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# THERMAL PROPERTIES OF POLYSACCHARIDES AT LOW MOISTURE
## II. Molecular order and control of dissolution temperature in agar*

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## Abstract

Differential scanning calorimetry (DSC) has been used to probe ordered structures and glassing behaviour for a range of agars containing <25% w/w water. Most commercial agars are supplied in an ordered (double-helical) state, show an endothermic helix-to-coil transition above 100°C at low-moisture, and require 90–100°C for solubilisation in excess water. Agars dried from the coil (single-chain) state show no corresponding endothermic transitions and only require a minimum of 45°C for aqueous dissolution. Evidence from helix-to-coil transition enthalpies, equilibrium water content as a function of relative humidity, and solid-state $^{13}C$ NMR spectroscopy suggests that water molecules are associated enthalpically with double-helical agar. Single-chain agar is apparently not obtained in a glassy state by direct drying from solution, but in common with double-helical forms, exhibits rubber/glass transition behaviour following heating (in a DSC pan) to 180°C.

**Keywords:** agar, dissolution temperature, glass transition, polysaccharide–water interactions

## Introduction

Agar is the name given to a broad range of polysaccharides extracted from a family of red seaweeds (*Rhodophyceae*), of which the bulk of commercial material is derived from *Gracilaria* and *Gelidium* species. The property for which it is valued is gel formation, with both food and non-food applications [1, 2]. Food uses in East Asia are widespread, with many specialty niches depending on gel texture [1]. Interest in the wider use of agar in foods is predicted [2], based on its high functionality, history of traditional use, and the development of reliable algal mariculture. One potential limitation to the further utilisation of agar is that most commercial materials require dissolution at 90–100°C prior

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to gel formation. This adds significantly to the cost and complexity of agar use in large-scale operations.

The chemical structure of agars is based on a repeating disaccharide (trivial name: agarobiose), with polymers containing alternating 1,3-linked β-*D*-galactopyranose and 1,4-linked 3,6-anhydro-α-*L*-galactopyranose residues and related derivatives (Fig. 1a). The regular repeating structure makes it possible for the polysaccharide chains to adopt ordered conformations, over extended regions of the chain, under appropriate environmental conditions. Drying from aqueous solutions near 100°C, followed by stretching of the resultant film at lower temperatures, gave a material with an X-ray diffraction pattern interpreted in terms of an extended single-helical structure [3]. Small-angle X-ray scattering showed [4] that solutions of agar are composed of single chains, and the segmental flexibility demonstrated by sharp signals in NMR spectra [5] are consistent with a single-chain random-coil state in solution at high temperatures. Cooling of hot aqueous agar solutions results in gelation. X-ray diffraction of fibres produced *via* low-temperature processing has been interpreted in terms of a double-helical model [6], although the number of X-ray reflections obtained was limited. Further support for the double-helix model

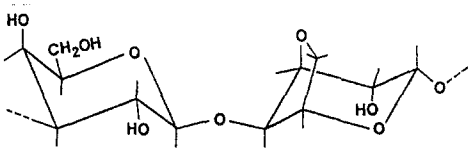


**Fig. 1** (a) Idealised disaccharide repeat structure of agar. Substituents such as 4,6 pyruvate ketal, 6 sulphate and 2 or 6 O-methyl groups on the left-hand galactose, and 2 O-methyl groups on the right-hand anhydrogalactose residue, can be present in varying, but usually low, amounts

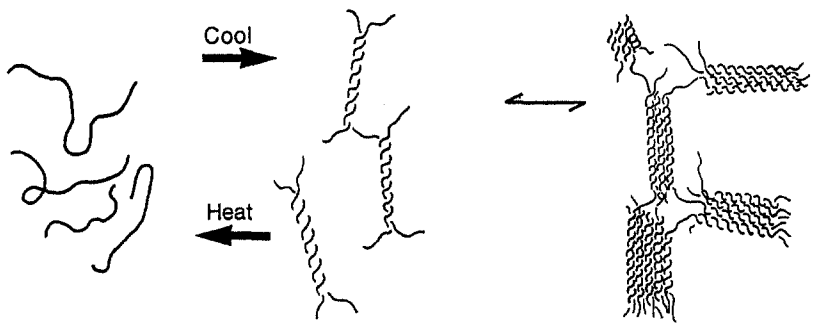


**Fig. 1** (b) Schematic illustration of agar's conformational states, showing the conversion of single chains to double helices and aggregates on cooling, and the reverse reaction on heating, which occurs at higher temperatures due to the stabilisation of helices within aggregates

comes from vacuum ultraviolet circular dichroism [7] and optical rotation [8] analyses. A number of methods can be used to follow the transition from the high-temperature, single-chain (coil) state to the low-temperature, ordered (hereafter presumed double-helix) state, including optical rotation [9], circular dichroism [10], small-angle X-ray scattering [4], and differential scanning calorimetry [11] (DSC). Gel setting is essentially coincident with double-helix formation [5, 11]. By all probes [4, 9–11], substantial hysteresis is observed, with the helix → coil or gel-melting transition being at higher temperatures (60–95°C) and somewhat broader than the coil → helix or gel-setting transition (40–30°C). This has been interpreted [10] in terms of substantial helix aggregation, with the 'melting' transition being the true equilibrium process of helix-aggregate conversion to the coil state, whereas lower temperatures are required in order to stabilise (nucleation of) the less-stable, isolated helices that subsequently aggregate. These states are illustrated schematically in Fig. 1b. Evidence from small-angle X-ray scattering [4] and electron microscopy [12, 13] supports the presence of significant aggregation in the gel state, with a range of final aggregate sizes. It is quite possible that the breadth of the melting transition is due to the heterogeneity of aggregate sizes, with larger bundles stabilised to higher temperatures. Studies of conformational transitions, in agars have concentrated on relatively water–rich systems (up to 40% w/w agar [11]). By analogy with other conformational transitions, e.g. starch gelatinisation [14], limiting water (< 50% w/w) would be expected to result in an elevation of the helix → coil transition to higher temperatures, most conveniently monitored by DSC. This might provide a direct probe of conformational state within low-moisture (solid) forms of agars, whereas other approaches, such as solid-state $^{13}C$ NMR, show only slight differences between coil and helix states [15, 16].

The purpose of this paper is to explore the thermal properties of aqueous agars at low moisture, to evaluate the calorimetric and dissolution/gelation properties of samples isolated/dried directly from the single-chain (coil) state, and to use the ability to manipulate agar conformational state, in order to improve general understanding of water–carbohydrate interactions under low-moisture conditions. With respect to applications in food processing, the hypothesis to be tested is that 90–100°C is required for dissolution of almost all commercial agar powders, because they are processed *via* the ordered (gel) state, and that reduced dissolution temperatures should be obtained for powders of agar in a single-chain conformation. This is analogous to control of gelatin solubility temperatures *via* definition of ordered state [25].

## Experimental

A typical gel-processed commercial agar (L11) was obtained from Oxoid (Basingstoke, U.K.). Roller-dried and spray-dried agar samples were derived

from Namibian *Gracilaria* spp [5] and supplied by Quest International (Cork, Ireland). Chemical compositions for each agar have been reported previously [5]. Water contents were manipulated in sealed chambers over saturated solutions of LiCl (11 % relative humidity (*r.h.*)), $MgCl_2$ (33% *r.h.*), NaBr (58% *r.h.*) and NaCl (75 % *r.h.*). DSC measurements were made using a Setaram microcalorimeter operating at 0.5°C $min^{-1}$ for 1% w/v agar (1–99°C, ca 0.7 g samples) and a Perkin-Elmer DSC 7 operating at 10°C $min^{-1}$ for low-moisture samples (5–180°C, ca. 20 mg samples), with data analysis performed using standard instrument software. Instruments were calibrated with indium or tripalmitin, with reference pans containing water (Setaram) or air (DSC 7). Solid-state $^{13}C$ NMR spectra were recorded on a Bruker MSL 300 instrument operating at 75.46 MHz and using cross-polarisation excitation (contact time 1 ms), dipolar decoupling, and magic-angle spinning (3–5 kHz), with an acquisition time of 0.05 s and a recycle time of 4 s. The force at fracture ('gel strength') of agar gels was determined using an Instron Universal Testing Machine in compression mode, with a cross-head speed of 10 mm $min^{-1}$, acting on cylindrical plugs (1 cm diameter, 1.2 cm depth) prepared in perspex moulds.

## Results and discussion

### *DSC as a probe of agar's conformational state under low-moisture conditions*

The DSC curves obtained on cooling and re-heating a dilute (1 % w/v) agar sample are shown in Fig. 2. Repeated heating and cooling cycles resulted in the same responses. The relatively broad transition observed on heating and the narrower transition at lower temperatures observed on cooling are similar to previous data for agar obtained by DSC [11, 17] and other techniques [4, 9, 10]. The enthalpy associated with both the exotherm on cooling and the endotherm on heating is 23±3 J $g^{-1}$ agar.

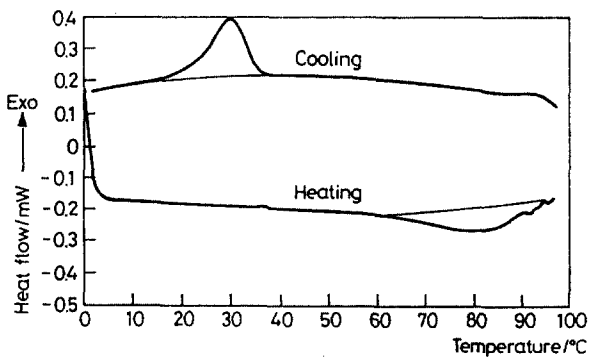


**Fig. 2** DSC traces obtained on cooling a 1% agar gel from 99 to 1°C at 0.5°C $min^{-1}$ and immediate re-heating at the same rate. Interpolated baselines used for calculation of transition enthalpies are also shown

DSC responses under low-moisture conditions, for agar produced *via* the ordered helical state (corresponding to almost all commercial agars), are exemplified in Fig. 3 for a sample containing 15.9% w/w water. On a first heating, a small endotherm in the range 55–70°C, characteristic of all low-moisture polysaccharides [18, 19], is observed. At higher temperatures, a larger endotherm is observed, with an associated enthalpy change (32 J $g^{-1}$) slightly greater than that observed at lower temperatures in more dilute conditions (Fig. 2) and assigned to the helix → coil transition. Cooling from 180°C indicates a second-order (glass) transition over the range 105–80°C, with no major exothermic event, as would have been expected (Fig. 2) for readoption of the ordered double-helical state. A subsequent heating process shows full reversibility of the apparent second-order transition, confirming its assignment to a glass transition, with no evidence for any endothermic melting process (Fig. 3). The general interpretation is, therefore, that double-helix dissociation into single-chains occurs at elevated (compared with excess-water conditions) temperatures, and that on subsequent cooling, readoption of the double helix does not occur, but a glass transition is evident at ca. 95°C.

The same general behaviour is found for different water contents (Fig. 4), with increasing water leading to decreases in the temperatures of both helix dissociation on the first heating and the glass transition observed on subsequent heating. Values for these parameters are given in Table 1. The increasing temperatures for helix dissociation with decreasing water contents follow a similar dependence to that previously characterised for starch gelatinisation [14], and suggest that, in general, polysaccharide order → disorder transitions observed below 100°C in excess water may be amenable to study in limited-water conditions at higher temperatures. The marked variation in transition enthalpy values

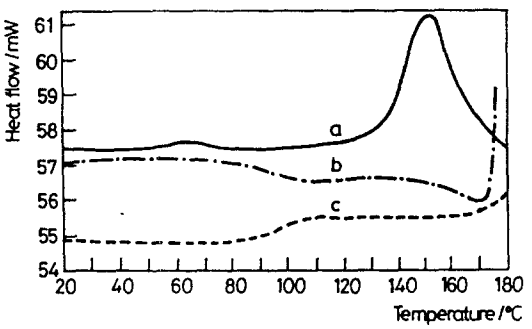


**Fig. 3** DSC traces, obtained for gel-derived, commercial agar annealed to contain 15.9% w/w water, (a) on heating from 5 to 180°C at 10°C $min^{-1}$ (continuous line), (b) on immediate cooling at the same rate (dot line), and (c) on subsequent immediate reheating at the same rate (dash line). Note that heat flow is plotted in the endothermic direction, opposite to that used in Fig. 2

**Table 1** Effect of relative humidity conditioning on equilibrium water contents (after at least 3 weeks equilibration), disordering temperature ranges and enthalpies (first heating in DSC), and glass transition temperatures (second heating in DSC, quoted to nearest 5°C) for agars in an ordered state (L11) or isolated by roller-drying or spray-drying (ex. Namibian *Gracilaria*)

| Isolation process | Relative humidity (%) conditioning | Water content/ % w/w | Glass transition temperature/°C | Disordering temperature range/°C | Disordering transition enthalpy/J $g^{-1}$ agar |
|---|---|---|---|---|---|
| *via* ordered state | 11 | 11.8 | 140 | 150–180 | 8.1 |
| | 33 | 15.9 | 95 | 135–175 | 32.2 |
| | 58 | 20.8 | 55 | 100–170 | 53.5 |
| | 75 | 24.6 | 30 | 85–165 | 57.6 |
| Roller-drying | 11 | 6.3 | 140 | – | – |
| | 33 | 10.2 | 105 | – | – |
| | 58 | 15.6 | 70 | – | – |
| | 75 | 21.6 | 35 | – | – |
| Spray-drying | 11 | 7.4 | 125 | – | – |
| | 33 | 10.7 | 85 | – | – |
| | 58 | 16.8 | 50 | – | – |
| | 75 | 22.6 | 25 | – | – |

with water content (Table 1) is interesting. We suggest that at the lower water contents (11.8 and 15.9% water), incomplete disordering occurs by 180°C, with the onset of pyrolysis exotherms in this temperature region precluding examination at higher temperatures. The similar (54 and 58 J $g^{-1}$) enthalpy values at 20.8 and 24.6% water are more than double the values obtained in excess water (23 J $g^{-1}$). We suggest that this may be due to enthalpic association of water molecules with the helix structure, causing an increase in the enthalpy per gram of agar. At higher (excess) water contents, such associations may not contribute to the enthalpy of helix melting, due to exchange with solvent water.

From the results and analysis presented above, it would be predicted that if agar is dried at temperatures higher than those required for the coil → helix transition to take place, then a single-chain state should result (Fig. 1b). To test this hypothesis, agars were isolated by either roller-drying or spray-drying directly from solution, annealed to various (low) moisture contents and analysed by DSC. Results for roller-dried samples are shown in Fig. 5. As for ordered agars (Figs 3 and 4), an endotherm is observed at 50–70°C for all samples on a first heating. In contrast to Figs 3 and 4, no major endotherm is observed above 100°C, consistent with the absence of a significant double-helical frac-

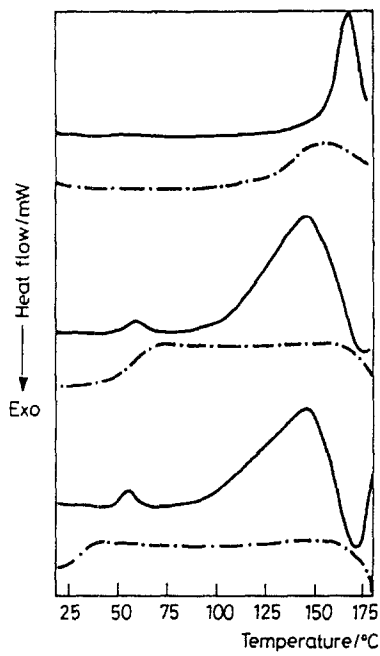


**Fig. 4** DSC traces obtained for gel-derived, commercial agar annealed to contain (a) 11.8%, (b) 20.8%, and (c) 24.6% w/w water. Upper traces (continuous lines) are for initial heating from 5 to 180°C at 10°C $min^{-1}$; lower traces (dot lines) are for a second heating (as in Fig.3(c))

tion following roller (drum)-drying. Pronounced exothermic behaviour is seen instead, which is assigned to the onset of pyrolytic degradation [20] that appears to be more significant for the single-chain, roller-dried material than for the gel-derived samples (Figs 3 and 4), thus suggesting that the double helix provides protection against pyrolysis in the DSC pan. Immediate second-heating scans reveal apparent glass transitions, with transition temperatures decreasing (as expected [20]) with increasing water content. Closely similar behaviour was found for spray-dried agars. Parameters extracted from all DSC traces are collected in Table 1.

It is interesting that equilibrium water contents, for the same *r.h.* environments, are higher for the ordered form than for either roller-dried or spray-dried materials (Table 1). We propose that this is due to the presence of water molecules tightly associated with the double helix, possibly in the interior hydrophillic cavity proposed from X-ray diffraction analysis [6]. The differences in apparent glass transition temperatures for similar water contents, among agars isolated by different means (Table 1), are difficult to interpret and may reflect varying responses to the initial heating to 180°C. Thus, moisture contents refer to materials at the start of the experiment, and take no account of any water that may vaporise on heating but not re-condense homogeneously

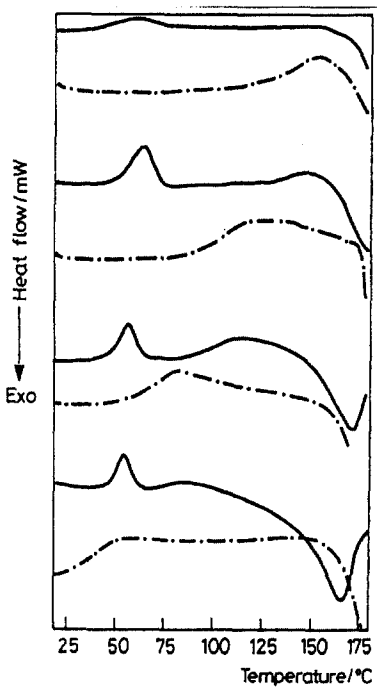


**Fig. 5** DSC traces obtained for roller-dried agar annealed to contain (a) 6.3%, (b) 10.2%, (c) 15.6%, and (d) 21.6% w/w water. Upper and lower traces are as in Fig. 4

with agar on cooling. Furthermore, pyrolytic degradation (particularly for single-chain forms) may alter molecular size sufficiently to affect glass transition temperatures.

DSC appears to be the method of choice for the direct determination of conformational state in solid particulate agars. Solid-state $^{13}C$ NMR is not very sensitive to conformational state [15, 16], c.f. Figs 7 and 8 below, and other conformational probes require processing into either solution, gel or film states. DSC may become useful in selection of ingredients on the basis of dissolution behaviour (see below).

### *Effect of conformational state on dissolution temperatures*

To obtain aqueous solutions, agars are required to be in the single-chain (random-coil) form (Fig. 1b). This can, in principle, be achieved either by heating (aggregated) double-helical forms above the temperature required for complete helix-to-coil transformation (typically 90–100°C, Fig. 2), or by dissolving single-chain forms directly. If the latter option is taken, however, care would be needed to ensure that temperatures below that required for the coil-to-helix transition are not sampled, as this would be predicted to result in adoption of (aggregated) helical forms that would require treatment at 90–100°C for dissolution. In order to test these proposals, agar powders dried from the ordered state and roller-dried or spray-dried from the solution state were mixed with 99 parts of water, at various temperatures, with prior thermal equilibration of both components. As a convenient (and practically important) index of solubilisation, mixed samples were poured into cylindrical gel moulds, allowed to cool to room temperature to form a gel, stored at 4°C for 24 h, and then tested for gel strength (force to fracture). Results are shown in Fig. 6. For temperatures up to 75°C, no gelation resulted for the ordered sample, whereas 100°C mixing resulted in significant gelation on cooling, due to agar solubility at 100°C (data not shown). For both roller-dried and spray-dried agars, similar gel strengths were observed following hydration at 45, 55, 65, 75 and 100°C, with reduced (spray-drying) or no (roller-drying) gel strength at 35°C (Fig. 6). These results are entirely consistent with single-chain solubility at temperatures higher than the coil → helix transition, since DSC (Fig. 2) shows that the transition onset is close to 35°C. The similarity of resultant gel strengths, following mixing at 45 and 100°C for single-chain materials, suggests that complete dissolution occurs at 45°C.

A previous report [21] described the use of drum (roller)-drying to produce agar that was found to be fully soluble at 55°C. Freeze-drying, by contrast, resulted in only limited solubility at 55°C, consistent with the suggestion [21] that gelation was not avoided during freezing of the agar solution in liquid nitrogen. The present work provides a mechanistic framework for understanding agar's

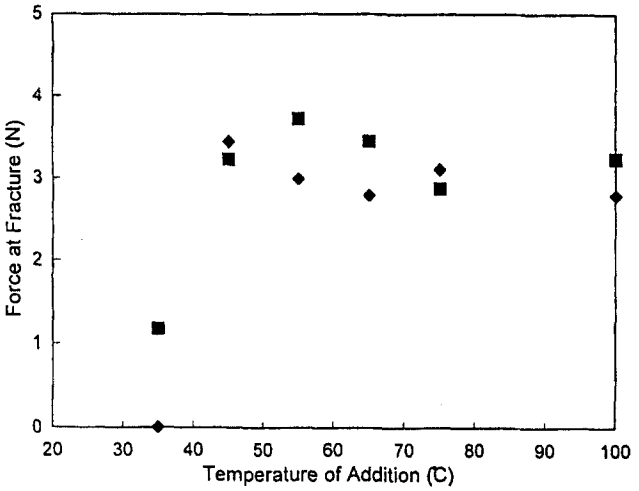


**Fig. 6** Force at fracture, measured by compression of 1% w/v roller-dried (diamonds) or spray-dried (squares) agar, following mixing of powder with water pre-heated to the temperatures shown. Resulting mixtures (35°C mixing) or solutions (45°C and above) were poured into cylindrical moulds and left to cool at room temperature for 24 h prior to testing. Fracture strengths shown are means of 6–12 replicates, with a typical standard deviation of ±5–10%. A number of commercial (gel-derived) agars showed significant fracture strengths only after 100°C mixing

dissolution temperatures and suggests that any isolation mechanism that results in single chains in the dry state would give full solubility at temperatures higher than the onset of the coil-helix transition.

## *Effect of water on agar's molecular order*

In order to further characterise low-moisture agar systems, $^{13}C$ CP/MAS (solid-state) NMR was used as a molecular-level probe. Although diffraction methods are widely applied to yield information about secondary structures of crystalline materials, commercial agars (like most polysaccharides in the solid state) show no detectable diffraction. NMR is a shorter distance-scale probe than X-ray or electron diffraction, and is sensitive to molecular order in the absence of crystalline order [22]. $^{13}C$ CP/MAS NMR spectra for ordered and roller-dried samples are shown in Figs 7 and 8, respectively, as a function of water content.

For ordered agar samples, peak chemical shifts are unchanged over the range of water contents, with all resonances becoming sharper with increasing moisture (Fig. 7). The same chemical shifts, with even sharper resonances, are observed for an agar gel [16], in agreement with other published data [15]. Under magic-angle spinning and dipolar-decoupling conditions, $^{13}C$ NMR signals

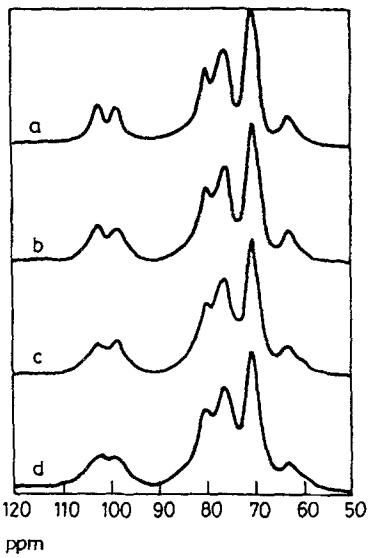


**Fig. 7** $^{13}C$ NMR spectra, of ordered (gel-derived) agar containing (a) 24.6%, (b) 20.8%, (c) 15.9%, and (d) 11.8% w/w water, obtained using cross polarisation, dipolar decoupling and magic-angle spinning

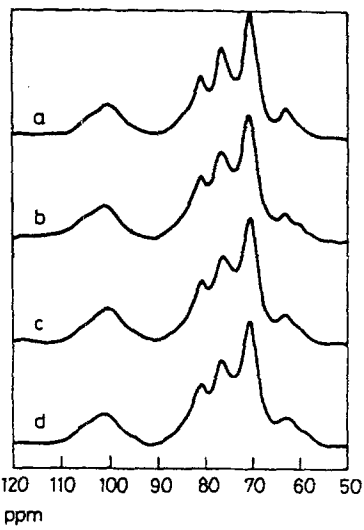


**Fig. 8** $^{13}C$ NMR spectra, of single-chain (roller-dried) agar containing (a) 21.6%, (b) 15.6%, (c) 10.2%, and (d) 6.3% w/w water, obtained as for Fig. 7

reflect the dispersion of chemical shifts experienced by specific atomic sites throughout the sample. A narrowing of signals is, therefore, interpreted in terms of a more homogeneous microenvironment at the atomic level, i.e. a better-de-

fined conformation. This is entirely consistent with the suggestion, from the data in Table 1, that increasing (low) water levels result in hydration of double-helix cavities, since this would be expected to result in a better definition of the agar helix at the level of chain conformation.

NMR spectra of roller-dried agar, as a function of water content (Fig. 8), also show sharper signals with increasing moisture, but the effect is much less marked than for double-helical agar (Fig. 7), suggesting less conformational annealing for the single-chain state in the presence of water. Chemical shifts in the 70–80 ppm range (70.5, 76.5 and 80.8 ppm) are closely similar to those for the double-helix form (70.5, 76.5 and 80.2 ppm), but there is more difference apparent in the C-1 region of the spectrum at ca. 100 ppm. This region should contain two signals of equal intensity, corresponding to the two C-1 sites in the disaccharide repeat unit (Fig. 1a), as observed for the double-helix state (at 98.5 and 102.6 ppm). For the single-chain state, one signal appears to have a maximum at 100–101 ppm, with a second broader signal noticeable at lower field (higher ppm values). The difference in C-1 chemical shifts between Figs 7 and 8 is consistent with a conformational change, since glycosidic conformation is known to be a major contributor to polysaccharide chemical shifts [23], and single-chain and double-helix glycosidic conformations differ significantly [8, 24]. The greater breadth of C-1 resonances for the single-chain state is taken as evidence for the presence of a wider range of glycosidic conformations than those found in the ordered double-helical state.

### *General features of polysaccharide-water interactions at low-moisture*

The thermal properties of agar polysaccharides in different conformational states (single-chain, double-helix) have been examined in parallel in this work. From a comparison of behaviours for 'ordered' (double-helix) and 'disordered' (single-chain) states, some general observations can be made, which serve to limit mechanistic interpretations of polysaccharide behaviour at low-moisture.

DSC and solubility properties show that roller-dried and spray-dried agar are in a single-chain ('disordered') state. However, glass transition behaviour is not seen until after these 'disordered' materials have been thermally treated and cooled. This suggests the presence of some residual structuring in the isolated single-chain state, which requires 'melting' by subsequent thermal treatment, in order that a state that is sufficiently disordered to glass is obtained. NMR (Fig. 8) shows that water causes single-chain conformations to become (slightly) better defined. We propose that hydrogen bonding between adjacent carbohydrate groups in the backbone serves to stiffen the backbone, with immobilisation occurring by this mechanism, rather than by kinetic trapping of non-ordered chains (glassing). This explanation is consistent with the finding that it is only those polysaccharides containing 3-bond, $1 \rightarrow 6$ glycosidic linkages that

show a glass-to-rubber transition on initial heating, with all other (2-bond) glycosidic linkages requiring some thermal pre-treatment [18]. It is proposed that inter-residue hydrogen bonding occurs to a significant extent for 2-bond glycosidic linkages, but not for 3-bond glycosidic linkages that consequently show classic glassing behaviour. There is no obvious DSC event corresponding to loss of the proposed hydrogen-bond structuring, demonstrating that, if this is the correct explanation, destructuring does not occur cooperatively.

The endotherm centred at 50–70°C is proposed to be a probe of the interaction of limited water with hydrophillic materials [19] (not just carbohydrates). The invariance of endotherm temperature with both water content and agar conformation fits the general behaviour observed for many polysaccharides [18, 19], and shows that the factors controlling the temperature of this calorimetric feature are independent of the degree of structural order within polysaccharides under low-moisture conditions.

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