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Phase Diagram of Gelatin Plasticized by Water and Glycerol

Mara Coppola,[1] Madeleine Djabourov,[1] Maria Ferrand[2]*

Summary: Gelatin is widely used in capsules manufacturing. Most of the capsules in pharmaceutical applications are hard capsules made out of concentrated solutions of gelatin, where water has been progressively removed during the drying process. More recently soft capsules found an increasing interest in pharmaceutical and cosmetic applications where they are filled and sealed with a liquid substance. In order to keep the shells of capsules flexible after drying at room temperature, plasticizer is added to the gelatin aqueous solutions. We present in this paper a systematic investigation of gelatin films, equilibrated under a range of relative humidity (RH). The films contain glycerol as plasticizer P or only water and gelatin, (G). In order to analyze the role of the plasticizer, we fixed various P/G ratios and measured the water retention versus RH. Films were characterized by DSC (Mettler Toledo DSC823). Glass transition temperature T_g, melting temperature T_m and enthalpy associated with helix-coil transition were determined. The role of water and glycerol was examined in relation with the large variations of these transition temperatures with film composition. Non equilibrium effects are also discussed, in particular concerning the glass transition temperature, the relaxation effects and the water repartition between amorphous coils and helical structure. In conclusion, we propose a unique phase diagram of the gelatin films with any proportion of water and glycerol.

Keywords: enthalpy helix-coil; gelatin films; glass transition; glycerol plasticizer; melting temperature

Introduction

Gelatin is one of the most versatile and used gelling agents in food application but it has found many other applications in the pharmaceutical and photographic field[1] as it is an abundant raw material, produced all over the world at low cost and with excellent film forming properties.[2]

Gelatin is obtained by thermal denaturation of collagen, which is the most common protein in the animal kingdom and consists of three polypeptide chains, each one twisted in a left-handed helix and supercoiled together to form a right-handed triple helix.[3] There are two main types of gelatin. Type A is extracted from collagen by acid treatment, type B by alkaline one. During the denaturation process the triple helix structure is broken to form a random-coil gelatin. In aqueous solutions, the chains coil around each other to form a collagen-like triple helix, when the solution is cooled below its helix-coil transition temperature.[4] In this way a three dimensional network is created: if the solution concentration is around 1 to 10 wt%, the system is used as a gel, which is a soft solid well know in food applications. If the solution concentration is much higher (around 30 wt%) the system is in general aimed to be used as a film for capsule formation and it contains various amounts of plasticizer, in addition to the water, which is natural solvent of the protein. As the films are let to dry at room

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[1] Laboratoire de Physique Thermique, ESPCI, 10 Rue Vauquelin, 75231 Paris Cedex 05, France
E-mail: mara.coppola@espci.fr
[2] Capsugel, 10 Rue Timken, 68027 Colmar, France

temperature under controlled atmospheres, they become brittle in absence of a plasticizer. For the production of soft capsules for pharmaceutical and cosmetic applications glycerol, special grades of non-crystallizing aqueous sorbitol and sorbitol-sorbitan solutions are the most used plasticizers.[5] In this paper we examine the phase diagrams of gelatin films containing various amounts of glycerol as plasticizer. The water content of the films varies during dehydration; glycerol is a non volatile component and has a constant mass during aging of the films. The role of the plasticizer is controlled by the ratio P/G, the mass of plasticizer (glycerol) over the mass of gelatin. The experimental work is based on Differential Scanning Calorimetry (DSC) measurements which characterize the states of the films after equilibration under controlled atmospheres. Firstly, we present the water retention versus relative humidity (RH); then the films are characterized by their glass transition temperatures, T_g, the triple helix melting temperatures T_m and the associated enthalpy. The role of water and glycerol is examined in order to explain the variation of T_g and T_m with the film composition. Non equilibrium effects are also discussed, in particular concerning the glass transition temperature, the relaxation effects and the water repartition between amorphous coils and helical structure. In conclusion, we propose a unique phase diagram of the gelatin films with any proportion of water and glycerol.

Material and Methods

Gelatin extracted from bovine hide was used in this study and it was supplied by Weishardt International SRO and has the following characteristics: 160 g Bloom, 11% moisture content and a viscosity of 3.86 mPa.s at $c = 6.67\%$ and 60 °C. Gelatin (G) was used without any previous treatment. Glycerol 98% purity (P) and deionised water (resistivity 16 MΩm) were used to prepare gelatin solution. P designates glycerol which is used to plasticize the films. Gelatin films were obtained from a 30 wt% gelatin solution where solvent was either pure water or a mixture of water and glycerol. In order to study the effect of the plasticizer (glycerol) on thermal properties, six ratios P/G were chosen: P/G = 0, 0.1, 0.3, 0.5, 0.7 and 1. Gelatin was let swelling in its solvent during 18 hours at 3 °C and then dissolved at 60 °C during 3 hours in a thermostatic oven, without any stirring to avoid bubble formation. The solution was then spread between two 20 × 20 glass plates and a 0.8 mm thickness of the film was achieved by placing two calibrated spacers between the plates. The film was kept covered overnight to let the gel formation with no evaporation phenomena occurring. Afterwards, gelatin films were finally cut in several parts and conditioned at 23 °C in desiccators using saturated solutions, to keep the relative humidity constant. The saturated salt solutions were chosen in order to vary relative humidity RH between 12% and 94%. An additional desiccator containing silica gel was used to achieve the highest level of dryness of the film (RH = 1%). Films were prepared in duplicates and weighed before and after drying. After 2 weeks, as the weight of the films remained constant, it was considered that the equilibrium was achieved. All films were analyzed through Differential Scanning Calorimetry (DSC), by using Mettler Toledo DSC823. The samples were stamped out of the films and were hermetically sealed in 40 μl aluminium pans. The reference was an empty pan and the system was calibrated with indium ($T_m = 156.5\,°C$, $\Delta H_m = 28.68\ Jg^{-1}$) and zinc ($T_m = 419.47\,°C$, $\Delta H_m = 109.98\ Jg^{-1}$). The thermal protocol was identical for all samples: the samples were introduced at room temperature, then they were rapidly cooled (under a 50 ml/min flow of N_2) to the lowest temperature which can be achieved in our instrument, −50 °C, then the heating ramp started between −50 °C and T_{max} °C with a rate of +10 °C/min allowing measurements of the glass transition and melting temperatures. The final temperature T_{max} was adjusted between 110 and 180 °C according to the composition of sample. Afterwards, a cooling ramp with a rate of -10 °C/min was performed to bring the film at -50 °C, then the samples were let for 5 minutes at this temperature before starting a second heating ramp at $+10$ °C/min during which the measurements were again performed. The glass transition temperature (T_g) and the melting temperature (T_m) were calculated as the onset of the specific heat step change and the onset of the endothermic peak respectively. The enthalpy associated to melting was calculated as the area of its endothermic peak with a linear baseline, tangent to the peak on both sides, outside the onset and endset temperatures.

Results and Discussion

Water Desorption Versus Relative Humidity RH and Plasticizer

The mass of water retained by the films per gram of gelatin W:

$$W = \frac{m_w}{m_{gel}}$$

was calculated at the end of the maturation time (water desorption) for each sample at different RH. W is related to gelatin concentration in films through the equation:

$$c_{gel} = \frac{m_{gel}}{m_{gel} + m_w + m_{glyc}} \qquad \frac{1}{c_{gel}} = 1 + W + \frac{m_{glyc}}{m_{gel}}$$

where:

m_w = mass of water
m_{gel} = mass of gelatin
m_{glyc} = mass of glycerol
c_{gel} = gelatin concentration (wt%)

The desorption isotherms (T = 23 °C) are shown in Figure 1 (low RH) and Figure 2 (1% < RH < 100%). One can notice that W varies between W = 0.05 and W = 1.5 according to the composition of the film and to the RH. The desorption isotherm of native collagen (in a kangaroo tendon) is shown for comparison with the dehydrated films of gelatin.[6] Native collagen has a fibrillar structure with a specific supermolecular structure, which is absent in gelatin dry films. Native collagen retains more water than gelatin films in pure water. The difference means that the triple helix conformation and the organisation in fibres favour the retention of water molecules in the vicinity of the protein. Indeed, water

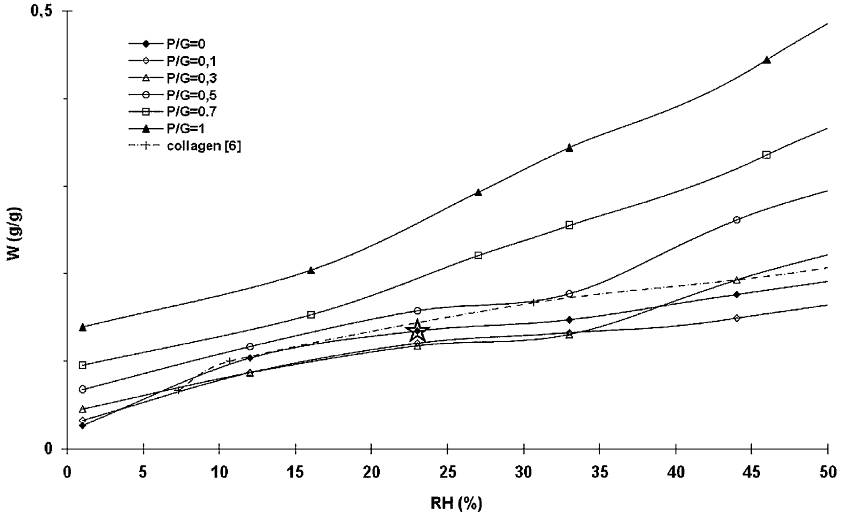

Figure 1.
Desorption isotherms of gelatin films at 23 °C - low RH.

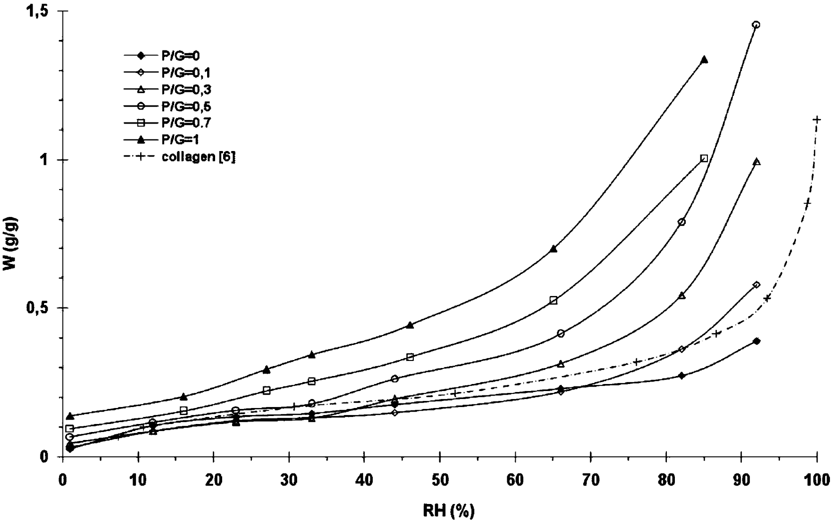

Figure 2.
Desorption isotherms of gelatin films at 23 °C - 1% < RH < 100%.

molecules placed at interstitial positions inside the triple helix contribute to the hydrogen bonding and stabilisation of the triple helices. These molecules are very strongly bound to the protein. Besides, the early investigation of collagen fibers[6] estimated the amount of water bound to the polar side-chains, carbonyl groups and peptide linkages to give a total contribution $W = 0.136\ g_{water}/g_{protein}$ for the primary layer of water uptake. The primary layer of sorption is completed at RH = 20% for collagen fibers and RH = 23% for films with only water as solvent. Films containing glycerol with a ratio of P/G = 0.1 and P/G = 0.3 show a water retention slightly below non plasticized films (P/G = 0) respectively up to RH = 40% and RH = 70%. Similar observations were made by Godbillot *et al.*[7] and R.A. Talja *et al.*,[8] who analyzed water binding in starch films plasticized by glycerol; the authors suggest a saturation of starch binding sites with glycerol when plasticizer is preponderant at low relative humidity. Above these values, plasticized gelatin films retain more water because of the hygroscopic property of glycerol. When P/G = 0.5, 0.7 and 1 films present always a higher moisture content in the whole range of relative humidity. W of pure glycerol varies exponentially between 0.05 and 1.5 g_w/g_{glyc} for 10% < RH < 90%, this is the reason why only at high moisture content its hygroscopic property has a large influence on water uptake of plasticized gelatin films.

Glass Transitions: 1st Scan - 2nd Scan P/G = 0

In Figure 3 a typical DSC scan is shown during a heating ramp from −50 °C to 120 °C. The heat flow exhibits two important transitions during the first scan: the glass transition T_g which is characterized by a change on the position of the base line (change of heat capacity) and in this case, by an endothermic recovery (glass transition with relaxation), followed by an important endothermic peak corresponding to the helix-coil transition. On the trace of the second scan, only a glass transition appears, at a temperature which is lower than the one obtained during the first scan. Sobral *et al.*[9] studied thermal properties of pigskin gelatin films by DSC and they also found out that during the 1st scan, the films present both glass transition and melting,

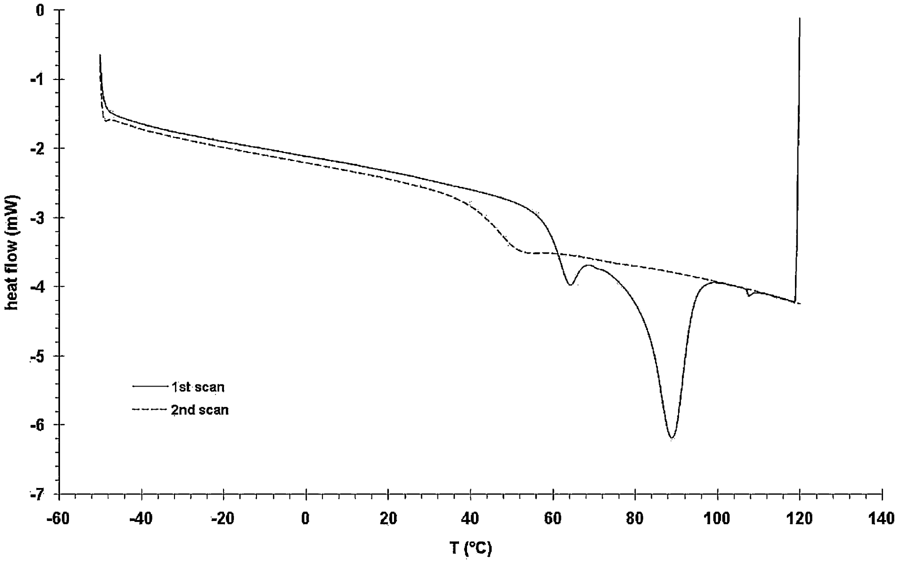

Figure 3.
DSC traces of a non plasticized gelatin film (P/G = 0) conditioned at RH = 44%.

whereas during the second heating ramp the samples show only a glass transition at a temperature, which is lower than the one of the 1st scan.

The width of the glass transition region is 20 °C. Between T_g and T_m, the film is flexible and contains a semi-crystalline structure, due to the presence of triple helices, during the 1st scan. Above T_m, all helices are melted and the film is in a viscous, liquid state (a melt, with a small quantity of solvent). By cooling down the sample again at −10 °C/min we do not allow the film reform the helices and the gelatin presents a purely amorphous state (coil conformation) during the second heating ramp. The shift observed between T_g of the 1st and of the 2nd heating scan may be related to a different repartition of water in the sample: in the sample heated the first time, part of water is immobilized among the peptide chains which constitute the triple helices. Once the helix-coil transition occurs, this part of water is totally available for the amorphous phase which softens at a lower temperature because water acts as a plasticizer. Chi-An Dai *et al.*[10] analyzed the thermal properties of renaturated gelatin by using conventional and modulated DSC: they also found out that gelatin films stored at room conditions posses a semi-crystalline structure, showing a glass transition temperature followed by an endothermic peak associated with the helix-coil transition during the first heating ramp. The authors[10] studied gelatin films dried at 50 °C and stored at 30% of relative humidity which were completely amorphous and they found that the glass transition temperatures of the two heating ramps are nearly identical.

The comparison between the 1st scan and the 2nd scan reveals other differences which are shown in Figure 4. In this figure, an enlargement of the heat flow in the glass transition temperature zone is shown during the 1st scan. The curves belong to gelatin films with no plasticizer (P/G = 0) dried at different relative humidity. Two main aspects can be observed. First, the onset of glass transition is shifted to lower values as RH increases (plasticizing effect of water). The corresponding values for W are indicated, they increase with RH. On the other hand, an increasing W gives raise to the appearance of a relaxation endothermic peak in the glass transition

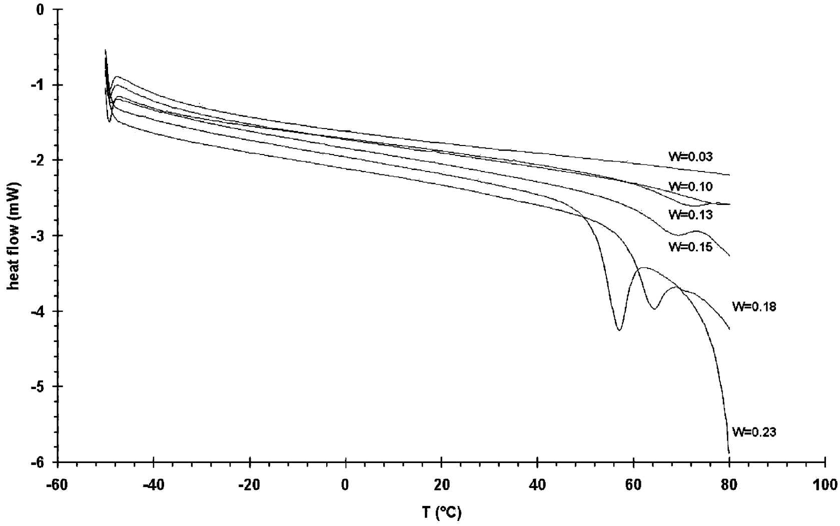

Figure 4.
Effect of bound water on the relaxation of the glass transition (P/G = 0), 1st scan.

during the first ramp of heating, which corresponds to the release of the energy stored by the sample during the equilibration period in desiccator.

The amplitude of the change of heat capacity is ΔC_p during the 1st scan is shown in Figure 5. ΔC_p goes through a maximum with increasing gelatin concentration or increasing T_g. Two limits are observed: i) at low concentrations $c_{gel} = 72$ wt% ($W = 0.39$), the glass transition temperature is low ($T_g = -11\,°C$), the overall mobility of the chains is reduced and the change of heat capacity ΔC_p is small ($\Delta C_p = 0.26$ J/Kg$_{gel}$), ii) at high concentrations, when $c_{gel} = 88$ wt% ($W = 0.13$) the amount of water is low, less than one layer adsorbed, the glass transition temperature

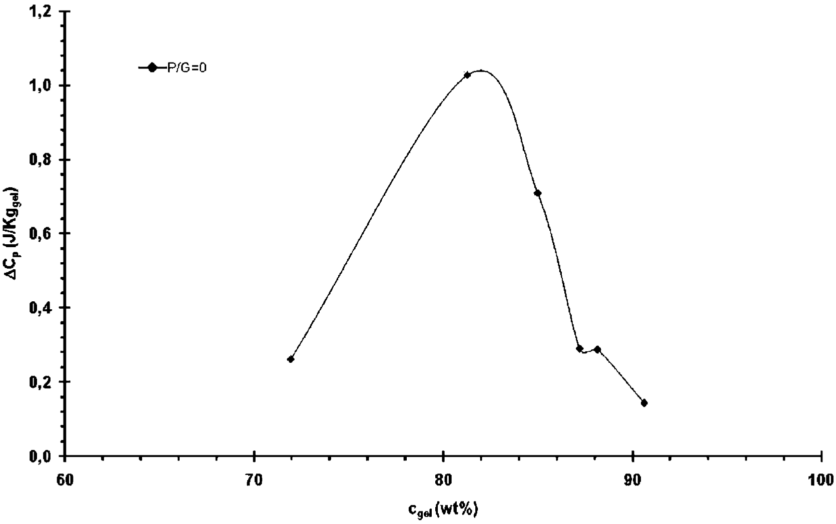

Figure 5.
Amplitude of the change of the heat capacity ΔC_p versus gelatin concentration.

is high, $T_g = 60\,°C$ and the free volume allowed for the protein to recover more mobility is reduced; again, ΔC_p is small ($\Delta C_p = 0.28$ J/Kg$_{gel}$). iii) At intermediate concentrations, $c_{gel} = 80$ wt% ($W = 0.25$), ΔC_p increases up to $\Delta C_p = 1$ J/Kg$_{gel}$ and the glass transition is around $T_g = 50\,°C$. The free volume is optimum for this amount of water and the relaxation amplitude is also significantly larger. The qualitative difference between the relaxed and the non relaxed glass transitions is known as an aging effect mainly in bulk polymers. For these protein films, it appears to be mainly related to the amount of water bound to the gelatin or plasticizer.

Glass Transitions in Presence of Glycerol

The glass transitions during 2nd scans of the amorphous films are summarized in Figure 6 for various amounts of plasticizer. The glass transitions of 2nd scans do not show relaxation effects, the vitreous state is a very "new" one, there was no time allowed for maturation of the structure. It is well known that this metastable state depends on the whole history of the sample, glass transition temperatures are not constant values, but can evolve in time (the maturation effects can be present also in these films, in addition to the new water reparation after melting of the triple-helices). For films containing only water T_g increases from $-20\,°C$ $c_{gel} = 72$ wt% to $160\,°C$ for $c_{gel} = 98$ wt%. Water acts as a plasticizer and decreases the glass transition temperatures (low concentrations). Films dried in desiccators with very low relative humidity are very brittle, and not easy to handle at room temperature, when $P/G = 0$ or 0.1, whereas those with $P/G = 0.3$ to 0.7 are soft at room temperature except for very dry atmospheres. The large amount of plasticizer allows keeping the films flexible at room temperature, in almost all atmospheres. The composition which corresponds to the first layer of adsorbed water is shown with a star in Figure 6.

In presence of plasticizer, the glass transition temperatures observed at $W = 0.13$ are lower and lower, which is another way to illustrate the substitution of glycerol to water in the vicinity of the protein.

Melting Temperatures and Enthalpies

It has been shown in our previous investigations that the enthalpy associated to the helix-coil transition $\Delta H = 56$ J/g of helix[11] is independent of the concentration of the films in a range up to $c_{gel} = 30$ wt% in

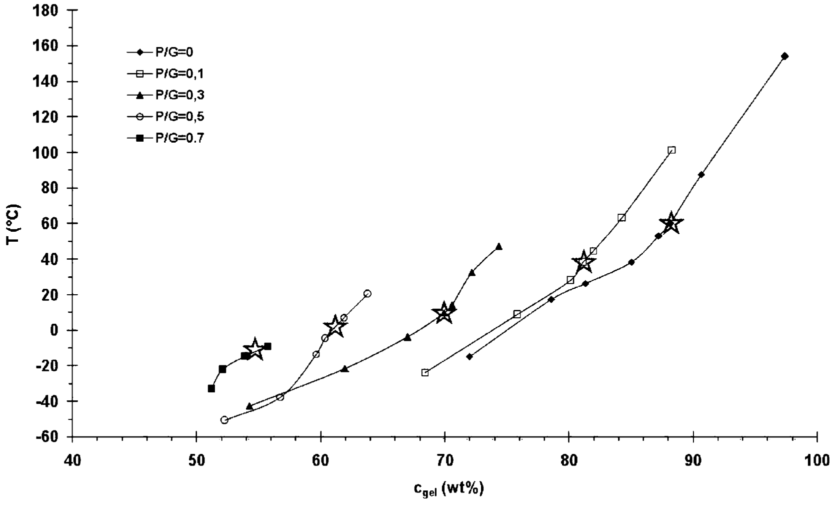

Figure 6.
Glass transition temperatures of gelatin films with various amounts of plasticizer (2nd scans).

presence of glycerol or water. Therefore, the area of the peaks is related only to the amount of helices present in the film. This in turn is controlled by the history of the film and in particular to the drying rate at the beginning, when the films are put in desiccators. Generally, the enthalpy varies between 20 and 30 J/g_{gel} meaning that the percentage of helices formed represents 35% to 53% of the weight of gelatin, which is in agreement with experiments performed in gels with various concentrations of protein cooled at room temperatures.

In Figure 7 melting temperature of films are plotted versus the concentration of gelatin in the film for different ratios P/G. For non-plasticized films (P/G = 0), T_m dropped from 180 °C to 50 °C when gelatin concentration varies between 98 wt% and 72 wt%. For $c_{gel} = 88$wt% the amount of bound water to gelatin is W = 0.13 and corresponds to the first layer of water sorption, with higher gelatin concentrations, the amount of bound water is almost one layer and one can see that the melting temperatures increase very strongly.

By adding glycerol, the range of gelatin concentrations which can be reached under drying conditions is obviously reduced from a maximum $c_{gel} = 98$ wt% for P/G = 0, to a minimum $c_{gel} = 32$ wt% when P/G = 0.5. The range of melting temperatures is also reduced: it is only between 35 and 80 °C for P/G = 0.5, while it is between 50 to 180 °C for P/G = 0.

In Figure 7 we also indicate the concentration which corresponds to the first layer of water by a star. When P/G increases, the melting temperatures of the films with W = 0.13 decrease accordingly, meaning that glycerol contributes itself to lower the melting temperatures.

There is a range of gelatin concentrations approximately $c_{gel} < 50$ wt% where T_m is constant at $T_m = 35$ °C, thus all binding sites on gelatin are saturated by water and glycerol and this state corresponds to a gel. We expect the triple helices to be randomly oriented in the gel state (see below) and to melt at a temperature which is below collagen melting temperature.

The Phase Diagram of Gelatin Films with Water and Glycerol as Plasticizers

In order to summarize the overall behavior of the gelatin films with various amounts of glycerol, P/G varying between 0 and 1, stabilized in various atmospheres with relative humidity varying between 1 and 100%, we propose to evaluate the efficiency of the two plasticizers, water and glycerol, by adding the contributions of the OH

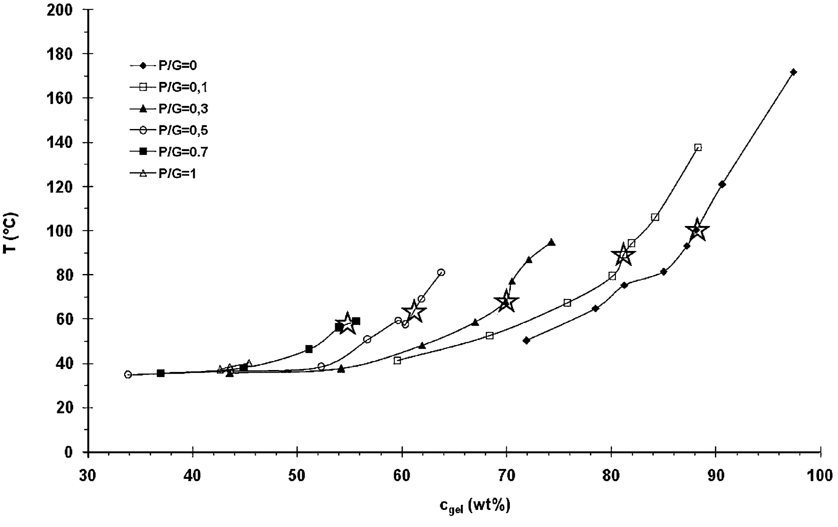

Figure 7.
Effect of gelatin concentration on melting temperatures.

groups resulting either from one or the other of the solvents. Therefore, we choose as a unique variable, the ratio r defined as:

$$r = \frac{number\ of\ OH\ groups}{number\ of\ protein\ residues}$$

Taking the molecular weights:

$M_{water} = 18$ g/mole, a water molecule containing 2 OH groups
$M_{glycerol} = 92$ g/mole, a glycerol molecule containing 3 OH groups
$M_{gelatin\ residue} \approx 100$ g/mole

The ratio r is given by the following relation:

$$r = 2\frac{m_w}{m_{gel}}\frac{1}{18}100 + 3\frac{m_{glyc}}{m_{gel}}\frac{1}{92}100$$

In absence of glycerol, r is related to W by:

$$r = \frac{200}{18}\ W = 11.1\ W$$

In Figure 8 we have plotted the melting temperatures T_m of the 1st scan and the glass transitions T_g of the 2nd scan, versus the ratio r. It appears that all the data collapse almost perfectly for the melting temperatures T_m and it is also very close for the T_g. As we already pointed out, the glass transitions being measured for recently cooled amorphous state, we believe that the scatter may be inherent to the metastable nature of the vitreous state. It is possible that by keeping the films in amorphous state for longer periods of maturation we could observe different values for T_g, which could be narrower with the respect of the amount of OH groups. It is remarkable that water and glycerol are interchangeable in the vicinity of the protein, at least concerning their effect on melting and glass transition of the films, over a very wide range of temperatures.

The different structures of the film are indicated on the Figure 8:

- Above T_m, the polymer is in random conformation, it is a very viscous liquid state, like a melt or a very highly concentrated solution with various amounts of solvents.
- Between T_m and T_g (considering T_g of 2nd scan as a border line of the amorphous state) the film is a flexible semicrystalline material, the amorphous part (coil conformation) is the plasticized by the presence of OH groups of the sol-

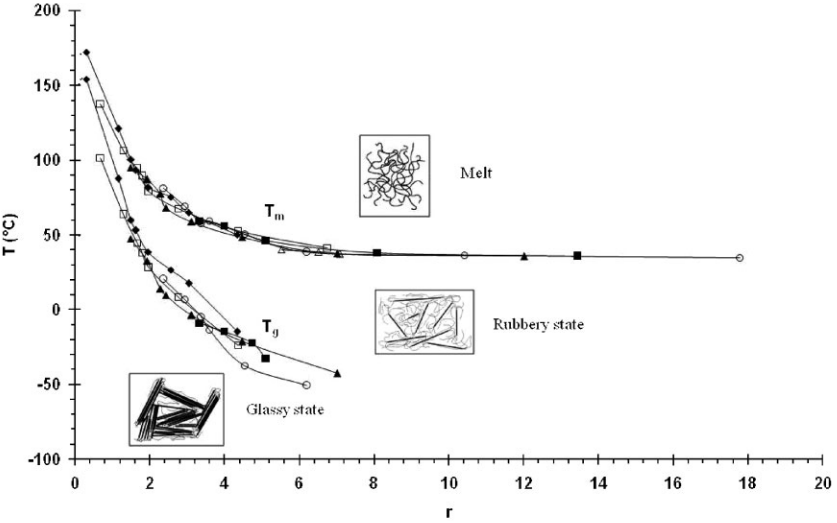

Figure 8.
Master curve for melting and glass transition temperatures versus the ratio *r*.

vents. For $r < 7$, the triple helices are probably packed into bundles due to steric interactions.[12] In this state of aggregation, the enthalpy of melting is equal to the one measured in gels[11] however the melting temperatures are considerably higher. In the concentration range $r > 7$, the melting temperature of the film become constant (at 35 °C at the scanning rate of $+10\,^{\circ}$C/min) and comparable to the denaturation temperature of collagen rods dispersed in aqueous solutions (slightly lower because of imperfections or loops of the renatured triple helices[4]). On this side of the diagram, corresponding to the less concentrated films or gels, the triple helices are not aggregated (randomly oriented triple helices).

o Below T_g, ($r < 7$) the films are in the glassy state: they are brittle, with bundles of triple helices and amorphous sequences; the plasticizers are not able to provide flexibility to the film. For practical applications, these plasticizers (water and glycerol) are efficient in the intermediate range of temperatures, between T_g and T_m.

Conclusion

We have investigated the phase diagram of gelatin films containing various amounts of water and glycerol. The films were equilibrated in atmospheres with controlled humidity at room temperature. The films are semi-crystalline: during the heating ramps starting at low temperatures, the glass transition and the melting temperature were both well determined. The measurements show the large effect of the amount of plasticizer on these temperatures. We propose for the first time a unique phase diagram of gelatin films based on the ratio of OH groups (water or glycerol) per residue, which controls both the melting and the glass transition. This analysis could to be used for other types of plasticizers.

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