FINAL REPORT

"A Comparative study of the physiochemical properties of the Hyaluronic Acids ViscoPlus and Chondrovital"

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PROPOSED OBJECTIVES:

The specific objectives of this project are:
1.-Refine and carry out rheological characterization using the elastic and viscous modules ***(G'*** and G'') of the **"ViscoPlus"** AH.
2.- HPLC measurement of the Molecular Exclusion the PM o said AH's.

MATERIALS USED

In order to complete the objectives stated above, we followed the proposed protocol in which three copies of the measurement were taken for each assay of the samples provided by ViscoBasic: ViscoPlus (LOT VGN34300A, CAD 2016-02).

1.- Rheological characterization:

In order to carry out the rheological characterization, the oscillating module was used as this is the most appropriate module for viscoelastic products. Ascending frequencies from 0.01 Hz to 20 Hz were applied with level of tension (of 20 Pa) that was chosen for the study according to the results obtained in a preliminary study in which tension in the linear viscoelastic region (LVR) was measured as the average tension between the various consistencies of the liquids being measured. The cone-plate (40 mm) geometry used was the most appropriate for obtaining good repeatability of the measurements.

Parameters such as the elastic dynamic modules or the storage module (G') (which means recoverable elastic energy storage of the deformation in an elastic solid) and the viscous or loss module (G'') (which describes the viscous dissipation or energy loss through a permanent deformation in the fluid) are very useful when characterizing the viscoelastic properties of polymer solutions (hyaluronic acid in our study). We also wanted to focus on the biomechanical function of these liquids which are intended to supplement synovial fluid.

Assays of the hyaluronic samples mentioned above were performed in the laboratory. Average values were obtained from the various assays.

The graphs show the results:

Regarding viscoelastic behavior, a graph of the viscous and elastic modules versus frequency reveals two components of the viscoelastic products (Table 1 and Fig. 1):

Fig. 1 shows the rheograms together, compared with other results obtained by our experimental group for other commercially available hyaluronic acids (indicated as A, B, C, D, E). Keep in mind that the lower the frequencies at which the crossover point is reached, the greater the elastic properties. It is expected that as the molecular weight increases, the crossover point will be reached at lower frequencies. In our case, there is a synergistic effect from both parameters (high MW and high concentration).

"Crossover Point"

Products	Freq (Hz)	G' = G'' (Pa)
A	0.07	28.21
B	0.185	83.10
C	0.165	30.43
D	0.51	111.93
E	0.60	268.10
ViscoPlus	0.06 ± 0.006	182.23 ± 6.40

Table 1

2. Degradation resistance tests:

2.1-Chemical degradation with OH radicals using the Fenton reaction.

200 mg of adjusted sample was taken and incubated at 37°C for 24 hours. Then 10 pL of H2O2 was added and the sample was shaken vigorously for 1 minute. Degradation kinetics was monitored in the HPLC described in the project. The measurements were carried out at different times between 0 and 90 minutes (0, 15, 30, 45, 60, 75, and 90) after the addition of H2O2. The assays were performed in triplicate.

The comparative graph of all of samples obtained an average result of the three copies as shown below: Table 2:

reaction t	% rem prom
0	100
15	93.5 ± 4.8
30	92.6 ± 4.6
45	91.5 ± 4.9
60	91.2 ± 4.4
75	90.4 ± 4.6
90	90.2 ± 4.5

2.2.- Enzyme degradation through reaction with hyaluronidase.

150 mg of each adjusted sample was taken and placed in a reaction with a final volume of 50 IU of hyaluronidase from bovine testicles (H3884 - Sigma) where the reaction kinetics was quantified every 15 minutes in the HPLC at a temperature of 37 °C in the injector. At the start of the reaction, a vial was prepared without subjecting it to the reaction. This was considered to be a reaction value of 0 (t_0). The degradation percentages are shown in Table 3:

reaction t	% rem prom
0	100
15	95.1 ± 3.4
30	88.6 ± 3.0
45	86.3 ± 3.1
60	84.6 ± 3.0
75	83.5 ± 2.7
90	82.6 ± 2.2

3.- Molecular weight measurement:

The molecular weight is calculated using the construction before the control line in which hyaluronic acid controls with known molecular weights (from Life Score) were used in the molecular exclusion HPLC (GPC), which is based on the chromatograph retention time for each of the molecular weights used.

A linear representation was obtained by representing the Log PM versus retention time.

The equation obtained for this control line was:

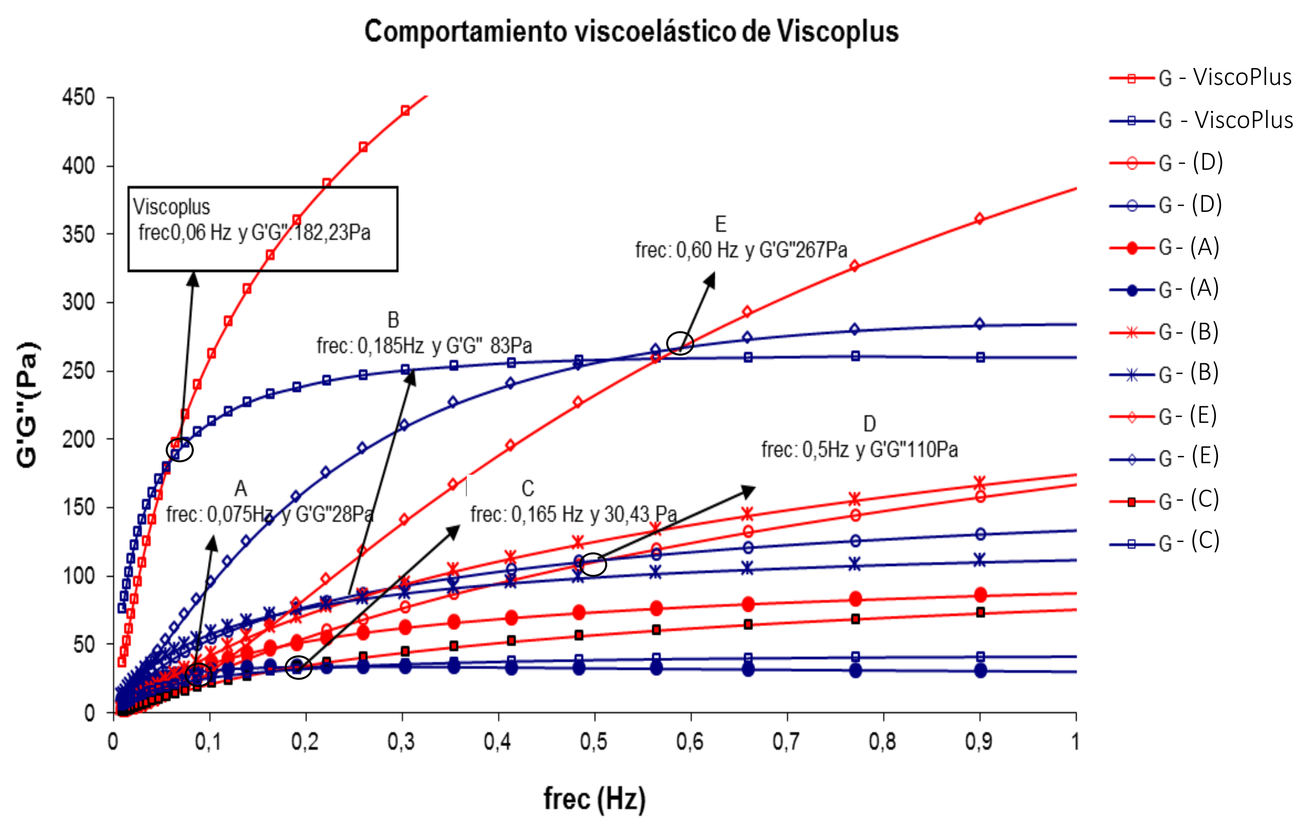
Y (Log PM) = -0.4829 x Tr + 6.5644 (R^2= 0.9943)

As with ViscoPlus, the Tr was 6.59. Application of the above equation gives us an approximate molecular weight value of 2410 KDA.

Conclusions

The results obtained from the rheological study reveal very favorable viscoelastic behavior as a result of the synergistic effect of a high concentration of hyaluronic acid and a relatively high molecular weight without being a product resulting from cross-linking processes. It also has a good hardness (182.23 Pa) at the crossover point where there is a switch from "viscous" to "elastic" behavior. This high level of hardness would indicate a shear capacity that would occur in a joint when walking and offers greater lubrication in order to prevent cartilage involvement, thereby protecting it.

Regarding the elastic properties of the product, the results reveal a high level of elasticity when changing from viscous to elastic behavior at a very low frequency (0.06 Hz). This puts the product at the forefront of the hyaluronics currently on the market for elasticity (Figure 1).



Viscoelastic behavior of Viscoplus

[remainder of graph does not require any translation with the exception of changing "frec" to "freq" wherever it appears].