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Investigation into the ageing process in gels of gelatin/water systems by the measurement of their dynamic moduli

Part II: Mechanism of the ageing process

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Summary

This second paper on the ageing of aqueous gelatin gels (1–5%) deals with the mechanism of the renaturation process. The maximum gelation temperature, i. e. the gel temperature, increases with increasing concentration. For the heat of cross-linking a value of $\Delta H^\circ = -442$ kJ/mol was found, corresponding with mono-helices of 24 windings and a length of 230 Å. The influence of the thermal history is considerable, and the heat of activation of the rate determining step is very small. Hence, the temperature dependence of the degree of ageing is not caused by temperature dependence of the reaction rate constant, but by the increase in stability of small structures which accompanies a decrease in temperature.

Zusammenfassung

Diese zweite Veröffentlichung betreffs der Alterung von 1–5% Lösungen von Gelatin in Wasser behandelt den Mechanismus des Renaturationsprozesses. Die maximale Gelierungstemperatur, d. h. die Geltemperatur, nimmt zu mit zunehmender Konzentration. Für die Verknüpfungswärme wurde gefunden $\Delta H^\circ = -442$ kJ/mol. Dieser Wert korrespondiert mit Monohelices von 24 Windungen und einer Länge von 230 Å. Die Abhängigkeit von der thermischen Geschichte ist bedeutend und die Aktivierungsenergie des geschwindigkeitsbestimmenden Reaktionsschrittes ist sehr klein. Demnach wird die Temperaturabhängigkeit des Alterungsgrades nicht verursacht durch die Temperaturabhängigkeit der Reaktionsgeschwindigkeitskonstante, sondern durch die Zunahme der Stabilität kleiner Strukturen mit abnehmender Temperatur.

Key words

Gelatin, visco elasticity, physical ageing, gels, gel temperature.

Introduction

In the preceding paper (1) the phenomenology of the ageing (renaturation) process of 1–5%

aqueous gelatin solutions was described. During the ageing process the dynamic moduli (i. e. G' and G'') were measured in the temperature range from -1.2 °C to 25.2 °C. Some of the mentioned results are:

- the renaturation process of gelatin molecules into collagen like structures appears to be strongly dependent on temperature and concentration: the rate of the process increases if the temperature decreases and/or the concentration increases;

- the frequency dependence of the storage modulus is negligibly small, whereas the loss modulus is very small in comparison with the storage modulus; both facts point to a rubber-like character of the studied gels;

- pre-ageing at a higher temperature has a strongly accelerating influence on the excess-ageing at lower temperatures;

- the renaturation process of gelatin into collagen like triple-helix structures appears to be of 3rd order, at least at 25.2 °C, and the rate determining step of this process is the aggregation of the fastly formed mono-helices into triple-helices.

In the present paper attention will be paid to the mechanism of the renaturation process of the gelatin gels.

Experimental

Determination of the dynamic moduli

For the determination of the dynamic moduli use is made of a dynamic rheometer developed at our laboratory (1–5). This apparatus enables us to determine very accurately the dynamic moduli in a range of about 8 decades ($-2 \leq \log G'$

≤ 5.7 , $-3 \leq \log G'' \leq 5.8$, G' and G'' in N/m^2) and in a frequency range of almost 7 decades ($-4 \leq \log \omega \leq 2.8$, ω in rad/s).

Material

The gelatin used was a gelatin from Gelatin Delft N. V. Its weight average molecular weight $\bar{M}_w = 66,500$ was determined (by Mrs. Dipl. ing. J. Spangenberg-Oskerova) by means of light scattering at 40°C in 0.15 M NaCl solution, $\text{pH} = 6.40$ (6). The iso electric point $\text{pH} = 4.83$ was determined by means of viscosity measurements. The amino acid composition determined in our Organic Department is given in table 1 of reference (1). Also in reference (1) it is described in which way the aqueous solutions of gelatin were prepared.

Determination of the maximum gelation temperature and the heat of reaction of the ageing process

Preamble

According to Eldridge and Ferry (7) the relationship between the melting temperature T_m of gelatin gels and the concentration is given by:

$$(d \ln c / d T_m)_{\bar{M}_w} = - \Delta H^\circ / (R T_m^2) \quad [1]$$

where:

- c = the concentration of gelatin (kg/m^3);
- T_m = the melting temperature (K);
- \bar{M}_w = weight average molecular weight;
- ΔH° = the heat of reaction for breaking a cross-link in two potential junctions (J/mol);
- R = the gas constant ($= 8.316 \text{ J}/(\text{mol K})$).

The following assumptions were made by the authors in deriving this relationship:

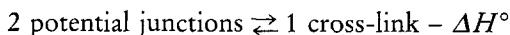
– The fraction of the total number of junctions in the gel that contribute to the elasticity, hence the fraction of the number of elastically effective cross-links, is a function of the concentration, but not of the temperature. This assumption is questionable since the degree of intramolecular cross-linking will depend on the rate of the ageing process, being smaller if the ageing temperature is higher. However, in the following it will appear that the variation in T_m is small, so that the question about the strict validity of this assumption is rather irrelevant;

– The number of potential junctions per unit of volume of the gel is proportional to the concentration. However, this is only true if the number of junctions already coupled in cross-links, is small with respect to the original

number of potential junctions. In other words, this holds if the ageing process has not proceeded very far. In view of the method of determination of T_m at least of T_{gel} (i. e. the maximum gelation temperature), this demand will certainly be satisfied;

– The number of potential junctions still available is not a function of the temperature. Also in this case the argumentation, mentioned in the previous point, is valid.

Eldridge and Ferry, in deriving equation [1], started their calculation from the following equilibrium:



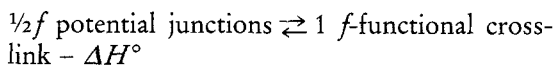
with the equilibrium constant:

$$K = m_{cl} / (m_j^2) \quad [2]$$

where:

- m_j = the concentration of potential junctions;
- m_{cl} = the concentration of cross-links.

Hence, the cross-links they consider are tetra-functional. Extension of the treatment to a functionality $f > 4$, where the following equilibrium is envisaged:



yields as an equilibrium constant:

$$K = m_{cl} / (m_j)^{\frac{1}{2}f} \quad [3]$$

In working out this relationship, in the way it was shown by Eldridge and Ferry for tetra-functional cross-links, one obtains the following equation:

$$(d \ln c / d T_m)_{\bar{M}_w} = \Delta H^\circ / [R T_m^2 (1 - \frac{1}{2} f)] \quad [4]$$

or

$$[d \log c / d (1/T_m)]_{\bar{M}_w} = - \Delta H^\circ / [2.303 R (1 - \frac{1}{2} f)] \quad [5]$$

where ΔH° is the heat of reaction for breaking 1 cross-link in $\frac{1}{2} f$ potential junctions. A plot of $\log c$ vs. $1/T_m$ yields a straight line with a slope equal to $\Delta H^\circ / [2.303 R (\frac{1}{2} f - 1)]$, provided both ΔH° and the functionality do not depend on the melting temperature and, as a conse-

quence, also not on the concentration (in reality, T_m appears to be dependent on the concentration).

Determination of the maximum gelation temperature

It appears from figure 4 of reference (1) that the induction period, i. e. the time needed for the development of a measurable increase of the storage modulus, increases strongly with the ageing temperature. At the maximum gelation temperature, T_{gel} , the induction period becomes infinite and hence, its reciprocal value goes to zero. In an empirical way it is found that, if the square root of the reciprocal induction period is plotted vs. the ageing temperature, a straight line is obtained. This is shown in figure 1 for a 1.95% (w/w) solution of gelatin. In fact, one does not need much phantasy to draw a straight line through the experimental points (the correlation coefficient amounts to $\rho = -0.998$). Extrapolation to $t_{ind} \rightarrow \infty$ yields $T_{gel} = 26.3^\circ\text{C}$. Why the relation between $t_{ind}^{-1/2}$ and T_a is linear, is still unclear to the author. In an analogous way the maximum gelation temperatures of the other gelatin solutions were determined. The results are mentioned in table 1. It appears that the maximum gelation temperature increases with the concentration.

Table 1. The maximum gelation temperature, T_{gel} , of aqueous gelatin solutions as a function of the concentration

c% (w/w)	5.00	4.00	3.00	1.95
T_{gel} °C	29.6	28.4	27.4	26.3

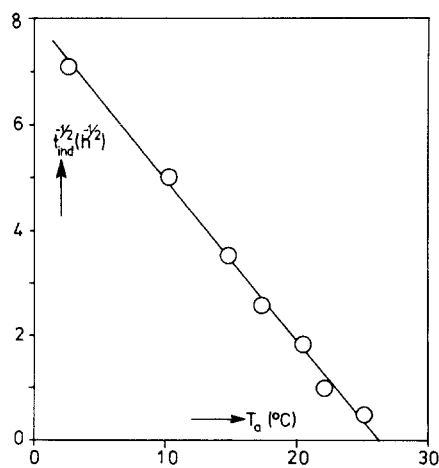


Fig. 1. Reciprocal square root of the induction period of 1.95% (w/w) gelatin/water plotted against ageing temperature

Calculation of the heat of reaction of the cross-linking process

In figure 2 $\log c$ is plotted vs. the reciprocal maximum gelation temperature. The slope of the straight line (correlation coefficient $\rho = -0.991$) yields:

$$\Delta H^\circ / (1/2 f - 1) = -221 \text{ kJ per mol of potential junction regions.}$$

Measurements of the melting temperature of gelatin gels, as reported by Eldridge and Ferry (7), yield values for this quantity varying from -206 to -924 kJ/mol, depending on the molecular weight and on the melting temperature. Analogous measurements taken by Tar (8) yield -252 to -317 kJ/mol. From measurements by Flory and Garrett (9) one can derive a value of -150 kJ/mol.

Since the cross-linking in gelatin gels consists of the formation of triple-helices the functionality f of the resulting cross-links is equal to 6, so that $\Delta H^\circ = -442$ kJ/mol. Harrington and Rao (10) report values varying from -170 to -340 kJ/mol.

From their measurements of the temperature dependence of the optical rotation of very dilute α -gelatin solutions Eagland c. s. (11) conclude that ΔH° of a folding process has a value of -18.8 kJ/mol. Oth and coworkers (12) conclude to a value of -17.6 kJ/mol and Flory and Garrett (9) to a value of -18.8 kJ/mol. In fact, measurements of the optical rotation yield a value of ΔH° corresponding to the formation of a repeating unit of three windings for the

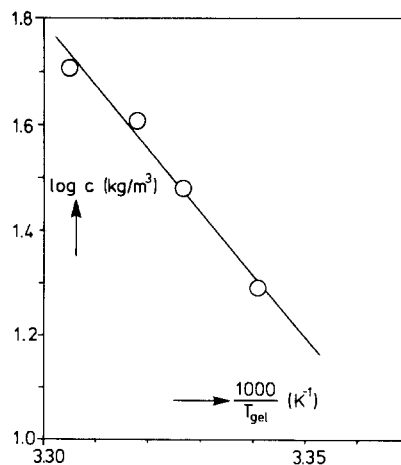


Fig. 2. Concentration of gelatin/water plotted against reciprocal maximum gelatin temperature

formation of a triple-helix. With the aid of the mean value, i. e. -18.3 kJ/mol, the mean number of windings per helix in a cross-link may be calculated to be $442/18.3 = 24$. Harrington and Rao (10, 13) state that for the formation of a stable helix at low temperature 7 to 11 windings are needed, whereas at higher temperatures this number will be considerably higher. Hence, a mean value of 24 in the temperature region of $26-30$ °C is not unreasonable. As 9 windings correspond with a length of 86 Å (14-16), for the length of the cross-links a value of 230 Å can be calculated.

The influence of the temperature history

The effect of a temperature decrease

In figure 9 of reference (1) the effect is demonstrated of a temperature decrease to -1.2 °C, as imposed to solutions of 1.95% after pre-ageing at 17.4 °C during various time intervals. At that occasion it has already been shown, that pre-ageing has a tremendous influence on the continued ageing at lower temperatures. In figure 3 the effect of the excess-ageing at -1.2 °C is shown again, but now in the overall ageing curves: G_r' plotted vs. $\log t_a$. G_r' is the reduced storage modulus, which is defined as (17):

$$G_r' = G'(T_0) = G'(T)c_0T_0/(cT) \quad [7]$$

where c_0 resp. c are the (w/v) concentrations of the dissolved gelatin at the reference temperature T_0 (i. e. -1.2 °C) resp. at the temperature T . The influence of pre-ageing at 17.4 °C on the overall ageing is more pronounced if the pre-ageing has proceeded for a longer time: the effect of excess-ageing after short times of pre-ageing is quickly overruled by that of excess-ageing after longer pre-ageing! Hence, in the same time a stiffer gel is obtained, if the pre-ageing at higher temperature has occurred during a longer time.

In figure 4 two ageing processes are compared. Both processes occur at -1.2 °C after 1.5 h of pre-ageing at various temperatures. One is pre-aged only at 17.4 °C, the other one is pre-aged at 17.4 °C during half an hour and, subsequently, at 8.6 °C during one hour. From the results it appears that the intermediate ageing process at 8.6 °C has a retarding effect on the excess-ageing process at -1.2 °C.

In figure 5 the influence of various pre-ageing processes, as carried out during 1.5 h at temperatures varying from 6.5 °C to 22.4 °C, on the excess-ageing at -1.2 °C is demonstrated for a 1.95% solution. On the overall ageing pre-ageing at 17.4 °C has the largest and pre-ageing at 6.5 °C has the smallest effect. The storage modulus after 5 h of excess-ageing at -1.2 °C is plotted vs. the pre-ageing temperature T_{pa} in figure 6. According to this figure the maximum

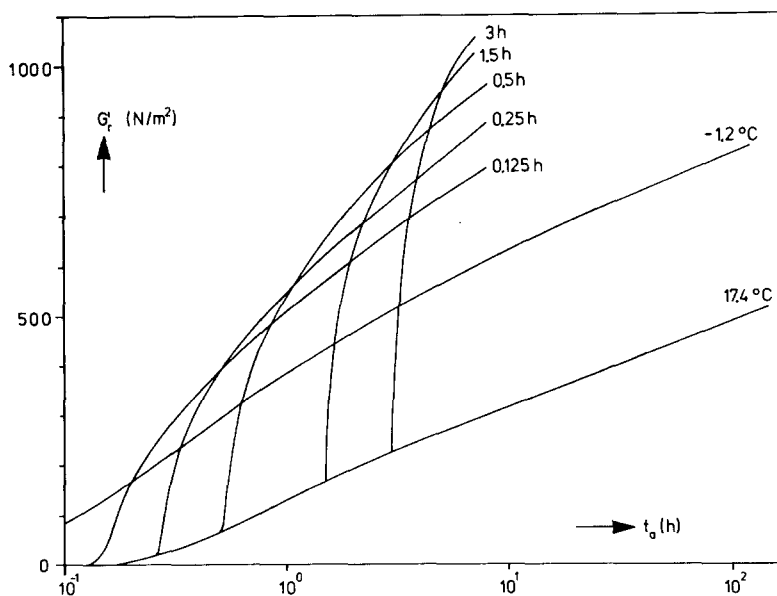


Fig. 3. Reduced storage modulus of 1.95% (w/w) gelatin/water of several ageing histories (see text) plotted against ageing time. Angular frequency 0.393 rad/s. Reference temperature -1.2 °C

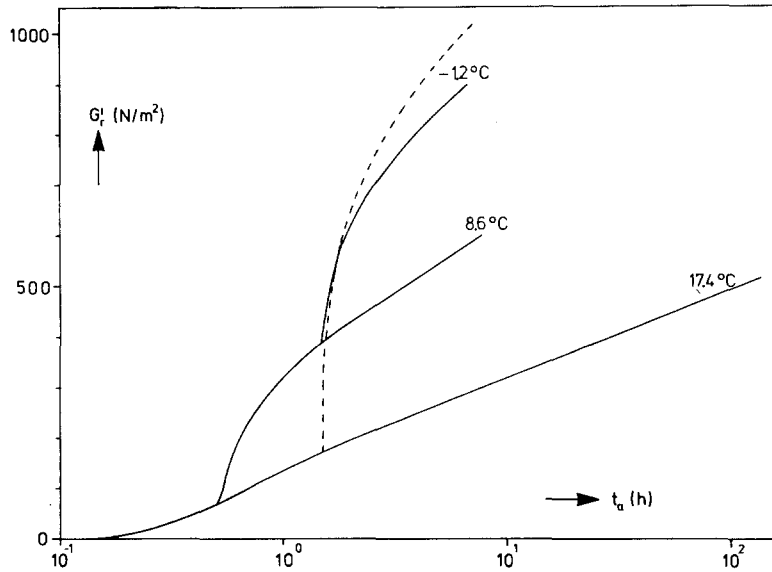


Fig. 4. Reduced storage modulus of 1.95% (w/w) gelatin/water with several ageing histories, as indicated, plotted against ageing time. Angular frequency 0.393 rad/s. Reference temperature -1.2°C

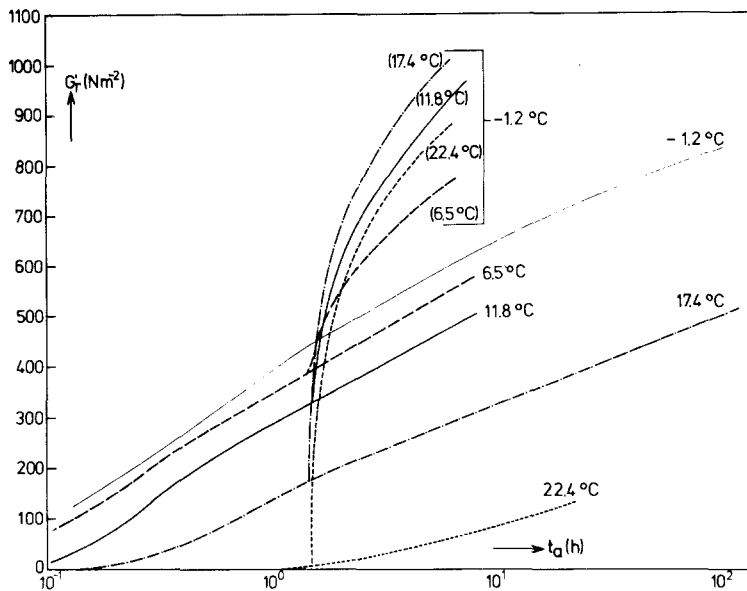


Fig. 5. Reduced storage modulus of 1.95% (w/w) gelatin/water plotted against ageing time for several ageing temperatures, on the one hand, and at -1.2°C after 1.5 h of ageing at the mentioned ageing temperatures, on the other hand. Angular frequency 0.393 rad/s. Reference temperature -1.2°C

effect of pre-ageing may be expected at 16.2°C . In this figure also $\Delta G'_r$ is plotted vs. the pre-ageing temperature, where $\Delta G'_r$ is defined as the difference between the storage modulus obtained after 1.5 h of ageing at T_{pa} and 5 h of subsequent ageing at -1.2°C , and the storage modulus obtained after 6.5 h plain ageing at the pre-ageing temperature. A monotonous increase

of $\Delta G'_r$ with pre-ageing temperature is perceptible. The curve seems to go to a maximum value at approximately 24°C . It is conceivable that also in this case a maximum has to occur: the overall storage modulus decreases for pre-ageing temperatures higher than 16.2°C , whereas the storage modulus for an unchanged ageing temperature T_{pa} becomes very small at high temper-

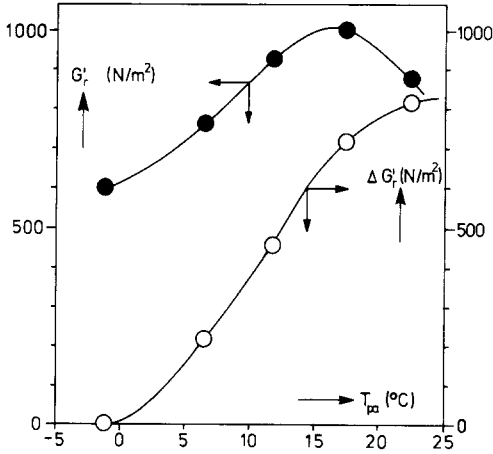


Fig. 6. (●) ... Reduced storage modulus of 1.95% (w/w) gelatin/water after 5 h of ageing at -1.2°C , preceded by 1.5 h of ageing at a higher pre-ageing temperature, plotted against pre-ageing temperature. (○) ... The difference between the mentioned reduced storage modulus and the reduced storage modulus after 6.5 h of ageing at the corresponding pre-ageing temperature plotted against the pre-ageing temperature. Angular frequency 0.393 rad/s . Reference temperature -1.2°C

atures T_{pa} . Stainsby (14) reports for the position of this maximum on the temperature scale a value of 24.2°C . Unfortunately, the concentration of the gelatin solution was not mentioned; the excess-ageing temperature is probably 10°C .

The effect of a temperature increase

It has already been observed that a temperature increase causes a quick decrease of the storage modulus to the value it should have at that higher temperature (see fig. 11 of ref. (1)). In figure 7 this is again demonstrated for a 1.95% solution, that was aged consecutively at 17.4°C (1.5 h), at -1.2°C (5 h) and, again, at 17.4°C . The pre-ageing process at 17.4°C causes a strong increase of the modulus during the excess-ageing at -1.2°C . The subsequent temperature increase to 17.4°C causes an initially very rapid decrease of the storage modulus, whereas, with proceeding time, another increase becomes observable, by which the curve for plain ageing at 17.4°C is approached gradually. This is shown in another way in figure 8. In this figure the plain ageing process at 17.4°C is shown, together with the excess-ageing at 17.4°C . As zero time of the excess-ageing process the instant is chosen, at which the temperature is increased from -1.2°C to 17.4°C (hence, after 6.5 h of ageing). The storage modulus decreases to a minimum value after approximately 2 h. Subsequently, a gradual increase takes place, by which the curve gradually approaches the curve for 17.4°C . From these measurements it appears clearly that an intermediate ageing at a lower temperature does not affect the final ageing at the higher temperature:

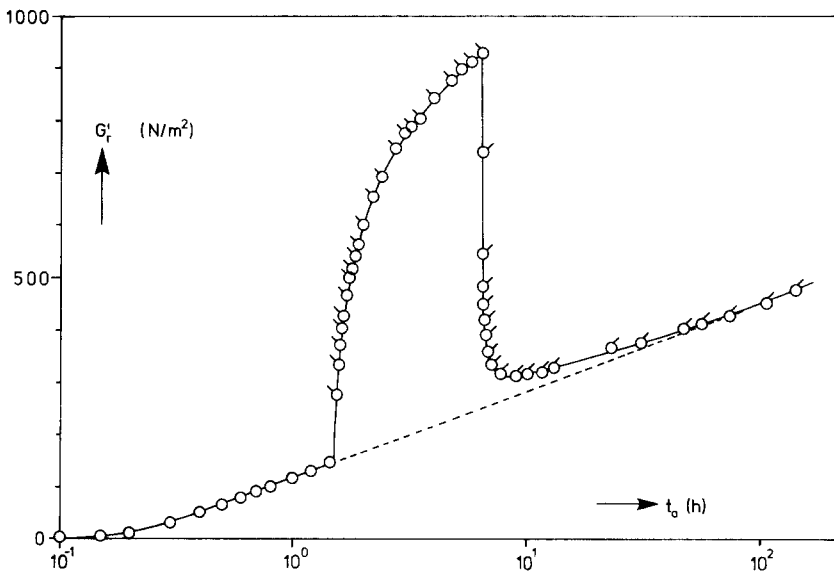


Fig. 7. Reduced storage modulus of 1.95% (w/w) gelatin/water with a special ageing history plotted against ageing time. (○) and (○) ... 17.4°C ; (⊖) ... -1.2°C . Angular frequency 0.393 rad/s . Reference temperature -1.2°C

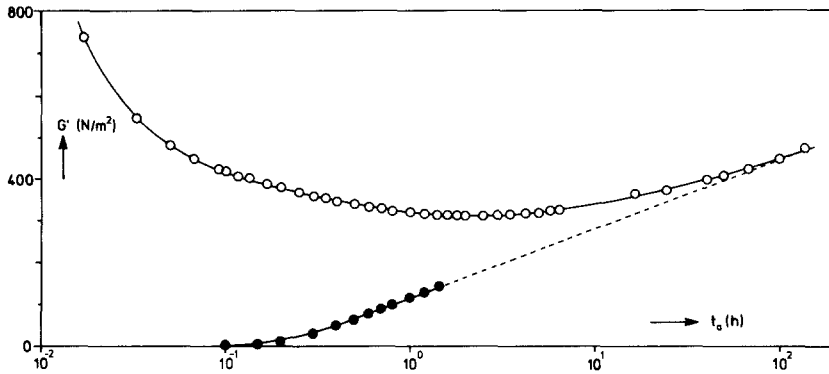


Fig. 8. Storage modulus of 1.95% (w/w) gelatin/water at 17.4 °C (●) and with a special ageing history (see text) (○), plotted against (excess-) ageing time. Angular frequency 0.393 rad/s

the influence of the intermediate ageing fades away within approximately 40 h.

In this latter experiment the final ageing temperature was equal to the initial one. In this case a kind of memory for the old situation might exist, by which the original state is finally reached again. However, the process is more complicated, as is revealed by figure 9. In this figure, the result of ageing of a 5% gelatin solution is shown. This solution was originally aged at 17.4 °C, subsequently during 5 h at -1.2 °C, and finally at 25.2 °C. In this figure the ageing curves for 17.4 °C and 25.2 °C are also shown. Apparently, the excess-ageing at 25.2 °C causes the storage modulus to drop to

the level of the plain ageing process at these latter temperature, though at this temperature no pre-ageing occurred. From this figure it appears also that the relative effect of pre-ageing at 17.4 °C on the ageing process at -1.2 °C is smaller for the 5% solution than for the 1.95% solution: for the 5% solution the storage modulus increases during 5 h from 1800 to 6600 N/m² (i. e. 270% increase) and for the 1.95% solution from 170 to 1000 N/m² (i. e. 490% increase, fig. 7). For a 3% solution an increase of 380% was found.

It should be remarked, however, as also appears from figure 10, that for a concentration of 5% pre-ageing at 17.4 °C, strictly speaking,

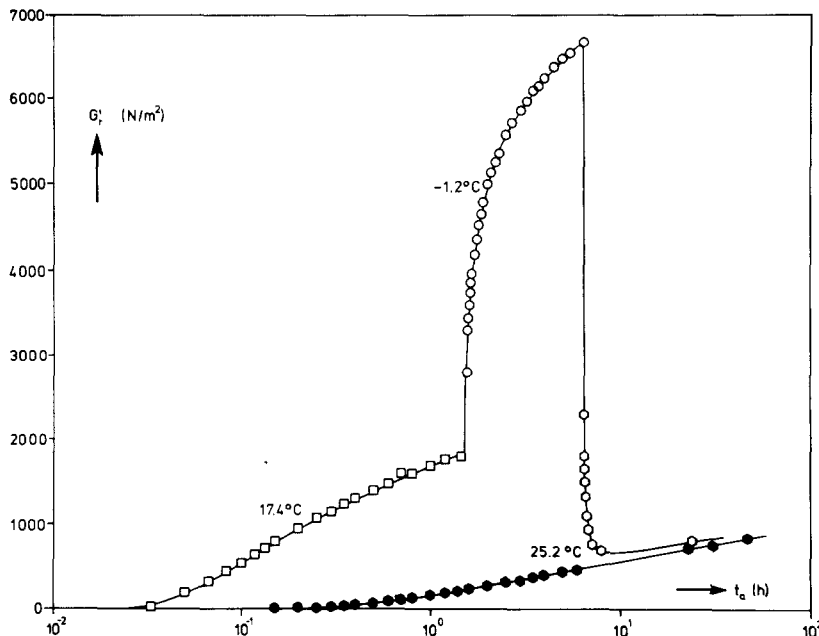


Fig. 9. Reduced storage modulus of 5.00% (w/w) gelatin/water with different ageing histories (as indicated) plotted against ageing time. Angular frequency 0.393 rad/s. Reference temperature -1.2 °C

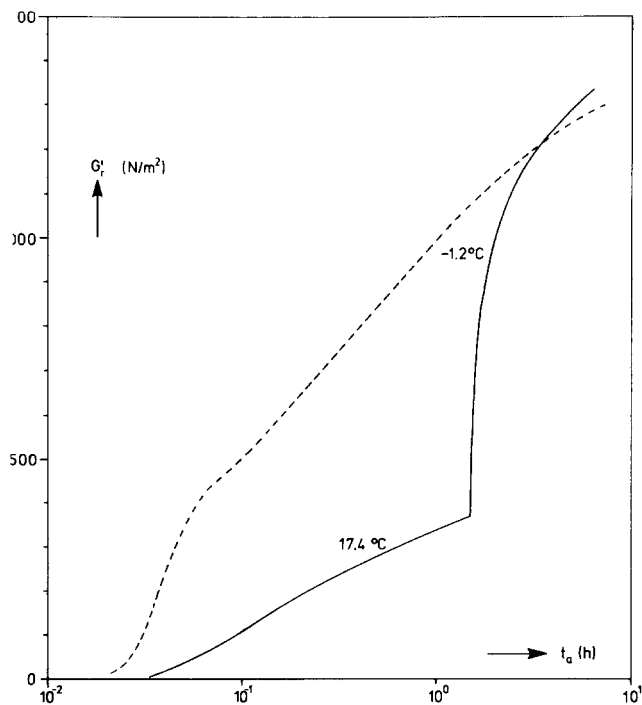


Fig. 10. Reduced storage modulus of 5.00% (w/w) gelatin/water with different ageing histories (as indicated) plotted against ageing time. Angular frequency 0.393 rad/s. Reference temperature -1.2°C

has only little effect on the overall ageing at -1.2°C : after 6.5 h of ageing the storage modulus of the pre-aged sample is only 4% higher than that of the sample which was plainly aged at -1.2°C . For 3% and 2% solutions this percentage is 31 resp. 71. It may be concluded that pre-ageing at high temperature is of great influence on the overall ageing at low temperature if the concentration is low (e. g. 2%), whereas this influence becomes smaller if the gelatin concentration becomes higher. It almost disappears at a concentration of 5%.

Discussion of the results

Pre-ageing of a 2% gelatin solution at a higher temperature has an accelerating effect on the excess-ageing at -1.2°C . This is revealed by the figures 3, 4 and 5. From this fact we might suppose that two processes are involved: a nucleation process, which takes place quickly at high temperatures, followed by a growth of structures (crystals, helices) which is fast at -1.2°C . An optimum effect is obtained by pre-ageing at 16.2°C (cf. fig. 6).

Comparison of the mentioned figures with figures 10, however, falsifies this conclusion: pre-ageing of a 5% solution at 17.4°C has a negligible influence on the overall ageing at -1.2°C . There rather seems to exist a temperature dependent dynamic equilibrium, which, however, is never completely approached in the course of the (ageing) time. This is supported by the figures 7, 8 and 9. From these figures it appears that heating up gels with different ageing histories quickly leads to a state of ageing, or at least to a storage modulus, which is also reached without pre-ageing at low temperatures.

According to Stainsby (18) a gel network is formed during cooling of a gelatin solution, due to the rearrangement of disordered gelatin molecules into collagen like structures. However, the structures which are formed, are far from perfect: at a certain temperature T_a only those structures are obtained which are stable in the temperature range $T_a < T < T_{\text{gel}}$. If this gel network is cooled to a lower temperature $T_{ea} < T_a$, first the structures already formed will grow and structures will be formed that are stable in the region $T_a < T < T_{\text{gel}}$ but, moreover, structures will be formed that are stable in the temperature region $T_{ea} < T < T_a$. The latter mentioned structures will be less stable than the first mentioned ones. On heating to the temperature T_a , they will melt again. The first mentioned structures will persist. This appears from figure 7 e. g., where an intermediate lowering of the temperature causes the storage modulus to increase strongly: structures, that are stable in the temperature region $-1.2^{\circ}\text{C} < T < 17.4^{\circ}\text{C}$, will be superposed on the network that is stable in the temperature region $17.4^{\circ}\text{C} < T < T_{\text{gel}}$. On heating to 17.4°C these structures, formed in second instance, will melt again, causing a strong decrease of the storage modulus. Since also during the time interval at -1.2°C structures which are stable at 17.4°C are formed and extended, the storage modulus is nevertheless higher than at the moment, when the temperature was lowered to -1.2°C . Heating a gel, that has been formed at T_a , whether or not followed by excess-ageing at a lower temperature T_{ea} , to a temperature T_c between T_a and T_{gel} will cause a melting of those structures that are stable in the temperature region $T < T_c$ (see fig. 11). The structures that are stable in this temperature region, will remain

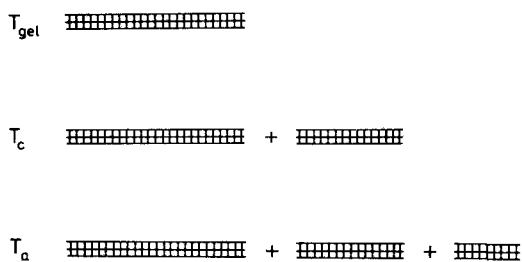


Fig. 11. Schematic view of the stability of collagen like structures as a function of temperature: $T_a < T_c < T_{gel}$. Besides large stable structures smaller ones will become stable with decreasing temperature

existing. This appears from figure 9 e. g. (also from fig. 11 of ref. (1)): heating a gel, that has originally been formed at 17.4 °C and subsequently has been excess-aged at -1.2 °C, to 25.2 °C, causes melting of structures that are stable below 25.2 °C. Only those structures that are stable in the temperature region $25.2\text{ °C} < T < T_{gel}$, remain existing.

It is remarkable that intermediate ageing at -1.2 °C (cf. fig. 7) as well as pre-ageing at 17.4 °C followed by excess-ageing at -1.2 °C (cf. fig. 9), has no effect on the growth of the structures at 17.4 °C resp. 25.2 °C: the formation and growth of the structures that are stable at the respective temperatures, was started and continued at the lower temperatures with the same rate as would have been the case at the higher temperatures. *Hence we have to conclude that formation and growth of certain structures is almost independent of temperature.* This means that the heat of activation of the rate determining step in the ageing process is rather small, e. g. smaller than 3 kJ/mol. This is in complete agreement with the conclusions of Eagland and coworkers (11). On the basis of their measurements of optical rotation and viscosity at 5 to 15 °C on very dilute gelatin solutions, these authors conclude that the reaction rate constant of the rate determining step in the ageing process of gelatin is almost independent of temperature.

Hence, the difference in degree of ageing at the various temperatures is not caused by a difference in reaction rate constant, but by increasing stability of small structures with decreasing temperature.

Finally, some remarks will be made about the results of the calculation of the heat of cross-linking. In the foregoing it was advocated that,

with decreasing temperatures, smaller structures will become stable. In determining the heat of reaction use has been made of the change of the maximum gelation temperature with the concentration. However, with the maximum gelation temperature also the extensiveness of the structures will change. Moreover, above the maximum gelation temperature also structures will be present. Their dimensions will be larger than those of the structures that are stable at and below the maximum gelation temperature. However, their number is too small to enable gelation. Hence, the melting point of a gel to a sol is not the temperature where all structures will disappear. ΔH° is the heat of formation of those structures, that are stable at the maximum gelation temperature. Pouradier (19) calculated ΔH , which corresponds to our $\Delta H^\circ / (\frac{1}{2} f - 1)$, with the aid of the temperature dependence of the shear modulus, as was shown by Eldridge and Ferry (7). He found values varying from -20 kJ/mol at low temperatures to -250 kJ/mol at high temperatures. The phenomenon, that at low concentrations intramolecular cross-linking plays an important role, has also to be taken into account. As a consequence of intramolecular cross-linking, the maximum gelation temperature (and also the melting temperature) will have lower values than those to be expected from the total cross-link density (if this quantity would be known). Hence, the results in table 1 will be too low. This inaccuracy will become more important with lower concentrations. As a consequence, the value of $-\Delta H^\circ$ will be larger than 442 kJ/mol and, as a consequence, the number of windings will be larger than 24. Accurate measurements of the maximum gelation temperature and/or the melting temperature, which are useful for the determination of the heat of reaction, have therefore to be taken at concentrations of at least 5%.

From the figures 7 and 8 it becomes obvious that, after a temperature increase, approximately 150 h will elapse before all non-stable crystallites have disappeared: in fact, the difference between the storage modulus measured after an increase of the temperature to a certain value and the storage modulus obtained with plain ageing at this value of the temperature, only disappears after 150 h. This means that the real melting temperature of a gelatin gel can only be measured, if each of many samples, which are aged in the same way, is heated to a constant

specific temperature and kept at this temperature during a long time (e. g. 150 h). In this way the melting temperature can probably be encircled. The usual methods of heating, – say – with 10 °C per h or even slower, will yield too high values for the real melting temperature of the gel (not for all structures present in the system). What the consequence of this wrong determination of the melting temperature is on the value of ΔH° is difficult to anticipate.

One final remark has to be made, however: it is difficult to understand that a melting process takes 150 h, especially if the liquid phase is present. We have to conclude that also a reorganization takes place, which causes a decrease of the number of elastically effective cross-links. It is probably not possible to distinguish between the two processes only with the aid of dynamic mechanical measurements. For this purpose other techniques will have to be used, like optical rotation, light scattering etc.

Conclusions

The mechanism of the network growth in aqueous gelatin solutions is examined:

– the maximum gelation temperature, determined by extrapolation of the induction period to infinite time, increases with increasing concentration;

– the heat of reaction of the cross-linking process was determined with the aid of the method of Eldridge and Ferry (7), but modified for hexafunctional cross-linking; a value $\Delta H^\circ = -442$ kJ/mol of cross-links, corresponding with mono-helices of 24 windings, was found; as a consequence for the length of the cross-links is calculated 230 Å;

– although the heat of reaction of the cross-linking process is high, the heat of activation of the rate determining step is very small;

– as a result the temperature dependence of the degree of ageing is not caused by temperature variation of the reaction rate constant, but

by the increase in stability of small structures which accompanies a decrease in temperature.

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