WHITE PAPER

AN INNOVATIVE WAY TO THERMALLY STERILIZE HYALURONIC ACID PRE-FILLED SYRINGES

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Several reports on degradation of HA involving microwaves, UV or γ-rays have been published. Rheological studies also show that HA solutions degrade at high temperature: viscosity of the solutions decreases in time as a function of temperature. Therefore, the thermal sterilization treatment of HA solutions represents a great challenge. An optimized moist-heat sterilization cycle can minimize product degradation (and change of molecular weight) maintaining the required viscosity for the specific application.

KEYWORDS

Dynamic viscosity determination

Peak cycle

Counterpressure treatment

Moist-heat sterilization

Sodium Hyaluronate

Pre-filled Syringes (PFS)

FEDEGARI GROUP

1. SODIUM HYALURONATE PFS

The Sodium Hyaluronate used to prepare the batch had an average molecular weight equal to 1.6 x 10^6 Dalton. The formulations were prepared by hydrating the polysaccharide in a physiological phosphate buffer solution for 12 hours at 50°C. The bulk preparations were divided in pre-filled syringes.

Glass PFS containing 50 mg / 2.5 ml of Sodium Hyaluronate.

Prefilled syringes with Hyaluronate Sodium provided by IBSA.

2. PROCESS SELECTED

Samples were treated using an Air over Steam autoclave (Fedegari FOA); this process equipment works with counterpressure in the chamber to balance pressure increase inside the syringes during the sterilization and to avoid the risk of plunger movement.

The air initially contained in the chamber is not extracted - as in a pure saturated steam cycle - but it is only "controlled" by increasing or decreasing its quantity (i.e. the pressure) during the heating/sterilization/cooling phase according to the characteristics of the load. The pressure of chamber air increases as it heats (with the relation of the absolute sterilization and cycle start temperatures). The total pressure in the chamber during the sterilization phase is given by the partial pressure of the heated air plus the partial steam pressure; the final pressure in the chamber will be therefore much higher than the one obtained with pure saturated steam autoclaves.

3. COUNTER-PRESSURE

The counter-pressure inside the autoclave was adjusted by setting the air pressure correction coefficient at 1.2. This value is a little higher than the standard value (1.08) to avoid plunger expulsion. At 121°C, 115°C and 130°C, the pressure of the vessel was 3.56 abs, 3.18 abs, and 4.25 abs, respectively. These values were calculated by using the counter-pressure equation (equation 1) and also verifying the results on Thema 4 in the final phase data section.

Equation 1

$$(P_{Total}) = (P_{Vapor}) + (k * 0.971 * \frac{T + 273.15}{30 + 273.15})$$

LOADS CONFIGURATION

Image 1 - PFS with TC inside

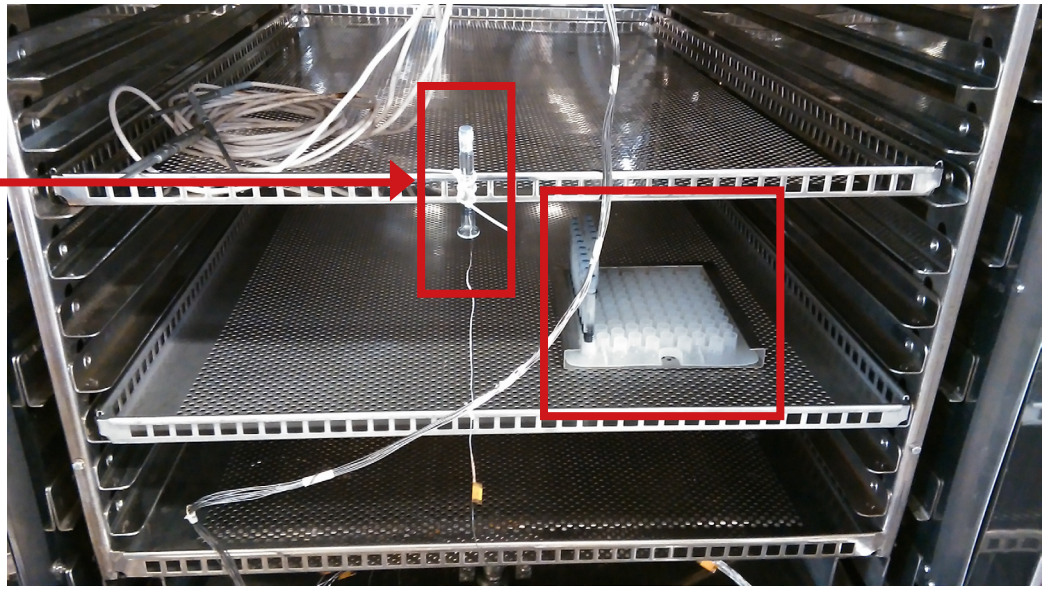


Image 2 - PFS with TC inside and 15 PFS placed in a tray

4. F_0 CALCULATION

The sterilization process was controlled by an F_0 target of 15' using a Kaye validator. It was calculated in the sample with a thermocouple device (TC) inserted into a "reference syringe". The accumulation period started once the temperature reached 100°C. The sterilization phase was manually stopped at F_0 < 15' so that at the end of the thermal process, the total F_0 accumulation - all cycle long - was roughly 15 minutes for each cycle.

5. LOADS CONFIGURATION

Load Type 1:

• 1 PFS was vertically attached to the side of the tray inside the machine using zip-ties. The thermocouple was placed inside the solution, ensuring that it was not touching any side of the glass syringe (Image 1).

• The autoclave temperature probes were free in the chamber.

Load Type 2:

• This load is the same as Type 1 but we also introduced 15 PFS placed vertically in a tray (Image 2).

6. STRUCTURE OF THE STUDY

Fedegari R&D laboratory performed several thermal sterilization cycles on Sodium hyaluronate PFS:

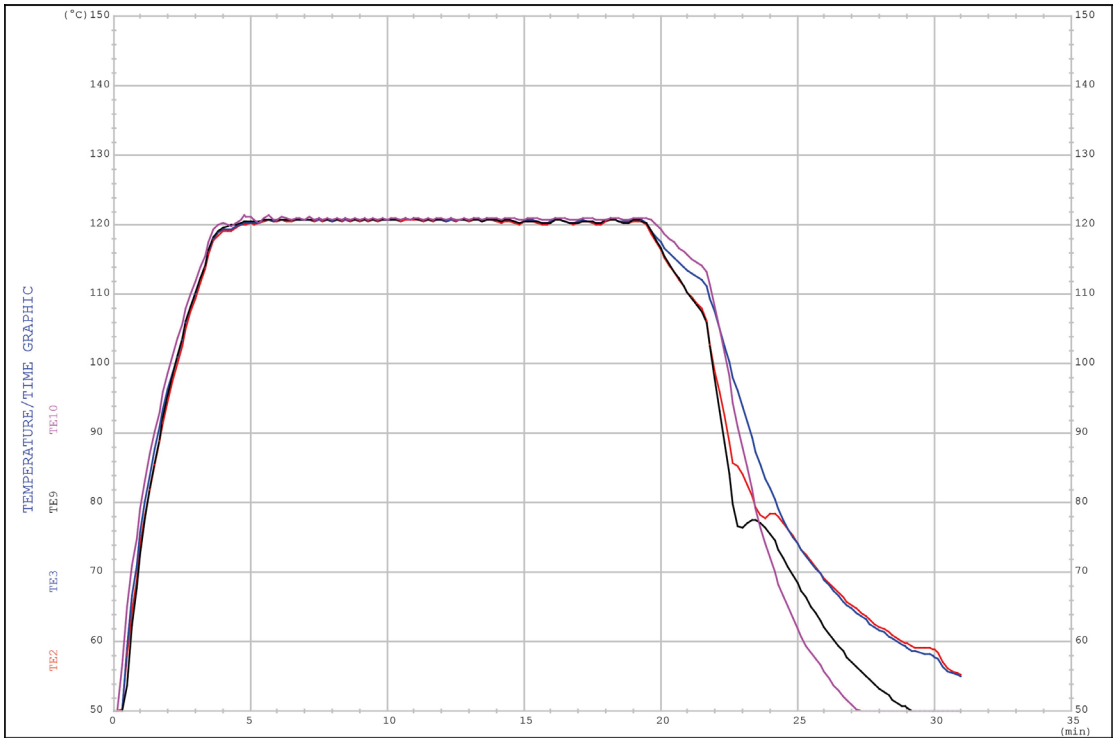
a) at 121°C, with a F_0 = 15 minutes;

b) at a higher temperature (130°C) and a shorter dwell time, F_0 = 15 minutes;

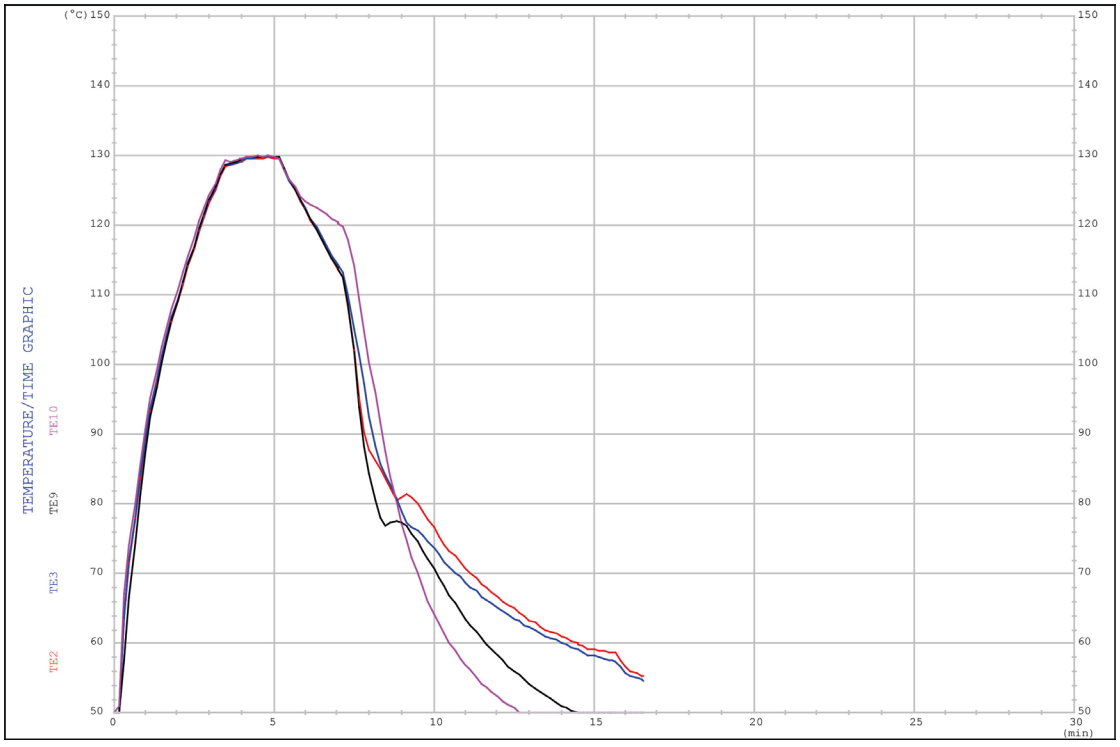
c) at a lower temperature (115°C) and a longer exposure time, F_0 = 15 minutes.

Temperature/time profiles

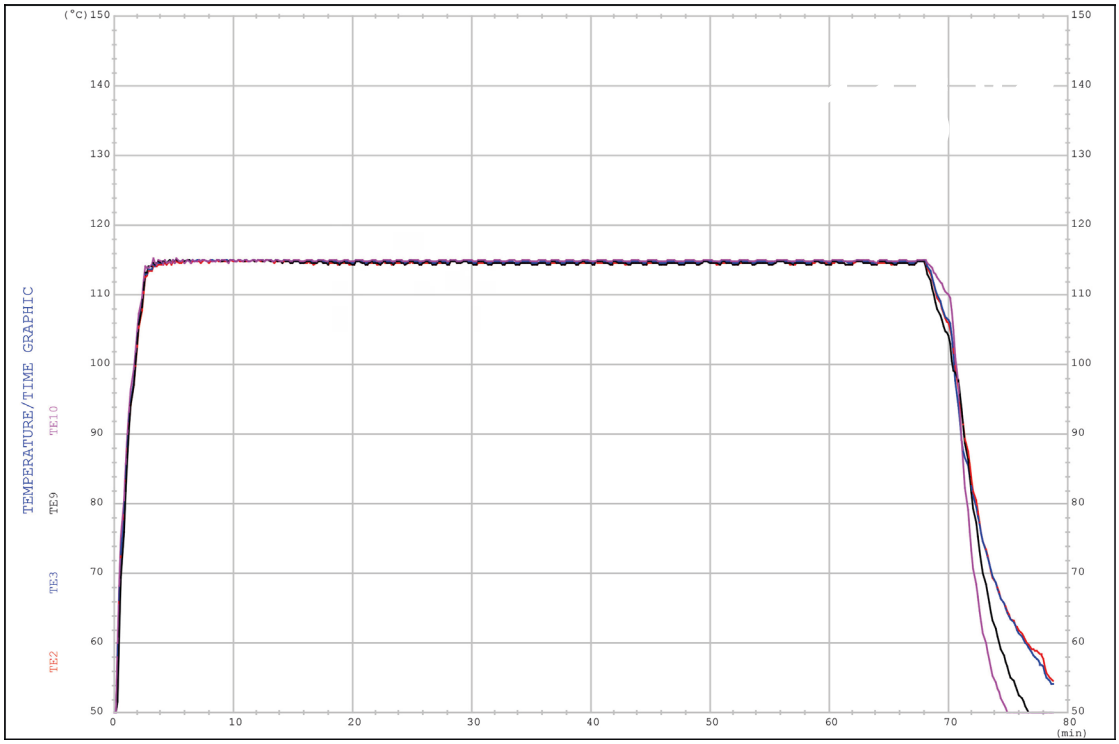
121°C



PEAK



115°C



7. TEST PERFORMED

For each temperature tested:

a) a preliminary trial including a PFS with a thermocouple inserted to obtain the F_0 accumulated during each of the three phases: heating, sterilization and cooling.

b) at least, two replicates with a tray containing 15 PFS.

8. VISCOSITY ANALYSIS

All the samples were analyzed at IBSA laboratories and compared with a non-treated product.

Sterilization Temperature in the chamber (°C)	Cycle	Load	Sample Sterilization Time	*Total F0 (min)
121	1.01.1	1 PFS	13 min 02 sec1	3.81
	1.02.1	15 PFS	14 min 29 sec1	4.69
	1.02.2	15 PFS	14 min 58 sec1	4.88
115	2.01.1	1 PFS	65 min 43 sec1	5.46
	2.02.1	15 PFS	64 min 33 sec1	4.79
	2.02.2	15 PFS	63 min 48 sec1	4.83
130	3.01.1	1 PFS	0 min 57 sec	14.26
	3.02.1	1 PFS	1 min 01 sec	15.05
	3.03.1	15 PFS	0 min 53 sec	14.84
	3.03.2	15 PFS	1 min 03 sec	15.51
	3.03.3	15 PFS	0 min 46 sec	15.74
	3.03.4	15 PFS	1 min 01 sec	15.08

8.1 Dynamic viscosity determination

A Rheological investigation was performed: a "Cone on Plate" rheomether was used to determine the solution viscosity. In detail, the diameter of the plate was 50 mm and the cone angle was 1°. The dynamic viscosity of each formulation was evaluated carrying out a rotational test at controlled shear rate (from 0.01 to 10 $^{s-1}$).

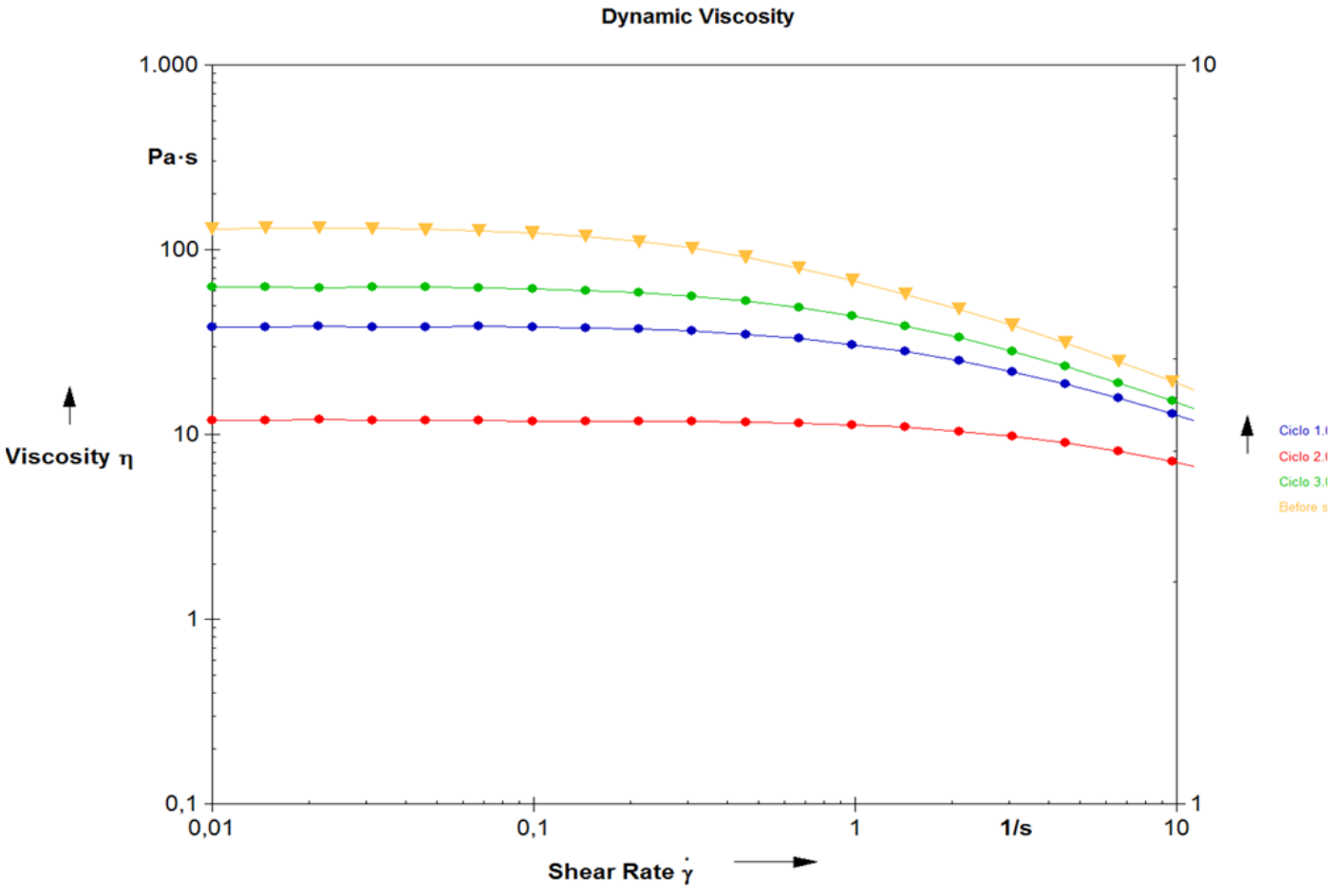
Two main shear rate intervals were identified:

1) a low shear range (from 0.01 to aprox. 1 $^{s-1}$) with a plateau value of viscosity corresponding to a "zero shear rate".

2) a medium shear range (from aprox. 1 to 10 $^{s-1}$) which corresponds to a "flow range" where the formulation starts to flow due to viscosity decrease.

During shearing, in fact, a certain number of macromolecules are oriented into shear direction (dis – entanglements). As a consequence, the viscosity tends to decrease. Simultaneously, however, other molecules, previously dis-entangled, are recoiling and re- entangling because of their viscoelastic behavior. At low shear conditions, the overlapping caused by these effects (dis - entanglements and re –entanglements) zeros the shear rate.

On the other side, at increased shear rate, the number of dis-entanglements exceeds the number of re-entanglements: the curve of viscosity decreases continuously.



9. CONCLUSION

Sodium Hyaluronate formulation presented viscosity decrease during all the sterilization cycles tested: the heat amount given to the specific formulation decreased its viscosity, independently of the sterilization temperature used. On the other side, the degree of damage and, as a consequence, of the viscosity change, was reduced during a peak cycle: the higher is the temperature and the lower is the exposure time, the lower is product degradation. In spite of the lower temperature, the worst cycle is the one run at 115°C for a long period. The long exposure time seems to be the cause of sample degradation.

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Notes

1. Technical details about trials and cycles can be provided by Fedegari R&D department upon request.

2. A special thanks to IBSA Farmaceutici for providing the PFS with Hyaluronic Sodium for this study and for making available their R&D laboratory for the additional rheological investigation.

3. A special thanks to Amphenol for making available their Kaye Validator AVS for process control.

About IBSA

IBSA is a privately owned, pharmaceutical company based in Lugano, Switzerland. Throughout its history, the company has developed a series of proprietary innovative technologies that have significantly improved the quality of its own formulations. Today IBSA markets its products in more than 70 countries worldwide. IBSA currently has a head count of about 1800 employees and its products cover 7 main therapeutic areas in 4 continents and more than 70 countries. Each year the company manufactures about 12 millions vials of hormones, more than 200 millions soft gel capsules, about 4.5 millions of pre-filled syringes and about 60 millions packages of finished products.

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