Molecular Weight Influence on Gelatin Gels: Structure, Enthalpy and Rheology

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Summary: This paper deals with the gelation properties of gelatins with various molecular weights. The samples are 4 different extracts from limed bones. We investigated firstly the structural properties: the helix amounts by optical rotation measurements and the enthalpy of helix formation and melting, with the same thermal protocols and for various concentrations. Comparison between the two methods allowed deriving the enthalpy of helix-coil transition and melting. Rheology was performed under the same conditions with a stress controlled rheometer working with a fixed, very small, strain during gel formation, maturation and melting. Correlation between all the measurements confirms the existence of the master curve for storage modulus versus gelatin concentration for all samples investigated.

Keywords: coil-helix transition; enthalpy; gelatin; molecular weights; rheology

Introduction

It is well established that the physical (thermoreversible) gelation of gelatin is due to the coil to triple helix transition of the gelatin chains.[1] The structural parameters of the renatured triple helices are identical to native collagen (as it can be seen, for instance, by wide angle X-ray diffraction), with however some important differences, which are intrinsically related to gelatin. In native collagen, the single chains (α-chains) have a well defined molecular weight (circa 110 000 g/mole), but extraction of gelatin from bones or skins involves some molecular degradation giving rise to a distribution of molecular weighs, containing sub-units of α-chains. In addition, some crosslinks between chains, which develop with aging of the animals, will survive to the chemical treatments during extraction and provide a fraction of higher molecular weights, called β-chains (two crosslinked chains) or γ-chains (three crosslinked chains) and even higher orders, called microgels. Thus the molecular weight distribution of gelatin is related to the processing of the raw material and to native state of collagen in tissues. It is also known that the molecular weight distribution has a great influence on gel properties (functional properties). At a given concentration, low molecular weights will form soft gels, which are used in general in food applications, high molecular weights are used in photographic and pharmaceutical applications because they provide stronger gels. Besides, very low molecular weights, known as hydrolysed gelatin, do not gel at all. The effect of the molecular weight is not easy to understand. It cannot not be analysed independently of the concentration of the solutions and of the kinetics of gelation, because there is no equilibrium of the gel state. In this paper we compare the properties of gelatins from four different extracts of the same raw material (limed bone).

Materials and Methods

The gelatin samples, kindly provided by Gelita AG, are various extracts from limed bone (LB), mainly from beef bones. The

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Table 1.
Characteristics of the gelatin samples.

Gelatin sample	Bloom (g)	Viscosity	Isoelec. point	Mw	I_p	α	β	γ	sub-units
	(g)	(mPa s)		g/mole		(%)	(%)	(%)	(%)
LB1	327	4.98	5.2	157 204	1.9	38.3	18.8	14.7	21.6
LB2	303	4.68	5.2	159 053	2.2	27.1	18.9	16.5	29.7
LB3	277	4.75	5.2	166 953	2.4	21.4	19.3	18.2	31.8
LB4	233	4.45	5.2	160 698	2.6	18.6	19.3	17.6	35.7

various extracts were characterised by their Bloom, their viscosity at a concentration of 6.67% g/g at a temperature of 60 °C, their molecular weight distribution and their isoelectric point. The data is summarized in Table 1. The molecular weight distribution is also shown in Figure 1. By increasing the number of the extract, from 1 to 4, one notices that the fraction of α chains decreases continuously, while the fraction of subunits increases, because of an extensive hydrolysis of the protein. In the molecular weight distribution of gelatins, the "α chain" contribution lies between 70 000 and 125 000 g/mole range. The extracts also contain a larger proportion of high molecular weights, above γ chains, which are the less soluble fractions of the raw material. The average M_w is not changing too much in this series, but the index of polydispersity I_p increases from 1.9 to 2.6. In the extract LB 4 the distribution is almost flat, and the presence of α chains is hardly visible.

Optical rotation measurements were performed with a Perkin Elmer 341 polarimeter equipped with a PC computer with software allowing simultaneous measurements of the optical rotation angle and temperature (the wavelength was 436 nm). The enthalpy of helix formation and melting, (cooling or heating at a rate of 0.05 °C/min) was measured on a micro-DSC III microcalorimeter from Setaram, Caluire, (France) using vessels with volumes of 1 mL. Rheology was performed under the same thermal protocols with a stress controlled rheometer (AR 1000 from TA Instruments) working with a fixed, very small, strain (0.5%) during gel formation, maturation and melting and the frequency is 1 Hz.

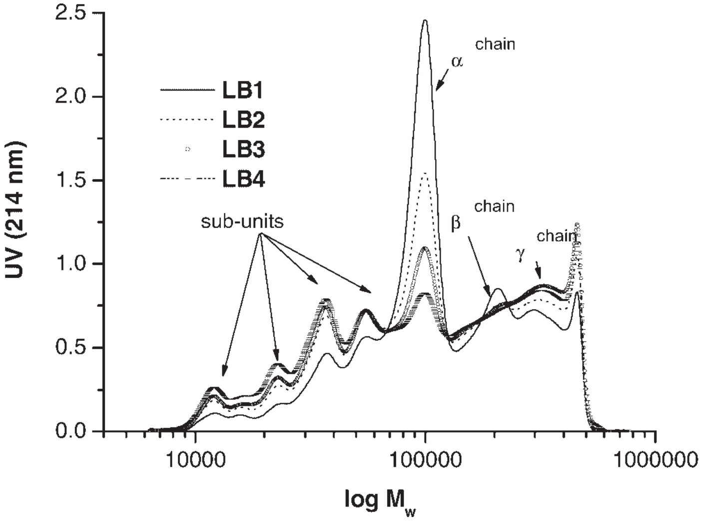

Figure 1.
The molecular weight distribution of the 4 extracts of limed bone LB.

Results and Discussion

1. Helix Formation

We report on Figure 2 helix formation of the four extracts during cooling at –0.1 °C/min and maturation. The concentration was of 4.5% g/g. The method of evaluation of the helix content is based on collagen optical rotation and it was published elsewhere.[2] The helix amount χ is the percentage of residues which are in the helical conformation. The helix amounts χ of the various samples evolve at different rates. The difference between samples is quite large at the end of the maturation (40 000 s or 11 hours) ranging from $\chi = 0.47$ for LB1 to $\chi = 0.32$ for LB4.

The time evolution of the helix amount is still visible even after 11 hours for all samples, there no limit which can be anticipated from the kinetics shown in Figure 2. The measurements illustrate the large influence of the molecular weight distributions, where the presence of important amounts of sub-units and large molecular weights hinders the helix renaturation. The chains with large molecular weights are β, γ and highly branched chains, not linear chains. The sub-units are too short to create entanglements and this obviously has an effect on the nucleation of the triple helices.

The melting curves obtained by raising temperature from 20 to 45 °C and after taking the derivative of χ (T) are shown in Figure 3.

The curves superpose between 20 and 25 °C, showing the same amounts of less stable helices for all samples, while towards the high temperature side, there is a higher stability for LB1. The peak position corresponds to 29 °C for LB1 and the half height is $\Delta T = 6.5$ °C and respectively to 27.4 °C and $\Delta T = 8$ °C for LB4. The melting temperatures reflect the distribution of lengths of the renatured triple helices and are strongly dependent on the index of polydispersity of the samples. Concentration effects are shown in Figure 4 for LB1: the helix amount is larger for larger concentrations. However, during the cooling ramp (–0.1 °C/min) the difference is visible mostly between 4.5 and 13.5% g/g, while it tends to be less pronounced between 13.5 and 22% g/g. At the end of maturation for these extracts, the values of the helix amount are $\chi = 0.47$, 0.54, 0.67, when concentration is increased by a factor of 5, the helix amount is multiplied by a factor 1.4.

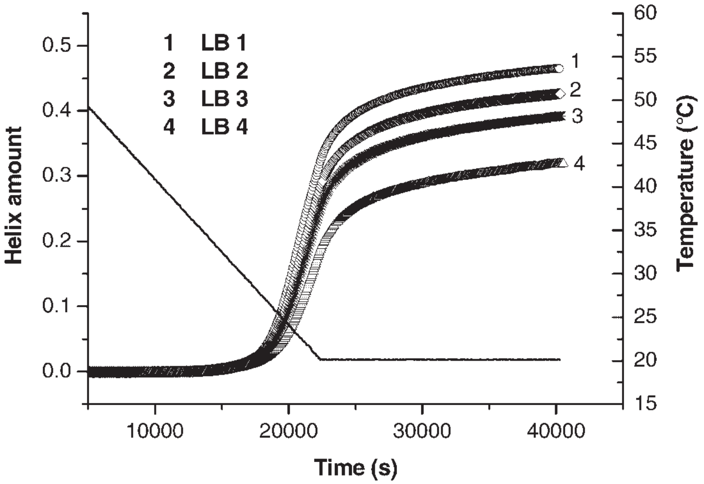

Figure 2.
Kinetics of helix formation during cooling and maturation at 20 °C for LB with various molecular weights ($c = 4.5\%$ g/g). The helix amount is called χ.

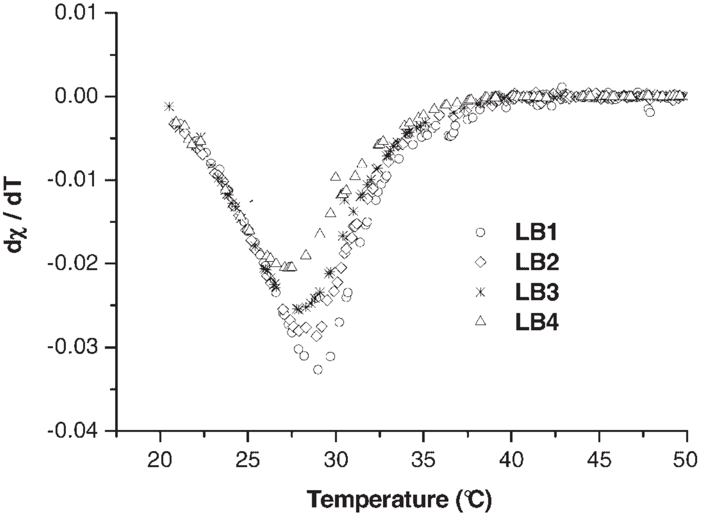

Figure 3.
Melting of helices obtained after 5 hours of maturation at 20 °C for different extracts ($c = 4.5\%$ g/g). The Y axis is the derivative of χ versus temperature.

These curves illustrate the complex kinetics of helix growth: during the cooling step the probability of nucleation of sequences is very sensitive to concentration, at the very beginning, as the triple helices appear first for the higher concentrations, while the kinetics during the ramp and the isothermal part of the plot are less concentration dependent (in Figure 4 the χ(time) curves for different concentrations are parallel). Annealing allows more helices to develop, through a process of elimination of loops or defects and growth of existing sequences. In the annealing step,

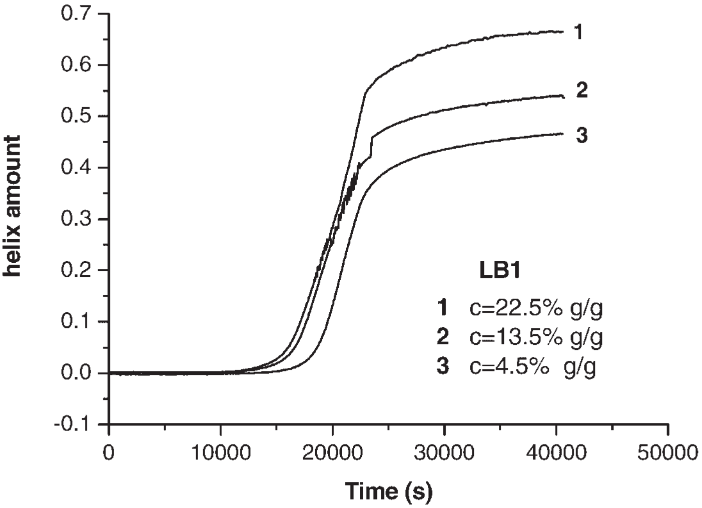

Figure 4.
Helix amounts χ versus temperature during formation and maturation for LB1 at different concentrations.

the thermal stability of the triple helices also increases, as it has been shown in the past in the literature.

2. Enthalpy

Because optical rotation measurements are limited in principle to low concentrations, we decided to develop the microcalorimetric approach which could extend the practical range of measurement of the structure from gels to almost dry films. The heat exchanged during gelation or melting is related to the hydrogen bonds which stabilise the triple helix. The pioneer work of Privalov and coworkers[3] is the reference for native collagen in solution. The denaturation temperature is well defined and the transition from native state to random coil is very sharp. For gels, there is a large spread in temperatures for helix formation and melting and equilibrium doesn't exist. The comparison between the helix concentration and the corresponding enthalpies must be performed with great caution, as this is not a classical situation for phase transitions in thermodynamics.

The thermal protocol was identical for cooling and heating at a fixed rate ±0.05 °C/min. The exothermic heat flux during cooling is shown in Figure 5 corrected by the base line measured in the high temperature range. The heat flux shows a maximum around 25 °C then decreases, indicating that the rate of conformational change decreases after a certain temperature or a certain amount of helix.

On the low temperature range, one does not reach the base line again, because there is still a positive heat flux which indicates that helix formation continues. Integration of the signal versus time gives the total heat exchanged, Q (mJ), from the moment when the renaturation starts. The heat increases with time or decreasing temperatures. In parallel, the optical rotation measurements in Figure 6 show the increase of the helical concentration c_{hel} (g/cm^3) in the solution. The helical contcentration c_{hel} is defined as the product of the helix amount χ by the gelatin concentration, c. It is expressed in (g/cm^3):

$$c_{hel} = \chi c$$

The derivative of this curve, dc_{hel}/dT is similar to heat flux. By dividing the total heat Q by the weight of the sample, we have a normalised value Q_{norm}(mJ/g) which may be compared to the concentration of helices c_{hel} (g/cm^3). The density of the solution being close to 1, the ratio of the two sets of data allows calculating the enthalpy of helix

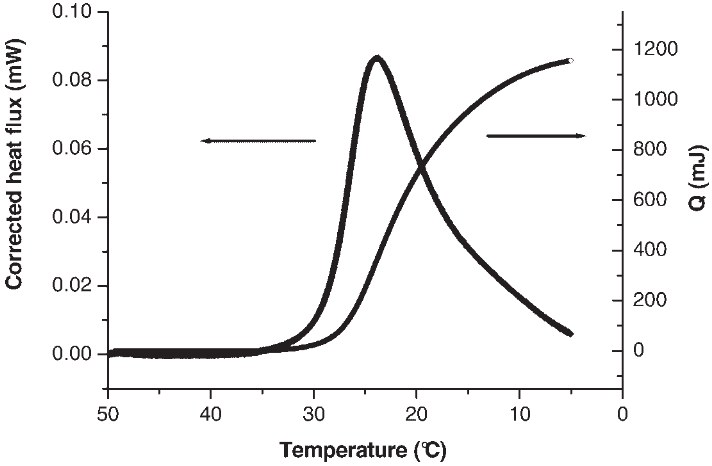

Figure 5.
Heat flow corrected for the base line and heat released Q as a function of time for LB 1, c = 4.5% g/g.

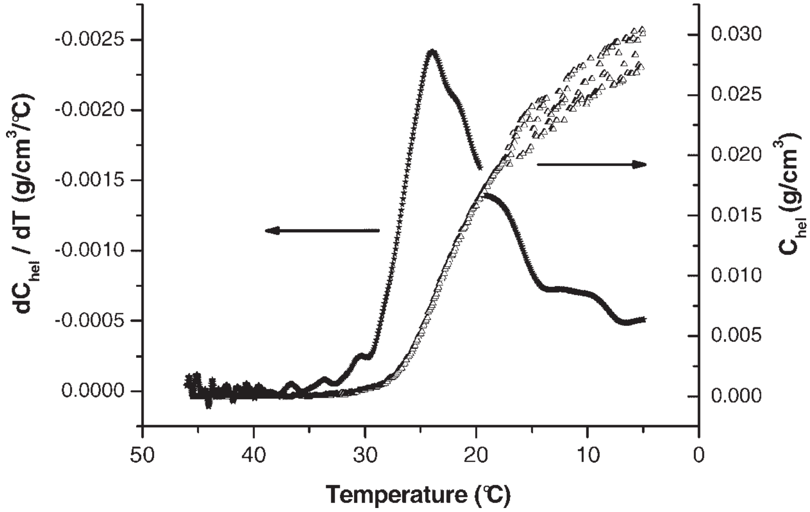

Figure 6.
Helical concentration and its derivative for LB1 (c = 4.5% g/g) at a cooling rate of −0.05 °C/min.

formation per gram of helix, as shown in Figure 7.

The experiments were performed at four concentrations, from 4.5% to 17.8% g/g for LB1 sample and at two concentrations, 4.5 and 13.5% g/g, for LB4. The enthalpies are summarized in Table 2. One finds an average of -55 ± 2 J/g of helices, during cooling ramps.

The data shows that the enthalpy of helix formation per gram of helix is independent of gelatin concentration and of the molecular weight of the extract: it is constant during the cooling ramp, which means that

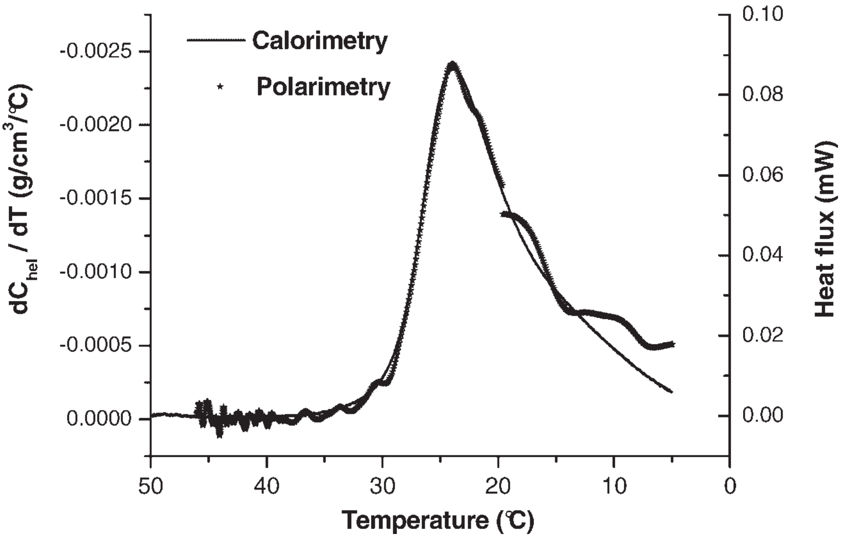

Figure 7.
Superposition of the heat flow and derivative of the helical concentration versus temperature during the cooling ramp for LB 1 (c = 4.5% g/g).

Table 2.
Enthalpy of helix formation at different concentrations and for various extracts of limed bone.

Samples	Concentration (g/g)	ΔH (J/g of helices) (during the cooling ramp)
LB1	4.5%	-55 ± 1
	8.9%	-56 ± 1
	13.4%	-55 ± 1
	17.8%	-55 ± 1
LB4	4.5%	-55 ± 1
	13.5%	-57 ± 1

there is no measurable effect related to the temperature of formation. The range of melting temperatures of the gel changes with concentration (data not shown). However the full length of the collagen rod and its degree of perfection is never reached in gelatin gels: the melting temperature of the helices is below 36 °C for concentrations below 20% g/g. The difference in the length of the triple helix sequences should influence the enthalpy per helix turn, as the ends of sequences lack of some hydrogen bonds which are necessary to the stability of the triple helix. On average, one should see a difference in enthalpy between helices formed at low temperature and for polydisperse samples, compared to the others. The results are unexpected also because the quality of the 'solvent' which includes more and more gelatin chains, as concentration is increased, also changes. None of these parameters seem to affect the enthalpy of helix formation, which remains constant. There are not many comparisons that we could find for such simple results. In a quite different area, there is an experimental investigation of the crystallisation of paraffins in crude oils which contain various proportions and various lengths of n-alkanes which crystallize progressively during a cooling process.[4] It was shown that the enthalpy of melting or crystallisation of the n-alkanes could be taken as constant in all these liquid mixtures, because these behave as ideal solutions.[5] It is difficult to use this argument in our case; therefore we have no simple explanation for our results.

3. Rheology

With the same thermal protocols used in optical rotation and calorimetry, we investigated the rheological properties during the three steps, which are cooling, maturation and heating. Helix concentration was systematically correlated to the shear modulus of the gels. The results are shown in Figure 8. The frequency is 1 Hz.

We expected a good correlation between the shear modulus G′ and the helix concentration c_{hel} (g/cm^3) based on the data published in a previous paper.[6] For LB1 at various concentrations, ranging between 4.5% and 22.5% g/g a single curve is observed for G′, while for G″ the data depends on the gelatin concentration. The G′ curve is independent on the frequency, while G″ is much smaller and frequency dependent. The data was obtained during the cooling step. A similar correlation was observed for the same samples during maturation and heating (data not shown). The master curve for G′ can be interpreted in terms of a schematic picture, where the shear modulus is totally determined by helix concentration. The gel structure is network of entangled rods connected by flexible links. The average length of these rods and their distance of entanglement are derived from a statistical mechanical model from c_{hel}.[6] In this paper, we have extended the range of validity of the master curve up to gelatin concentrations $c = 22.5\%$ g/g (instead of 8% g/g in ref. 6). The effect of the molecular weight distribution was also investigated, and as shown in Figure 9, for LB1 to LB4, all the data collapse on a single curve, the master curve, as before.

Conclusion

The experiments presented in this paper demonstrate that the changes of the

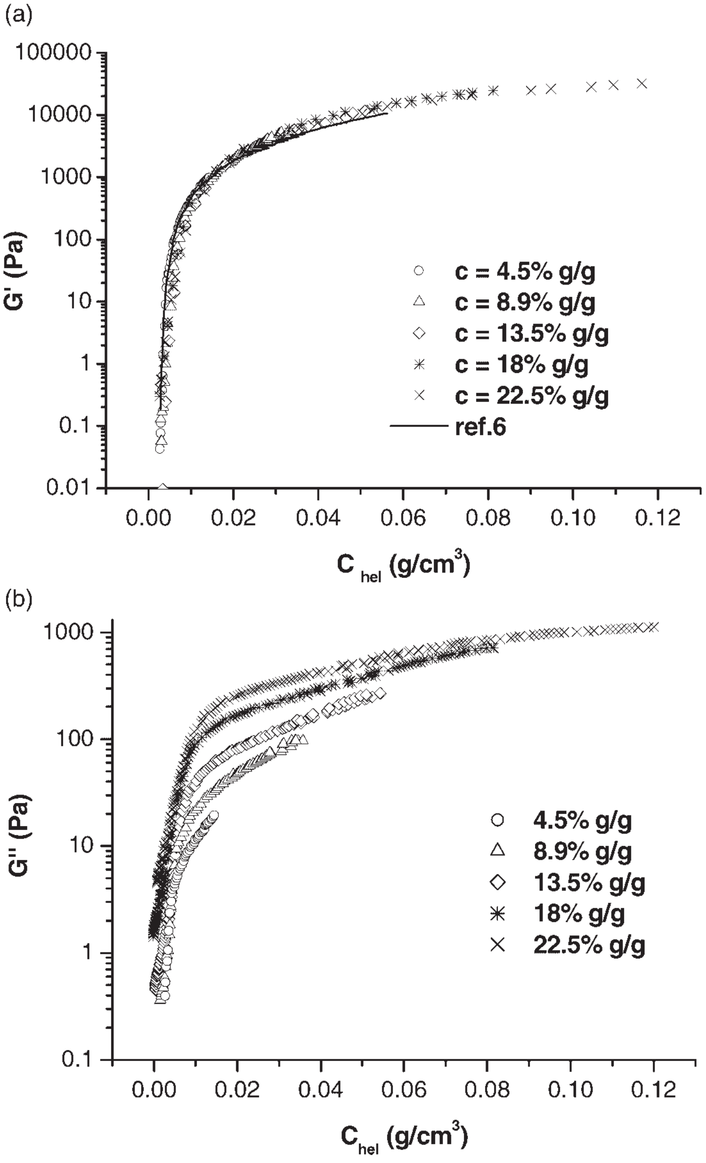

Figure 8.

(a) Storage modulus G' versus helical concentration for limed bone gelatin (LB1) at different gelatin concentrations (frequency 1 Hz). (b) Loss modulus G'' versus helical concentration for limed bone gelatin (LB1) at different gelatin concentrations (frequency 1 Hz).

structural and rheological properties of the gels, when the molecular weight and polydispersity of the samples change, can be analysed within a unique master curve relating the shear modulus to the helix concentration. Moreover, very simple results were established for the enthalpy of helix formation and melting for all samples despite the significant changes of the many other properties, such as the range of temperatures, the quality of the solvent, the molecular weight… A theore-

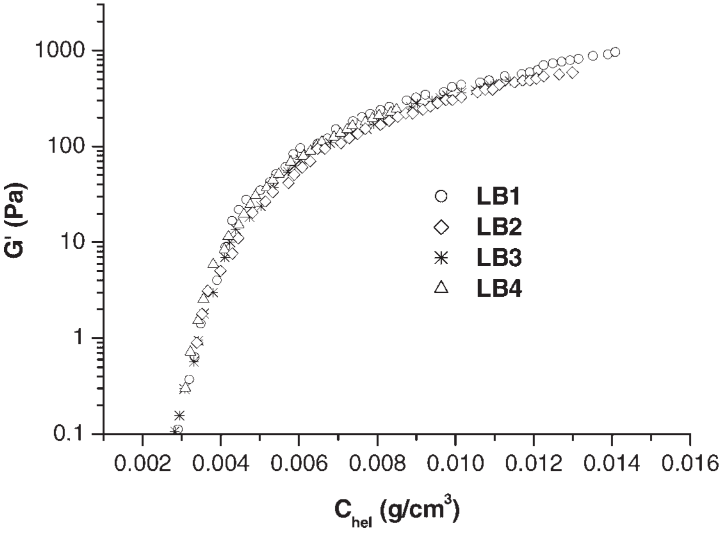

Figure 9.
Storage modulus G′ versus helical concentration during cooling ramp for the 4 extracts of limed bone gelatin.

tical interpretation of the enthalpic properties of the gels is needed.

[1] K. te Nijenhuis, "Thermoreversible Networks. Viscoelastic properties and structure of gels", *Adv. Polym. Sci.* **1997**, *130*, 1.

[2] C. Joly-Duhamel, D. Hellio, M. Djabourov, *Langmuir* **2002**, *18*, 7208.

[3] P. L. Privalov, E. I. Tiktopulo, *Biopolymers* **1970**, *9*, 127.

[4] J. M. Létoffé, P. Claudy, M. V. Kok, M. Garcin, J. L. Volle, *Fuel* **1995**, *74*, 810.

[5] P. M. Gjogomu, M. Bouroukba, J. Dellacherie, D. Balesdent, M. Dirand, *Thermochem. Acta* **1997**, *306*, 69.

[6] C. Joly-Duhamel, D. Hellio, A. Ajdari, M. Djabourov, *Langmuir* **2002**, *18*, 7188.