Table 10. nPWD and CDR scores for the dominance matrix from Table 9

Individual	nPWD	CDR
D	0.40	1.00
A	0.31	1.20
E	−0.14	2.10
C	−0.31	2.50
B	−0.53	3.00
Pearson's correlation	nPWD	CDR
nPWD	1.00	−1.00
CDR	−1.00	1.00

of the eigenvalue solution[1] and then uses the power and weakness scores to derive either scale-based or interval-based indices which are unique.

For another interesting illustrative example we consider Boyd and Silk's[12] method of assigning cardinal dominance ranks (CDR) with nine data sets of dominance interactions among five captive male cockroaches, *Nauphoeta cinerea*, originally reported by Bell and Gorton[13]. Table 9 displays the matrix showing dominance interactions for the third data set among cockroaches with the the animals ranked according to their cardinal dominance rank. Table 10 gives the nPWD from Ram's protocol and CDR scores for the dominance matrix from Table 9. Table 10 also shows the remarkable negative correlation between nPWD and CDR scores and this is graphically represented in Figure 2. Again, all five individuals have unique scores. CDR, which is rarely used as a dominance measure is apparently an interval scale measure like nPWD, with the ranking score inversely related to the nPWD score.

Ramanujacharyulu's tournament metaphor gives us two new dominance indices. Both are dimensionless rating measures. The PWR is an indicator on a ratio scale like CBI and the FDI. The nPWD is an indicator on an interval scale and is similar to DS. Both PWR and nPWD seem to yield unique ranks.

From the point of view of naturalness, which is a desirable property of mathematical models, Ram's procedure is simple and direct. It treats wins and losses which are asymmetrical in a symmetric fashion by the separate row-wise and column-wise handling of the eigenvalue solution[1] and then uses the power and weakness scores to derive either scale based or interval based indices which are unique. No assumptions need be made about a linear or near-linear hierarchy or about the probabilities of winning or losing[11].

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Molecular association and gelling characteristics of curdlan

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Curdlan, a polysaccharide known to be used in the food packaging industry that gels out when exposed to higher temperatures, leading to different kinds of gel structures. The triple-helical structural formations of the

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curdlan gels are studied in the present work with varying concentrations and heating rate. The gel strength and associativeness of the molecules are analysed using rheology as a tool. The gelation behaviour of various curdlan gels that were derived from different species could be distinguished using the modified Hill equation parameters.

Keywords: Curdlan, gelling characteristics, molecular association, nitrogen-limiting conditions, rheology.

CURDLAN, a high molecular weight polysaccharide mainly consisting of β-(1→3) glucose residues, is used in various food and pharmaceutical applications. It is usually a secondary metabolite synthesized by *Agrobacterium* species, and *Alcaligenes faecalis* var. *myxogenes* under nitrogen-limiting conditions. The intra- and inter-molecular interactions of the curdlan molecules result in gels upon heating to a higher temperature. Evolution of these viscoelastic gels is by the interaction of triple-helical structures formed from the long chains of curdlan molecules.

The present study analyses the gel behaviour of curdlan synthesized by *Agrobacterium* species 31750 and its mutant strains, and compares the properties with commercial curdlan. The structural formations of these curdlan gels are estimated using rheology as a tool. Heating rate, gelation time, concentration of curdlan and associativeness of the molecules are the significant factors that influence the type of gel formed. The influencing factors are measured as material properties and are compared for various curdlan samples. Modified Hill equation is applied to analyse the molecular associations of these gels. Gel strength and association of molecules are considered to be interrelated and can be estimated using the modified Hill equation.

Curdlan is a high molecular weight polysaccharide consisting mainly of β-(1→3) glucose residues. It is water insoluble in nature but soluble in alkali solution, which was first discovered by Saito *et al.*[1]. The molecular weight of the synthesized curdlan molecules vary from 580,000 to 660,000 Da. Recent advances show the production of curdlan with *Agrobacterium* and *Alcaligenes* under nitrogen-limiting conditions[2]. Extensive studies were done to optimize the important factors like temperature, type of microorganism, pH, agitation, aeration, and nutrient and nitrogen level in the production of curdlan[3,4]. Post stationary growth phases are the crucial process steps that produce curdlan as a secondary metabolite, where nitrogen level is the key influencing factor. The optimization of curdlan production thus depends on the pre- and post-growth phases, that are again influenced by the type of stain used[5,6]. It is also reported that optimal growth for pre-stationary phase occurs at pH 7.0 and curdlan production phase at an optimum pH value of 5.5 (ref. 7).

Curdlan is essentially a neutral, linear β-(1→3) glucon that may consist of inter-chain β-(1→6) linkages (Figure 1). Various forms of curdlan are being reported in the literature – hydrated and anhydrous forms which are structurally different at room temperature[8,9]. It was found that curdlan consisting of single and triple helical molecules in the ratio 6 : 1, at room temperature, could be converted to 1 : 6 ratio in the hydrated and anhydrous forms upon heating. These triple helical coils undergo aggregations in extended junctions forming gels[10].

Food gels are formed from dispersed macromolecular particles that undergo liquid–solid transition and form complex structures having solid-like properties. Majority of the food gels originate from macromolecular self-assembly of food constituents which include polysaccharides, proteins and lipids that play a significant role in processing and storage of food. Hence, measurement of gel strength is an important indication of the structural strength of the material.

Curdlan is used as a thermal stabilizer in various food additives such as noodles, sauces, frozen foods and packaged meat. The use of curdlan as a drug gel is based on its biological activity[11]. Curdlan was proposed as the additive for 'super workable concrete', where it improves the segregation of cement and stones. It was also proposed as an organic binding agent in the ceramic industry[12,13]. Various medicinal and pharmaceutical applications of curdlan include anti-infective against bacterial, fungal and viral agents, anti-radiant, anti-coagulant and wound-repairing activities, and anti-HIV. Curdlan mixed with epichlorohydrin is used as an immobilizating agent for hydroxyl or sulphydryl groups of enzymes[5].

Curdlan produced from various microorganisms can be characterized by the respective molecular weights, reacting groups and ability to form gels with increase in temperature. It is known that at temperatures above 80°C, these materials form thermo-irreversible gels called highest gels. On the other hand, temperatures close to 55–60°C lead to the formation of lowset gel (reversible gels). The molecular organizations of both gels are different from each other[14]. In the highset gels, curdlan micelles are the key factors that are formed by crosslinking among triple or multiple stranded helicages, mostly through hydrophobic interactions, which are considered to be
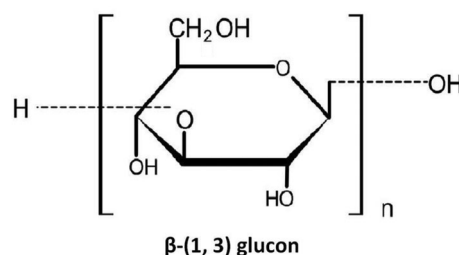

Figure 1. Structure of curdlan.

responsible for gel formation. On the other hand, in the lowset gels the micelles are made of single helicages through hydrogen bonding[10,15]. It has been argued that the strength of the gel will be influenced by the concentration of curdlan, and scanning rate of temperature[15]. Appreciable efforts have been made to compare and contrast the properties of curdlan by gelation rheology[15].

In the present study, curdlan synthesized from *Agrobacterium* species 31750 and its mutant strains, and commercial curdlan are considered. Solutions of these materials are prepared with known concentrations and analysed for gelation time and gel strength using rheology as a tool. Modified Hill equation is used to estimate the associativeness of the molecules for different varieties of curdlan.

Curdlan, a (1, 3)-beta-D-glucan obtained from *Alcaligenes faecalis* var. *myxogenes* IFO13140, was supplied by Sigma Aldrich. *Agrobacterium* sp. ATCC 31750 (formerly *Alcaligens faecalis* subsp. *mycogenes*) and mutants derived from the wild strain were used to produce curdlan[16]. The seed medium containing sucrose 20 g l^{-1}, yeast extract 5 g l^{-1} and peptone 5 g l^{-1} (pH 7.0) was inoculated with a single colony and incubated at 30°C for 17 h. Next, 10% (v/v) of seed culture was used to inoculate the fermentation medium containing sucrose 100 g l^{-1}; $(NH_4)_2PO_4$ 2.3 g l^{-1}; KH_2PO_4 1 g l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.4 g l^{-1} and 10 ml of trace element solution with the following composition (per litre of 0.01 N HCl): $FeSO_4 \cdot 7H_2O$ 5 g, $MnSO_4 \cdot H_2O$ 2 g, $CoCl_2 \cdot 6H_2O$ 1 g, $ZnCl_2$ 1 g and 0.3% (w/v) calcium carbonate. The batch fermentation was performed in a 7.5 l stirred bioreactor (Bioflo 110, New Brunswick Scientific, USA) equipped with a dissolved oxygen probe, pH controller and temperature sensor. Seed inoculum (300 ml) cultivated at 30°C for 17 h in a shake flask was transferred to the fermenter containing 2.7 l of production medium. Then 4N NaOH and 4N HCl were used to adjust the pH. The culture pH was controlled at 7.0 during growth phase and was lowered to 5.5 immediately after nitrogen limitation. The agitation speed and aeration rate were maintained at 700 rpm and 1.0 vvm respectively. After 120 h, the fermentation broth was centrifuged at 8000 *g* for 15 min to separate the supernatant (curdlan) and pellet (biomass). The supernatant was mixed rigorously with 3M HCl (1 : 1 ratio) to precipitate curdlan. The mixture containing the precipitated curdlan was centrifuged again at 8000 *g* for 15 min. The pelleted curdlan was washed thrice with distilled water to remove salts and dried to constant weight at 100°C.

All the other chemicals used were of laboratory-grade. A 6% curdlan solution of commercial, wild, M1 and M5 samples each was prepared by dissolving 6 g of the respective curdlan samples in 100 ml of 0.1 M NaOH solution. These solutions were tested for their rheological properties on Antonpaar Physica 301 stress-controlled rheometer. Cone and plate geometry with a cone angle of 1° and diameter of 25 mm was used for the analysis. To avoid solvent vaporization, a solvent trap was used.

To estimate the linear viscoelastic regime, a strain sweep was performed by keeping the frequency at 10 rad/s and by varying the strain%. To measure rheological properties, the peltier was heated to a higher temperature prior to addition of the sample to ensure that the temperature of the sample reaches the desired value. Sufficient time was allowed for the sample to become a gel. The geometry was then lowered and the experiment was performed by varying the frequency with strain% by continuously monitoring the modulus of the material.

To follow the variations of structure during gelation, the peltier of the rheometer was set to a temperature ramp rate and the modulus changes were followed at an appropriate frequency and in the linear viscoelastic strain%.

Production by mutant strain yielded 0.88 g curdlan g sucrose^{-1} (66 g l^{-1}) in 120 h, whereas the wild strain produced 41 g/l in 120 h with a yield of 0.62 in a stirred bioreactor. To determine the linear viscoelastic regime, a strain sweep was performed at a constant frequency of 10 rad/s on all the samples at 80°C, 85°C and 90°C and keeping them at the respective temperatures for a period of 30 min. Figure 2 *a–c* shows the strain sweep at 80°C, 85°C and 90°C respectively. Figure 2 *a* shows that the modulus of commercial, wild, M1 and M5 samples is consistent up to a strain% of 1, after which they start decreasing. From Figure 2 *b*, it is evident that the change in modulus is more after the application of 1% strain for the commercial, wild, M1 and M5 curdlan samples. Figure 2 *c* shows that the modulus after 1 strain% for commercial, wild, M1 and M5 curdlan samples decreases rapidly. One can infer that the linear viscoelastic regime will be evident till the application of 1 strain%, as the material is regaining its original modulus value. It was also observed that the modulus of each sample varied with temperature, within the same order of magnitude, indicating the variation of gel strength with temperature. Gel formation with commercial curdlan showed a higher value of modulus, indicating the greater association of curdlan molecules which become hard with increase in temperature (Figure 2 *a–c*). Figure 2 *c* shows that 90°C during the strain sweep, there is weak association of molecules at higher strain percentages above 1. This is an indication of the rigidity of these materials, where they break after elasticity of the materials is lost at a narrow strain range. On the other hand, wild, M1 and M5 samples seem to be softer in nature as they retain their structure even at higher strain percentages. It was also observed that the complete gel formation did not occur when the final temperature was kept at 80°C compared to the gel at 90°C. From Figure 2 *a–c*, it can be concluded that with increase in temperature, the structure of the material becomes harder resulting in the formation of hard gels due to the interactions of triple-helical structures.
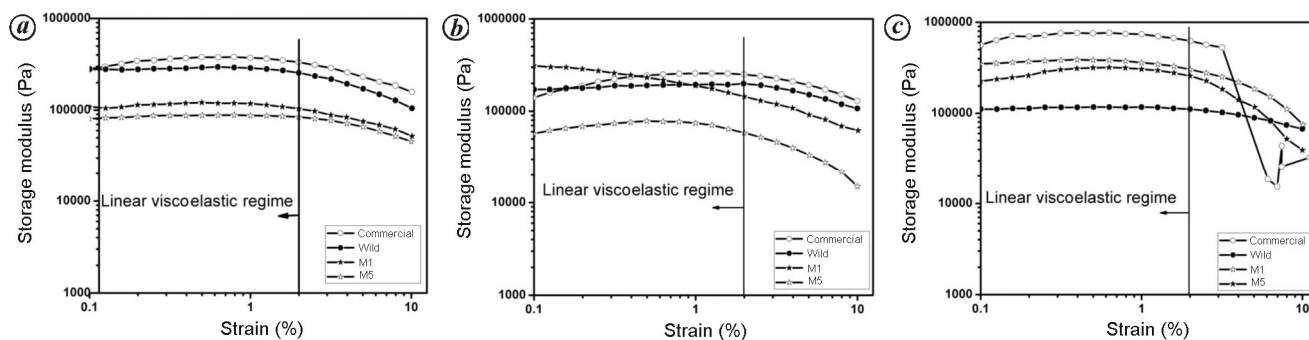
Figure 2. Storage modulus as a function of strain% of curdlan samples at (*a*) 80°C, (*b*) 85°C and (*c*) 90°C.
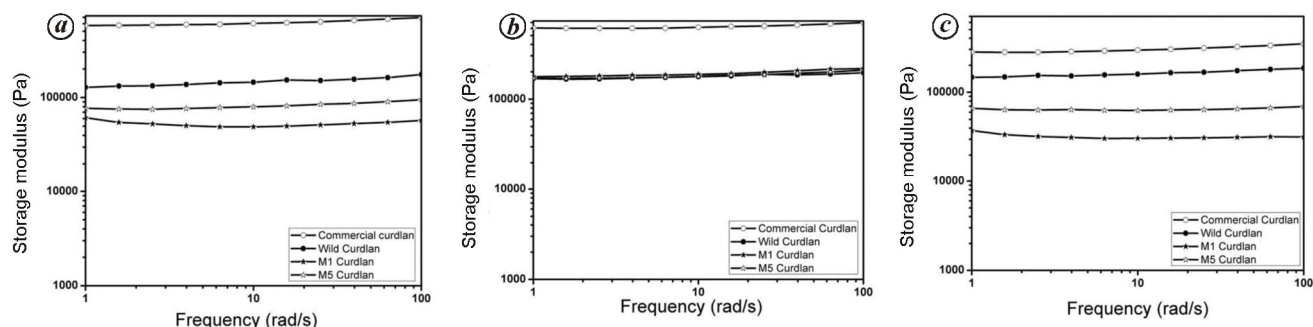

Figure 3. Frequency sweep of various curdlan samples at (*a*) 80°C, (*b*) 85°C and (*c*) 90°C.
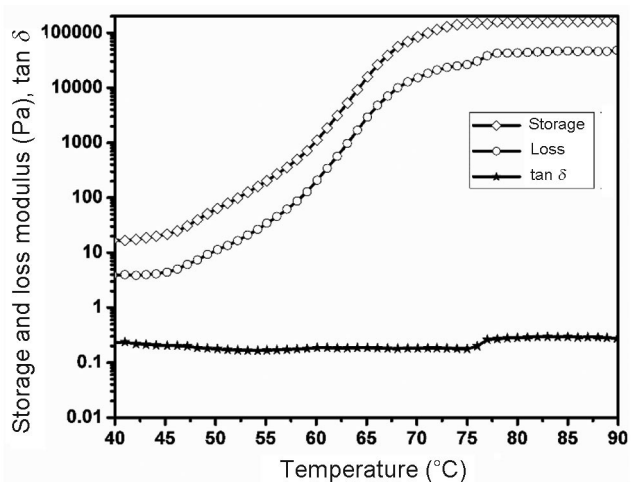

Figure 4. Storage and loss modulus, and tan δ variations with temperature for commercial curdlan sample.

To analyse gel strength, the samples were subjected to frequency sweep at 80°C, 85°C and 90°C, and keeping them at the respective temperatures for a period of 30 min, on the rheometer peltier. The frequency was varied from 1 to 100 rad/s with a constant strain rate of 1%. The strain% was chosen based on the linear viscoelastic regime where the modulus is same over the range of frequencies. It can be seen from Figure 3 *a* that the commercial curdlan sample clearly shows gel behaviour while

dominating the modulus compared to the other samples, when the gelation temperature on the peltier of the rheometer was maintained at 80°C. Though M1, M5 and wild curdlan samples showed gel behaviour, their modulus values were less than that of the commercial curdlan sample. Figure 3 *b* shows that there is no change in the modulus of commercial curdlan sample; however the wild, M1 and M5 samples showed improvement in their modulus. It is an indication that the commercial curdlan can gel and retain its properties at 80°C, whereas the wild, M1 and M5 samples are still undergoing molecular associations towards gelation. Further, when the temperature is increased to 90°C, all the samples showed different values of modulus (Figure 3 *c*). This is an indication of structural variations in the gel, where the multiple-stranded helicages dominate to form the permanent set. It can be concluded from Figure 3 *a–c* that the storage modulus of commercial curdlan is stable over a wide range of frequencies, it has a modulus of approx. 696,000 Pa and retains its value when the gel is made at 80°C and 85°C. Also, the decrease in modulus value can be due to the dissociation of hydrogen present in triple-helix molecules, resulting in the formation of single helical structures[17].

To analyse the variations of modulus with temperature, the corresponding samples were loaded on the rheometer and a temperature sweep was performed by keeping the amplitude at 1% and at different frequencies. An example
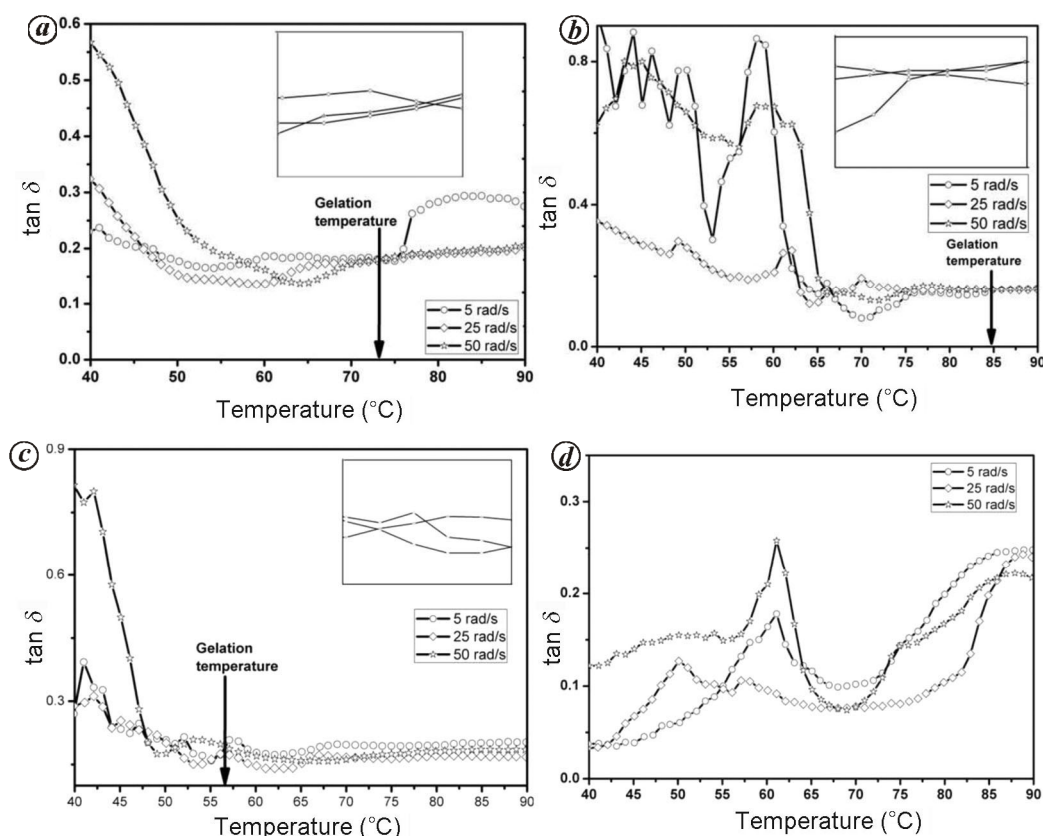
Figure 5. Variation of tan δ with temperature for (**a**) commercial, (**b**) wild, (**c**) M1 and (**d**) M5 curdlan samples.

is shown in Figure 4 for commercial curdlan at a frequency of 10 rad/s. A series of such tests were performed on commercial, wild, M1 and M5 curdlan samples, and the storage and loss modulus evolution was monitored by varying the temperature at the rate of 2°C/min. The temperature ramp rate at 2°C/min is an optimal value that has been observed in the literature. To find the gel temperature, multi-frequency plot of tan δ versus temperature was used, where tan δ is the damping coefficient which is the ratio of loss modulus to the storage modulus. The gel point might be identified where the modulus becomes independent of frequency. In this multi-frequency plot, the proposed common criterion for gel is that gelation temperature is a point where it becomes independent of frequency[18–20]. These variations are as shown in Figure 5 *a–d* for commercial, wild, M1 and M5 samples respectively. The commercial, wild and M1 samples showed a gelation temperature of 73.2°C, 66.5°C and 56.5°C respectively, whereas for the M5 sample the gelation temperature was beyond 90°C.

Temperature-induced gelation involves the formation of triple-helical structures of curdlan, which result with the association of single helicages[17]. More the association, more will be gel strength. It was also shown that the formation of thermo-irreversible gel is due to the formation of hydrophobic interactions among the triple-helical

structures[15]. The usual meat gel is made of soya protein isolates and egg white. But the addition of curdlan to these materials could improve their properties like elasticity and gelation temperature. Hence curdlan is an important additive in the food processing industry. In the present study, the association factor of curdlan gels was measured using the modified Hill equation, and the molecular association of the triple-helical structures using the Hill coefficient *n* for various types of curdlan samples was presented. Figure 6 is an example of such a graph which can be divided into three main parts.

1. At the initial stages, the storage modulus is low indicating the solution behaviour with free-moving molecules.
2. As the temperature is increased, the gelation begins and storage modulus increases steeply forming the network structure in molecules, indicating the sol–gel transition region.
3. Finally a plateau region appears, where storage modulus reaches a final equilibrium value, indicating completion of the molecular network.

The evolution of storage modulus plotted versus time results in a sigmoidal curve for the wild curdlan at a frequency of 10 rad/s. The Hill equation is fitted to the

Table 1. *n* and *K* values of curdlan samples

Sample	Commercial curdlan		Wild curdlan		M1 curdlan		M5 curdlan	
Frequency (rad/s)	*n*	*K*	*n*	*K*	*n*	*K*	*n*	*K*
5	12.69	14.81	6.03	24.45	9.78	13.57	4.70	120.34
10	5.67	15.26	9.00	19.92	5.86	17.50	3.62	262.16
25	11.20	12.28	8.60	22.25	5.64	15.94	4.14	259.20
50	12.53	15.57	16.70	18.97	5.59	17.38	5.28	22.85
Average	10.53	14.48	10.08	21.40	6.72	16.10	4.43	166.14
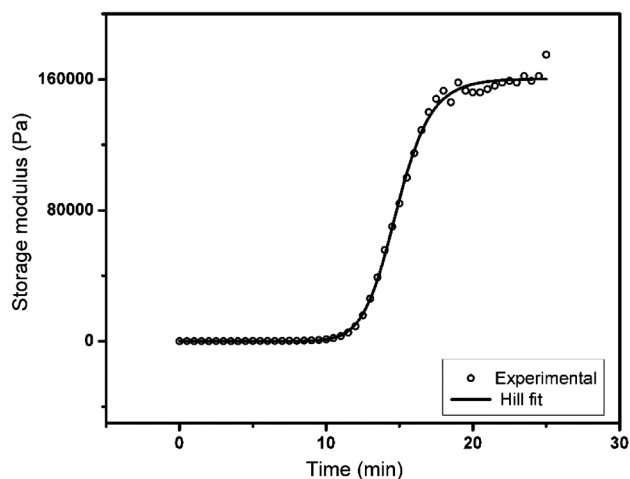
Figure 6. Evolution of *G′* with time for commercial curdlan sample at 5 rad/s.

experimental values of the storage modulus to evaluate the kinetics of modulus growth as

$$G'(t) = G'_{\infty} \frac{t^n}{t^n + K^n},$$

where G'_{∞} is the equilibrium storage modulus, *K* the time at which $G'(K) = G'_{\infty}/2$ and *n* is the slope at half gelation time *K*. It gives the fitted values of Hill coefficient *n* = 12.69 and half gelation time *K* = 14.80 (Figure 6). *n* is significant in it that represents the cooperativeness to form hydrogen bonds among the curdlan molecules. Table 1 lists the values of *n* and *K*. It can be seen from the table that the wild and commercial curdlan samples show associative bonding nature among the molecules to form permanent gel at an *n* value close to 10. It can be also observed that in the commercial sample, the *n* value is consistent at different frequencies, whereas it varies with frequency for the wild sample. It can be argued that the structure in commercial curdlan is rigid. However, for the wild sample, the gel is more responsive to frequency indicating viscoelasticity nature. The M1 and M5 samples show low values of *n*, indicating the poor association of curdlan molecules and hence the gel strength.

With the results obtained, we can now conclude that the rheology of materials provides clues about their structure and formation. This study reveals the cooperative bonding among curdlan molecules and their gel strengths at various temperatures. The method provides a broad understanding about the thermo-responsive gelation of curdlan samples utilizing the parameters *n* and *K*, when they are modelled using the modified Hill equation.

All the samples of curdlan can be modelled by following the change in modulus with change in temperature at a particular ramp rate. As the modulus evolution during this follows a sigmoidal curve, the modified Hill equation is best suited to assess the association of molecules. It is found that the commercial and wild curdlan samples display greater strength when they are made into gels. However, for the M1 and M5 samples, the association factor is less. This is evident from the low gel strength that these samples possess. By evaluating the value of *n* with this model, one can easily predict the gelation behaviour of a material using rheology as a tool.

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Source change of lead materials for mirror-making industry in Western Han, China: evidence from Nanyang bronze mirrors

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The circulation of lead materials has always been a major concern in the Bronze Age archaeology. The source of lead materials for mirror-making industry during the Han Era in China is still not comprehensible. In particular, the change of raw material sources from Western to Eastern Han has not been well explained. In this study, five bronze mirror samples that were excavated from Nanyang City, Henan Province, Central China, were analysed using optical microscope, scanning electron microscope-energy dispersive spectrometer and multi-collector inductively coupled plasma mass spectrometry methods. Further data on Han mirrors from Chang'an and Linzi in China and Japan were collected for a comparative study. The results show that lead ore sources in Western Han were multiple and complex, while those in Eastern Han were dominated by the Lower Yangtze. This type of change had begun to take place in Western Han, not in the transitional period as revealed in previous studies. The present study indicated that Nanyang played a pivotal role as a resource hub in this process.

THE history of the bronze mirror industry in China can be traced back to the Qijia culture (*c.* 4000 BP). In the early stage, bronze mirrors functioned more as religious objects than as reflectors of images[1]. As the first highlight in the history of ancient Chinese bronze mirrors, the Han Dynasty witnessed unprecedented production and use of such mirrors[2]. They became an indispensable part of the daily life of the Chinese, from emperors to the common people[3]. These mirrors have consequently become important representatives of the material culture at that time.

The initial decoration of the Western Han (WH: 202 BC–AD 8) mirrors was inherited from the Warring States period (475–221 BC); soon afterwards, the kaleidoscopic patterns emerged. Researchers have done a detailed analysis of various types and subtypes of these mirrors, and

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