DETERMINATION OF THE MOLECULAR WEIGHTS OF HUMIC ACIDS BY CRYOSCOPY IN STATIONARY PHASE

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Abstract:

This piece of work is a study of the molecular weight of the humic acids to determine their ability to penetrate into the leather and to subsequently establish chemical bonds between the fibres. The determination of the molecular weight was carried out by cryoscopy in stationary phase performed in a nitrogen atmosphere. According to the study, natural humic acids present the lowest molecular weight. Regenerated humic acids and sulphited humic acids present a higher molecular weight but they can still penetrate between the collagen fibres.

1 Introduction

Several studies have been carried out over the last years with the aim of developing new tanning products capable of fully or partially replacing basic chromium (III) salts and vegetable extracts1-14.

In line with the research mentioned above, previous studies explored the possibility of using humic derivatives as tanning and retanning agents. Such derivatives, obtained from a raw material commonly found in Spain, coals and lignites, are mainly used as fuels and very occasionally as humic acids. The use of such derivatives in tanning would increase the value of these products considerably. Also, their use would cause a low environmental impact15-17. This study is about the molecular weight of humic acids and aims to determine their ability to penetrate into the leather and to subsequently establish chemical bonds between the fibres. Since these compounds are not homogeneous, the molecular weight values obtained in the humic acids are always an average of the distribution of molecular masses of the sample. In this sense, we have distinguished between different fractions of humic acids: natural humic acids (NHA), regenerated humic acids (RHA) and sulphited humic acids (SHA).

Determination of the molecular weight was by cryoscopy in stationary phase. It was performed in a nitrogen atmosphere because it is fast and easy to handle, giving values that, depending on their origin and type, range between 1000 and 4600. The cryoscopic method measures solvent activity in solution relative to the activity of the pure solvent.

The theoretical part of the cryoscopy method in stationary phase starts with the observation of the cooling curve in Figure 1

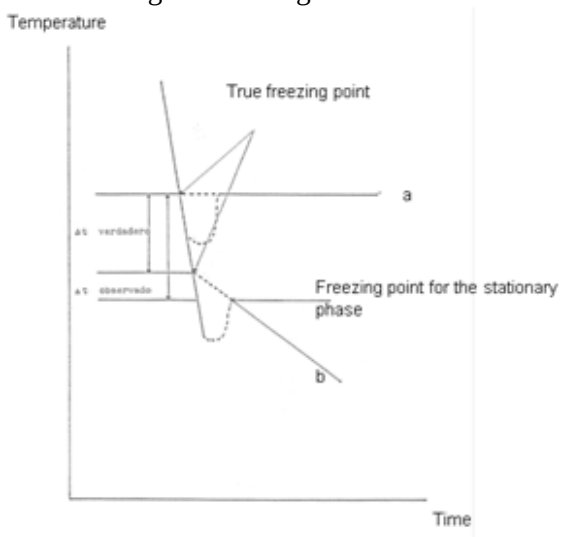


Fig. 1 Cooling curves

As can be seen in the figure, the temperature of the solution, initially above the freezing point, decreases at a speed which is as slow and constant as possible. It can also be seen that the temperature decreases below the expected point. Crystallization starts with this overcooling; it is subsequently followed by a quick increase of the temperature, and again it decreases slowly. The method of the stationary phase takes as the freezing point the maximum temperature after crystallization instead of the extrapolated value which would stand as the true one. A very important condition is the constant regulation of the cooling speed and degree of overcooling, as is the minimum error made when taking the freezing point, instead of the extrapolated value, as the maximum

value of the curve. This is included in the percentage of errors of the method itself when using the blank determination. In order to determine the molecular mass of the solute we need to know its mole fraction. The problem for the theoretical treatment is calculating the mole fraction of the sample from the action of the solvent in solution. Raoult's law is formulated this way: the activity of the solvent is obtained, after a first estimate, with the mole fraction of the solvent, all the terms of the coefficient of activity which change alongside the nature of the simple can be relegated to quadratic terms or higher in the mole fraction. The problem is expressing the activity as a function of the mole fraction and then separating the linear term from the higher ones.

The cryoscopic method assigns an activity to the pure solvent, and when the sample is added to the solvent, solvent activity in solution decreases. This method measures the change in temperature that is required to equate the solvent activity in the pure phase to the activity in solution which is being measured.

Lewis and Randall's equation for cryoscopic measurements is as follows:

$$\ln a = \frac{\Delta H}{RT_0^2}(T_0 - T)\left[1 + \left(\frac{1}{T_0} - \frac{\Delta C_p}{2\Delta H}\right)(T_0 - T) + \left(\frac{1}{T_0^2} - \frac{2\Delta C_p}{3T_0\Delta H}\right)(T_0 - T)^2\right]$$

Where:

a = activity

ΔH = solidification heat

R = gas constant

T_0 = freezing point of pure solvent

T = freezing point of solvent in solution

Cp = heat capacity at constant pressure

ΔCp = difference between heat capacities of solvent in solution and pure solvent.

Through extensive mathematical elaboration, an expression of the molecular mass of the solute can be obtained. In the limit dilution, both the solute weight and the cryoscopic decrease near zero. This becomes the traditional formula:

$$M = K\frac{1000 \cdot W}{t \cdot w}$$

where:

M = molecular mass

K = cryoscopic constant of the solvent

W = solute weight

t = cryoscopic decrease

w = solvent weight

With this formula, cryoscopy is based on the measurement of the decrease of the freezing point of a solvent, which originates with the addition of the sample. Cryoscopy makes use of the following methods to measure the freezing point: cooling curve method, heating curve method, stationary phase method and equilibrium method.

The method used in this study, the stationary phase method, measures the temperature at which the heat of an overcooled solution which is released by crystallization is counterbalanced exactly by the heat of the system that is lost. The measurement begins according to the procedure of the cooling curve. That is, the solution is initially above its freezing point and starts cooling at a slow and constant speed, the temperature decreases below its freezing point and then, through seeding or occurring spontaneously, the crystallization of this overcooled solution begins; after that, the temperature increases abruptly to a maximum value and slowly decreases again. The method followed (stationary phase) uses as a value the maximum temperature recorded after the crystallization. For this method to be applicable there must always be the same percentage of frozen solvent, provided that the maximum of the cooling curve is reached. This is controlled by keeping the cooling speed and degree of overcooling constant. The error made when noting the value of the maximum temperature is properly corrected by normalization and calibration of the method. This method is traditionally used for cryoscopic determinations because of the many advantages it offers over other cryoscopic methods: speed, ease of handling, and it does not require the plotting of the temperature vs. time graphs for each freezing point.

As mentioned previously, the value obtained is the value of the average linear molecular weight. This value, as is found in a mixture, is defined as the molecular weight obtained from averaging the molecular weights of each of the mole fractions which are homogenous with respect to the molecular weight. It is nothing more than the arithmetic mean.

Despite the ease of handling and speed, there are some limitations which restrict the use of this method:

i) Sample solubility. The sample must be soluble both above and below the freezing point for the solvent used. This is not a problem when the sample is pure since the freezing point no longer changes when beyond the solubility limit. However, when the sample is a mixture, one of the components might be beyond the solubility limit and not be properly detected by the measurements, which would render them useless.

ii) The sample must not form a solid solution with the solvent; the solid solution is formed when the sample is soluble in the solid phase and is distributed between this phase and the liquid phase.

iii) The solvent must not have a solid-solid transition phase within or near the area of cryoscopic decreases to be measured. This interference prevents us from observing the true freezing point of the solutions whose temperatures are within the area of solid transition temperature. From the data of these solutions, the values obtained of the cryoscopic constant are erroneous.

iv) The sample must not undergo associations within the solution, whether with the solvent or with the sample itself. When the association is with the solvent, we obtain greater values of the cryoscopic constant and lower values of molecular weight. To avoid erroneous measurements and to obtain the values of both he cryoscopic constant and the molecular weight, the extrapolation to infinite dilution is determined: there are practically no molecules of solute and association is considered inexistent. When association occurs with the sample itself, molecular aggregates which act as individual particles are formed. The molecular association occurs mainly in samples with –OH groups and by hydrogen bonding. The interference produced by association in the determination of molecular weights has been proven only for certain phenols and acids. Distinguishing between association and the effect of solid solution may be difficult since their effects are similar in the measurement of the molecular weight. They can be detected by comparing the results obtained with a polar solvent and with a non-polar solvent. The bonds by hydrogen bonding are expected to persist more with one than with the other. When determining a dilution to infinite, an interference-free value can be obtained by association.

v) Magnitude of the molecular weight. The best measurements are obtained for substances with a molecular weight up to 1000 and for those which slightly exceed 3000, since the influence of the molecules with low molecular weight on the total mean value is more sensible when the magnitude of the molecular mass of the superior components is greater.

vi) The fusion heat of the solvent does not have to be too low since solvents with low fusion heat tend to yield a high cryoscopic constant. These results in large decreases of the freezing point and major errors in the measurements since the lower the fusion heat of the solvent the more difficult it is to maintain and control the heat exchange between the cryoscopic tube and the cooling chamber.

2. EXPERIMENTAL

2.1. Material

The cryoscopic solvent used is Sulpholane (tetramethylene-sulphone); in accordance with the comparative study by Durwell & Langford, this solvent is the most appropriate due to its characteristics and to the values of its physical constants.

The most interesting values of its physical constants are:

-dielectric constant at 30º 44
-fusion point
28ºC
-boiling point (760 mm) 285ºC
-fusion point 2.73
0.03 cal/g.
-cryoscopic constant 66.2
0.6 ºC/ mol

This is why its use is recommended as the cryoscopic solvent for humic acids.

A disadvantage for the determination of molecular weights is the possible associations of the solute with the solvent. Durwell & Langford studied the behaviour of acetic acid, methanol and water with sulphane and found out that above concentrations of 0.01-0.1 molar, the acetic acid behaves like a monomer, methanol shows some associations and water is found as dimer. Given that the humic acids are acid-based and bear a greater similarity to the acetic acid than they do to methanol and water, it should be assumed that these acids behave in like manner in spite of the differences of behaviour due to their complex molecular structure and in contrast with the simple molecular structure of the acetic acid.

The presence of water in the solvent or in the sample is one of the worst interferences in cryoscopy. It can easily be eliminated from Sulpholane due to the difference in boiling points between Sulpholane and water. The effect that water has on the cryoscopic decreases, making them more acute and yielding much lower molecular weights. To achieve this, the Sulpholane we use is subjected to a bi-distillation to ensure its purity.

In contrast to other cryoscopic solvents, the cryoscopic constant of Sulpholane does not vary with the concentration. In addition, the value of its cryoscopic constant is high, which renders the Sulpholane solutions more sensitive to variations of the freezing point when altering the concentration of solute. The fact that the freezing point is around the area of 28ºC ensures a precise and comfortable testing. The solubility of humic acids in Sulpholane is good. The high higroscopicity of Sulpholane needs to be taken into consideration; it needs to be always handled in a stream of nitrogen.

The humic acids used for the determination of the molecular weight are divided into three types: natural humic acids, directly extracted from lignites from Meirama; regenerated humic acids obtained by oxidation of carbon by air; sulphited humic acids obtained by sulphonation of natural acids.

2.2. Experimental method

We used the cryoscopic device shown in Figure 2.

The operational method consists of two stages:

1st. Step: First, the freezing point of pure Sulpholane is found and recorded using the differential thermometer. A reference point is obtained from which to calculate the cryoscopic decreases. The cryoscopic tube is positioned and the air is removed using a stream of nitrogen; the agitation system and the thermometer are positioned, making sure the thermometer does not touch the tank wall. Next, Sulpholane is heated and fed into the system. When the temperature is approximately 6 ºC above the freezing point, the system is put into service; when the temperature of Sulpholane is at around 2 ºC above the freezing point, the float is prepared at approximately 4 ºC below the freezing point so that the cooling speed is approximately 0.2 ºC/ min. The maximum temperature can be obtained when the freezing is completed. Additionally, the temperature has been recorded every 30 seconds. We use these values to plot the graphs (Fig.3, Fig.4 and Fig.5). If the temperature of the cold float is changed, Sulpholane can be melted again and the measurements replicated desired.

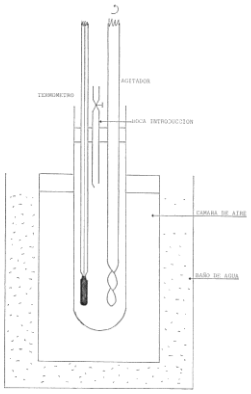
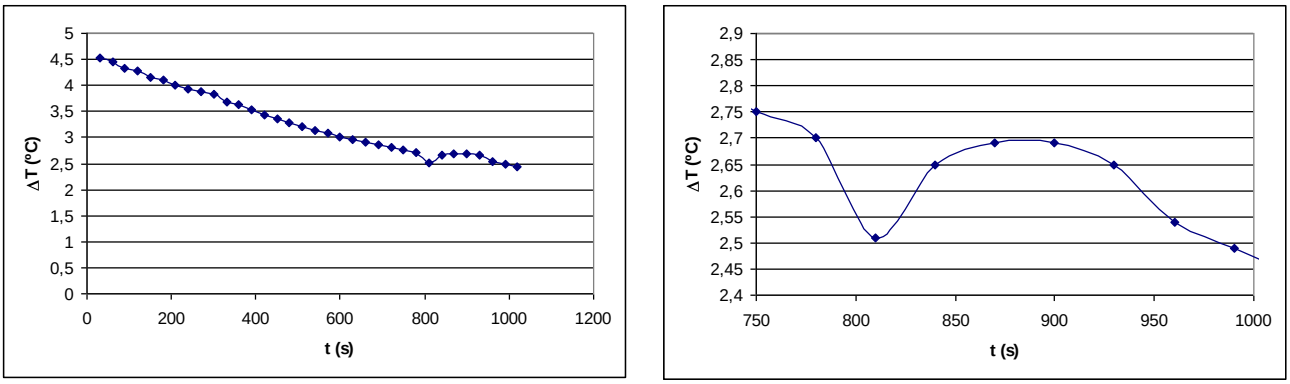


Fig. 2. Sketch of a cryoscope

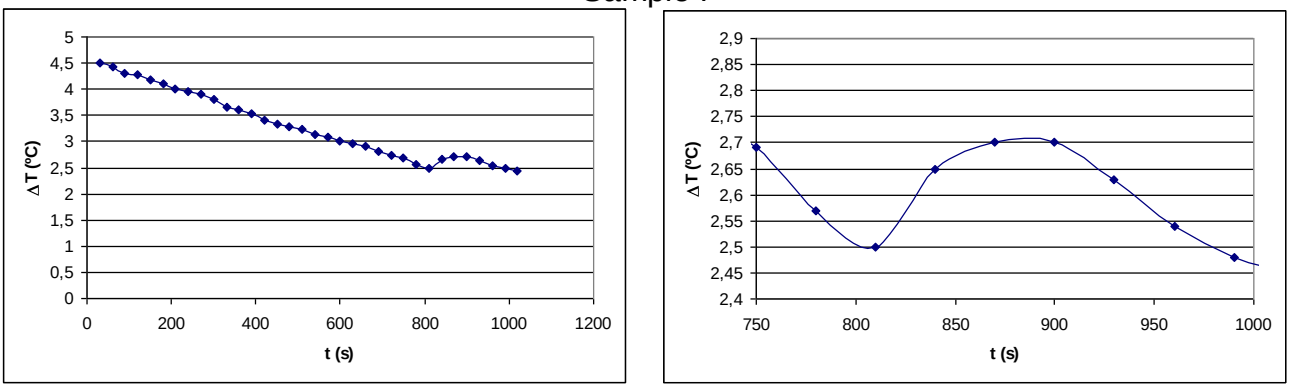
2nd step: the solutions of humic acids are prepared to measure the cryoscopic decreases. Firstly, the humidity in the humic acids has to be eliminated to avoid the interferences previously explained; a desiccator is used to that end, and the humic acids are left in there at 38-40 ºC for 40 hours. Next, the acids are left to cool at room temperature, weighed in a dry atmosphere (1 g approximately), placed inside an Erlenmeyer and sealed with an air-tight cap. The Sulpholane is added (100 g approximately) in a tared vessel, the whole set is weighed and the difference in weight tells us the exact weight of the solvent. A 3/1 mixture of acetone/water is prepared, poured into the solution at a rate of 70 cc per gram of humic acids and agitated for 12 hours. After this period, the humic acids form a homogenous solution. The acetone is eliminated from the solution under a slight vacuum and the water by distillation, heating up to a maximum of 50-60 ºC and at a high vacuum to prevent chemical changes in the molecule of the humic acids. When the distillation process is completed, a stream of dry nitrogen gas is applied to the water-free solution, which is then placed in the cryoscopic test tube to carry out the test. The conditions are the same as with the pure solvent.

3. RESULTS

Fig.3, Fig.4 and Fig.5 shows the plotting of the increase of cryoscopic temperature and the subsequent enlargement of the stationary phase for each of the three samples (N.H.A., R.H.A. and S.H.A.).



Sample I



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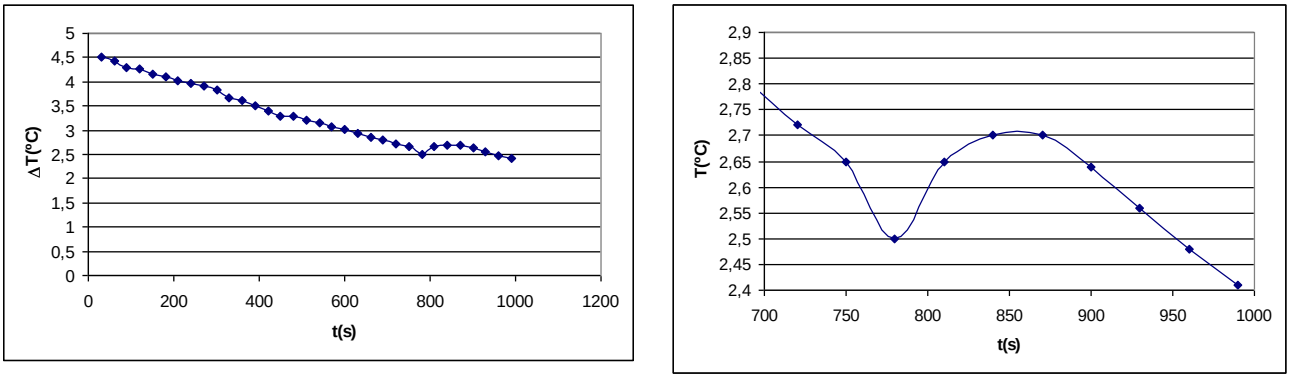
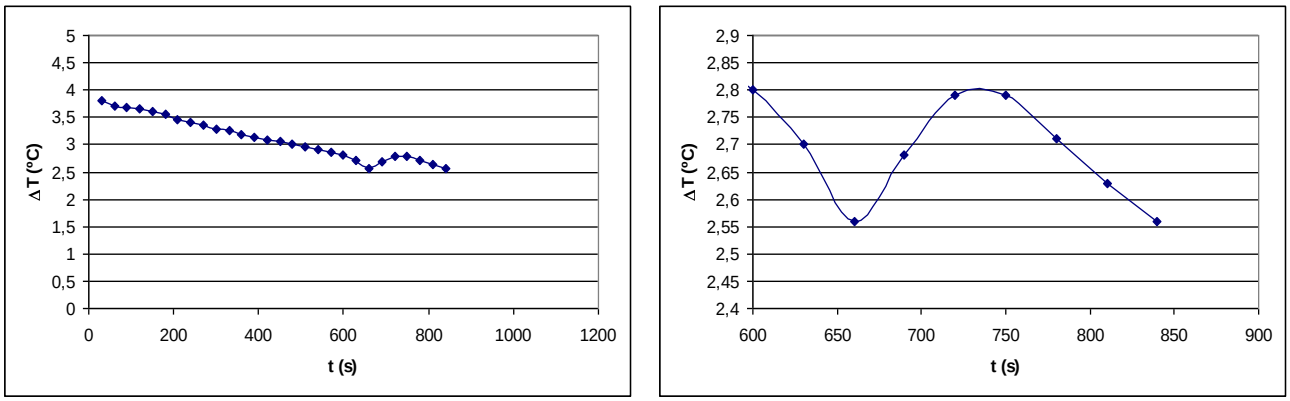
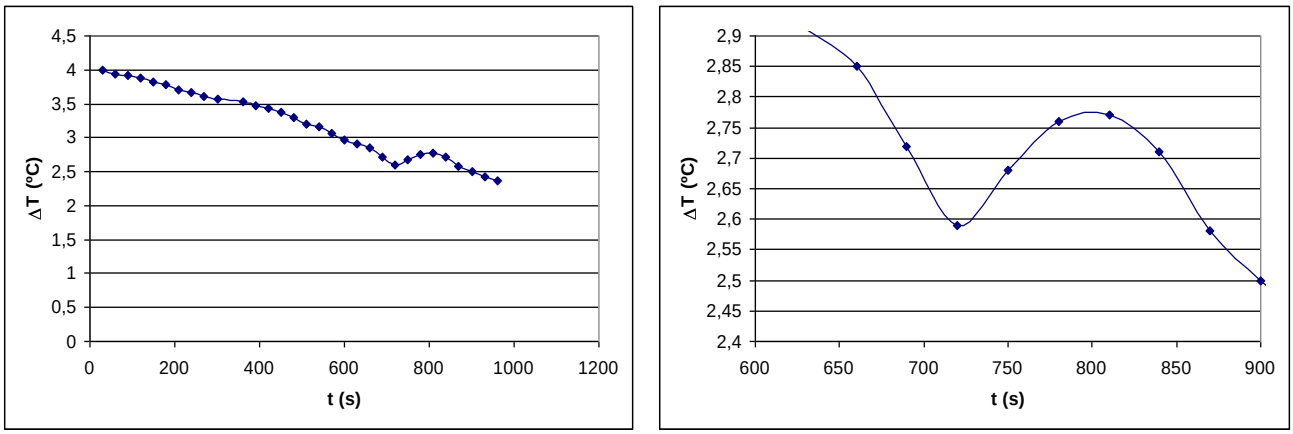


Sample III

Fig. 3. Cryoscopic temperature increase and expansion of stationary phase of N.H.A.. samples.



Sample I



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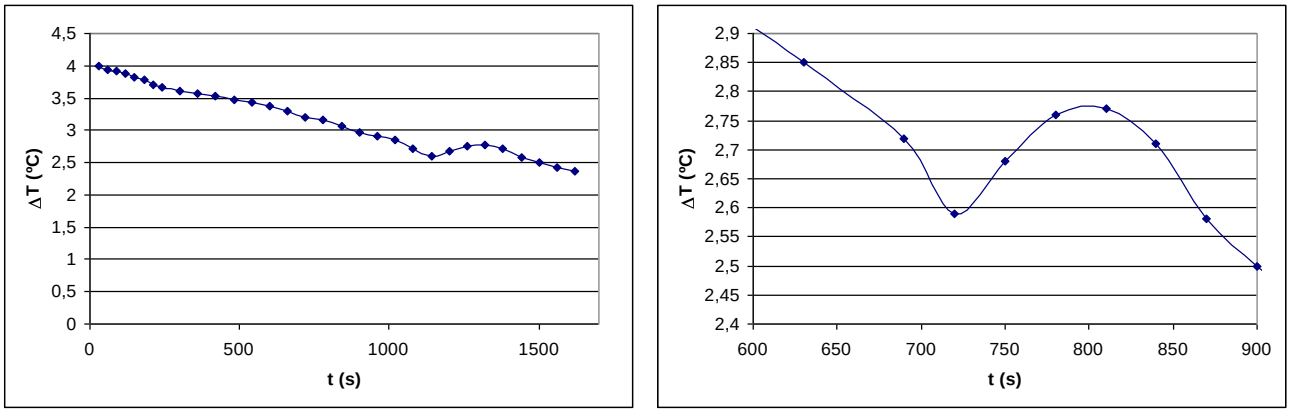
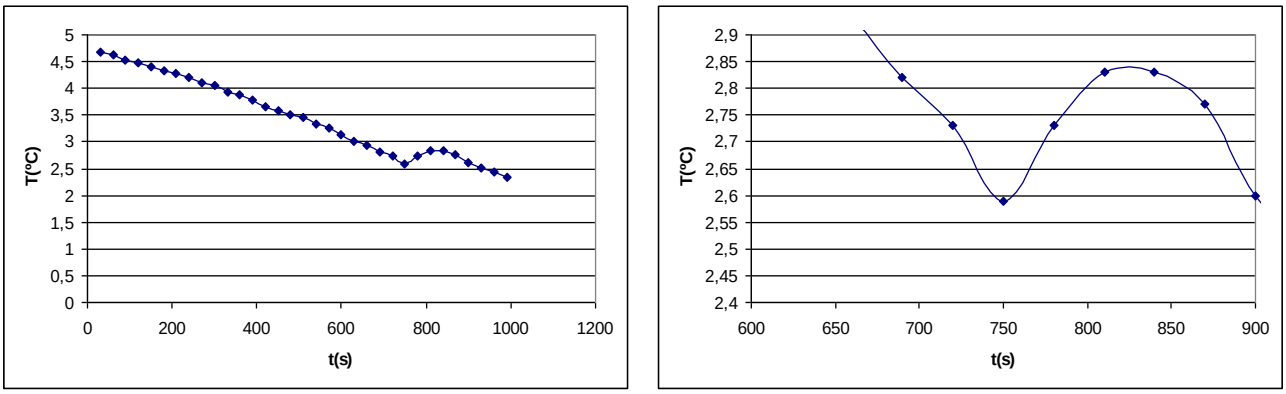
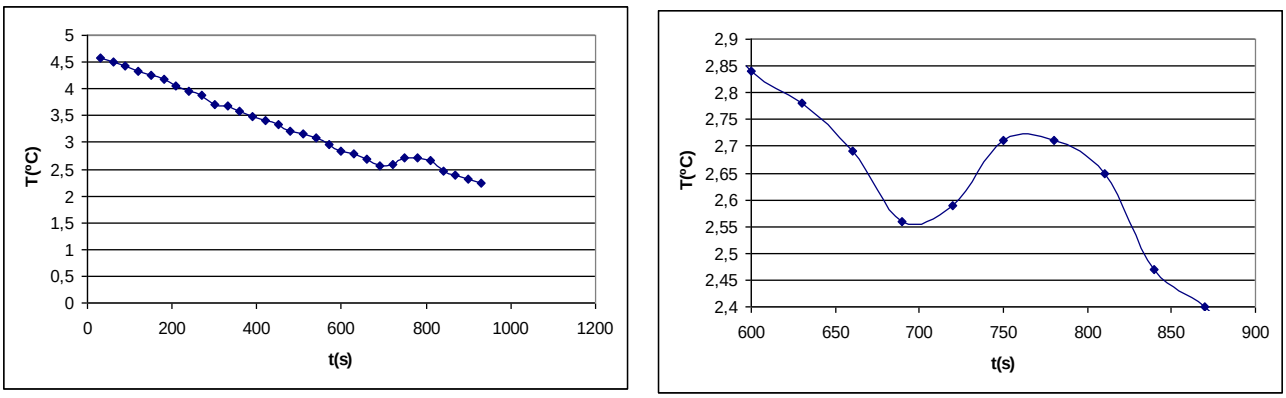


Sample

Fig. 4. I Cryoscopic temperature increase and expansion of stationary phase of R.H.A. samples.



Sample I



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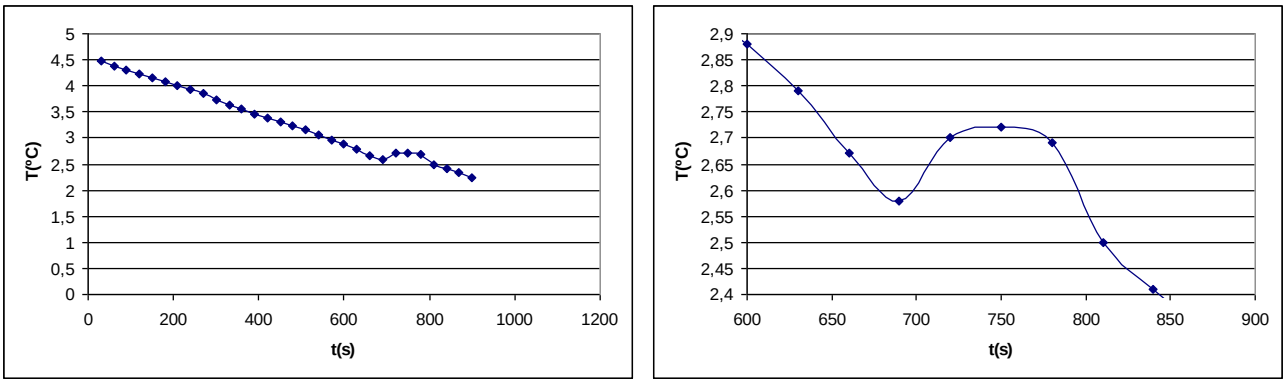

Sample

Fig. 5. Cryoscopic temperature increase and expansion of stationary phase of S.H.A. samples.

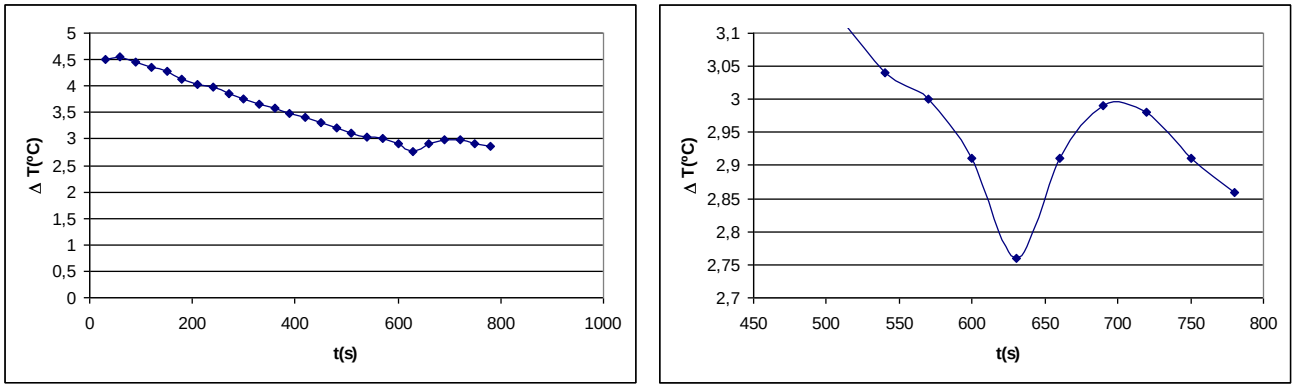

Fig. 6. Cryoscopic temperature increase and expansion of stationary phase of Sulpholane samples.

Table I shows the molecular weights obtained:

TYPE	g humic acid /100g Sulpholane	MOLECULAR WEIGHT
N.H.A. Sample I	1.0298	2309.2
N.H.A. Sample II	0.9971	2314.3
N.H.A. Sample III	1.0117	2316.1
R.H.A. Sample I	1.0048	3692.6
R.H.A. Sample II	1.0745	3645.0
R.H.A. Sample III	0.9992	3888.1
S.H.A. Sample I	1.0212	3937.7
S.H.A. Sample II	1.0409	3445.4
S.H.A. Sample III	0.9897	3639.9

As the graphs and tables show, the natural humic acids have the lower molecular weight. The regenerated and the sulphited humic acids have a higher molecular weight, but it is still enough to be able to penetrate between the collagen fibres.

4. CONCLUSIONS

- The method followed is easy, replicable and economically viable.
- The values of the molecular weights obtained of the three different types of humic acid analysed are sufficient to penetrate between the fibres and bond chemically with them. That is to say, the three types have tanning capacity.
- The natural humic acids have a lower molecular weight.
- The molecular weight of the regenerated humic acids, resulting from hydrolysis of humines and bitumens, is higher than that of natural humic acids, but not high enough to be able to penetrate between the fibres (they are in the top part of the interval of valid molecular weights).
- The sulphited humic acids contain sulphonated groups and thus have molecular weights similar to those of regenerated humic acids. This is likely to occur due to bonding between the molecules.

5. REFERENCES

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