Thermal treatment of Hyaluronic Acid Pre-filled Syringes: challenges to overcome

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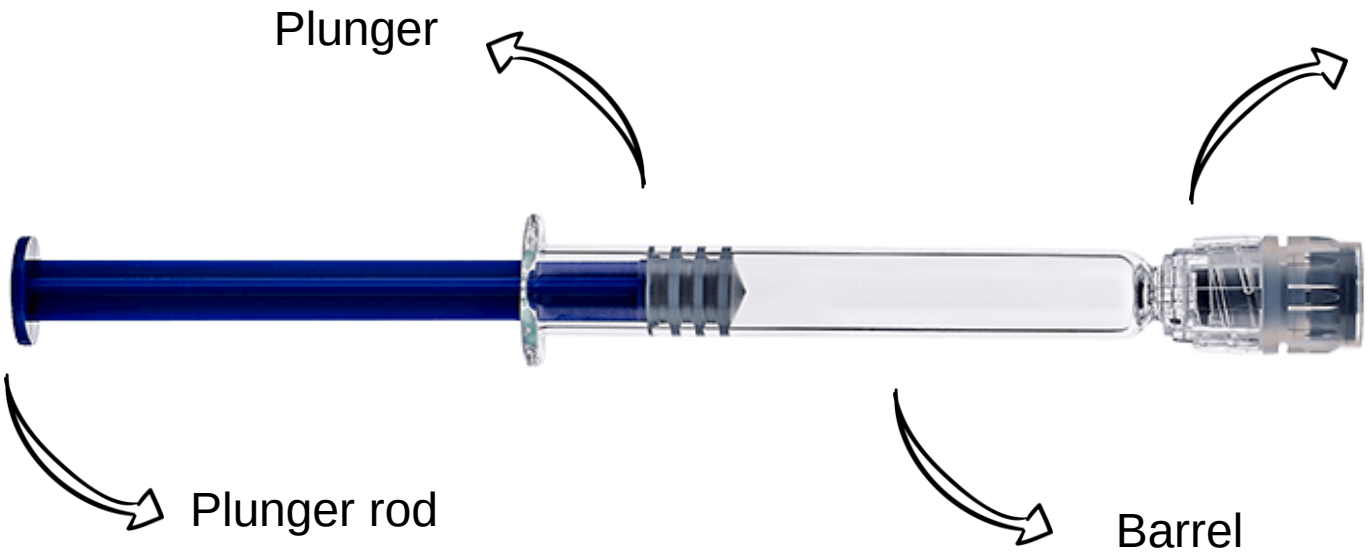
Anatomy of a syringe

LUER LOCK syringe

- requires **disposable needle**
- **insertion** and **twist** to form a locked connection



Open discussion

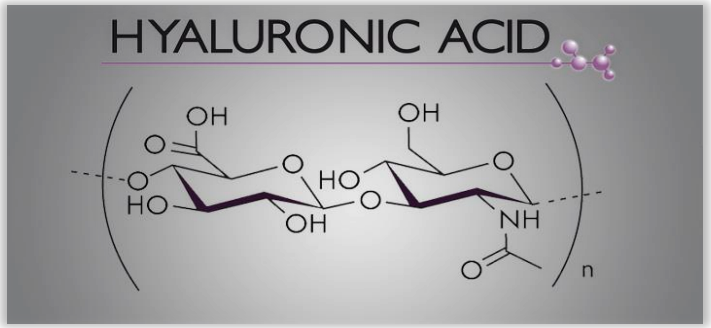
- What have we to sterilize?
- What have we to look at?
- What are the «critical parts»?
- What are the risks?

What we have to sterilize

The product

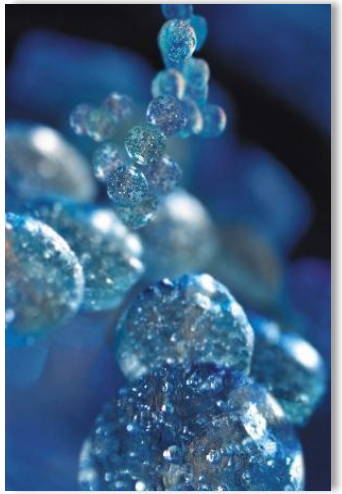
- First study of PFS with Sodium Hyaluronate (M.L. Bernuzzi, A. Giori): a review of it
- Sterilizing treatment based on temperature control
- Sterilizing treatment based on F_0 control
- Many applications for hyaluronic acid
- Counterpressure autoclaves
- What happens when an aqueous solution in a sealed container is heated

Hyaluronan



- Linear polysaccharide
- Repeating N-acetyl-D-glucosamine and D-glucuronic acid units
- High molecular mass
- Viscoelastic properties

Hyaluronic acid degradation

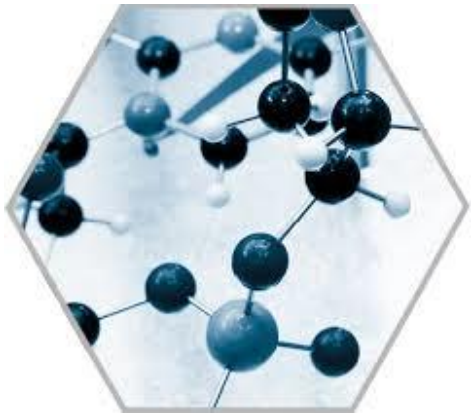


- Several sterilization methods:
  - *Microwaves*
  - *UV*
  - *Gamma rays*
  - *High Temperature*

The viscosity of the solutions decreases in time as a function of temperature

An optimized moist heat sterilization treatment can minimize the HA degradation

The study



Proving that a well-developed moist heat sterilization cycle can minimize HA degradation

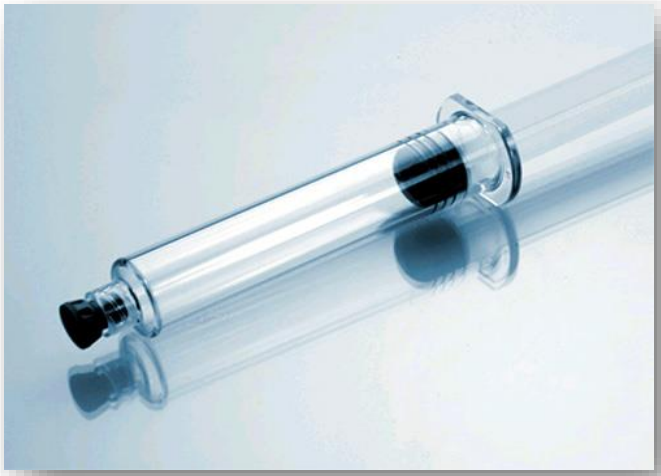
Sterilization treatment

Three sterilization treatments were performed on Sodium Hyaluronate prefilled syringes:

115 °C	121 °C	130 °C

The sterilization process was controlled by F_0 target = 15' using the Kaye validator.

Sodium hyaluronate PFS



- HA molecular weight = 1,6 x 10^6 Dalton
- Solution formulation: polysaccharide hydrated in a physiological phosphate buffer solution for 12 hours at 50 °C
- The solution was divided in glass prefilled syringes (PFS), containing 50 mg/2,5 ml of Sodium Hyaluronate

Moist heat sterilization

It is the method of choice used for the sterilization of PFS

«Sterilization by saturated steam under pressure is preferred, wherever applicable, especially for aqueous preparations».

European Pharmacopeia 10th edition

Physics of a syringe during a sterilization cycle

Increase of the internal pressure inside the syringes during the heating phase due to:

1. Water evaporation
2. Dissolved gases that leave the solution
3. Thermal expansion of the liquid
4. Air present in the head space

Counter-pressure steam sterilization

To balance the overpressure inside the syringes and to reduce the risk of plunger expulsion:

The air pressure contained in the chamber is not extracted but it is “controlled” during the entire cycle

Obviously, the chamber air pressure increases with the heating

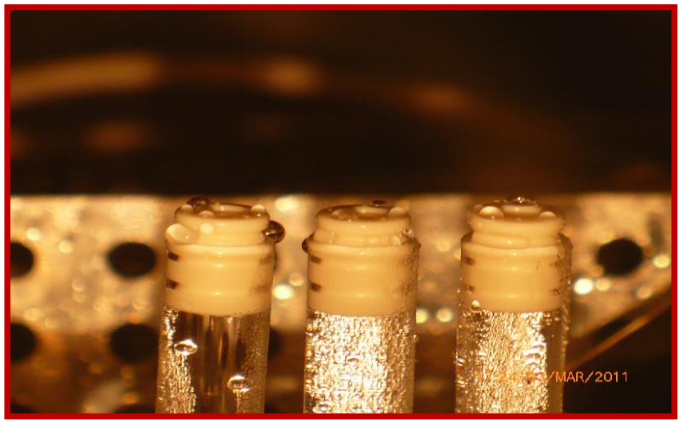
The total pressure in the chamber is given by the partial pressure of the heated air plus the partial steam pressure

Counter-pressure

Pressure inside the chamber at the different temperatures used in the cycles performed on PFS

Temperature	$P_{v(T)}$	$P_{v(T)} + P_{a(T)}$
121 °C	2,05 bar abs	3,56 bar abs
115 °C	1,69 bar abs	3,18 bar abs
130 °C	2,70 bar abs	4,25 bar abs

Syringe without head space



Both in the case of **glass and polycarbonate**, the pressure required to prevent any temporary movement of the plunger in a completely filled PFS exceeds by a factor of **one hundred** the pressure which can be applied in a moist-heat sterilizer.
The above is coherent with both the common sense (liquids are typically “incompressible” but under very high pressure)!
Polyethylene and polypropylene containers could fully compensate the thermal expansion of water, but both mechanical resistance and chemical stability of them depend a lot on the specific qualities of these plastics and they may be unsuitable for syringes to be exposed at 121 °C.
Enough length must be always provided at the open end of a glass or polycarbonate PFS to allow the plunger to “trip outwards” during the sterilization without ever protruding from the syringe. For treatment at 121 to 124 °C, the free length beyond the plunger must be at least 5.8% of the water length inside a glass PFS, and at least 4.2% in a polycarbonate.

F_0 calculation

$$F_0 = \sum \Delta t \, 10^{\frac{T-121}{z}}$$

- Algorithm used to measure the lethality of a thermal sterilization process
  It calculates, by measuring physical parameters (time and T) the amount of heat (lethal dose) delivered to the product

- *Δt= time interval between subsequent temperature measurements*
- *T= actual sterilization temperature*
- *z= temperature coefficient (10 °C for F_0 calculation)*

F_0 calculation

- F_0 target = **15 minutes**
- Beginning of F_0 accumulation at 111 °C
- F_0 accumulation during heating, sterilization and cooling
- Sterilization phase stopped at F_0 < 15’
- Thermocouple device (TC) inserted into a “reference syringe”

Structure of the study

Three sterilization treatments were performed on Sodium Hyaluronate PFS:

121 °C with a F_0 = 15’

Higher temperature (130 °C) and shorter dwell time, F_0 = 15’

Lower temperature (115 °C) and longer dwell time, F_0 = 15’

Structure of the study

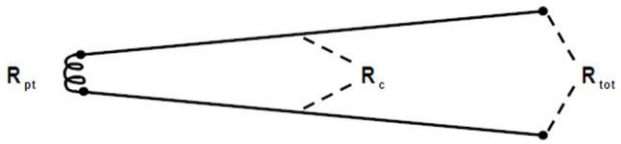
For each temperature tested:

a) Preliminary trial including a PFS with a TC

F_0 accumulated during heating, sterilization, cooling

b) At least two replicates with 15 PFS

Structure of the study

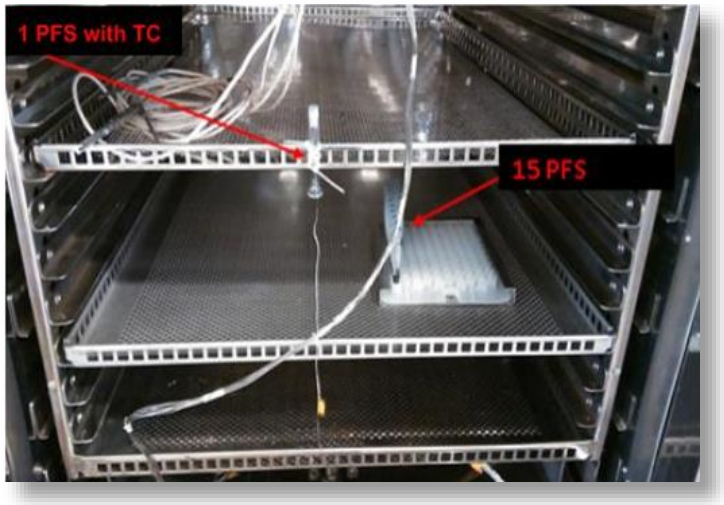


Pt 100 probes

Temperature probes locations

- 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.
- Other temperature probes (Pt100) were located within the autoclave chamber to additionally monitor the temperature during the sterilization process.
- The autoclave temperature probes were free in the chamber.

Loads configuration



- Load type 1

  1 PFS with TC inside

- Load type 2

  1 PFS with TC inside +

  15 PFS placed in a tray

‘Home-made’ method to insert the termocouple in the syringe

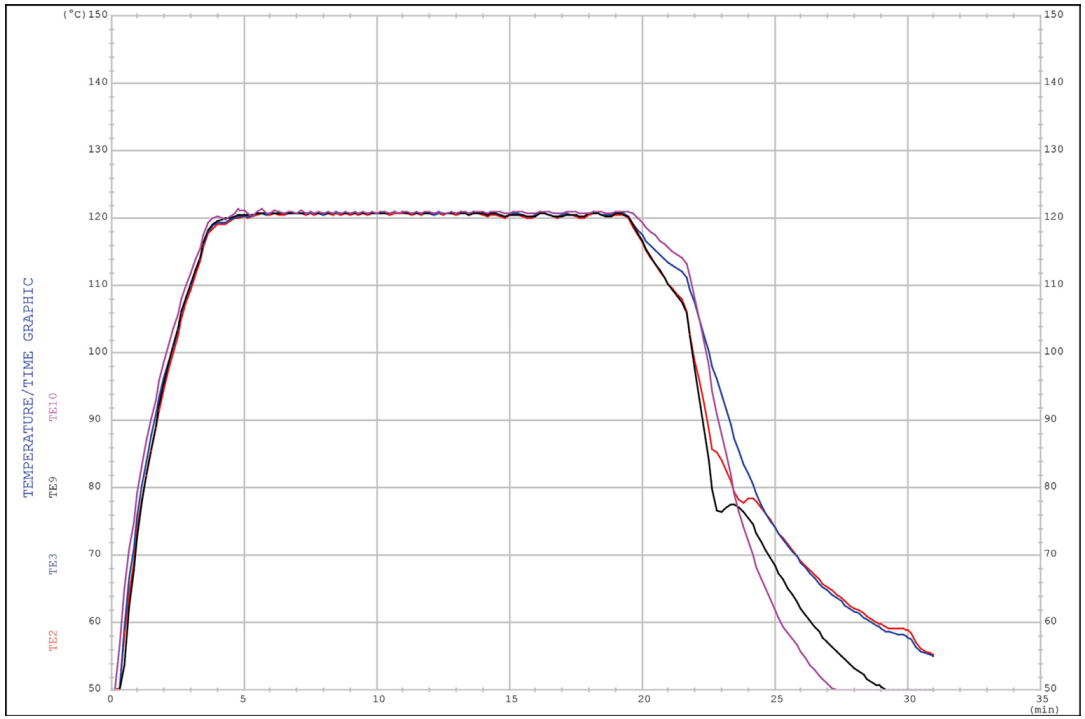
Sample with the TC inserted directly inside the syringe

Test performed

Sterilization temperature in the chamber (°C)	Cycle	Load	Sample Sterilization Time	*Total F_0 (min)
121	1.01.1	1 PFS	13 min 02 sec	13.81
	1.02.1	15 PFS	14 min 29 sec	14.69
	1.02.2	15 PFS	14 min 58 sec	14.88
115	2.01.1	1 PFS	65 min 43 sec	15.46
	2.02.1	15 PFS	64 min 33 sec	14.79
	2.02.2	15 PFS	63 min 48 sec	14.83
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

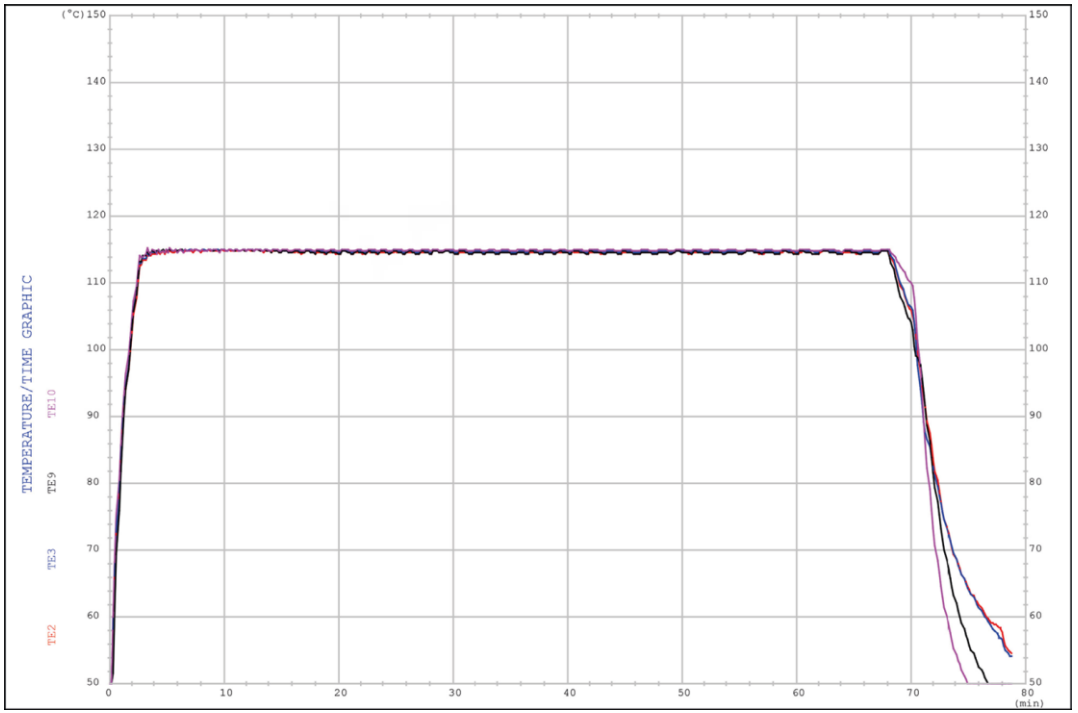
Temperature-Time profiles

121°C



Temperature-Time profiles

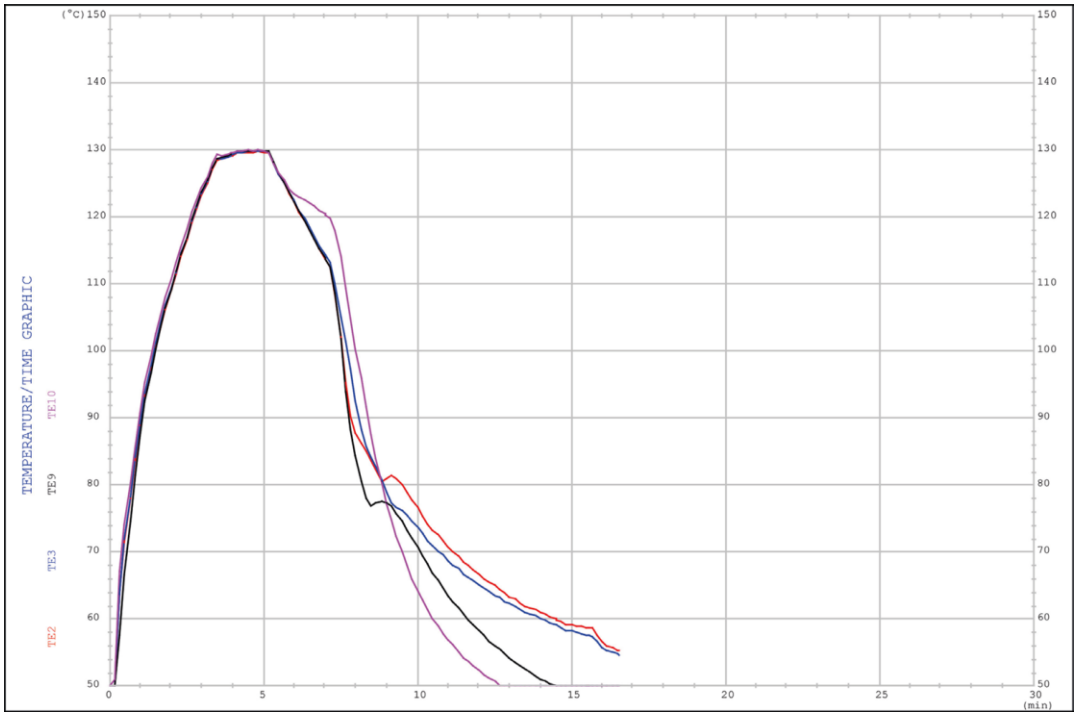
115°C



Temperature-Time profiles

130°C peak cycle

- ✓ Fast heating/cooling of the product
- ✓ Sterilization at high temperature and short holding time
- ✓ Higher counterpressure

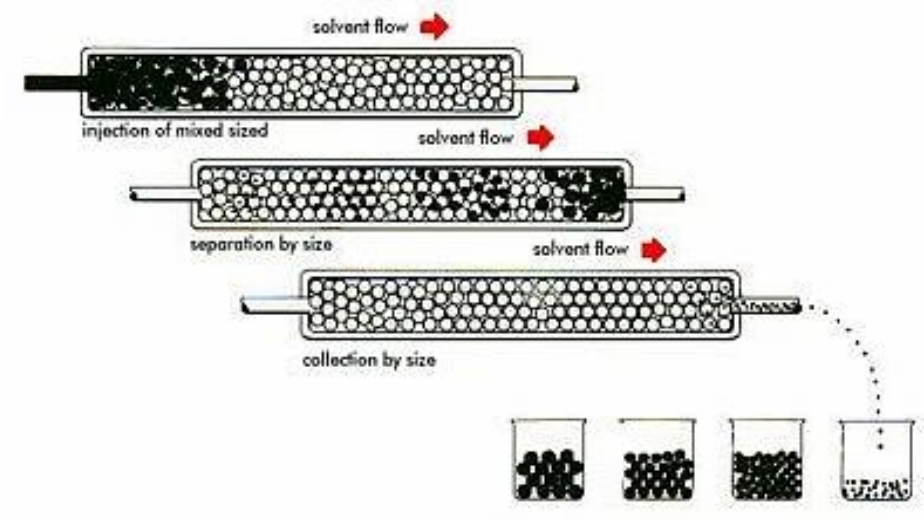


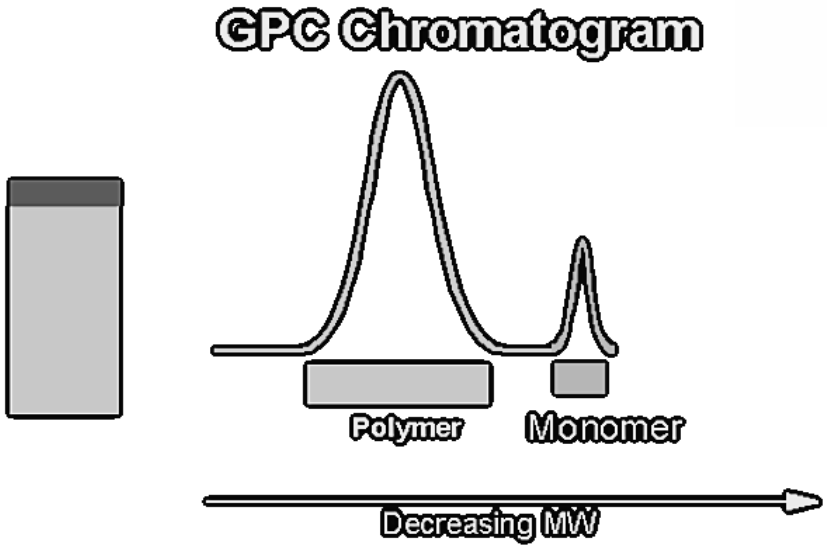
How to know about product degradation

GPC (Gel permeation chromatography)

Useful to know the molecular weight of the product and discover the degradation

How does GPC work



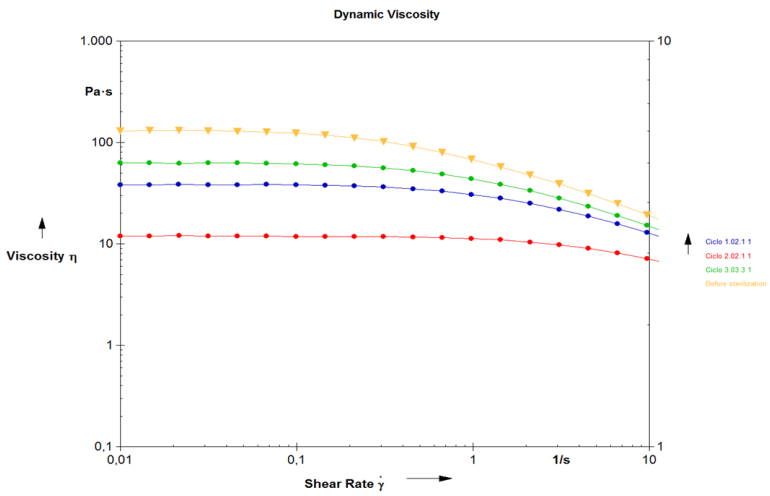


Rheological investigation

After the cycle, ***a rheological investigation*** was performed in order to understand if the different processes had damaged the product. Viscosity is a parameter used in order to understand the **degradation** of the product.

A *«cone on plate rheometer»* was used.

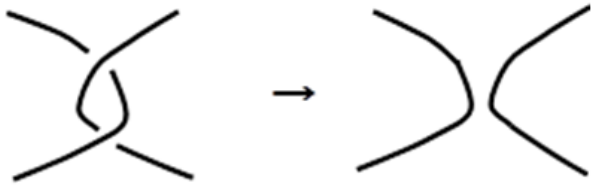
Viscosity analysis



Identification of two main shear rate intervals

Low shear range (from 0.01 to around 1 sec^{-1})	Medium shear range (from around 1 to 10 sec^{-1})
Plateau value of viscosity	"Flow range" = viscosity decrease

Viscosity analysis

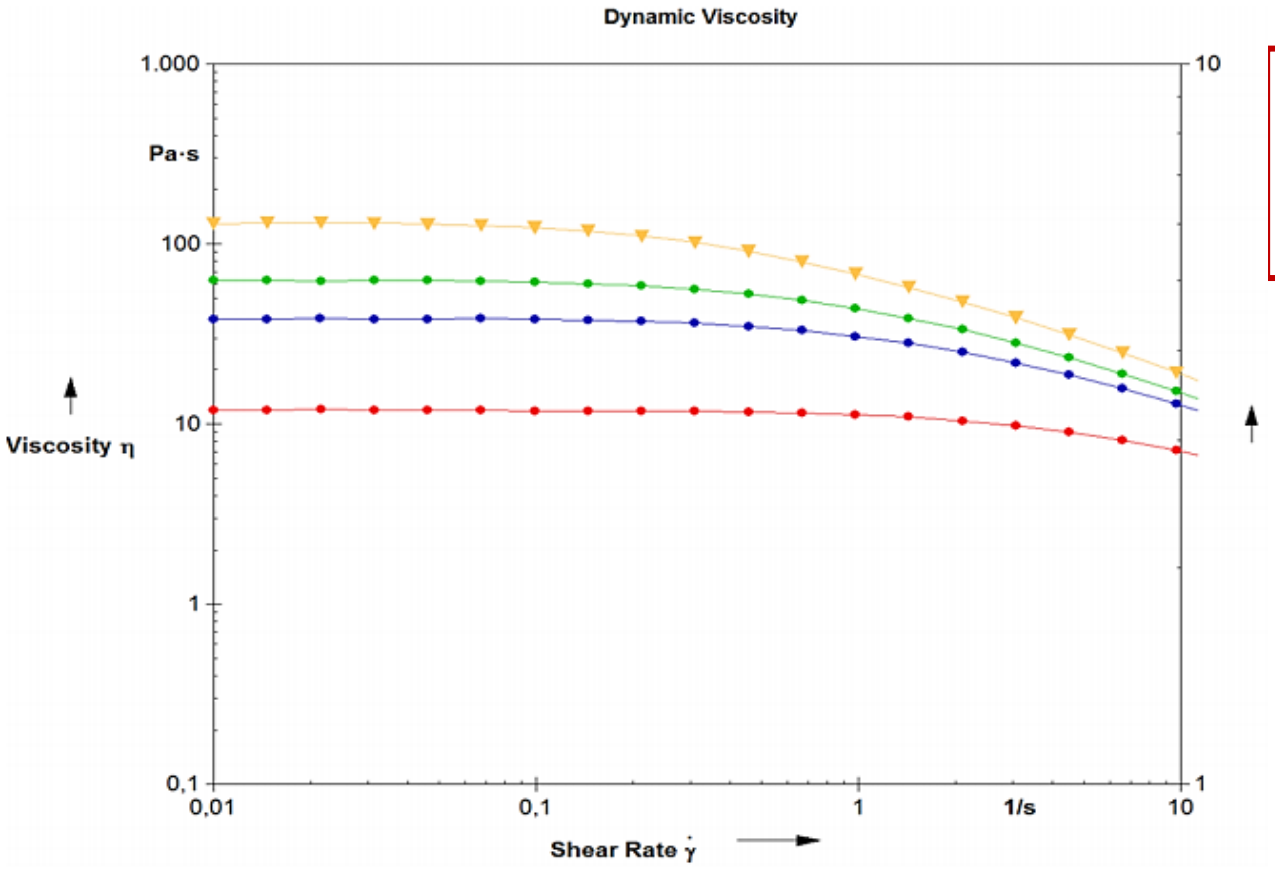


Entanglement and disentanglement

During shearing a certain number of macromolecules are oriented into shear direction (***dis-entanglements***). As consequence the viscosity tends to decrease in this parts of volume. Simultaneously however other molecules, previously disentangled, are ***recoiling and re-entangling*** again because of their viscoelastic behavior. At low shear conditions, the overlapping of these effects (dis- entenglements and re-entanglements) occurs as a costant limiting value of the viscosity function.

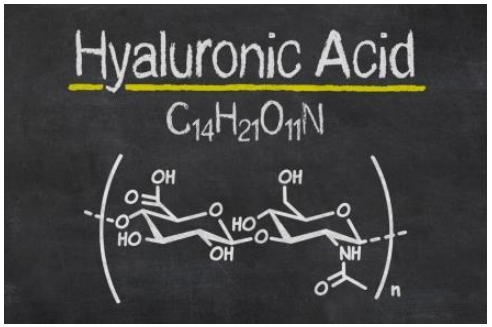
On the other side ***at increased shear rate***, the number of ***dis-entanglements*** is exceeding the number of re-entanglements: the curve of the viscosity is decreasing continuously.

Dynamic viscosity



Yellow: untreated sample
Green: cycle at 130°C
Blue: cycle at 121°C
Red: cycle at 115°C

Conclusions of the first study



- Viscosity reduction at the end of all cycles performed.
- The worst cycle is the one run at 115 °C.
- The viscosity change is reduced during a **peak cycle**

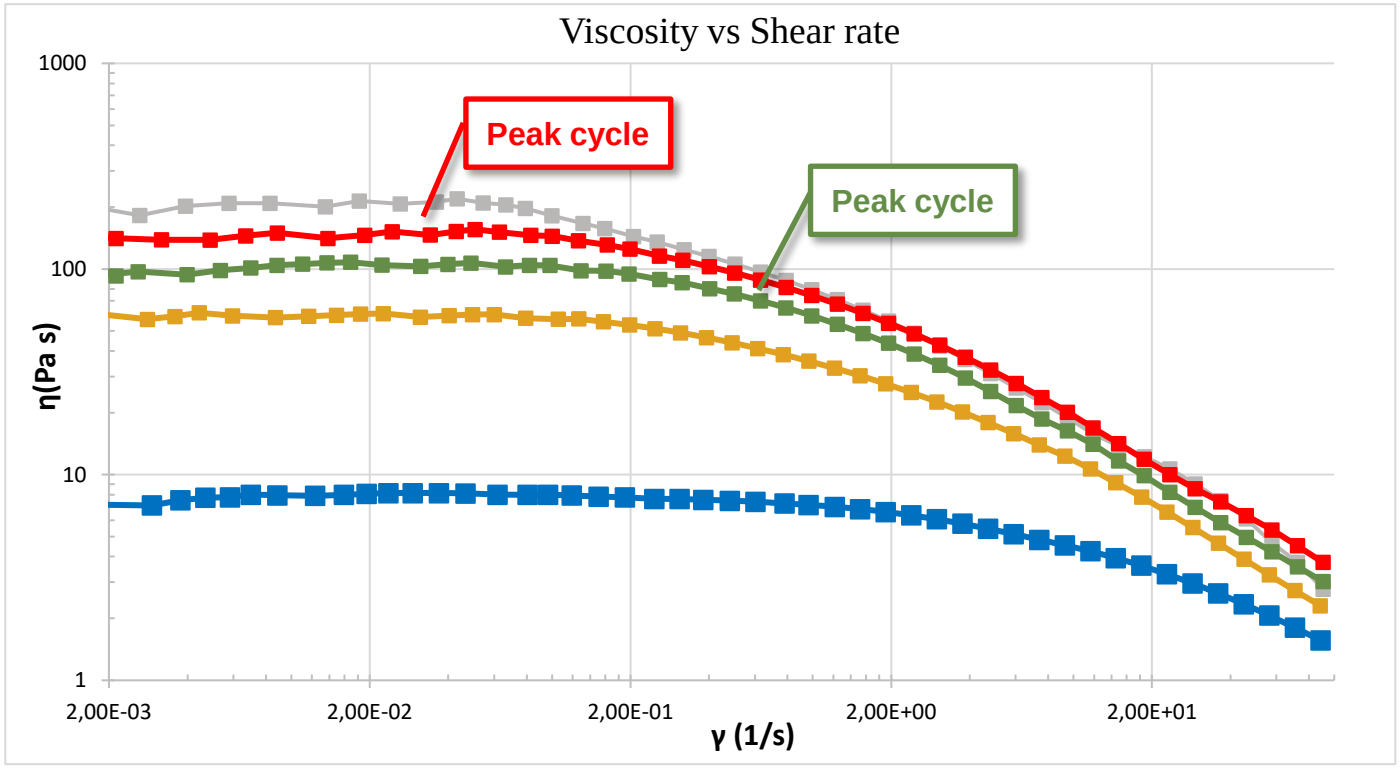
The higher is the temperature and the lower is the exposure time, the lower is product degradation

Sterilizing treatment based on temperature control

Contrary to what one could expect, the treating at lowest temperature and for longest time leads to significant reduction of polymer degradation and its viscosity.

A peak cycle at high temperature and short time preserves the integrity of the sample.

Sterilizing treatment based on temperature control

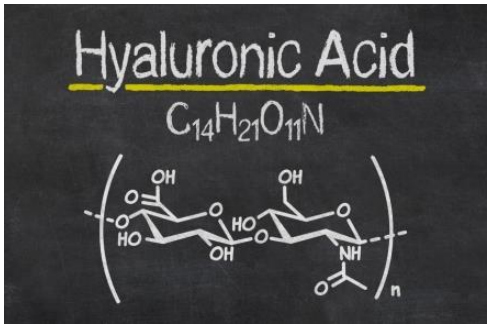


Sterilizing treatment based on temperature control

The customer may not have the proper autoclave to reach **130°C** because an over-rating machine is required.

In this case, the sterilization cycle at **125°C** could be selected as the best compromise.

PFS with sodium hyaluronate: viscosity reduction



Load treatment strategy: viscosity reduction

Possible strategies to treat heat-sensitive loads

a. To minimize the microbial load of the product to be sterilized, so as to be able to minimize the heat dose (temperature-time) required in order to achieve an adequate SAL (Sterility Assurance Level).
b. To make the heating/cooling phases as rapid as possible
c. To raise the sterilization temperature, adequately reducing its duration: this solution can be clearly implemented in combination with solution b.

How to have a further reduction of the product degradation

Since HA is easy to degrade, it's better to approach a sterilizing treatment based on the F_0 control.

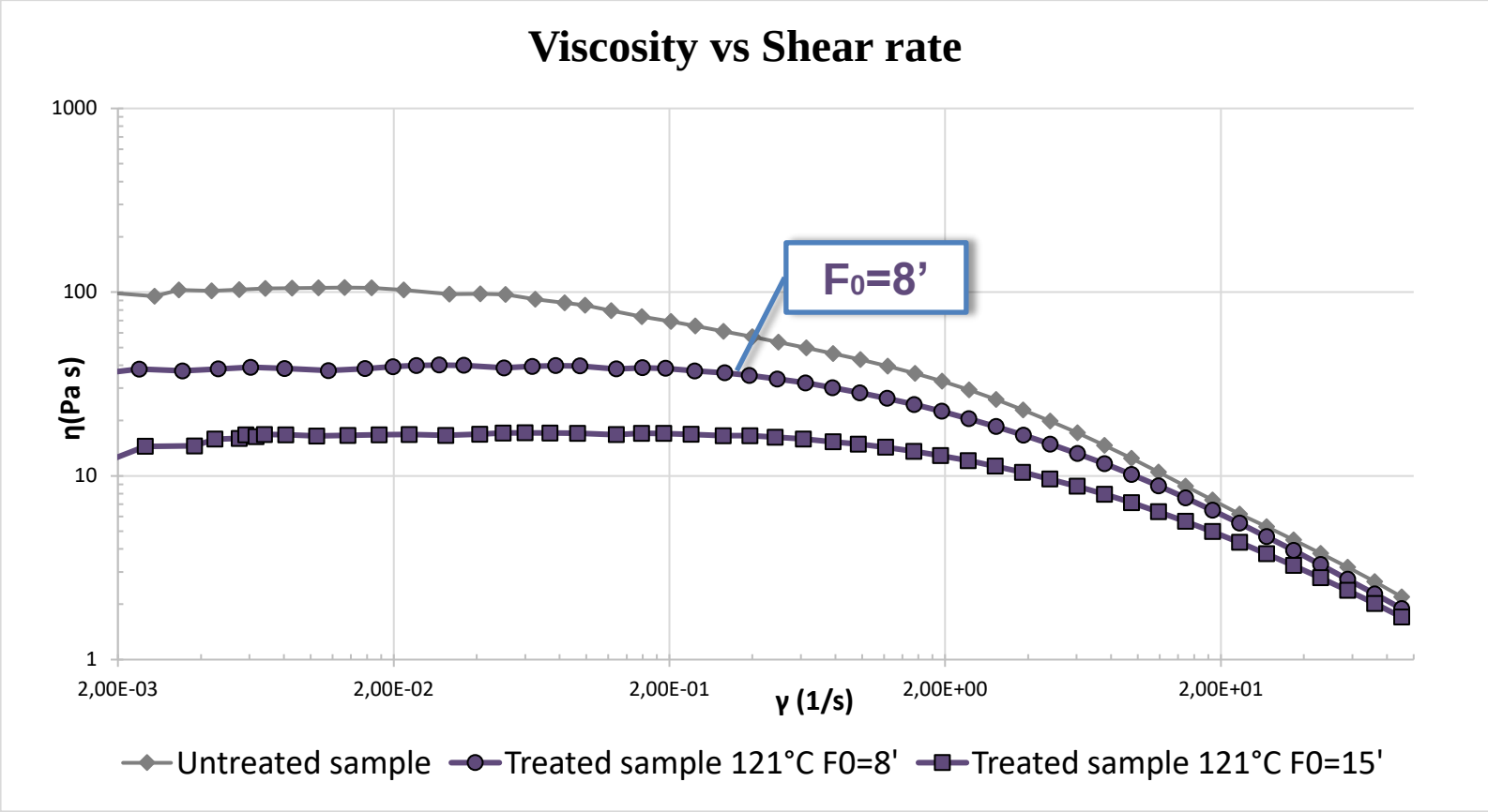
An approach with F_0 equal to 8'
is tested, in order to try if the degradation could be reduced.

Sterilizing treatment based on F_0 control

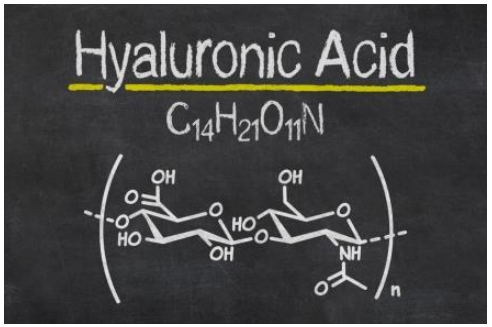
KAYE validator is used to control the reached F_0. It is connected with a set of termocouples, and one of them is inserted inside a syringe.

This is the best way to know the delay of the heat accumulation caused by the hyaluronic acid.

Viscosity vs Shear rate



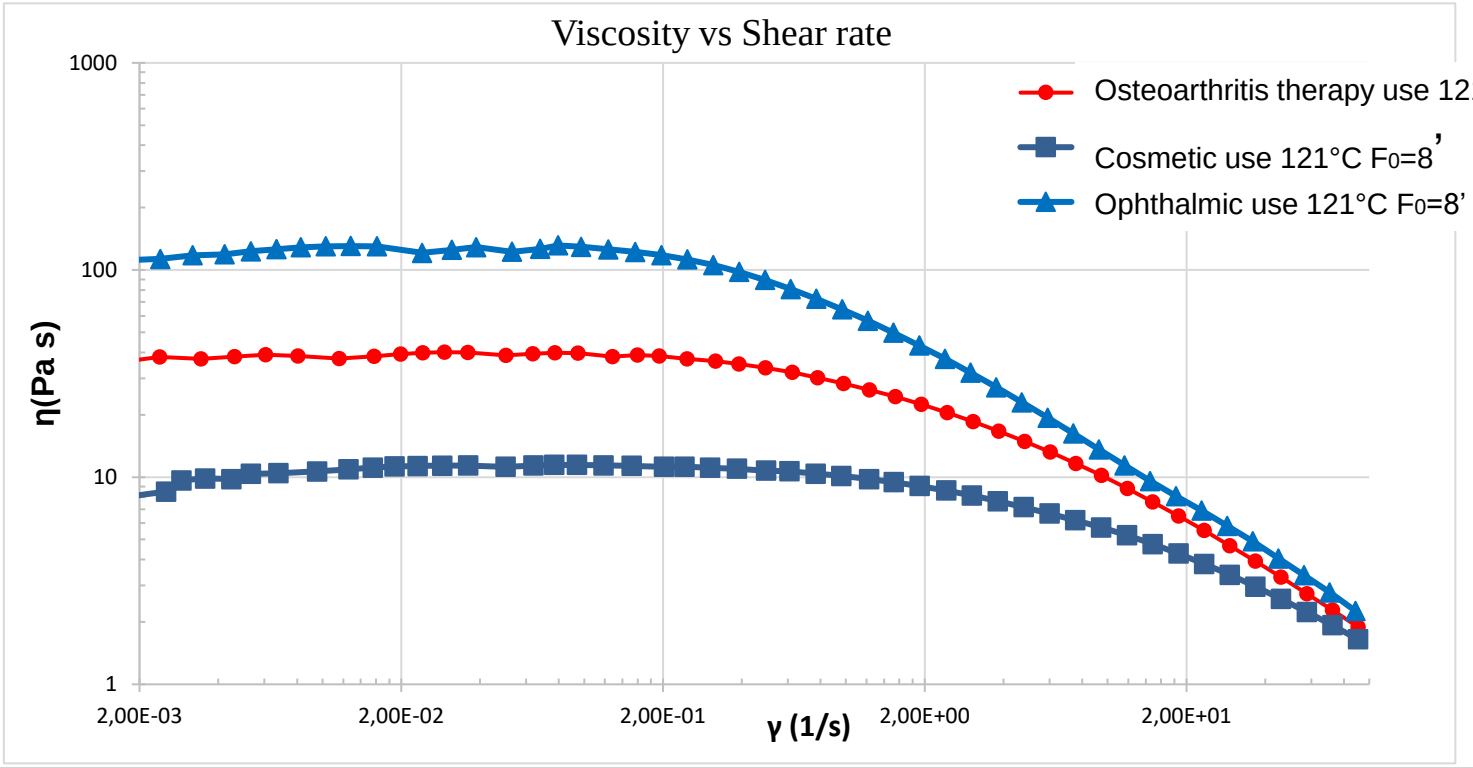
Many application for hyaluronic acid



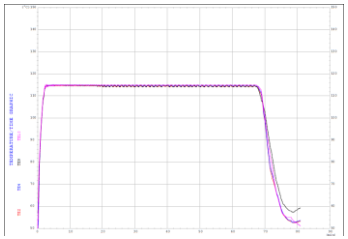
- Ophthalmic
- Cosmetic
- Osteoarthritis therapy

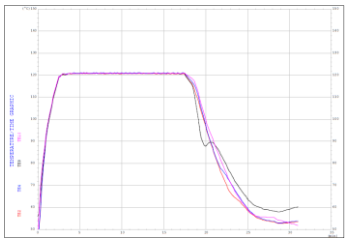
There are many different viscosity grade of hyaluronic acid and it's better to choose according to the proper use.

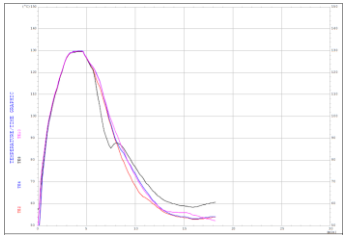
Differences depending on HA applications

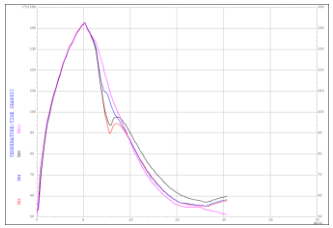


Autoclave (Air Over steam) sterilization of HA









115° C

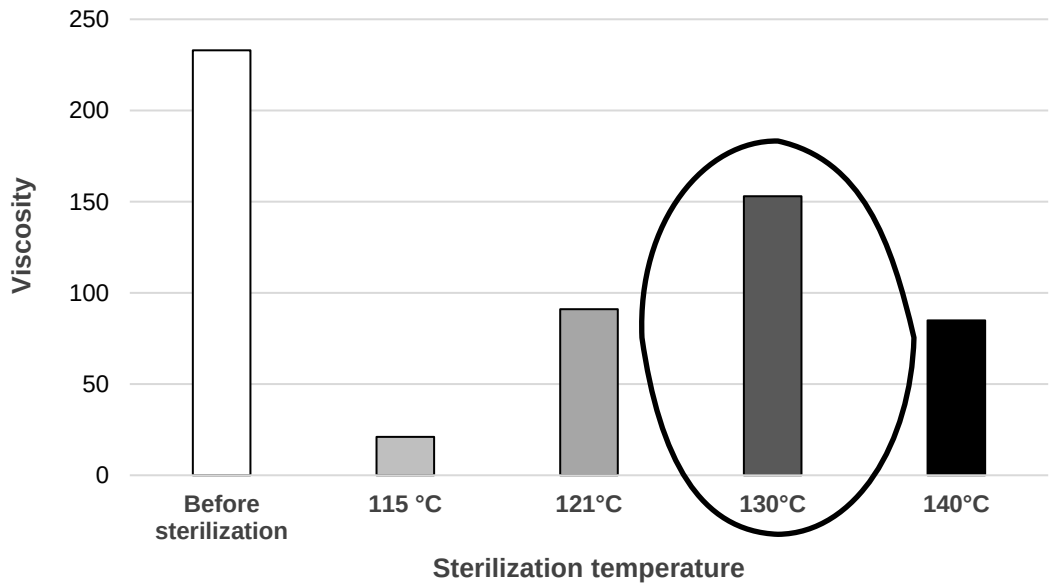
121° C

130° C

140° C

Different temperatures
Same lethality effect

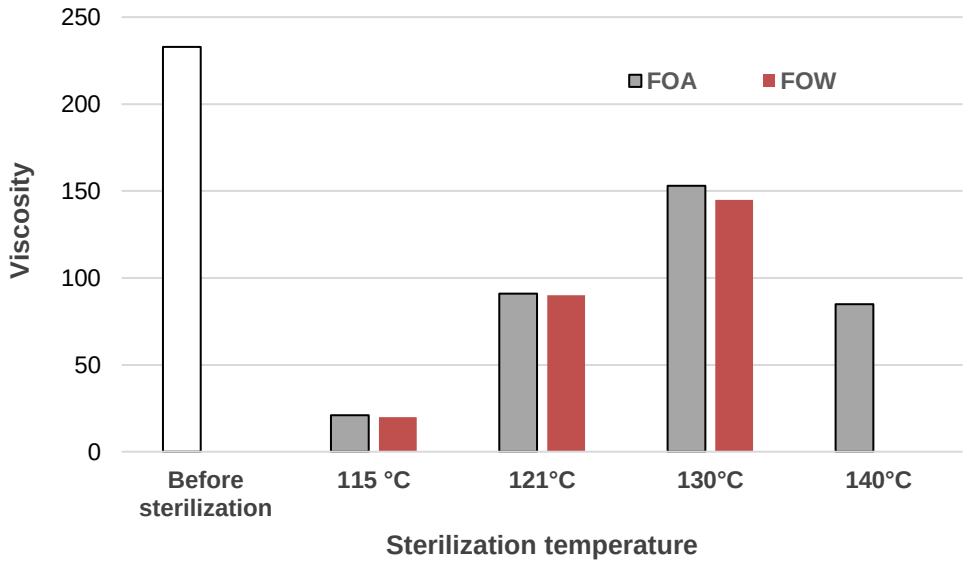
Choose the best FOA sterilization cycle



Minor degradation of HA at 130°C

Sterilization cycle with FOW

Sterilization cycles, using an FOW autoclave, were performed, in order to observe any **differences** in the **product degradation**.



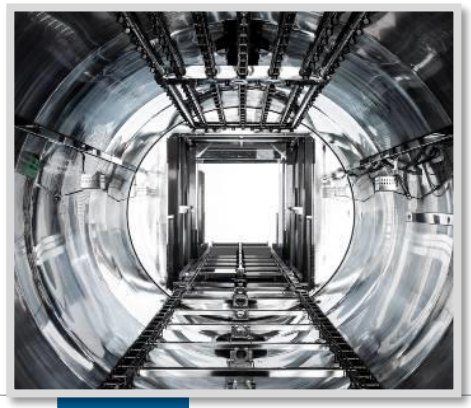
Remarks:

- No differences in terms of degradation between **FOW** and **FOA** at different temperatures with same lethality effect
- Cycle performed with a limited load, and small volume syringes

Counterpressure autoclaves

Steam-air mixture autoclave (FOA)

PFS treatment: which to choose?



Superheated water autoclave (FOW)

What we have to sterilize?

The container: a syringe with a LUER LOCK

Does a sterilization cycle affect the mechanical characteristic of a syringe?

Impact of the steam sterilization on the PFS

Does the steam sterilization cycle damage the primary packaging (syringe)?

Total Length test

Torque Force test

Pull Off Force test

Unscrewing test

The results of the sterilization process with autoclave at **different temperatures** (115°C, 121°C, 125°C, 130°C) are compared to the sterilization with Ethylene Oxide (EtO). The **EtO treatment** was used in the testing as **control**.

Syringes maintain the characteristics required by the manufacturer's customers

Mechanical characteristic of a syringe

	cycle 1 (115° C) empty syringe			cycle 2 (121° C) empty syringe			cycle 3 (125° C) empty syringe			cycle 4 (130° C) empty syringe		
	unscrewing force TC [Ncm]	torque force LLA [Ncm]	PoF LLA [N]	unscrewing force TC [Ncm]	torque force LLA [Ncm]	PoF LLA [N]	unscrewing force TC [Ncm]	torque force LLA [Ncm]	PoF LLA [N]	unscrewing force TC [Ncm]	torque force LLA [Ncm]	PoF LLA [N]
Specification:	**≥ 2**	**≥ 5**	**≥ 50**	**≥ 2**	**≥ 5**	**≥ 50**	**≥ 2**	**≥ 5**	**≥ 50**	**≥ 2**	**≥ 5**	**≥ 50**
Minimum:	1,73	2,34	63,37	1,98	2,44	67,11	1,84	2,75	73,51	1,83	2,85	72,04
Mean:	2,71	4,99	82,70	2,43	5,01	84,43	2,52	6,27	89,16	2,49	6,49	82,10
Maximum:	4,00	14,28	122,60	3,10	9,80	108,27	3,19	23,27	116,88	3,20	12,61	100,17
Std. Dev.:	0,455	1,929	10,524	0,224	1,471	7,352	0,270	3,787	9,997	0,300	2,029	6,561
Cpk (UT):	0,52	-0,87	1,04	0,64	0,00	1,56	0,64	-0,33	1,31	0,54	0,25	1,63

	cycle 1 (115° C) filled syringe			cycle 2 (121° C) filled syringe)			cycle 3 (125° C) filled syringe			cycle 4 (130° C) filled syringe		
	unscrewing force TC [Ncm]	torque force LLA [Ncm]	PoF LLA [N]	unscrewing force TC [Ncm]	torque force LLA [Ncm]	PoF LLA [N]	unscrewing force TC [Ncm]	torque force LLA [Ncm]	PoF LLA [N]	unscrewing force TC [Ncm]	torque force LLA [Ncm]	PoF LLA [N]
Specification:	**≥ 2**	**≥ 5**	**≥ 50**	**≥ 2**	**≥ 5**	**≥ 50**	**≥ 2**	**≥ 5**	**≥ 50**	**≥ 2**	**≥ 5**	**≥ 50**
Minimum:	1,72	5,13	50,51	1,58	4,02	56,65	1,54	3,44	62,10	1,42	4,68	50,34
Mean:	2,82	11,18	73,91	2,65	11,72	79,60	2,70	9,15	76,79	2,79	9,40	63,78
Maximum:	5,27	17,52	101,06	5,21	17,98	108,26	3,84	17,40	100,49	7,58	16,09	86,57
Std. Dev.:	0,528	3,729	9,766	0,536	3,070	10,296	0,427	3,536	8,939	0,847	2,678	7,738
Cpk (UT):	0,52	0,11	0,82	0,40	0,73	0,96	0,55	-0,08	1,00	0,31	0,55	0,59

https://www.youtube.com/watch?v=j1RwnFjHAHQ

The microbiology of a syringe

Key points:

- ❑ we have to sterilize a heat sensitive load
- ❑ we have to reduce its degradation
- ❑ a tailored cycle could be the best choice

HOW TO PROCEED

BIOBURDEN/BIs APPROACH

Purpose of the study

Determine the **most resistant bioburden isolate** and perform steam D_{121}-value of spores suspended in Hyaluronic Acid Injection

BIOBURDEN EVALUATION

Procedures

BIOBURDEN EVALUATION

Genetic identification with RNA sequencing technique of each isolate

Boil test. 1.0 x 10^4 spores inoculated into tubes and put in heat bath at 95-100°C for 10 min (Chapter <1229.2> USP 43). After heat treatment, two 1 mL aliquots of each isolate will be plated in Petri plates and incubated for >48h.

Initial screening

Crop production of the most resistant isolate; approximately 10^8 of the most resistant organism will be required to perform a D-value analysis.

Procedures

BIOBURDEN EVALUATION

One set of **40 syringes containing the most resistant isolate** suspended in Hyaluronic Acid Injection will be prepared.

Population assays will be performed on the syringes. The purpose of these assays is to **verify that the spore concentration in the syringes is stable** for the duration of the study.

D-value determination in the steam B.I.E.R. at 121°C.

Identification of the most resistant isolate

Another way to go

Do the customers really want to do this procedure every time?

NO!

It’s necessary to find the **Biological Indicator** which adapts better to the customers’ product

Procedures

Syringes inoculated with

B. subtilis

or

G. stearothermophilus

Determination of the D-value

The inoculated syringes are compared to BIs present in the market with *B.subtilis* or *G. stearothermophilus*.

If these BIs have a D-value > than the syringe, the customer will use them.

An example

- The most resistant microorganism, in the syringe, with HA, has a $D_{121°C}$ = 0,1'
- Bacillus Subtilis, 10^6 spores, in the syringe, with HA, has a $D_{121°C}$ = 0,3'
- Geobacillus Stearothermophilus, 10^6 spores, in the syringe, with HA, has a $D_{121°C}$ = 3,2'
- Geobacillus Stearothermophilus, 10^4 spores, in the syringe, with HA, has a $D_{121°C}$ = 2,8'
- BI in the market, with B. Subtilis, in an ampoule, 10^6 spores: $D_{121°C}$ = 0,4'

BI in the syringe in it

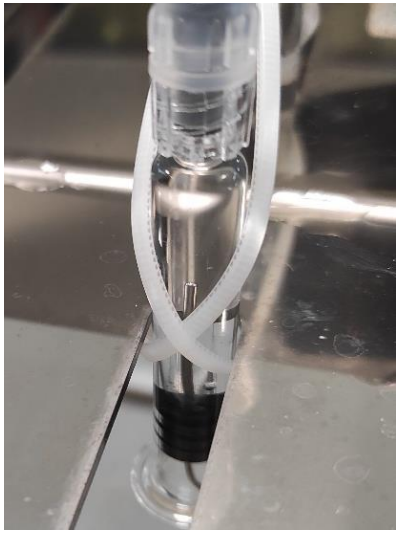
Table 1. Insertion of SterilAmp SASU Biological Indicator in Hyaluronic Acid syringes

Plunger removal with the help of sterile tweezers

Removal of about 0.3 mL of the product and insertion of the Biological Indicator

Insertion of the plunger: the syringe with Biological Indicator inside is ready

Syringe thermal behavior



Study of the syringe thermal behavior

A preliminary phase of the test session involves the study of the syringe thermal behavior, to understand the lag time, i.e. the time necessary to heat up the product until it reaches the same temperature of the chamber.

In order to perform this test, a sterilization cycle is performed. After the set sterilization temperature (121°C) is reached in the autoclave chamber (read by a temperature control probe), a calculation of the amount of time needed to reach the same temperature in the syringe (read by the TC connected to the KAYE validator) is carried out.

Several trials have been performed; the lag time was equal to 35''.

F_0 calculated: the minimum required, according to the US supplier of BIs

- We consider:

  - the D value of the BI
    we increase it a little bit to provide a certain safety level
    0,3’ → 0,4’
  - the lag time of the product
    to add the contribute of the packaging
  - N_0 = initial spores population
  - N = target of the cycle

Final $F_0 = D_{121}$-value ($\log_{10}N_0 - \log_{10}N$) + lag time

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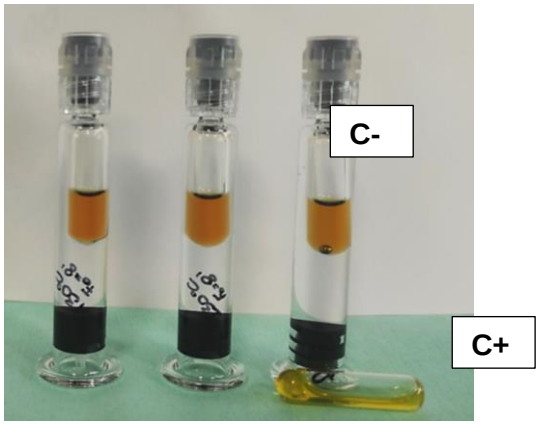

< 8 minutes

This cycle was performed, two samples with a BI were inserted into the autoclave, a sample with a negative control was also located into the chamber

At the end of the cycle they were removed and incubated

No growth

Thank you

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