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TEMPERATURE DEPENDENCE OF THE ELASTIC PROPERTIES OF ALGINATE GELS*

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ABSTRACT

The moduli of elasticity of calcium and lead alginate gels increase with time after preparation, and the temperature dependence of the rate of syneresis suggests an activation energy of 8–12 × 10^4 J mol^{-1} for the formation of new junctions At zero time, a negative temperature-dependence was found for the elastic force measured at a low degree of deformation (4%) Deformation of the gels was associated with an increase in entropy and internal energy When the calcium ions in a preformed calcium alginate gel were exchanged for lead ions, which have a higher affinity for alginate, the modulus increased due to an enhanced increase in internal energy with deformation Reversal of the sequence of introducing the two types of ions gave the opposite effect The data suggest that the junctions are "weak points" in the gels, and that even small deformations can cause partial rupture

INTRODUCTION

There has recently been discussion[1–3] concerning the applicability of the statistical theories for ideal, rubber-like elasticity[4,5] to biological gels having non-permanent junctions, such as alginate gels The basic concept in the classical theories[4,5], and in later refinements[6,7], is that the elasticity is entropic in origin This situation is explained mechanistically by the less-probable configuration taken up by the molecular chain in response to an applied external force

By some criteria, alginate gels seem to follow predictions from these theories For example, the dependence of the modulus of elasticity upon the degree of polymerization is of the correct form[2,3,8], the ratio between the loss and storage moduli is low[1], and the frequency dependence of the latter is small[1] On the other hand, there are indications that the number of statistical elements (Kuhn segments) in an average strand between junctions[1,2,8] is very much smaller than would be required to make the statistical theories applicable Moreover, a close correlation exists

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*Some of the work was performed (by I -L A) at the Department of Chemistry, The University of Trondheim, NLHT-Rosenborg, N-7000 Trondheim, Norway between the moduli of elasticity of alginate gels and the stability of the cross-linkages that are introduced by chelation of divalent metal ions between adjacent chains[2,8,10]. This correlation suggests[2] that the single-chain strands in between junctions are very restricted in their movements, and that the energy applied for deforming the gels is partly used to rupture junctions. If this conclusion is correct, the elasticity of alginate gels should be partly energetic in origin. The object of the present paper is to clarify this question.

The data needed are values of the modulus of elasticity at different temperatures, a positive temperature-dependence is characteristic for entropic elasticity, and a negative one for energetic elasticity[4]. It is confusing that both a positive and a negative temperature-dependence have been reported for calcium alginate gels[1,8,9]. Such contradictory results can be explained if it is remembered that alginate gels are most probably non-equilibrium gels[2,8,10]. The change in thermal energy with temperature could therefore alter the gel structure by changing the degree of cross-linking. Since this reaction must be time dependent, the time dependence of the modulus at different temperatures must be determined before its temperature dependence can be evaluated for gels of comparable structure. Hitherto, this has not been done, and hence little significance can be attached to the earlier data. We now report on the time dependence of the modulus of elasticity at different temperatures, and use values extrapolated to zero time for evaluation of the thermodynamic quantities. Any other time would represent an arbitrary position with respect to the cross-linking reaction.

THEORY

Systems like those treated here undergo a slow change with time[8], most probably because a cross-linking reaction continues after the gels have set. Simultaneously, a loss of water (syneresis) occurs. The systems are therefore not in true equilibrium. Since the first postulate in irreversible thermodynamics[11] can be used whenever a system is not too far from equilibrium, we assume that it is permissible to use the Gibbs equation. From the general equation for the Gibbs energy of a closed system without chemical reaction, and the Gibbs equation for a closed system with pressure/volume and elastic work, Flory[4] arrived at

$$F = (\partial U/\partial l)_{T,V} + T(\partial F/\partial T)_{V,l}, \tag{1}$$

where F is the equilibrium value of the elastic force (obtained at zero rate of compression), U is the internal energy of the system, and ∂l the change in length of the system during deformation.

The following cross-relation is also valid

$$(\partial F/\partial T)_{V,l} = -(\partial S/\partial l)_{T,V} \tag{2}$$

This means that data for the elastic force at constant length and volume, measured at different temperatures, permit calculation of the change in entropy and internal energy of the system caused by compression at a given temperature and volume These measurements are difficult because of thermal expansion The following approximation is valid for rubber[4]

$$-(\partial S/\partial l)_{T\,V} \approx (\partial F/\partial T)_{P\,\alpha}, \qquad (3)$$

where $\alpha = l/l_0$ = the ratio between the length of the piece of rubber at an applied elastic force, F, and at zero elastic force

We have found that the assumptions made during the derivation of equation *3* are also valid for our system Thus, no change in volume was recorded in the range of compression used here (4%), and the thermal expansion coefficient was found to be negative and small ($\approx = -0\;6 \times 10^{-3}$ cm^3/K) We conclude, therefore, that it is necessary to measure the elastic force at constant α and P, at a series of different temperatures, in order to obtain values for the entropy changes (equation *3*) and changes in internal energy (equation *1*)

EXPERIMENTAL

Materials — Alginate was prepared in the usual way[13] from *L hyperborea*, old stipe, harvested at Hustad 4/5-62 The ratio between D-mannuronic acid and L-guluronic acid residues was previously determined[14] to be 0 38, and the block structure to be 25% of "MG blocks" and 75% of "GG blocks" The intrinsic viscosity in 0 1M NaCl was 0 94 m^3/kg (9 4 dl/g) A 2% (w/w) solution of sodium alginate was prepared, and gels were made by dialysis in small cylinders as previously described[12] Four types of gel were made by dialysis to exhaustion for 2 days at room temperature against the following 0 34M aqueous salt solutions (1) $CaCl_2$ (Ca-gels), (2) $Pb(NO_3)_2$ (Pb-gels), (3) $CaCl_2$ then $Pb(NO_3)_2$ (Ca/Pb-gels), and (4) $Pb(NO_3)_2$ then $CaCl_2$ (Pb/Ca-gels)

Methods — Moduli of elasticity were determined by compression at a rate of 2 mm/min in an Instron TTK Universal Testing Machine, as previously described[12] The method[12] had a standard deviation of 7% The temperature was regulated to 10 20, 30, 40, and 50 ±0 5° in a thermostatic bath filled with the dialysis medium A set of thermistors was used to estimate the time taken for the gels to reach the desired temperature after immersion in the bath Stress–strain diagrams were recorded after this minimum time (600 sec), and at time intervals For each measurement at a given temperature or time, 3–6 pellets of gels were compressed, and average values of the modulus of elasticity were calculated with knowledge of the measured, geometrical size of the gels[12] The elastic force at a degree of compression of 4% was used for calculating thermodynamic data In some of the experiments, the elastic force was determined at a series of different rates of compression between 0 5 and 10 mm/min, providing a comparison[12] of the elastic force obtained at the standard rate of compression (2 mm/min) with that obtained at zero rate of compression (an extrapolated condition) The thermal expansion coefficient for calcium alginate gels was determined from the measured volume immediately after temperature-equilibration of the gels

The degree of ion exchange that had occurred in the Ca/Pb-gels and in the Pb/Ca-gels was determined in the following way The gels were dialysed four times, for at least 5 h, against large volumes of distilled water to remove the excess of divalent metal ions, and then three times for 24 h against 30 cm^3 of 0 2M HNO_3 to liberate the bound metal ions from the alginate A Perkin–Elmer atomic absorption spectrophotometer Model 290 B was used to analyse for the content of calcium and lead ions in the dialysates

RESULTS

Time dependence of the modulus of elasticity

Representative results are given for Ca-gels, Pb-gels, and Ca/Pb-gels at 50° in Table I and Fig 1 The data show a marked increase in modulus, and a decrease in volume, especially in the initial phase of the ageing process Similar results at other temperatures (not shown) indicated that the rate of ageing was markedly temperature-dependent For example, the ageing was hardly observable at 10° within the time scale used (20 h) Further, the rate of ageing increased by a factor of between 2 and 3 with an increase in temperature from 40 to 50° If we assume, as before[2 8 10], that the modulus is proportional to the concentration of junctions in the gel, and that the ageing is due to formation of more junctions, the observed temperature-dependence of the reaction corresponds to 8–12 × 10^4 J/mol (20–30 kcal/mol)

This high activation energy suggests that considerable alteration of the gel structure occurs during ageing, making extrapolation to zero time mandatory for elucidation of temperature effects on gels of comparable structure

The extrapolation to zero time was simplified by the fact[12] that the modulus of elasticity of alginate gels is proportional to the square of the alginate concentration Plots of $E \times (V/V_0')^2$ versus time, where E is the measured modulus of elasticity,

TABLE I

MODULI OF ELASTICITY, E, AND VOLUME OF GELS, V, AS FUNCTIONS OF TIME AT 50°

Time	*Ca-gels*[a] ($V_0'^b$ = *1 61 cm*^{3})		*Pb-gels*[a] (V_0' = *1 52 cm*^{3})		*Ca/Pb-gels*[a] (V_0' = *1.36 cm*^{3})	
(*h*)	E (*N/cm*^{2})	V (*cm*^{3})	E (*N/cm*^{2})	V (*cm*^{3})	E (*N/cm*^{2})	V (*cm*^{3})
0 17	17 2	1 57	15 7	1 42	41 2	1 33
0 5	18 7	1 53	18 6	1 36	42 9	1 29
1	19 1	1 50	21 6	1 32	45 1	1 26
1 5	18 6	1 47				
2	22 1	1 46				
4 5	23 1	1 45	26 5	1 17	48 8	1 23
18	23 2	1 45	27 4	1 14	52 9	1 23

[a] See Experimental, for explanation of the abbreviations [b] V_0' is the volume measured at room temperature immediately before the experiments

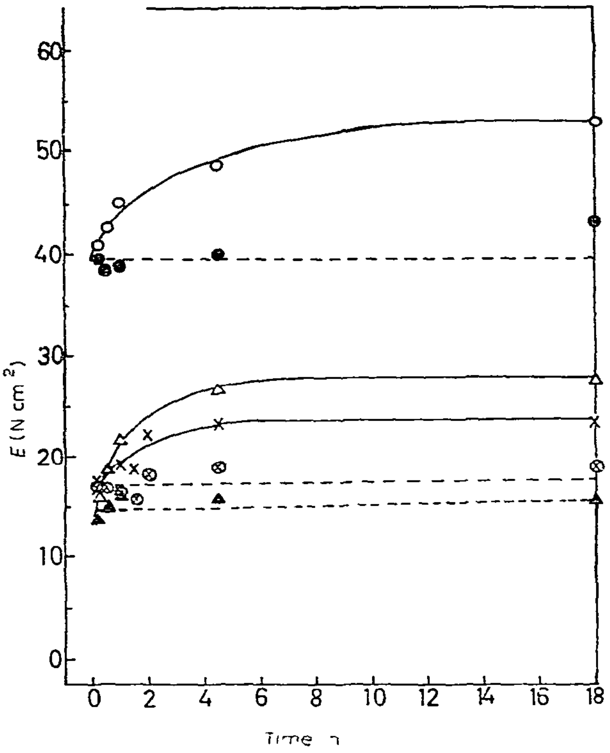

Fig 1 Modulus of elasticity, E, and $E \times (V/V_0')^2$ (see text) as a function of time at 50° E, ——— ×, Ca-gels, △, Pb-gels, ○, Ca/Pb-gels $E \times (V/V_0')^2$ – – –, ⊗, Ca-gels, ▲, Pb-gels, ●, Ca/Pb-gels

V is the volume of the gels at the time of measurement, and V_0' is the volume of the gels immediately before immersion into the thermostatic bath, yielded straight lines having a slope close to zero, as seen in Fig 1 Such plots were used to carry out the extrapolation to zero time

Modulus of elasticity, elastic force, and changes in entropy and internal energy during compression of gels

Moduli of elasticity of four different types of alginate gels (Ca-gels, Pb-gels, Ca/Pb-gels, and Pb/Ca-gels) extrapolated to zero time at different temperatures are given in Table II It should be pointed out that the exchange of calcium ions for lead ions in the gels prepared by first dialysing against calcium salt and subsequently against lead salt was complete, whereas the reverse sequence of dialysis gave only 50% exchange The volumes of the gels, measured at room temperature, are included in Table II

The volume of the sodium alginate solution prior to dialysis (V_0'') was, in all cases, 2 3 cm^3 Table II shows that a different amount of shrinkage has occurred during the dialysis procedures, consequently, the alginate concentration is also different in the gels In order to compare the gels at the same alginate concentration, all the moduli have been corrected[12] by multiplying them with $(V_0'/V_0'')^2$ before calculation of the elastic force, F The results are shown in Fig 2

From Fig 2, the changes in entropy and internal energy were calculated, and the results are given in Table III

TABLE II

MODULI OF ELASTICITY OF ALGINATE GELS EXTRAPOLATED TO ZERO TIME AT DIFFERENT TEMPERATURES

Temp (degrees)	*Ca-gels* ($V_0 = 1\ 61\ cm^3$) E (N/cm^2)	*Pb-gels* ($V_0' = 1\ 52\ cm^3$) E (N/cm^2)	*Ca/Pb-gels* ($V_0 = 1.36\ cm^3$) E (N/cm^2)	*Pb/Ca-gels* ($V_0 = 1\ 32\ cm^3$) E (N/cm^2)
10	26 ±1	31 ±1	65 ±6	25 ±1
20	24 ±3	25 ±2	49 ±4	20 ±5
30	21 ±2	25 ±3	47 ±3	22 ±2
40	18 ±2	19 ±3	43 ±1	13 ±2
50	17 ±1	14 ±2	40 ±2	15 ±1

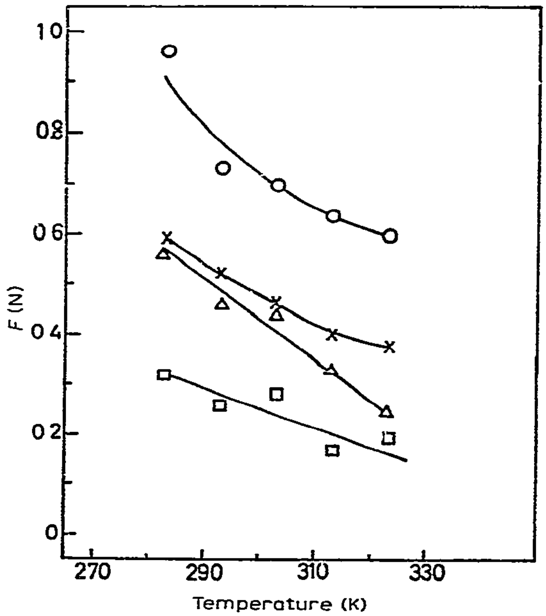

Fig 2 Elastic force, F, at a degree of compression of 4% as a function of temperature for 2% alginate gels ×, Ca-gels, △, Pb-gels, ○, Ca/Pb-gels, □, Pb/Ca-gels

TABLE III

CHANGES IN ENTROPY AND INTERNAL ENERGY DURING COMPRESSION OF 2% ALGINATE GELS

Type of gel	*Temp.*, T (*K*)	$F_{\alpha=0.96}$ (*N*)	$(\partial S/\partial l)_{T,V}$ $\left(\frac{N}{K}\times 1000\right)$	$T(\partial S/\partial l)_{T,V}$ (*N*)	$(\partial E/\partial l)_{T,V}$ (*N*)
Ca	283	0.59			
	293	0.52	7.0	2.1	2.6
	303	0.46	5.2	1.6	2.1
	313	0.40	4.0	1.3	1.7
	323	0.38			
Pb	283	0.56	8.5	2.4	3.0
	293	0.46	8.5	2.5	3.0
	303	0.44	8.5	2.6	3.0
	313	0.33	8.5	2.7	3.0
	323	0.25	8.5	2.7	3.0
Ca/Pb	283	0.96			
	293	0.73	9.2	2.7	3.4
	303	0.70	5.5	1.7	2.4
	313	0.64	3.9	1.2	1.9
	323	0.60			
Pb/Ca	283	0.32	3.7	1.1	1.4
	293	0.26	3.7	1.1	1.3
	303	0.28	3.7	1.1	1.4
	313	0.17	3.7	1.2	1.3
	323	0.19	3.7	1.2	1.4

DISCUSSION

The high rate of ageing at 50°, seen in Table I and Fig. 1, and the high activation energy for the ageing reaction strengthen the earlier conclusion[2,8,12] that alginate gels are non-equilibrium gels, and that a cross-linking reaction is continuing after the first setting of the gels.

The most important feature of the results is the negative temperature-dependence of the modulus of elasticity and the elastic force obtained at zero time, as seen in Fig. 2 and Table III. This result means that the entropy of the system increases during compression. This is in contrast to the well-known situation for rubber[4,5]. Simultaneously, the internal energy of the system increases. This behaviour is also in contrast to rubber elasticity, where $(\partial U/\partial l)_{T,V} \approx 0$. Because of the favourable change in entropy with compression, the elasticity must be energetic in origin. This situation may be due to distortion of chemical bonds in the stiff, alginate chain, but is most easily explained by assuming that some rupture of junctions occurs during compression, as suggested earlier[1,2,8 10]. Classical (entropic) rubber-elasticity theory should therefore not be applied to alginate gels. This conclusion confirms that no applicable theory has yet been presented for these types of gels[3,10], and that development of such theories stands out as an important, unsolved problem.

Before discussing the numerical values of the data in Tables II and III, it should be pointed out that the scattering of the points in Fig 2 suggests relatively large errors, so that only main tendencies should be regarded as significant Moreover, a limit of zero rate of compression should have been used to eliminate kinetic effects and to obtain the equilibrium value of the elastic force[4] As discussed earlier[1 2], it is not possible, for the present types of gels, to use a rate of compression lower than 2 mm/min, probably because of a pressing out of water at lower rates of compression In the present work, we performed experiments with a series of different rates of compression, and found that the *F*-values at 0 2 mm/min were 8–20% higher than those obtained at zero rate of compression (an extrapolated condition) Fortunately, however, the differences were found to be the same at 20 and 50° These control experiments therefore indicate that the *F*-values in Table III are systematically too high by 8–20% We have also checked that this type of error will not violate any conclusion to be given below For example, the *F*-values for the Ca-gels are too high by ~20%, whereas those for the Ca/Pb-gels are too high by ~8% This situation makes the difference between the two sets of elastic forces even greater than that indicated in Table III Finally, it should be stressed that the *F*-values in Table III have been scaled[1 2] to the same concentration of alginate, so that the effect of the ions in causing different amounts of shrinkage during dialysis has been eliminated

The data in Table II show that a twofold increase in modulus is observed when calcium ions are exchanged for lead ions Alginate is known to chelate lead ions more strongly than calcium ions[15 16] The increase in modulus accompanying the ion-exchange reaction is therefore in agreement with the close correlation observed earlier[2 8 10] between the affinity of alginate for an ion and its ability to form rigid gels Table III also suggests marked hysteresis with respect to gel structure, as (*a*) the Ca/Pb-gels gave higher moduli than the Pb-gels, and (*b*) even the very incomplete exchange of lead ions for calcium ions gave lower moduli in the Pb/Ca gels compared to the Ca-gels

A comparison of the values given in Table III indicates that the gels may be divided into two "groups" The Pb-gels and the Pb/Ca-gels represent the one "group", where the change in entropy and internal energy during compression is the same over the whole range tested The different elastic forces observed at different temperatures are due only to T in front of the entropic term (equations *1* and *2*) This may be interpreted in terms of a stable structure, it is unlikely that S and E of the gel change in the temperature interval tested The Ca-gels and the Ca/Pb-gels represent the other group, where the changes in entropy and internal energy during compression of the gels decrease with increasing temperature This behaviour is probably due to some instantaneous reorganization or rupture of junctions upon heating As discussed above, the structure of the network imposed by the lead and the calcium ions is most probably different It is therefore not unreasonable that the high affinity of alginate for lead ions located in the junction zones may lead to a more-stable structure with respect to temperature than do calcium ions

When comparing the numerical values within the two "groups" of data, it is seen that the exchange of calcium for lead ions leads to higher values of F and of both $(\partial S/\partial l)_{T,V}$ and $(\partial E/\partial l)_{T,V}$ The exchange of lead ions for calcium ions leads to a lowering of these parameters Because of the probable maintenance of the structure inside the two groups, these changes may most easily be explained by again assuming some rupture of junctions during the compression By this assumption, it is understandable that the energetic term is largest for ions that are more strongly chelated between adjacent chains in the junction-zones

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