

Chemically and Physically Cross-linked Gelatin Gels

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Gelatin gels are widely used in many applications including food, photographic, pharmaceutical, biomedical and technical applications. Using the same macromolecule in solution (collagen denatured during extraction and dissolved in water) two types of gels can be obtained: the “physical gel” which is thermo-reversible and which is obtained when the solutions are cooled, due to the conformational transition of the gelatin chains from coil to triple helices and a “chemical gel” which results from the cross-linking of the chains induced by certain reagents, which were added to the aqueous solution. In general, the chemical reaction proceeds in the temperature range where the physical gel is not observed, in the high temperature range, thus the two gelation mechanisms can be observed independently. For food and pharmaceutical applications, only the thermo-reversible gel is used, the gel is supposed to “melt” in the mouth or in the stomach. For photographic applications, both types of gels must be present : the physical gel is formed in fist place during film coating and it entraps the silver halide crystals in suspension, while the chemical cross-linking is necessary to preserve the gel from entire dissolution during the various steps of the photographic developments. In an intermediate range of temperatures, both gels can form simultaneously and the two mechanisms of gelation are in competition.

In this presentation we show the results obtained when the two mechanisms are independent. The experiments deal with both rheological (rheometer AR 1000 from TA instruments) and structural properties. For physical gels, from optical rotation measurements (Perkin Elmer 341 Polarimeter) one can calculate the concentration of helices for different types of gelatins, different gelatin concentrations, temperature treatments, time... It was found, for all these experiments, that the storage moduli of the gels, G' , measured in the same conditions as the optical rotation are uniquely related to the concentration of helices^(1,2). Figure 1 shows an example of the master curve obtained with both fish and mammalian gelatins.

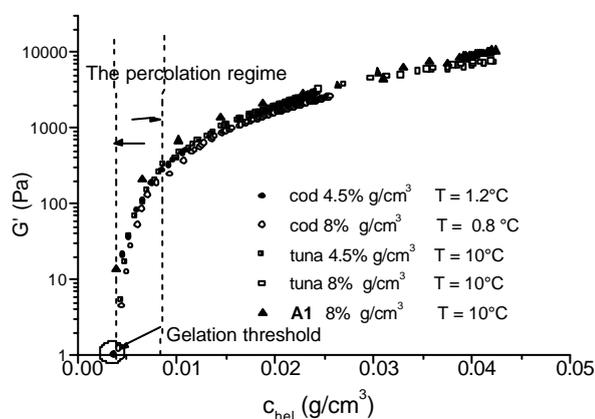


Fig.1 The master curve for the elasticity versus helical concentration for any type of gelatin.

The chemical reaction inducing gelation was followed by microcalorimetry (MicroDSC III from Setaram, Caluire, France). The cells used for these experiments are the mixing cells made of two chambers, one containing the gelatin solution and the other the dissolved reagent. The valve separating the two chambers is suddenly opened and the liquids are mixed. The exothermic reaction starts and the released heat is measured versus time. The reagent used in our case is the bis(vinylsulfonyl)methane (BVSM) kindly provided by Kodak Industries (France). The reagent combines with the amino-acid groups such as Lysine, Hydroxylysine and Histidine. Each BVSM molecule provides two covalent bonds. When the reaction is limited by the amount of reagent, one can establish a calibration curve which measures the enthalpy of formation per C-N bond. We found an average of -40.6 kJ/mole per C-N bond, as shown in figure 2.

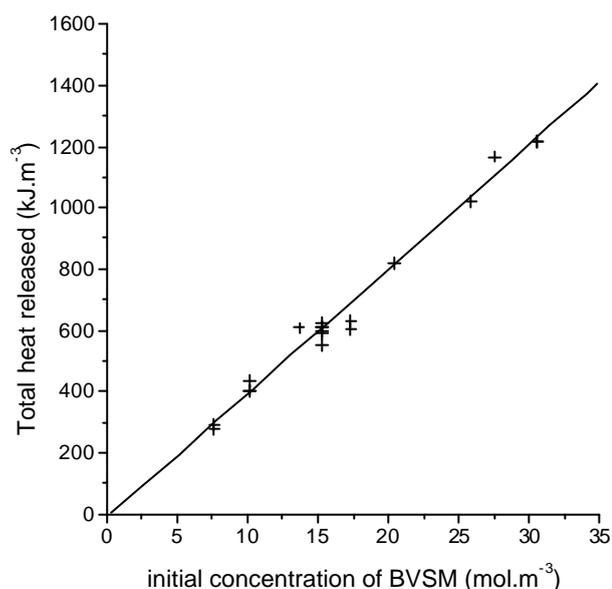


Fig. 2 The calibration curve giving the heat of reaction per C-N bond between BVSM and gelatin.

For different amounts of reagent and different gelatin concentrations, we were able to follow the rheological and thermodynamic aspects of the gelation process. The correlation between the two types of measurements puts into evidence the critical domain of the sol-gel transition for the chemical cross-linking of gelatin.

1. Christine Joly-Duhamel, Dominique Hellio and Madeleine Djabourov, *Langmuir*, **2002**, *18*, 7208
2. Christine Joly-Duhamel, Dominique Hellio, Armand Ajdari and Madeleine Djabourov, *Langmuir*, **2002**, *18*, 7158

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OUTLINE OF THE PRESENTATION

**INTRODUCTION : Bio-diversity of gelatins of various sources:
mammalian and fish gelatins**

- 1. The physical aspects of gel formation ; structure and rheology**
- 2. The master curve for elasticity of the physical gelatin gels. Interpretation**
- 3. Chemical gelation in presence of a cross-linker. Hints for interpretation**

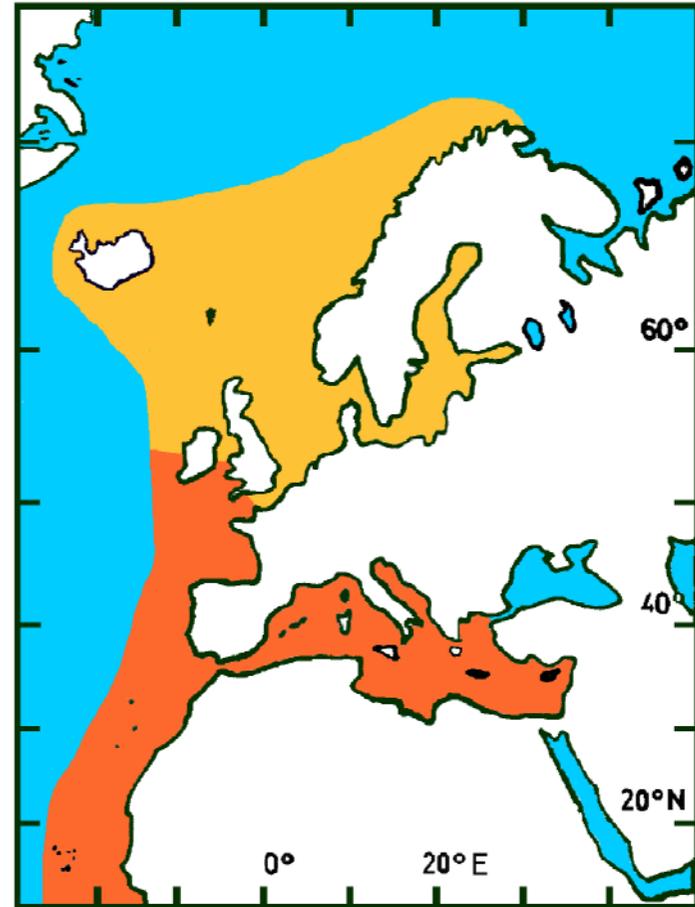
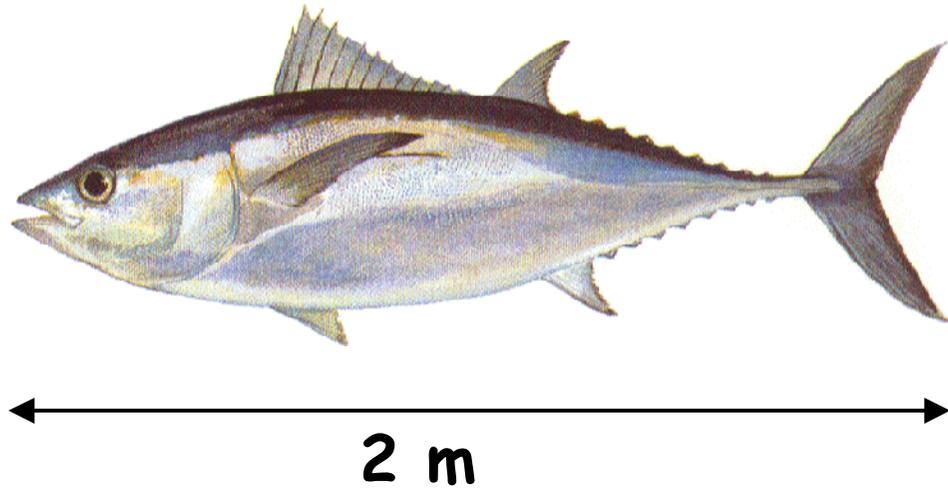
OUTLOOK and CONCLUSIONS

INTRODUCTION

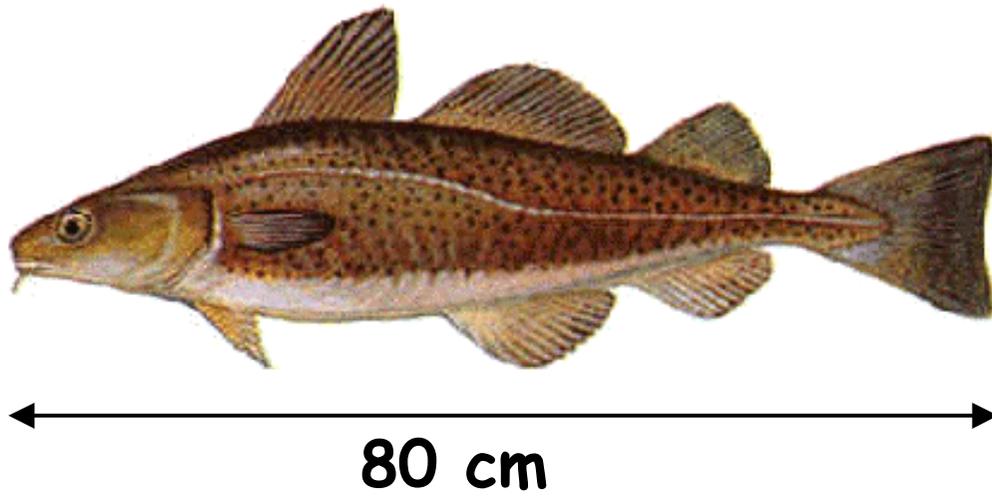
Biodiversity of gelatins

- ☐ Mammalian and fish collagens**
- ☐ The amino acid compositions of collagen and the physical properties of gels**

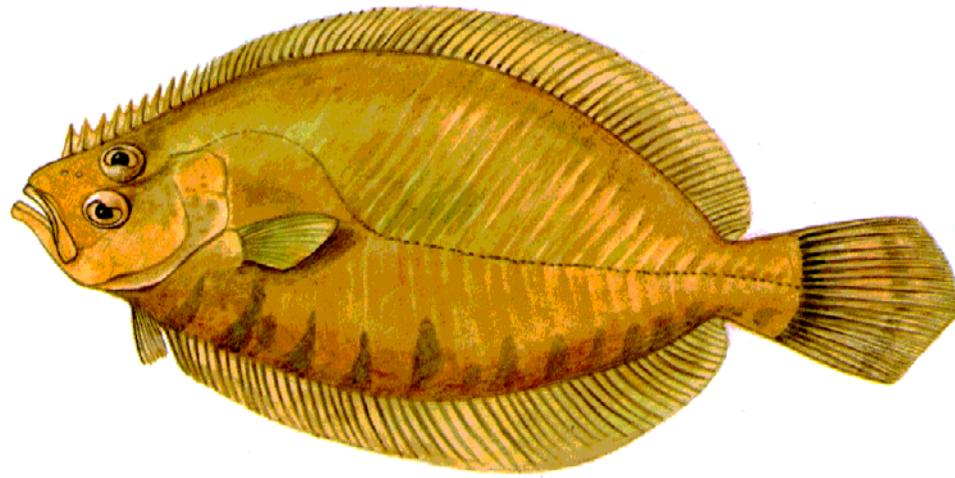
tuna



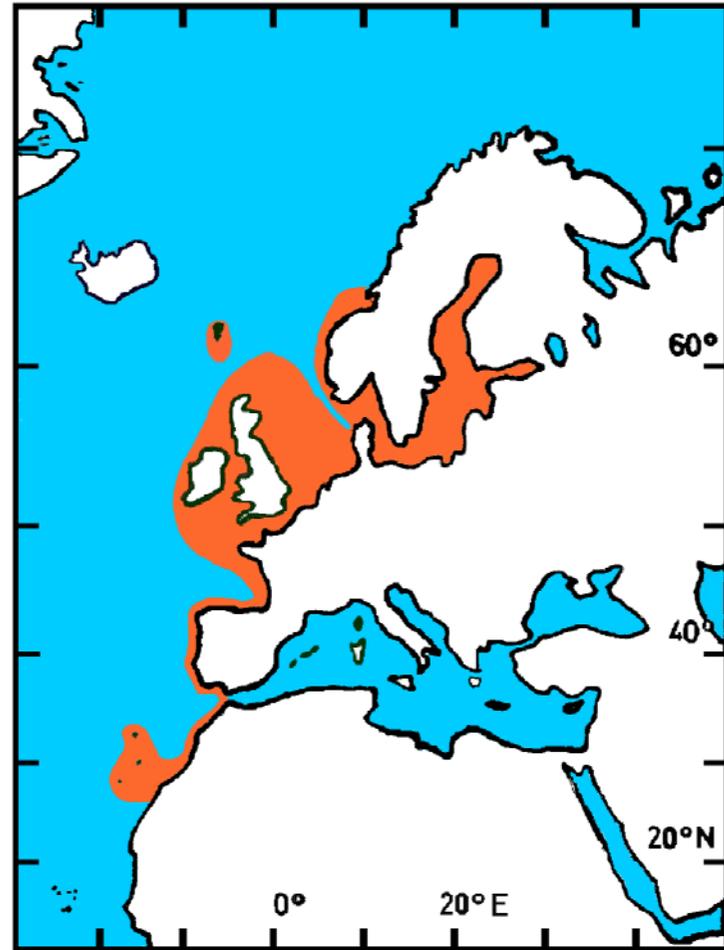
cod



megrim



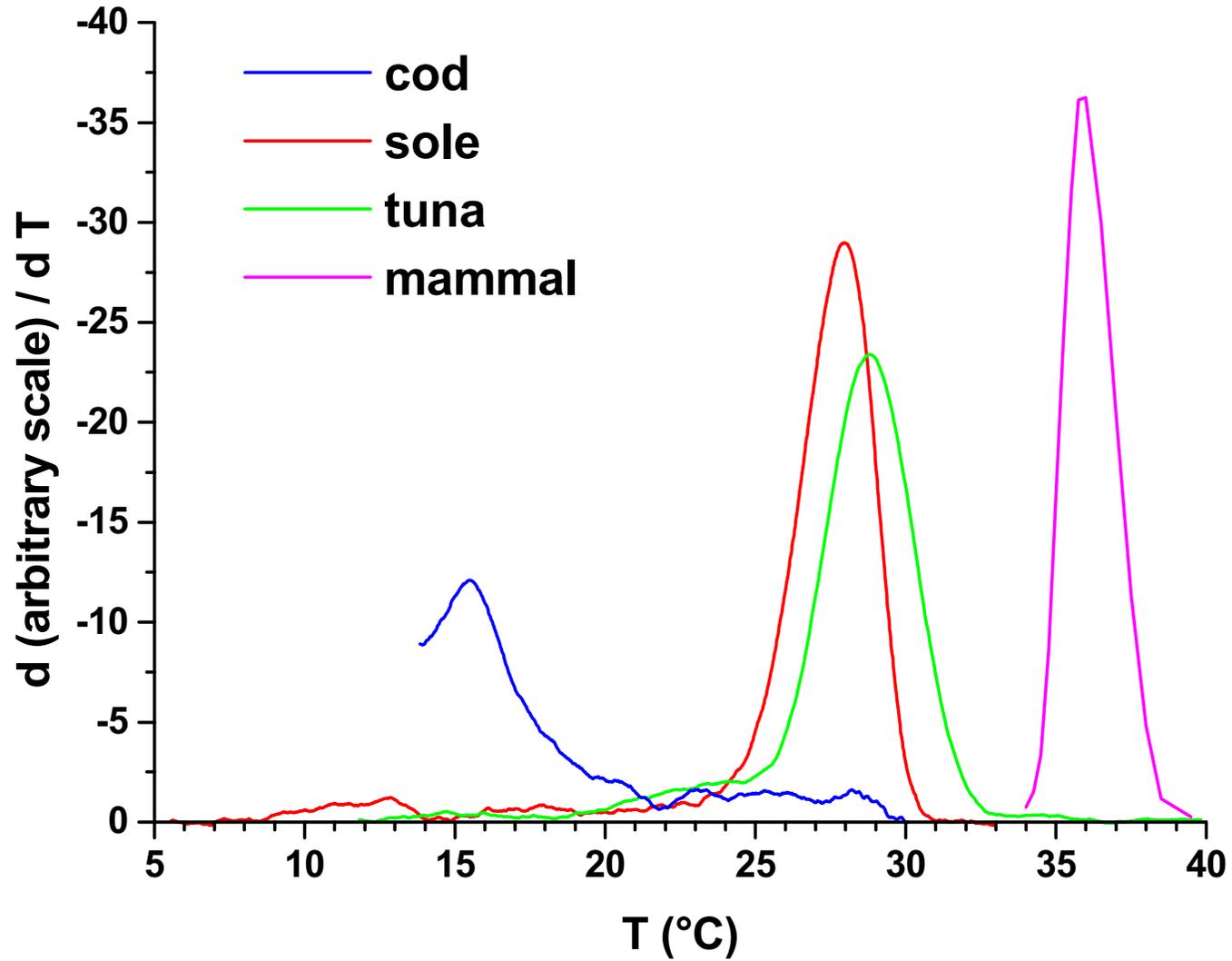
← 40 cm →



Imino-acid composition of gelatins and melting temperatures of corresponding collagens

Imino-acid (g/100g protein)	Bovine Gelatin	Pig skin gelatin	Tuna	Sole or megrin	Cod
Pro	13.03	13.99	11.61	12.86	10.29
Hypro	12.23	11.15	10.49	9.06	6.72
Total	25.26	25.14	22.1	21.92	17.01
T_m (collagen) °C	36*	36*	29	28	15

Collagen melting curves

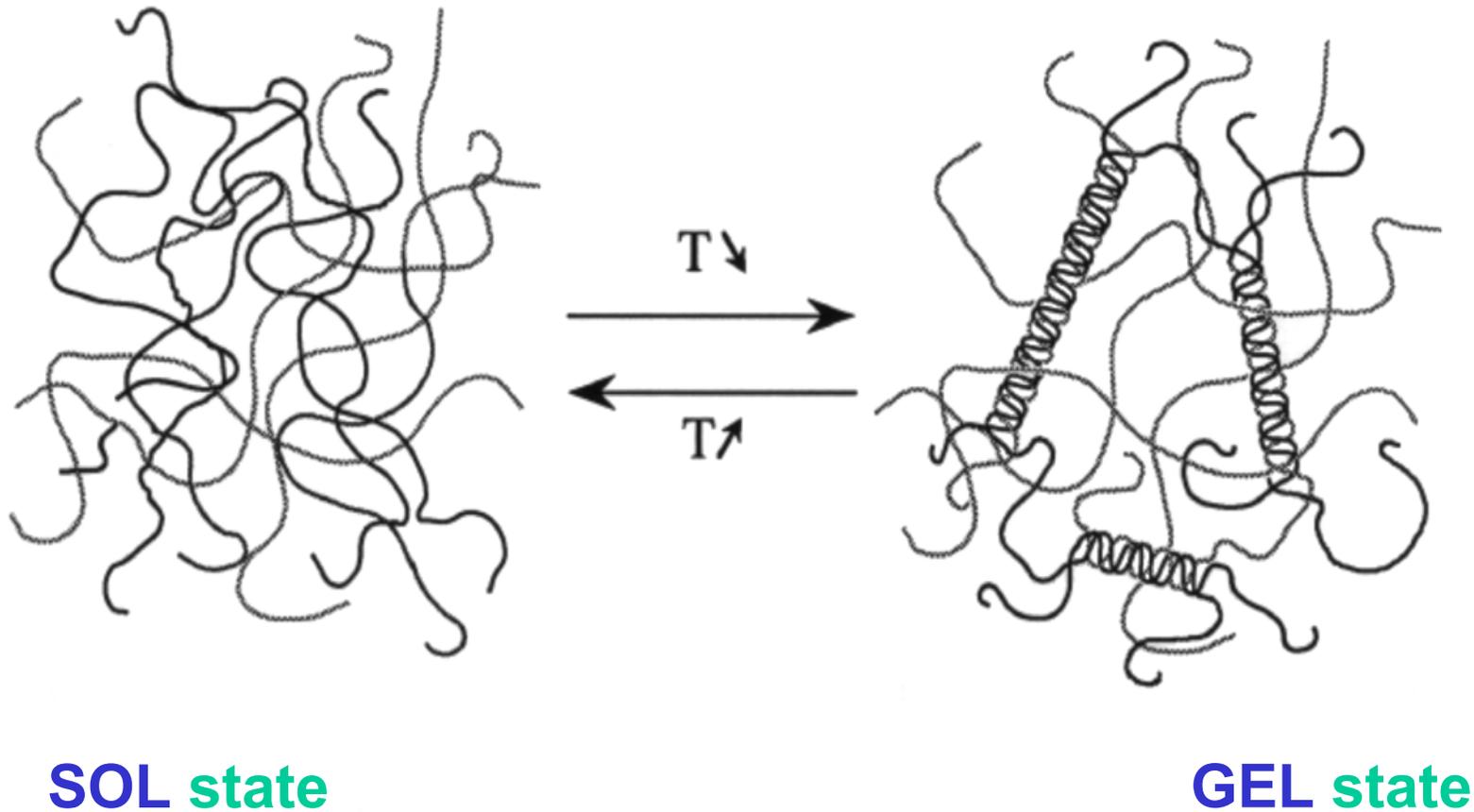


**1. The physical aspects of gel formation :
structure and rheology**

Mechanism of gelation

Gelatin gelation is related to the coil-triple helix transition.

Gelation and gel strengthening are kinetic processes



Molecular characteristics of various gelatins

gelatin	process	M_w (g/mole)	M_w/M_n	I_p
Beef, High M (A1)	alkaline	145 700	1.68	5.0
Beef, Low M (A2)	alkaline	102 200	1.95	5.0
Pig skin, High M (B1)	acid	168 500	1.91	8.7
Pig skin, Low M (B2)	acid	74 500	1.89	7.6
tuna	acid	122 600	1.66	8.9
megrin	acid	226 300	1.66	8.9
cod	acid	120 500	1.93	8.9

Experimental methods

- ❑ **Optical rotation:** continuous recording of the optical rotation angle versus time or temperature

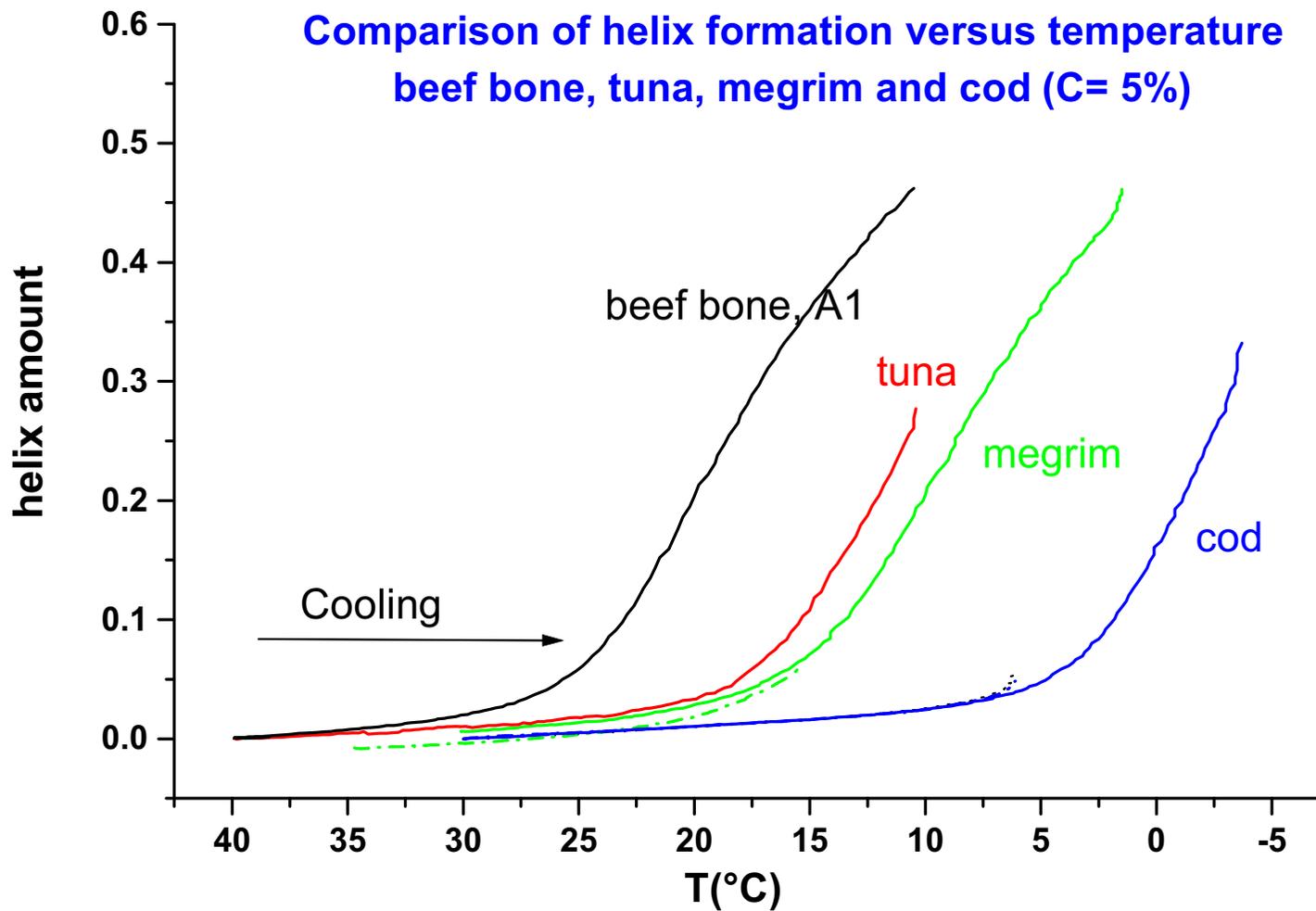
Calculation of the helix amount χ

Or

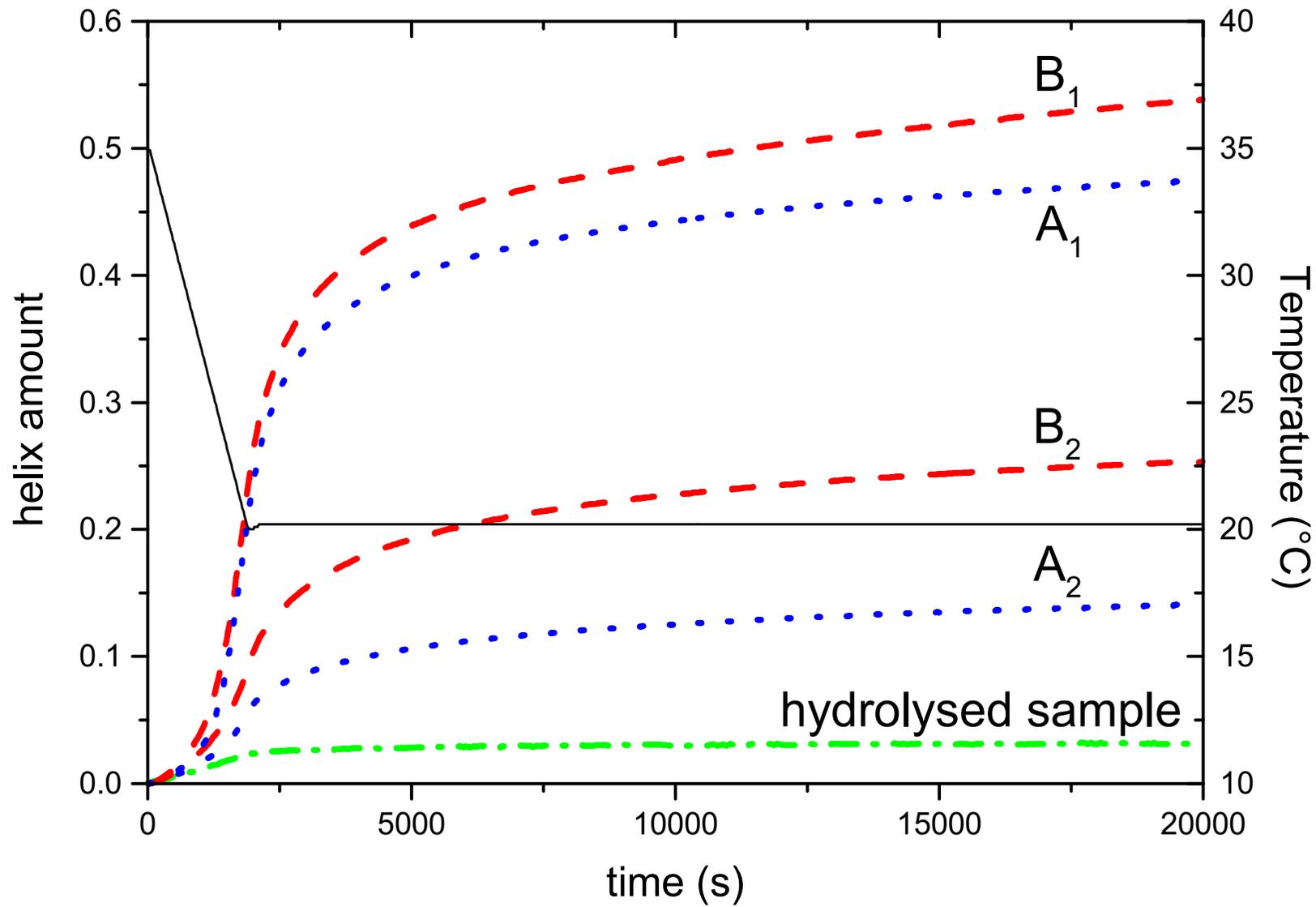
of the helical concentration c_{helix}

- ❑ **Microcalorimetry** to follow the chemical crosslinking (Micro DSC III Setaram, France)
- ❑ **Rheology** : G' and G'' at a fixed frequency (1 Hz) with very small deformations (0.5%) during gelation, maturation, melting...

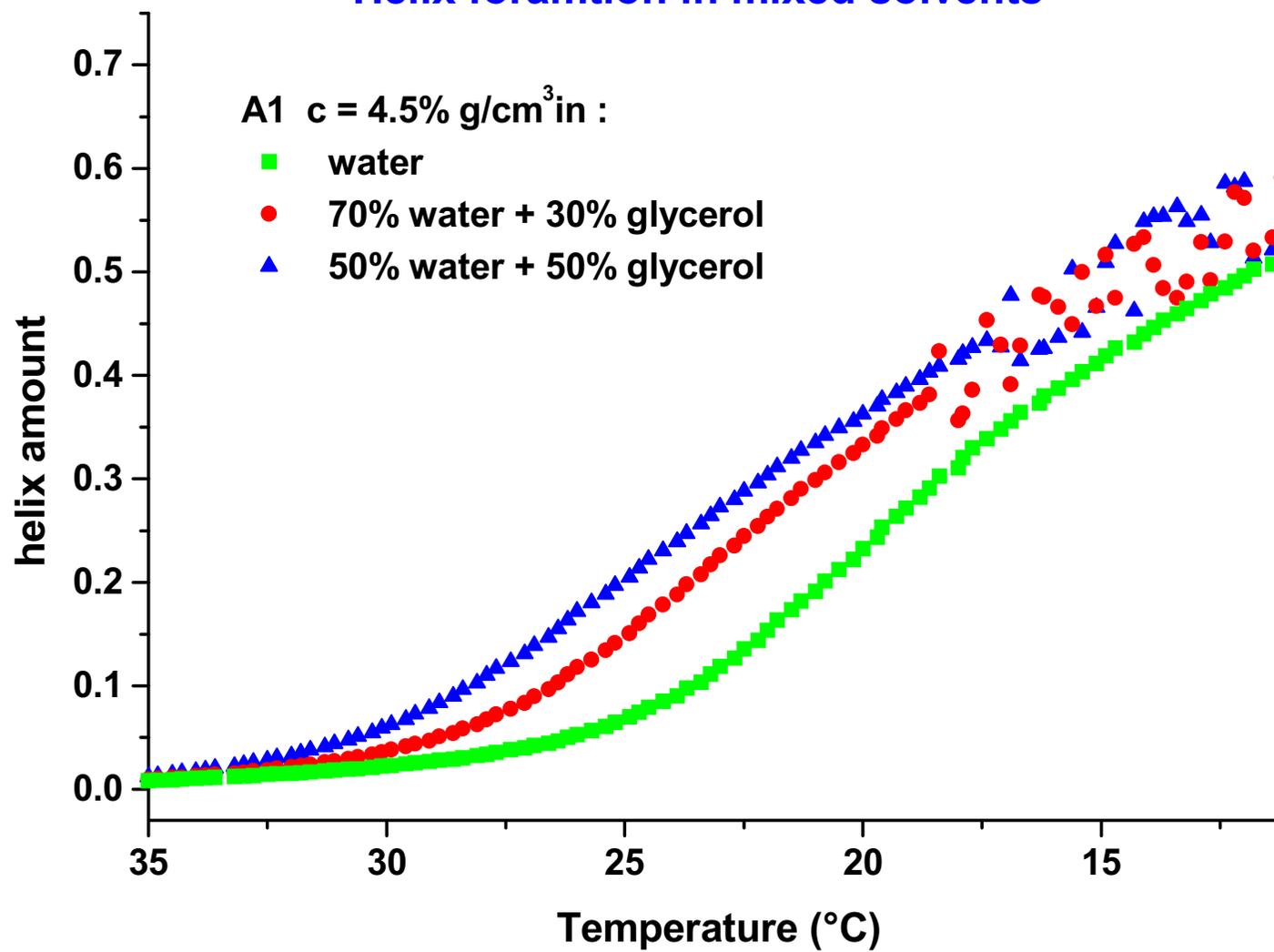
**Comparison of helix formation versus temperature
beef bone, tuna, megrim and cod (C= 5%)**



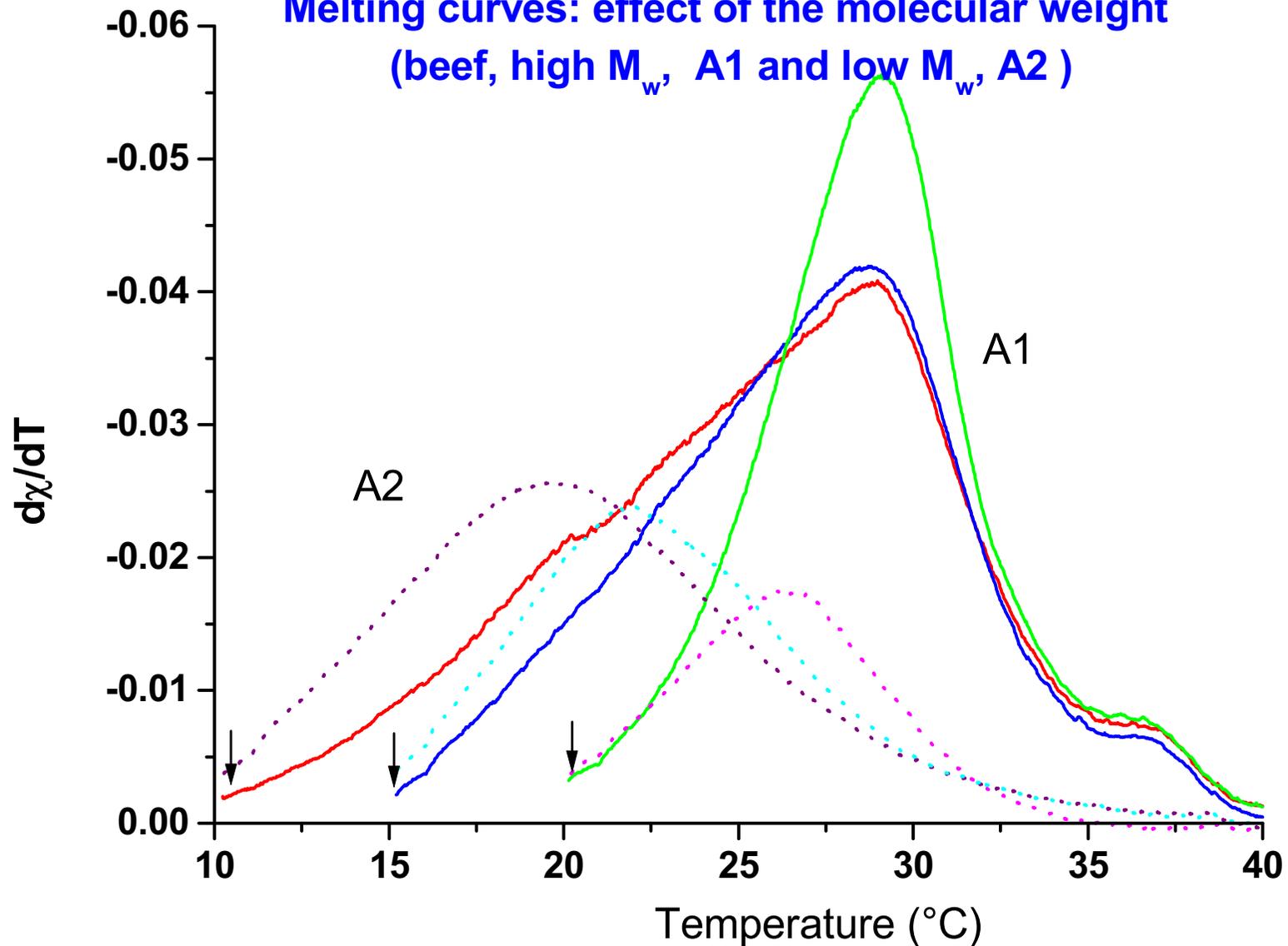
Kinetics of helix formation



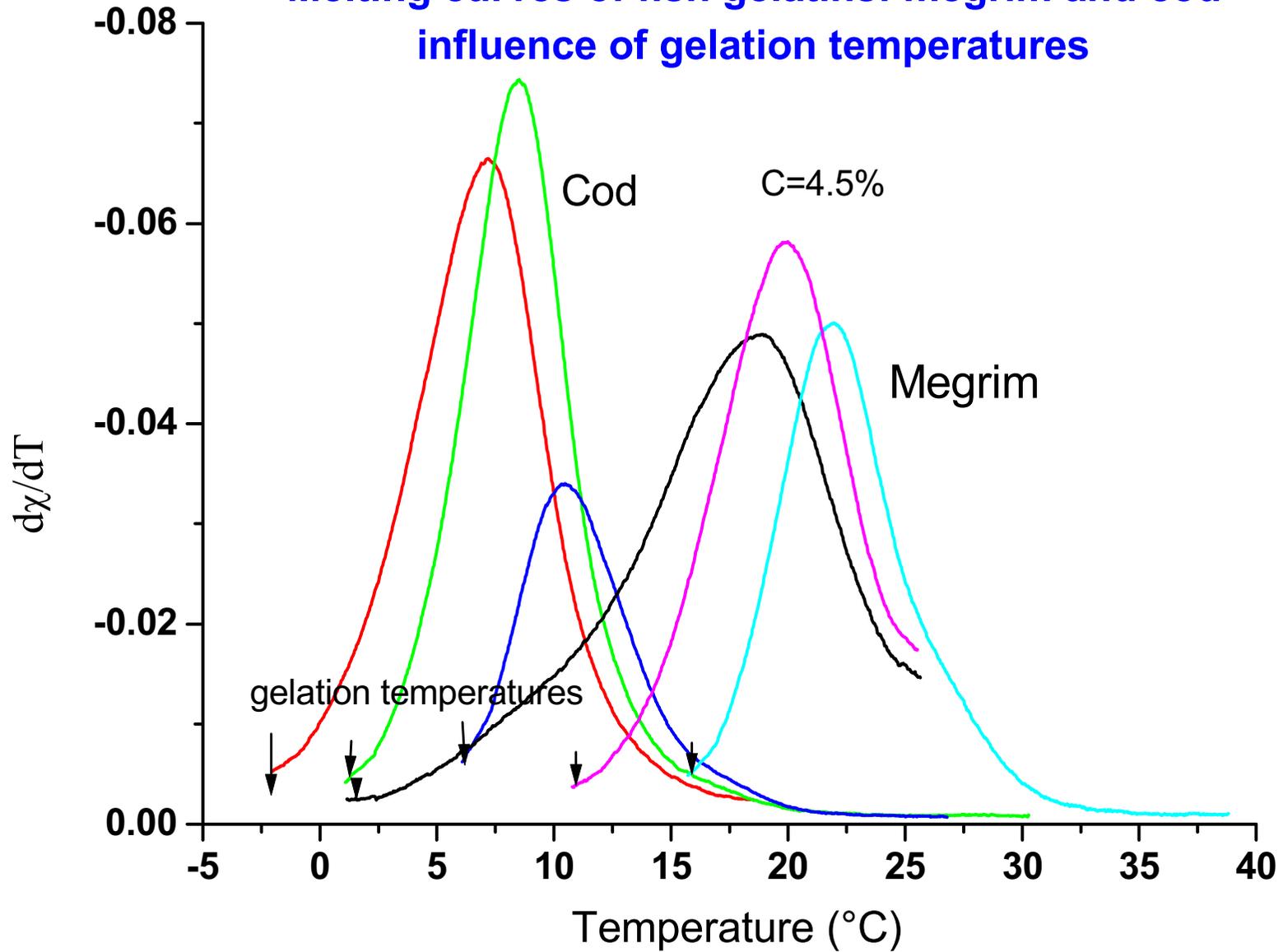
Helix formation in mixed solvents



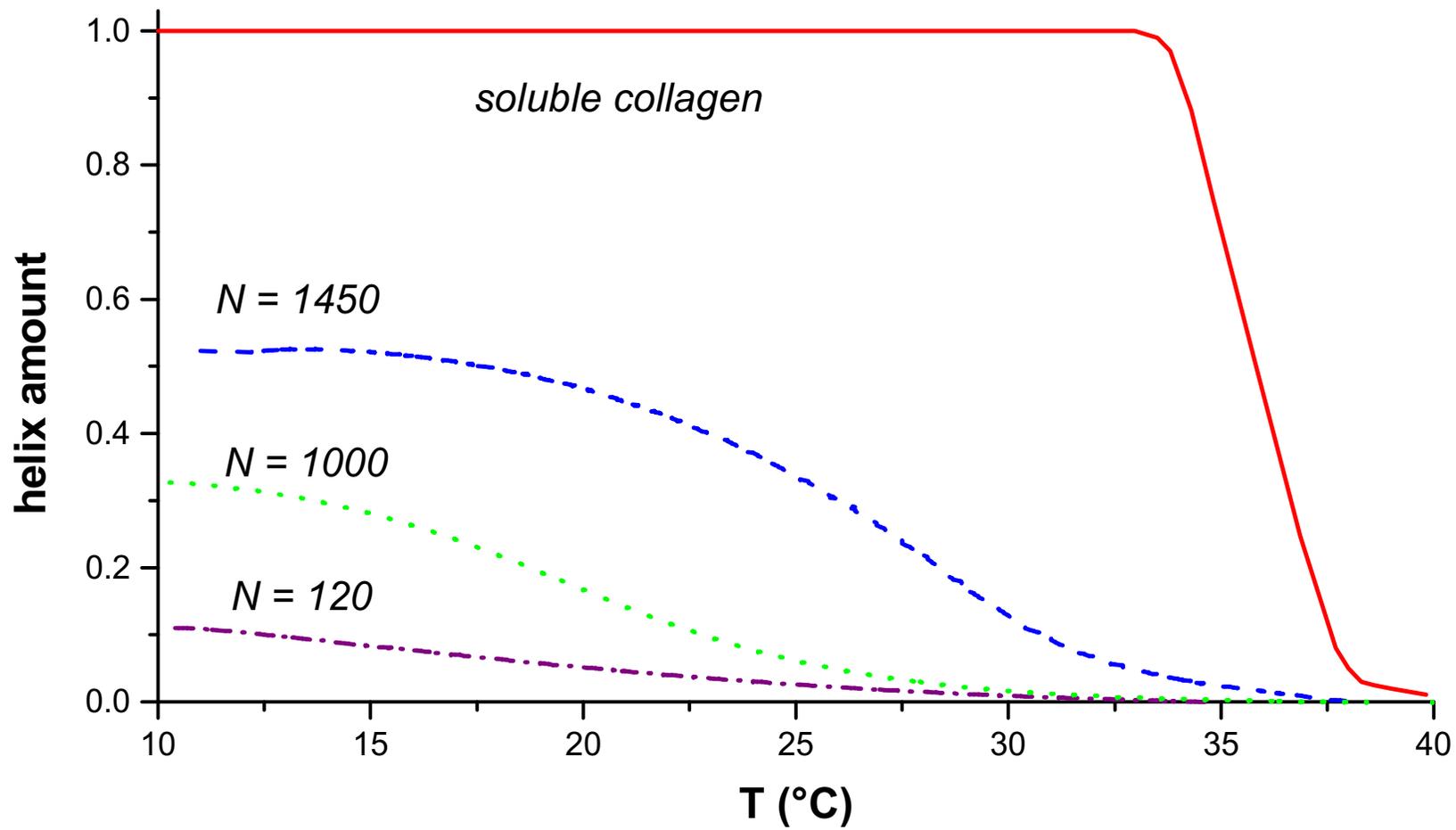
**Melting curves: effect of the molecular weight
(beef, high M_w , A1 and low M_w , A2)**



**Melting curves of fish gelatins: megrim and cod
influence of gelation temperatures**

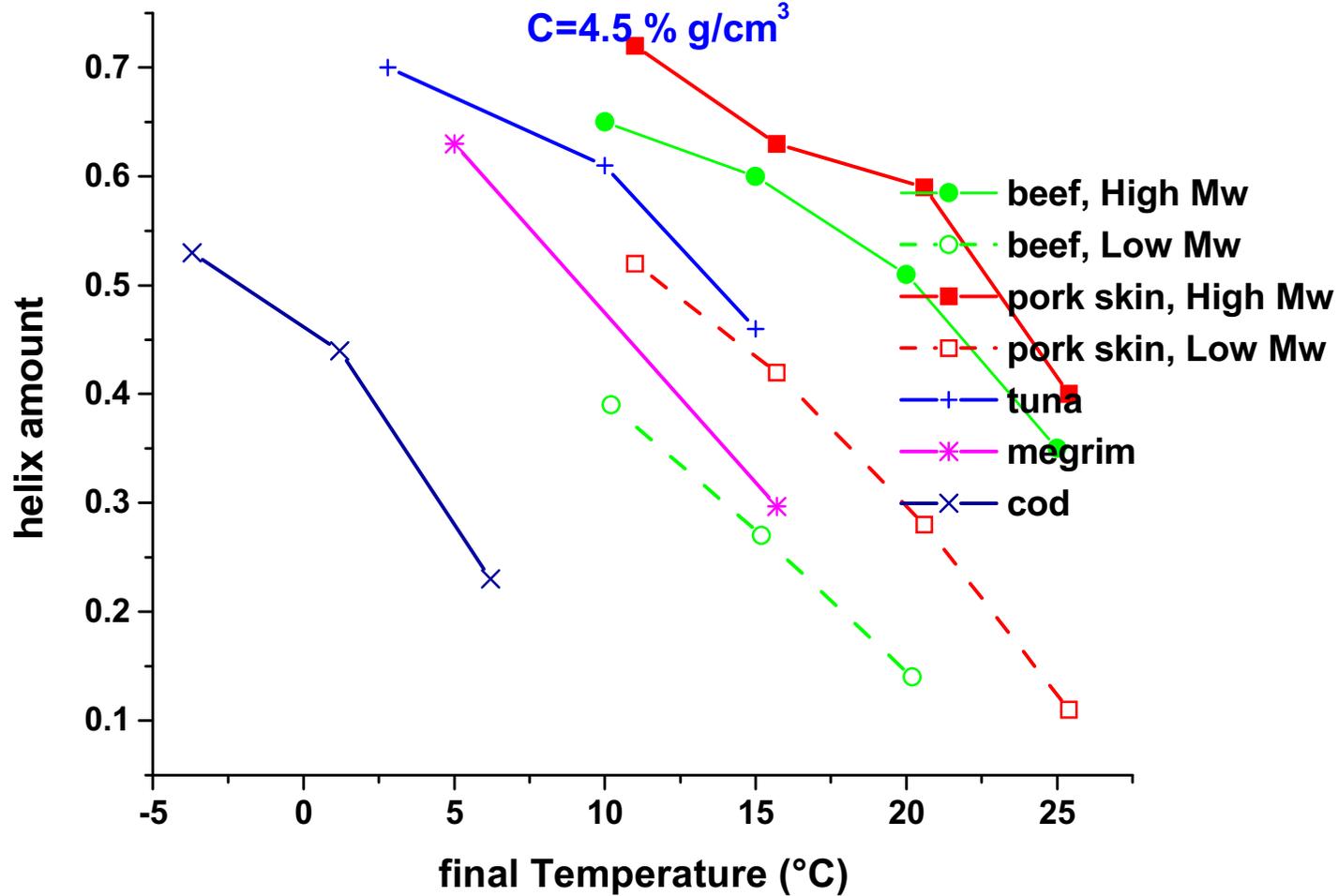


MELTING of HELICES in GELS :
effect of the molecular weight



Helix amounts versus temperature for various gelatins

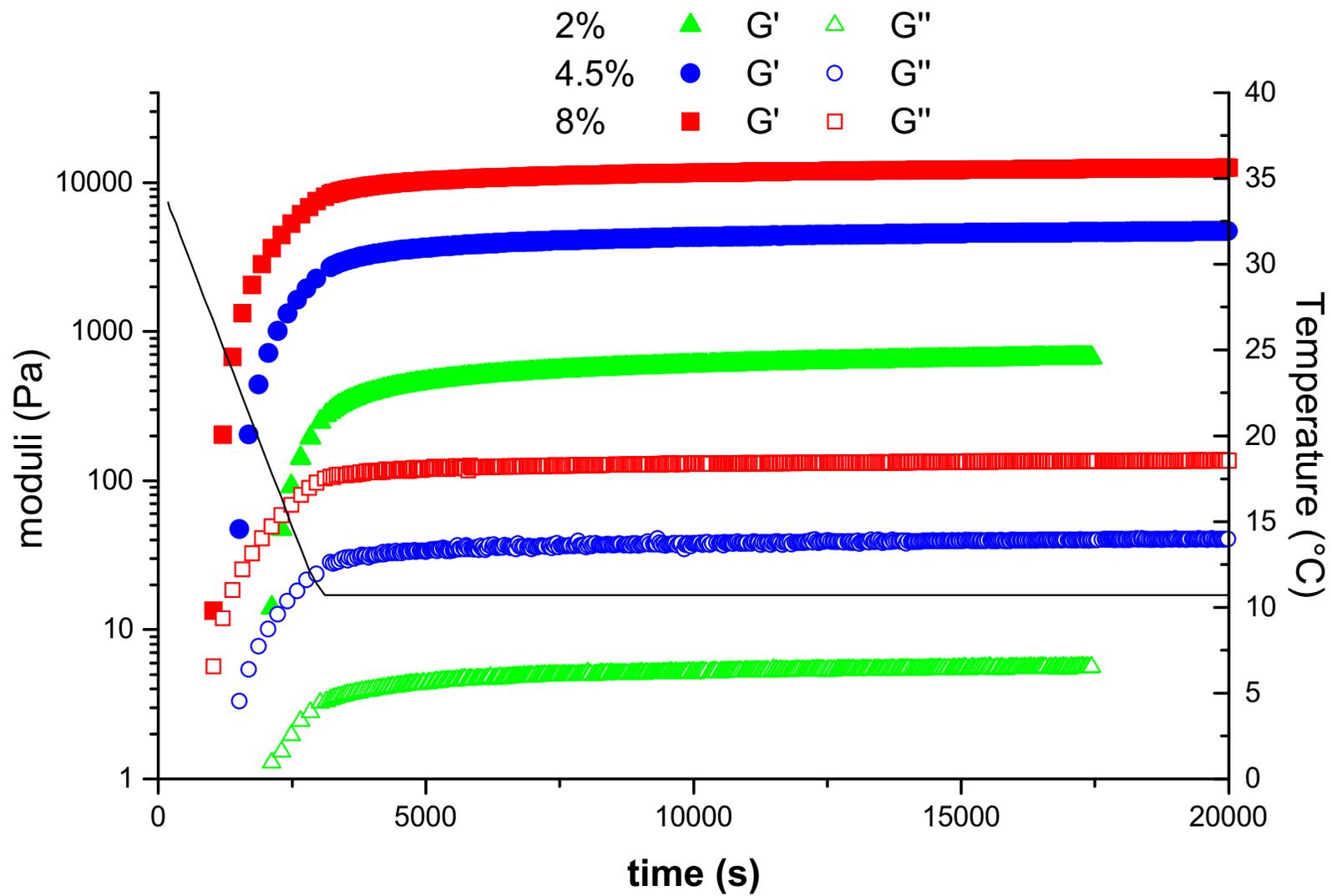
$C=4.5\% \text{ g/cm}^3$



2. The master curve for elasticity of the physical gelatin gels.

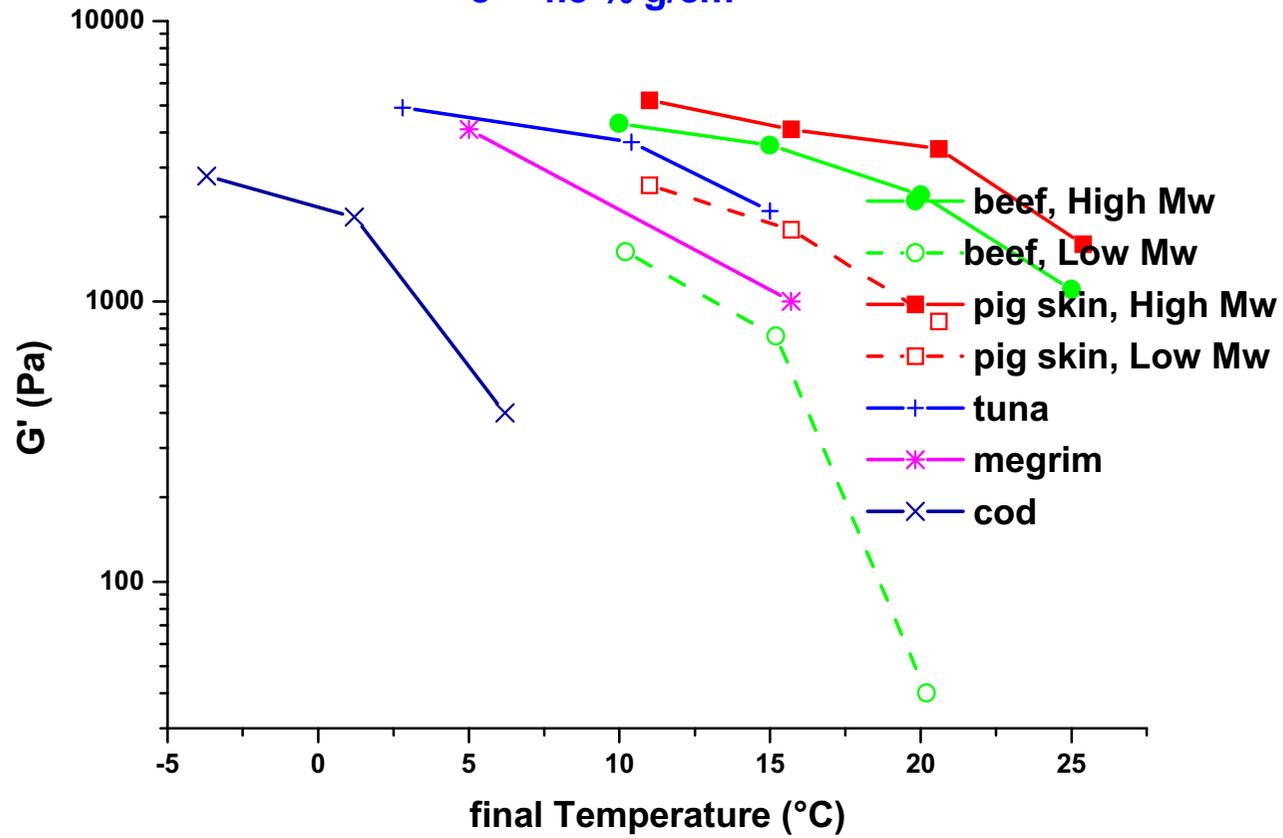
Interpretation

Kinetics of gelation: effect of concentration, A1



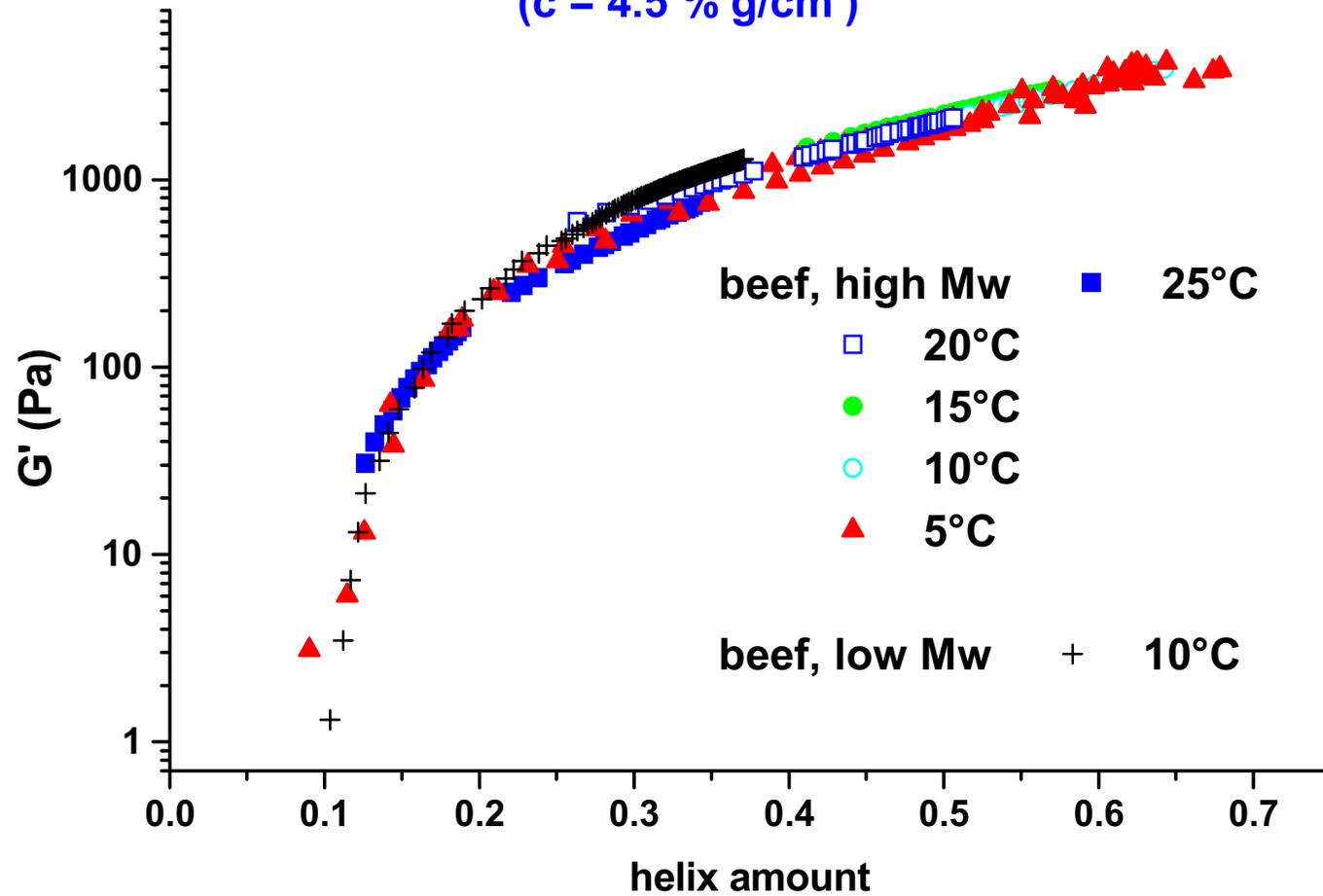
The shear moduli versus temperature for various gelatins

$c = 4.5 \% \text{ g/cm}^3$

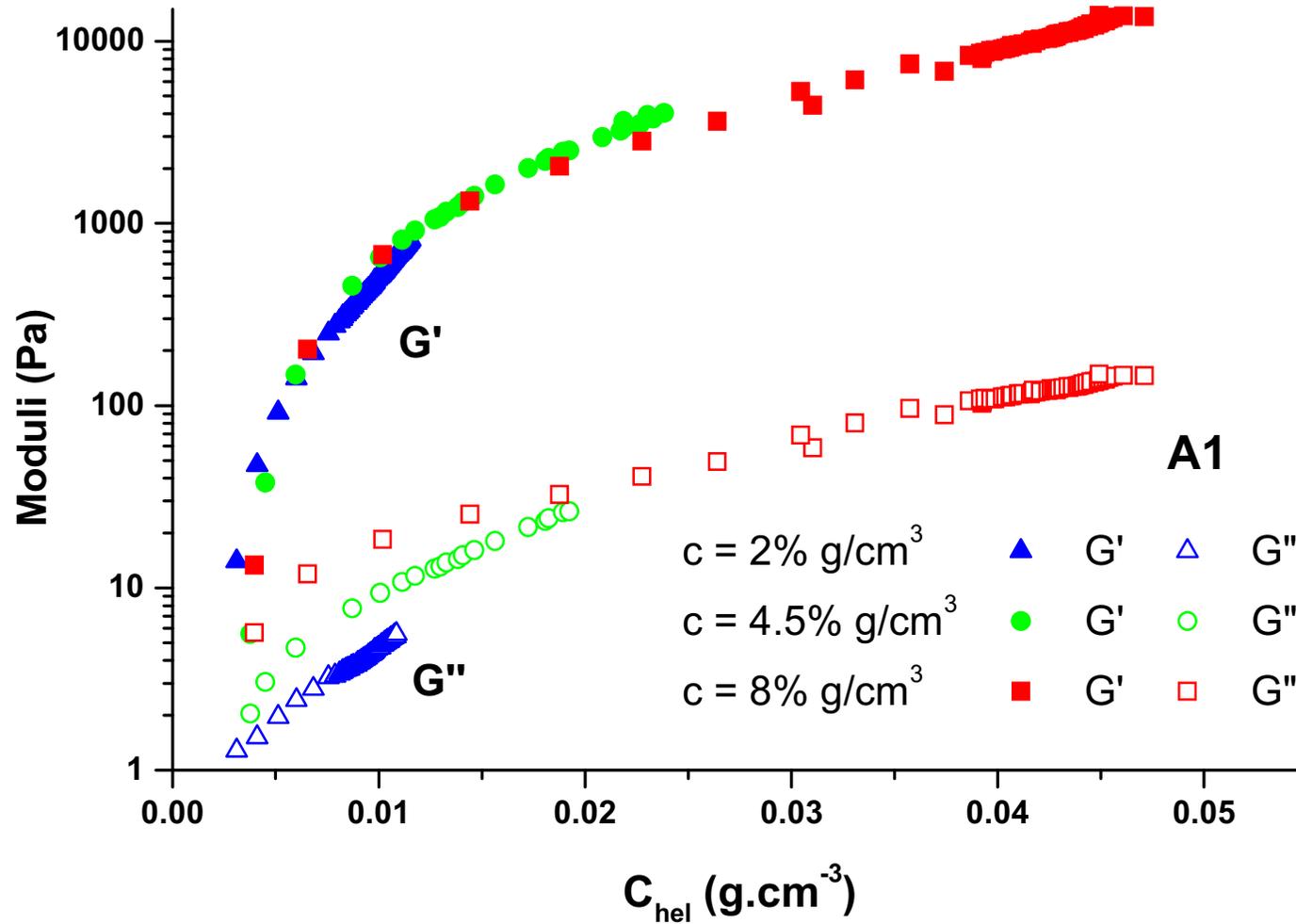


Elasticity versus helix amount:
effect of temperature and molecular weight

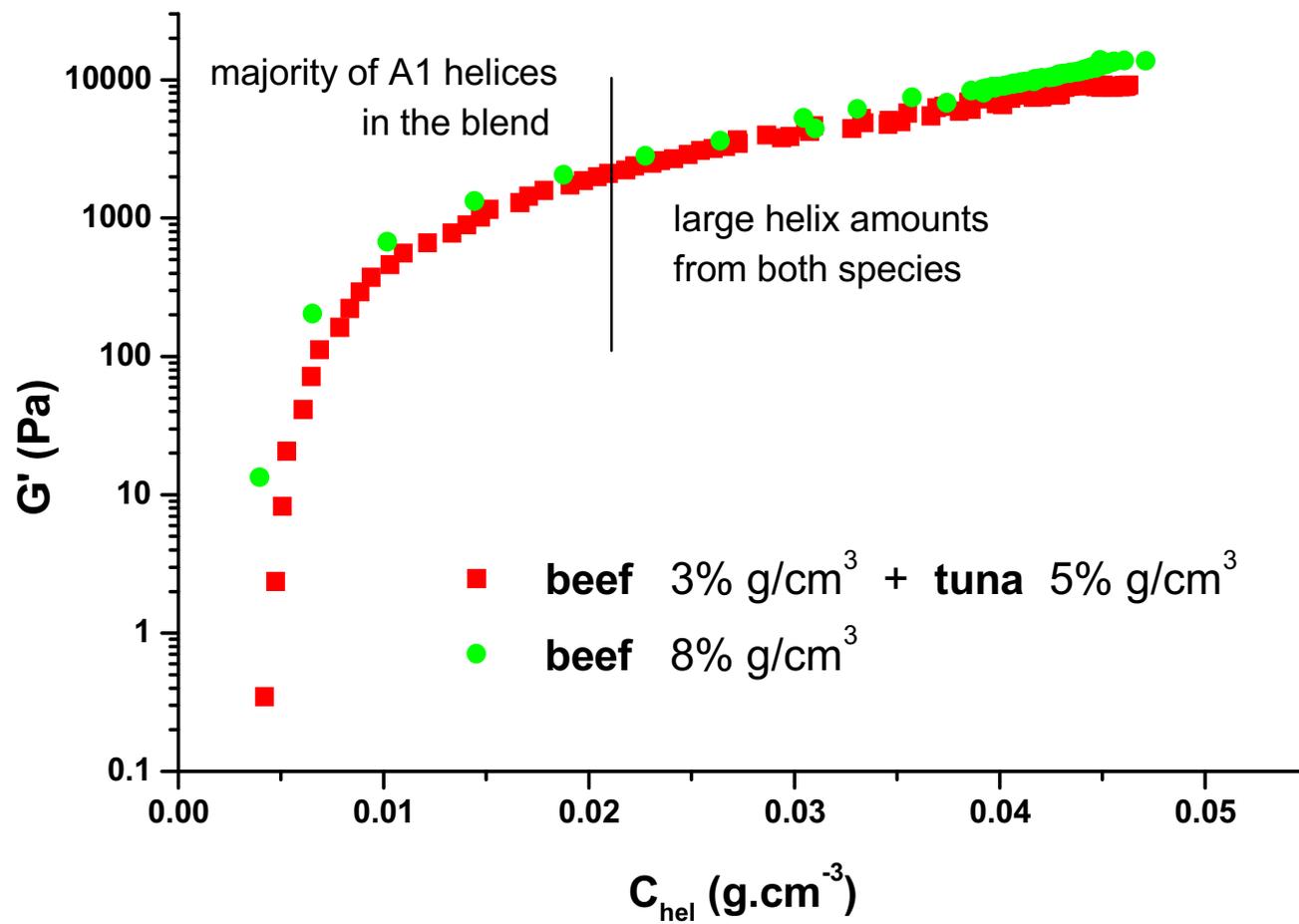
($c = 4.5 \text{ \% g/cm}^3$)



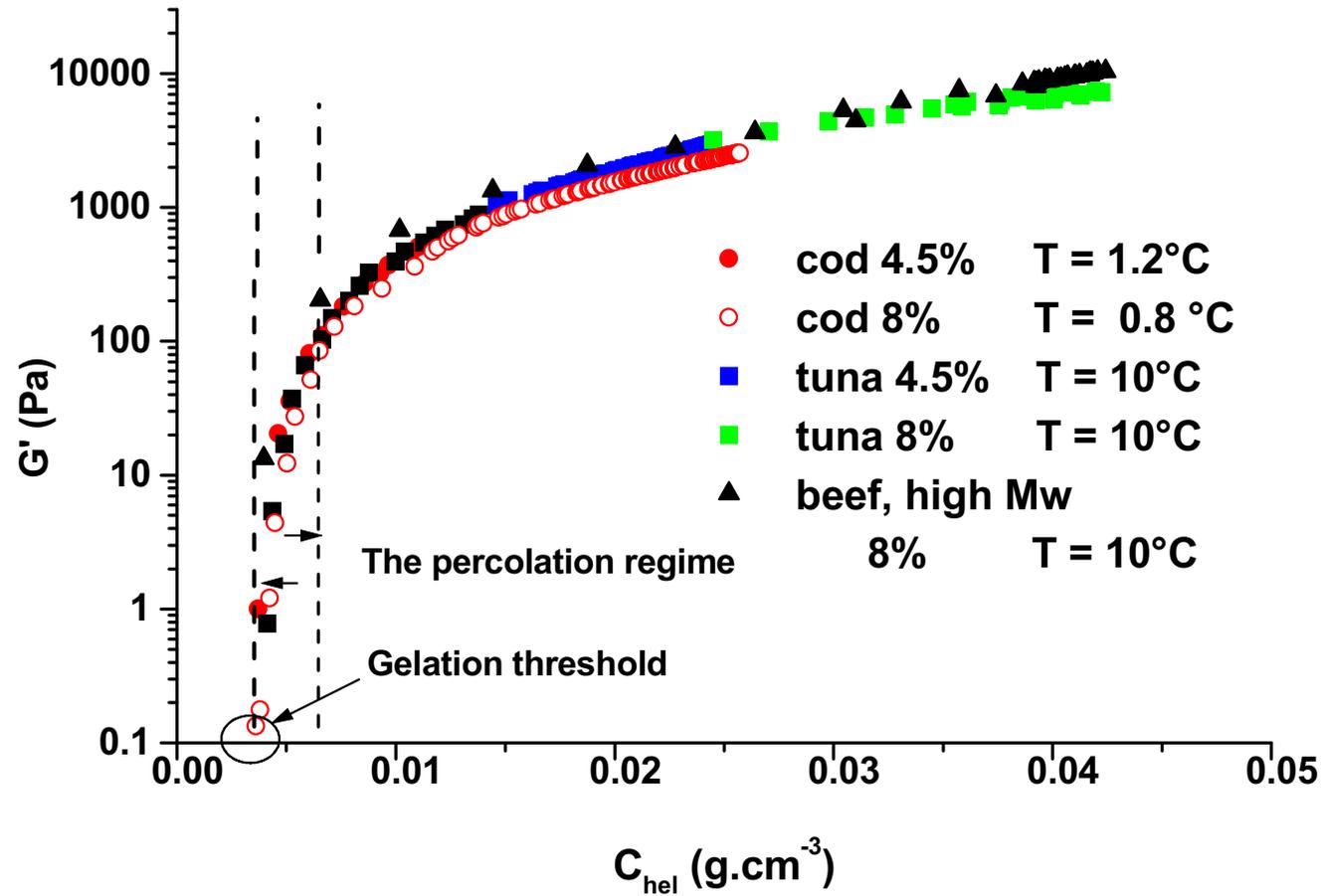
Shear moduli versus helix concentration



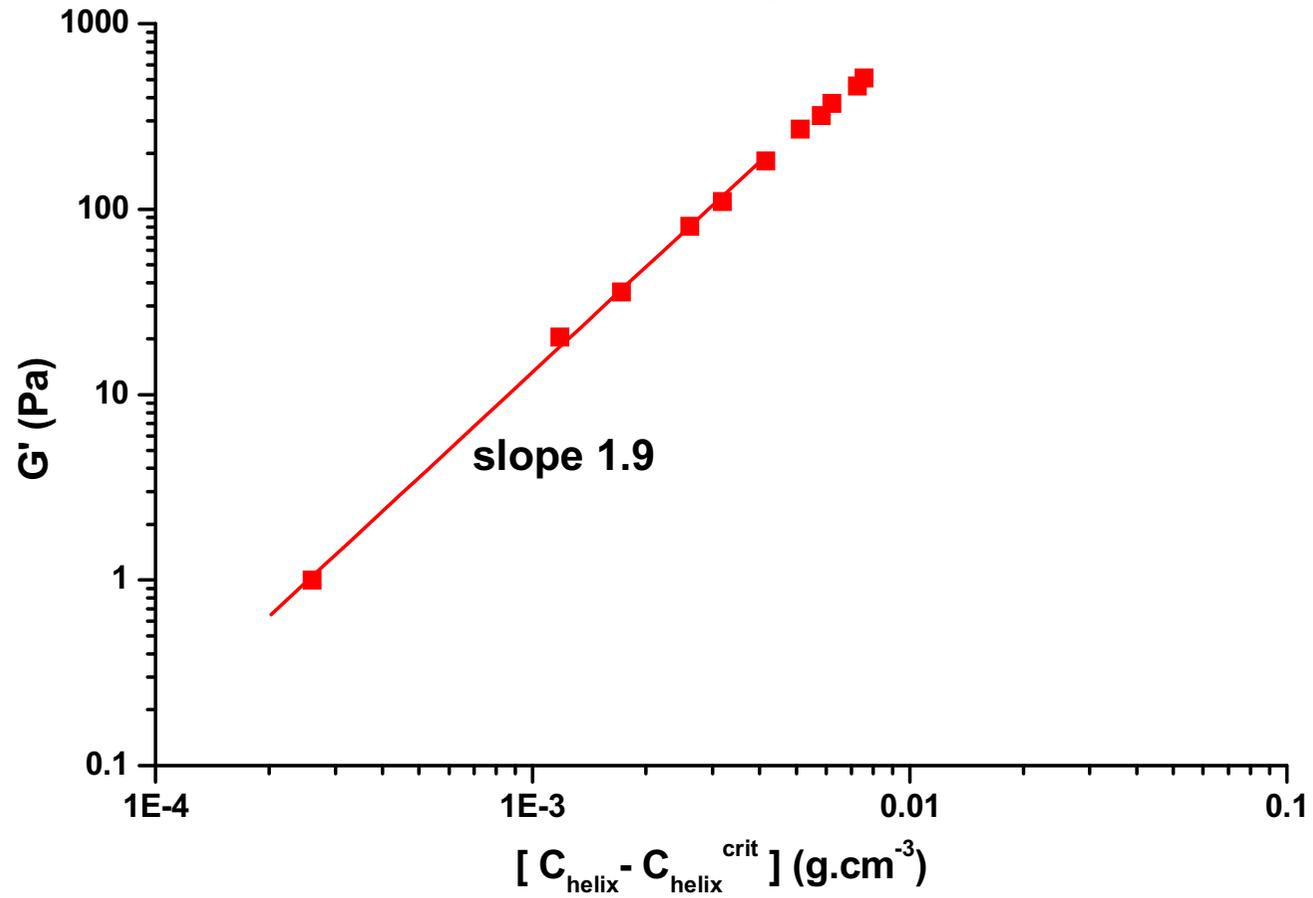
The master curve for elasticity:
blending of gelatins : (beef, High Mw) + tuna



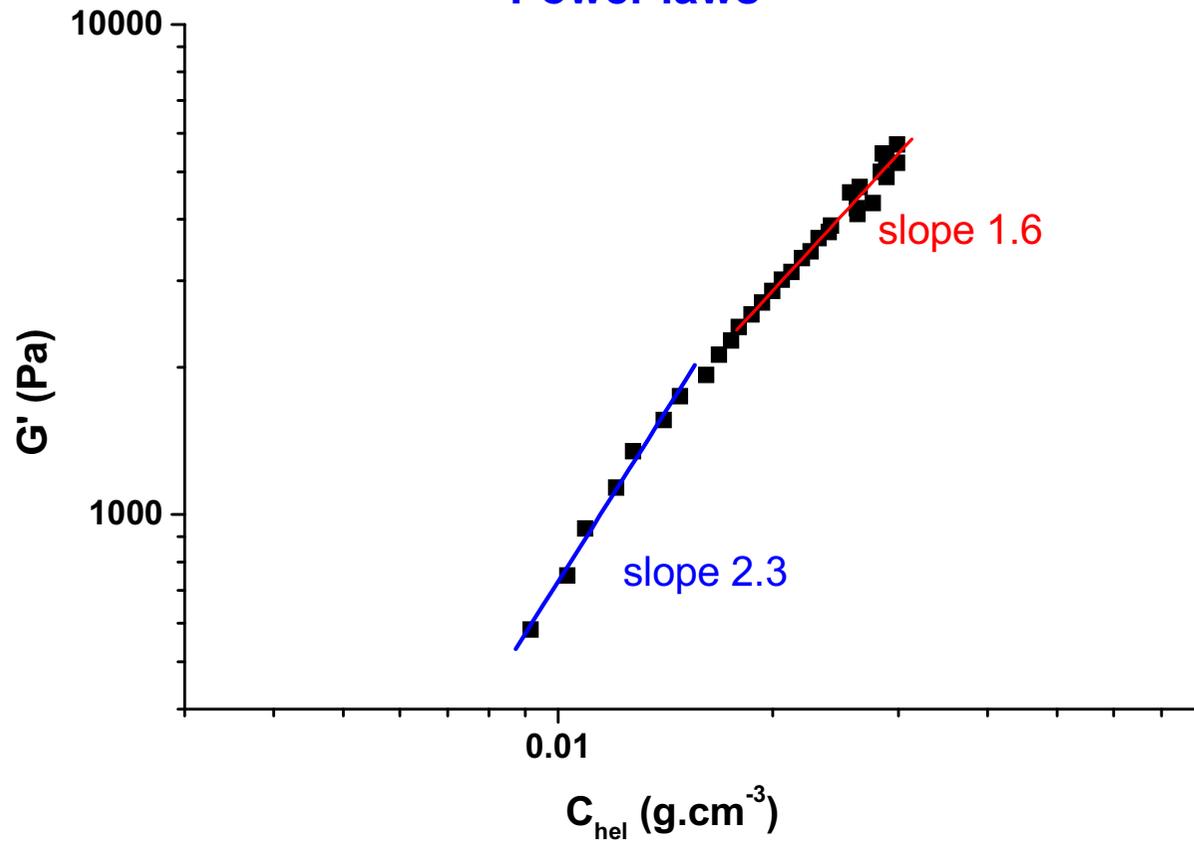
The master curve for elasticity : gelatins from various sources



Elasticity versus distance to the gel point :
the percolation regime



Elasticity versus helix concentration far from the gel point:
Power laws



A fibrous network containing helical sequences and coils:

Three domains:

- a. Below the gelation or percolation threshold, c_{hel}^{crit} no elastic modulus
- b. Between $c_{hel}^{crit} < c < 2 c_{hel}^{crit}$ the percolation regime

With a power law dependence of G' versus $c - c^{crit}$

$$G' \sim (c - c^{crit})^{1.9}$$

in agreement with the **scalar percolation**.

c. $c > 2 C_{hel}^{crit}$ a more homogeneous network is formed. There is **no simple power law dependence of G' versus helical concentration**. A progressive transition is observed.

Models dealing with networks of semi-flexible strands

Two parameters are required to define the elasticity:

- the length of the rods l
- the distance of entanglement of the rods d

If : absolutely rigid rods connected with very loose links (Jones and Marques 1990):

$$G' \approx k_B T l^{-1} d^{-2}$$

d is related to the total length of helices per unit volume, L_v , measured experimentally:

$$d = \left[\frac{1}{L_v} \right]^{-\frac{1}{2}}$$

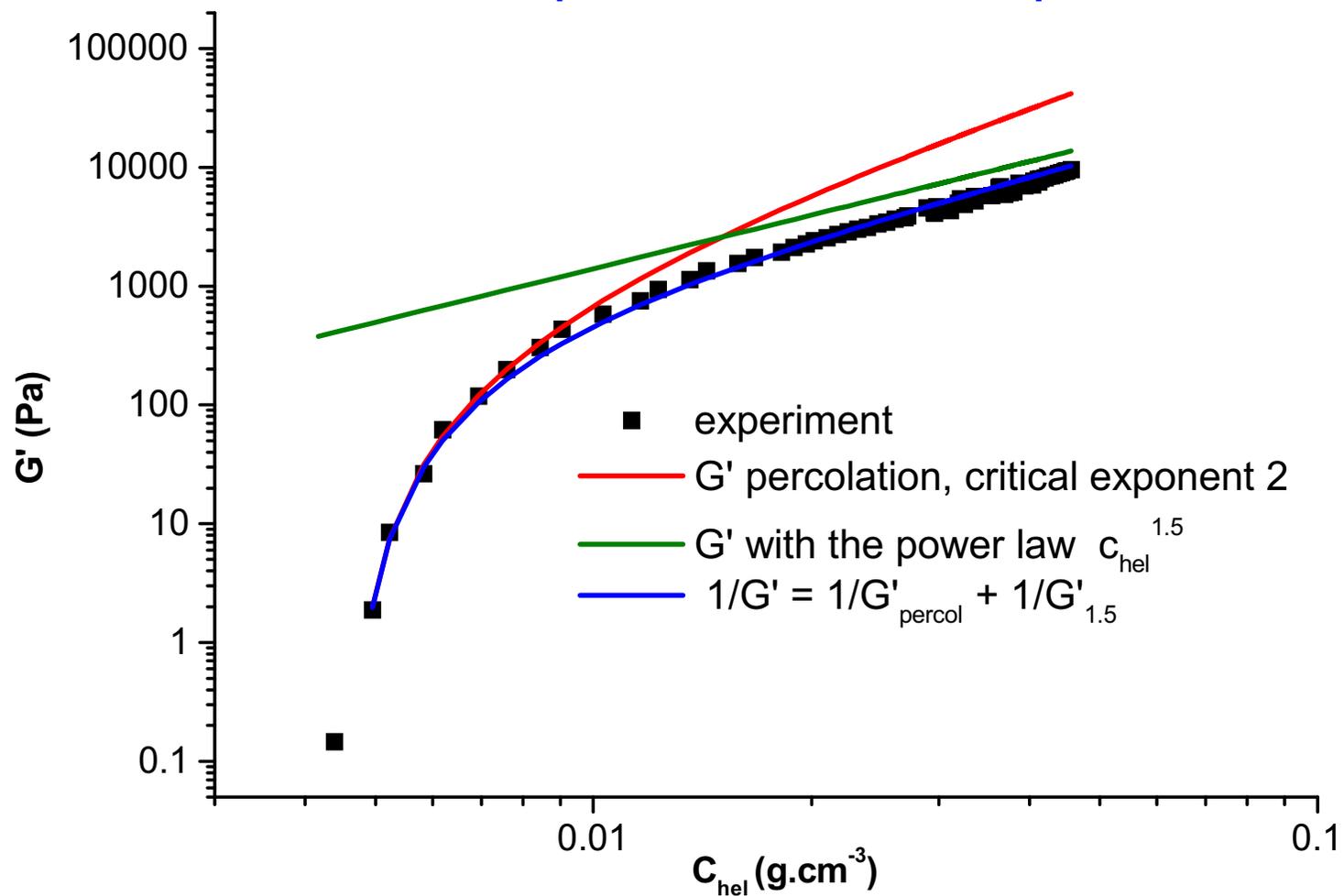
Then:

$$d \sim c_{hel}^{-1/2}$$

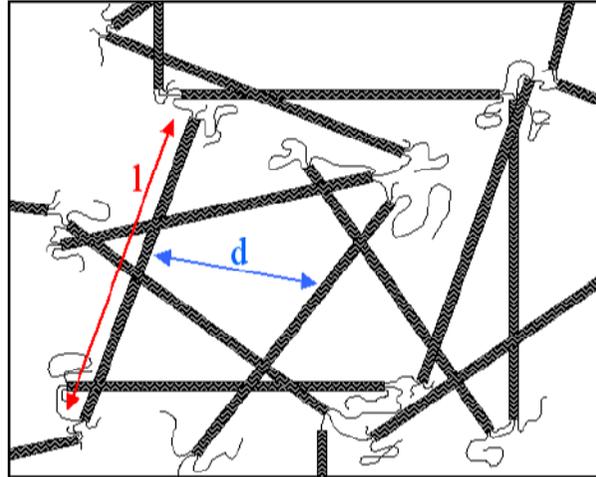
If one assumes that l and d are alike then one obtains:

$$G' \sim c_{hel}^{1.5}$$

Transition from percolation to well developed network



Gelatin physical gels



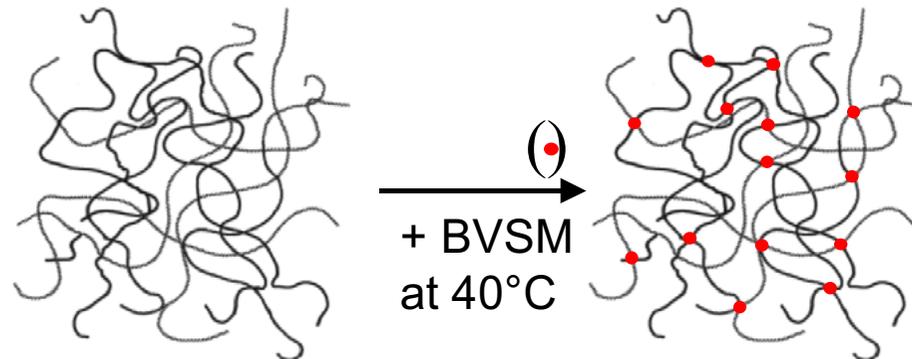
- The elasticity of gelatins networks is a unique function of the helix concentration
- There is no rubber like network involving large flexible coils connected by local junctions, at least in the linear regime.
- The fish gelatins are identical to the mammalian ones in this concept.

3. Chemical gelation in presence of a cross-linker.

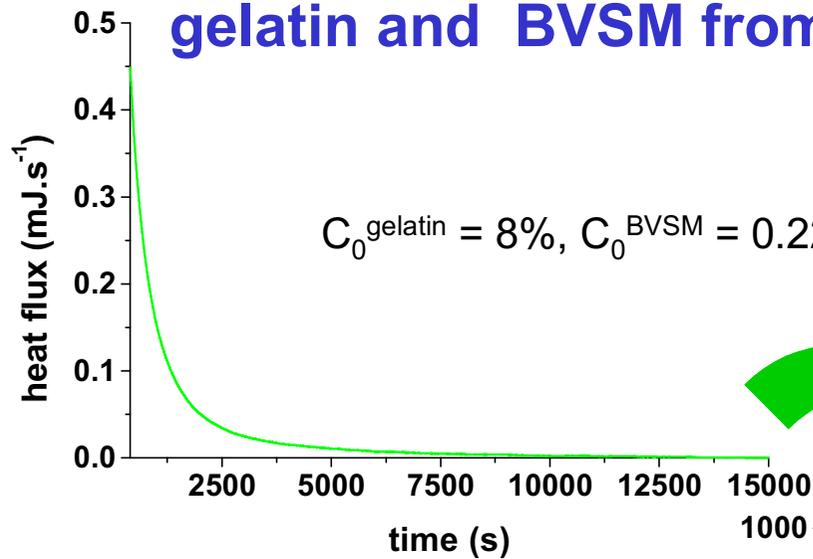
Hints for interpretation

Chemical gel : crosslinking

A permanent gel is obtained by using a crosslinker. Kodak uses the bis(vinylsulfonyl)methane BVSM. The crosslinker creates covalent links C-N with the amine groups of the gelatin coils (Lysine, Hydroxylysine and Histidine). A permanent network is created in the high temperature state (coils in solution) (beef gelatin, alkaline, in buffer)

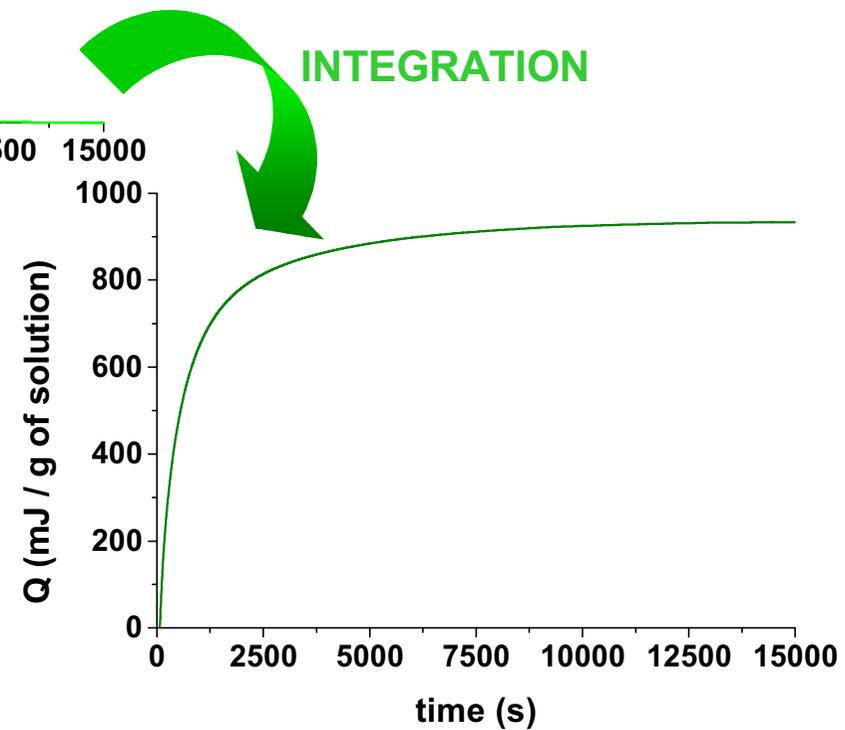


Formation of covalent links between gelatin and BVSM from microcalorimetry



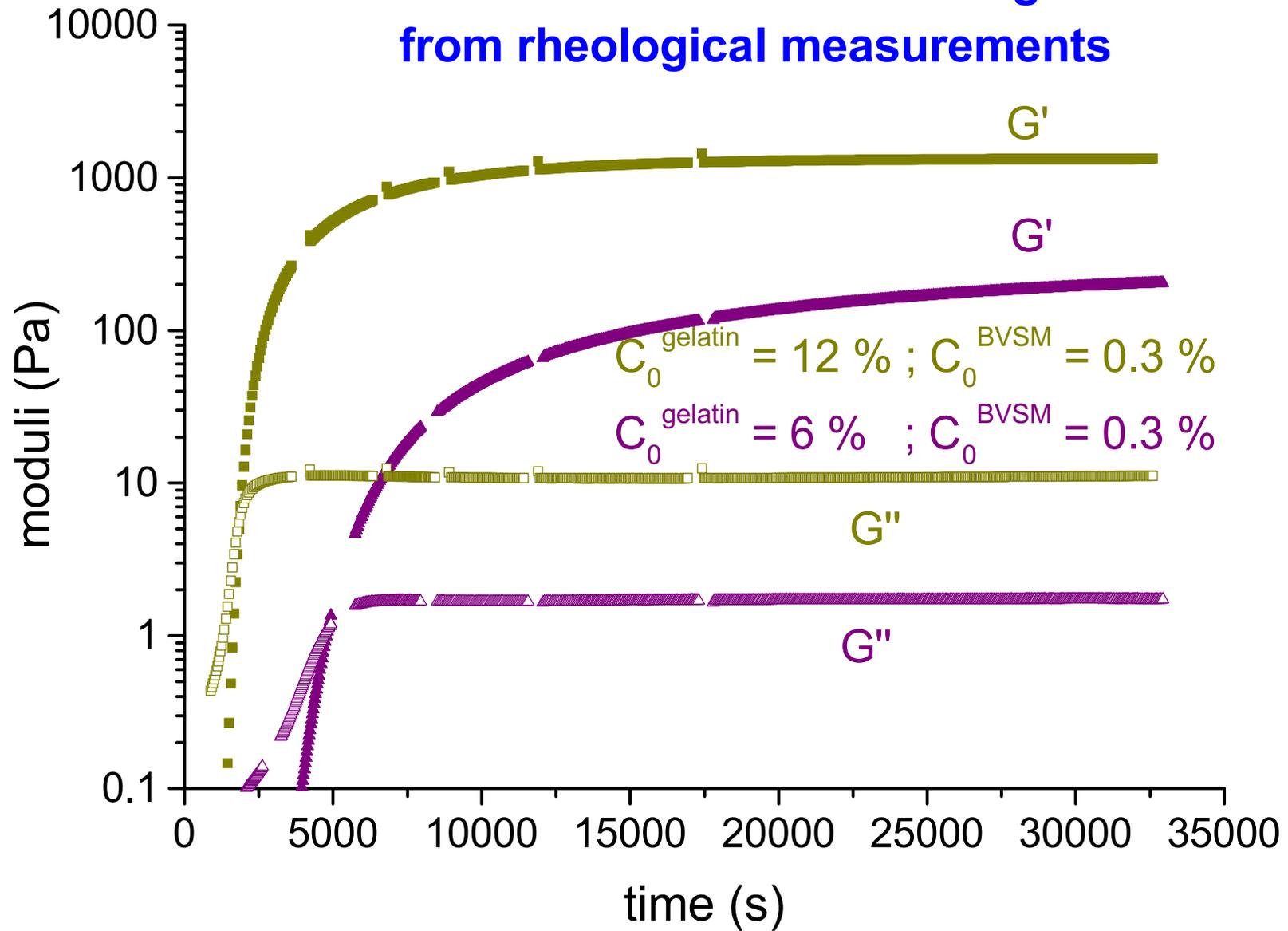
$C_0^{\text{gelatin}} = 8\%$, $C_0^{\text{BVSM}} = 0.225\%$, $\text{pH} = 6.79$, $T = 45^\circ\text{C}$

Formation of C-N links is **exothermic**

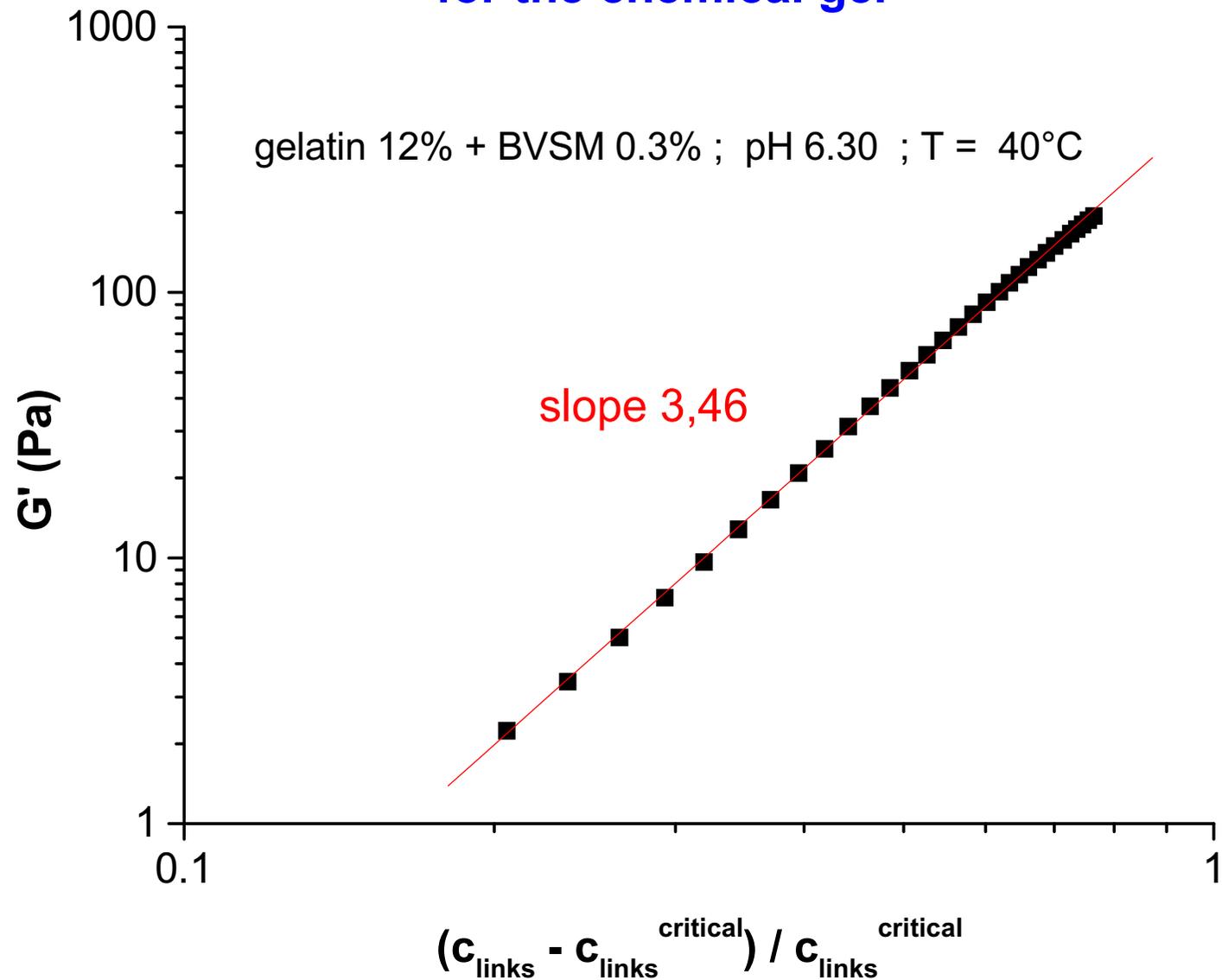


INTEGRATION

Kinetics of the crosslinking from rheological measurements



Critical behaviour versus the concentration of links for the chemical gel



Conclusions and outlook

- ❑ Physical gelation and chemical gelation of gelatin can be interpreted in the framework of critical phenomena.
- ❑ The two types of gels exhibit two different critical exponents of percolation.
- ❑ The physical gel reaches a state of a well developed network, while the chemical gel remains in the critical domain.
- ❑ Computer simulations are now under way.
- ❑ The gels crosslinked with both physical and chemical bonds are under study.

for more details see:

**C. Joly-Duhamel, D. Hellio and M. Djabourov
Langmuir, 2002, 18, p.7208**

and

**C. Joly-Duhamel, D. Hellio, A. Ajdari and M.
Djabourov,
Langmuir, 2002, 18, 7158**

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