistent with experimental observations showing that up to 60% sucrose,

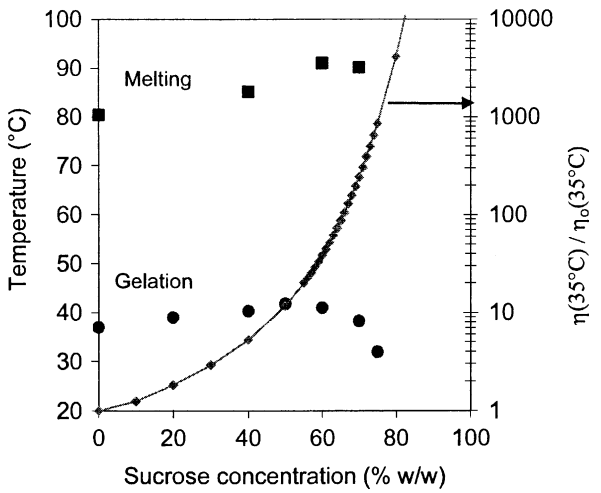

Fig. 15. Temperature of gelation and melting for agarose 2% w/w as function of the sucrose concentration, superimposed to the relative viscosity of the sucrose solution at 35 °C.

increasing sucrose content leads to a reduction of the aggregation degree (smaller correlation length, associated with smaller cross-section radii). This could also possibly explain why ordering, and hence gelation, occur at higher temperature in this sucrose concentration range. This is also consistent with previous observations, which report that the presence of sucrose reduces the quantity of helical aggregates, increases the number of cross-links, and decreases the length of the helices (Nishinari et al., 1992, 1995; Watase et al, 1990). Thus, the increase in the stress and strain at failure may be interpreted in term of melting and reforming of junction zones, on the basis of the interpretations made for gelatin gels (Groot, Bot, & Agterof, 1996a,b).

Above 60% sucrose, the dramatic increase in viscosity could have an inhibiting effect on network formation. At the local level, the degree of the coil-to-helix conversion decreases, and is associated with a slowing down of the gelation process. Reaching very high levels of viscosity could limit the growth process to an extent that would affect the global growth rate of the gel. In such concentrated conditions, the occurrence of the glass transition should be considered. Indeed, the increase in viscosity depends according to the WLF approach on the distance from the glass transition temperature (Ferry, 1980). Values of glass transition temperatures for sucrose:water with 0, 20, 40, 60, 70, 80% sucrose are respectively of −135, −125, −111, −87, −70 and −44 °C. These values are expected to be shifted upwards in presence of agarose, since the presence of polymers have been suggested to increase the glass transition temperature. Hence, it is likely that, at high sucrose content, the glass transition Tg becomes sufficiently close to the gelation temperatures to inhibit the growth process, thus slowing down the formation of the gel network. This would also explain why increasing the sucrose content leads to a gradual increase in the viscous behaviour of the gels (G'').

Secondly, solvent quality should also be considered, although this notion is complex in the case of entangled polymer solutions in a binary solvent. In the case of agarose gelation in water, the packing of agarose helices into thick bundles has often been interpreted as reflecting the poor solvent quality of water for the helices. The addition of moderate concentrations of cosolutes of similar chemical origin (i.e. the use of a sugar:water mixture as a solvent for a polysaccharide) could possibly increase the affinity of the helix for the binary solvent and limit the drive towards helix aggregation. This is however purely speculative and would be rather difficult to approach from an experimental point of view. Light scattering measurements could be used to evaluate the solvent quality of sucrose:water mixtures for agarose chains in the sol state, but upon ordering, the fast evolution of the system would prevent any measurement. However, at very high cosolutes content, a competition for water is expected. Agarose helices are known to be stabilised by H-bonds with water molecules, some occupying the inner part of the helix (Foord & Atkins, 1989). The reduction of available water molecules could possibly decrease their contribution to the stabilisation of the helices, and contribute to the observed decrease in the coil-to-helix conversion degree.

In conclusion, and as reported in previous studies, the influence of the addition of cosolutes on the gelation of helix-forming biopolymer is complex to describe. The experimental observations are in favour of a gradual evolution from a turbid, coarse and brittle network to a clear, fine-stranded and highly deformable network, similar to gelatin gels in water. This could arise from favoured nucleation with regards to growth. Whether this is driven by the increase in viscosity or by changes in the solvent quality remains to be determined. Furthermore, it is not obvious to picture why favouring nucleation rather than growth leads to stronger and more deformable gels. The modifications occurring at the scale of the network are not necessary linked to local modifications (i.e. the thickness of the fibres), but also depend on the type of association between these fibres as well as on their internal flexibility. It is thus likely that the deformable character of the sucrose gels arise from an increased internal flexibility of the fibres, which could be a consequence of the observed decrease in cross-sectional thickness.

Acknowledgements

The authors would like to thank Dr N.B. Johnson for his expertise in microscopy and Prof. I.T. Norton and A.H. Clark for fruitful discussions.

References

Amici, E., Clark, A. H., Normand, V., & Johnson, N. B. (2000). Interpenetrating network formation in gellan/agarose gel composite. *Biomacromolecules*, *1*(4), 721–729.

Aymard, P., Martin, D. R., Plucknett, K. P., Foster, T. J., Clark, A. H., & Norton, I. T. (2001). Influence of thermal history on the structural and the mechanical properties of agarose gels. *Biopolymers*, *59*(3), 131–144.

Camerini-Otero, R. D., & Day, L. A. (1978). The wavelength dependence of the turbidity of solutions of macromolecules. *Biopolymers*, *17*(9), 2241–2249.

Clark, A. H., & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymer gels. *Advance in Polymer Science*, *83*, 57–192.

Doty, P., & Steiner, R. F. (1950). Light scattering and spectrophotometry of colloidal solutions. *Journal of Chemical Physics*, *18*(9), 1211–1220.

Ferry, J. D. (1980). *Viscoelastic properties of polymers* (3rd ed). New York: Wiley.

Foord, S. A., & Atkins, E. D. T. (1989). New X-ray diffraction results from agarose: extended single helix structures and implications for gelation mechanism. *Biopolymers*, *28*(8), 1345–1365.

Groot, R. D., Bot, A., & Agterof, W. G. M. (1996a). Molecular theory of strain hardening of a polymer gel: application to gelatin. *Journal of Chemical Physics*, *104*(22), 9202–9219.

Groot, R. D., Bot, A., & Agterof, W. G. M. (1996b). Molecular theory of the yield behavior of a polymer gel: application to gelatin. *Journal of Chemical Physics*, *104*(22), 9220–9233.

Mathlouthi, M., Cholli, A. L., & Koenig, J. L. (1986). Spectroscopic study of the structure of sucrose in the amorphous state and in aqueous solution. *Carbohydrate Research*, *147*, 1–9.

Marvin, R. S. (1954). The dynamic mechanical properties of polyisobutylene. In V. G. W. Harrison (Ed.), (pp. 156–163). *Proceedings of the Second International Congress Rheology*, London: Butterworths.

Mc Evoy, H., Ross-Murphy, S. B., & Clark, A. H. (1985). Large deformation and ultimate properties of biopolymer gels, 1. Single biopolymer component systems. *Polymer*, *26*(10), 1483–1492.

Nishinari, K., Watase, M., Kohyama, K., Nishinari, N., Oakenfull, D., Koide, S., Ogino, K., Williams, P. A., & Phillips, G. O. (1992). The effect of sucrose on the thermo-reversible gel–sol transition in agarose and gelatin. *Polymer Journal*, *24*(9), 871–877.

Nishinari, K., Watase, M., Miyoshi, E., Takaya, T., & Oakenfull, D. (1995). Effects of sugar on the gel–sol transition of agarose and κ-carrageenan. *Food Technology Chicago*, *49*(10), 90–96.

Normand, V., Lootens, D., Amici, E., Plucknett, K. P., & Aymard, P. (2000). New insight into agarose gels mechanical behaviour. *Biomacromolecules*, *1*(4), 721–729.

Normand, V., & Ravey, J. C. (1997). Dynamic study of gelatin gels by creep measurements. *Rheologica Acta*, *36*(6), 610–617.

Norton, I. T., Jarvis, D. A., & Foster, T. J. (1999). A molecular model for the formation and properties of fluid gels. *International Journal of Biological Macromolecules*, *26*(4), 255–261.

Pezron, I., Herning, T., Djabourov, M., & Leblond, J. (1990). Scattering from a biopolymer solution in the sol and gel states: the gelatin example. In W. Burchard, & S. B. Ross-Murphy (Eds.), *Physical networks, polymers and gels* (pp. 231–252). Barking, UK: Elsevier.

Podesva, J., Prochazka, O., & Medin, A. (1995). Studies on agaroses: 1. specific refractive index increments in dimethyl sulfoxide and in water at various wavelengths and temperatures. *Polymer*, *36*(26), 4967–4970.

Sworn, G., & Kasapis, S. (1998). Effect of conformation and molecular weight of co-solute on the mechanical properties of gellan gum gels. *Food Hydrocolloids*, *12*(3), 283–290.

Thormaehlen, I., Straub, J., & Grigull, U. (1985). Refractive index of water and its dependence on wavelength, temperature, and density. *Journal of Physical Chemistry Ref. Data*, *14*(4), 933–945.

Tsoga, A., Kasapis, S., & Richardson, R. K. (1999). The rubber-to-glass transition in high sugar agarose systems. *Biopolymers*, *49*(4), 267–275.

Watase, M., Nishinari, K., Williams, P. A., & Phillips, G. O. (1990). Agarose gels: effect of sucrose, glucose, urea, and guanadine hydrochloride on the rheological and thermal properties. *Journal of Agricultural and Food Chemistry*, *38*(5), 1181–1187.

Winter, H. H., & Chambon, F. (1986). Analysis of linear viscoelasticity of a cross-linked polymer at the gel point. *Journal of Rheology*, *30*(2), 367–382.