Innovative method for the sterilization of heat sensitive products: NTS® – Non Thermal Stress®

N.Scacciati*[1], A.M. Piras[1], L. Zappolini[2], A. Dal Canto[2], A. Malventi[2] and C. Michelini[2]

* noemi.scacciati@phd.unipi.it
1 Department of Pharmacy, University of Pisa - Via Bonanno, 6 – 56126 Pisa (PI)
2 CMed Aesthetics S.p.A. - Via Panfilo Castaldi 4 - 56121 Pisa (PI)

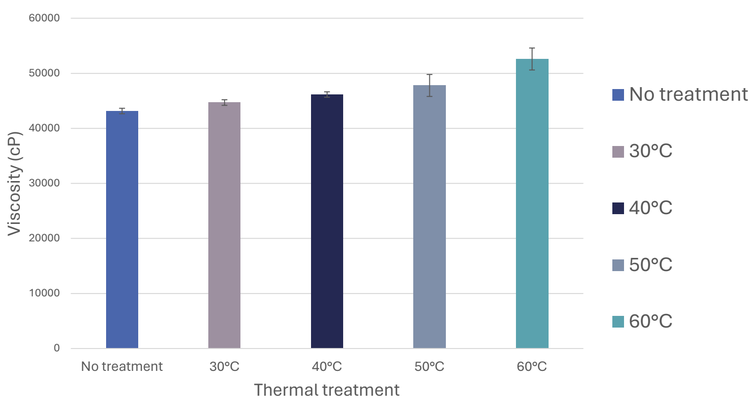
63 SIMPOSIO AFI RIMINI 5·6·7 GIUGNO2024

Introduction

In aesthetic medicine, ensuring the sterility of injectable and mesotherapy products is crucial, but traditional sterilization methods can alter their properties. To address this, the Non Thermal Stress® (NTS®) method uses sterile filtration to preserve the integrity, viscosity of solution and avoid the degradation of thermolabile substances. This technique maintains the original qualities of hyaluronic acid (HA) and other bioactive compounds, ensuring their efficacy and safety. Products tested with this method include MESO HAIR REV01 (**HB**), MESO SUCCINIC + HA + AA REV00 (**AK**), MESO ANTIAGE-C REV02 (**AA**), MESO WHITE REV01 (**LM**), MESO ANTIOX + HA REV01 (**AO**), SODIUM DEOXYCHOLATE REV02 (**TE**), and HA 1.6 MDa 0.5% (**HA-CTRL**).

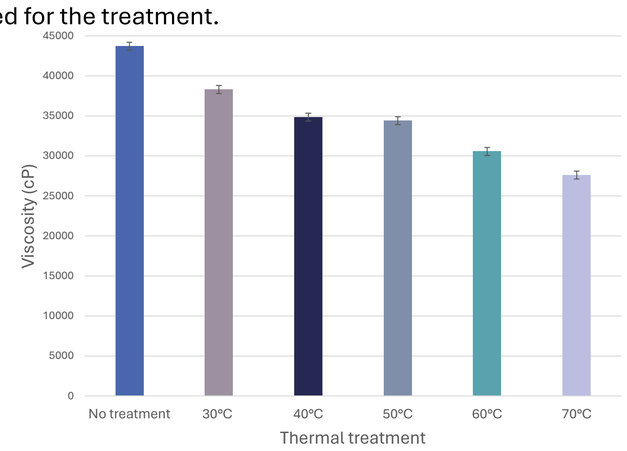
Optimization of thermal treatment

The viscosity of solutions were evaluated after 4 h of thermal treatment at different temperatures. The solutions were cooled at room temperature, the viscosity of samples were evaluated at 20°C.



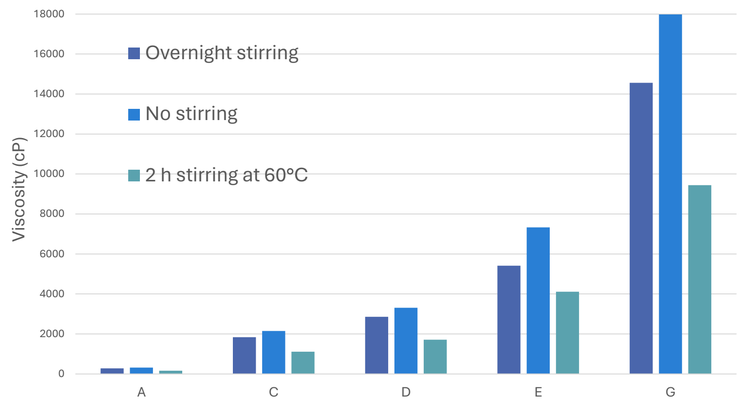
The viscosity of solutions cooled at room temperature is directly proportional to the thermal treatment used. The cooling at room temperature do not promote the filtration of solutions.

The viscosity of solutions were evaluated after 1 h of thermal treatment. It was evaluated for each solutions at the temperatures used for the treatment.



The viscosity of solutions decrease with the increase of the temperature of thermal treatment. Thermal treatment at 60°C facilitate filtration through 0.22µm pores filter and do not cause water evaporation and the degradation of hyaluronic acid.

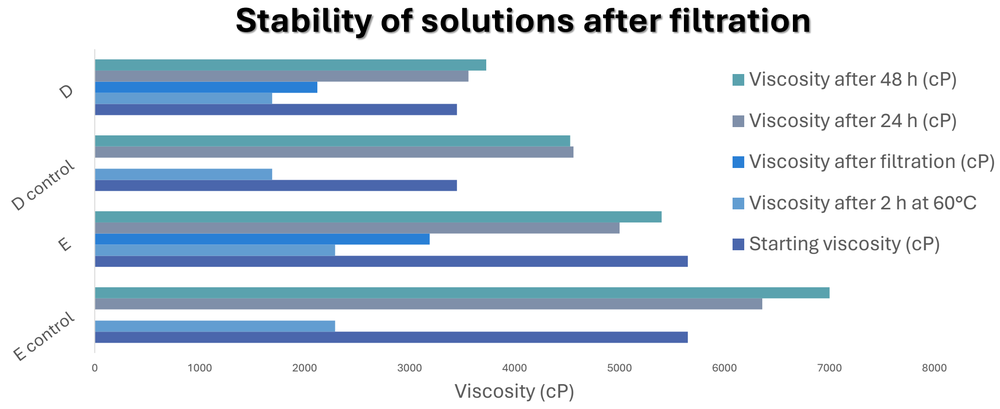
Stirring and temperature have been evaluated as factors that can change the viscosity of the solution. Different hyaluronic acid solutions have been tested for various conditions.



Solutions without stirring overnight show a viscosity increase from 14% to 35% compared to the initial sample; while heat treatment reduces viscosity from 50% to 56%.

Following these preliminary tests, the protocol to be applied was defined, namely overnight stirring and subsequent heating to 60 °C for 2h before filtration.

Stability of solutions after filtration



The viscosity of the solutions was measured before and after filtration, and at 24 and 48 hours to assess stability. The pre-filtered sample's viscosity remained constant post-filtration. In contrast, unfiltered solutions showed increased viscosity over time, likely due to interactions between hyaluronic acid chains [1]. This increase was not observed in filtered solutions, probably because the chains are stretched through small pores during filtration.

It can be concluded that filtration not only preserves the rheological characteristics of the initial solution but also results in a more stable compound over time compared to the untreated solution.

Optimization of pressure filtration

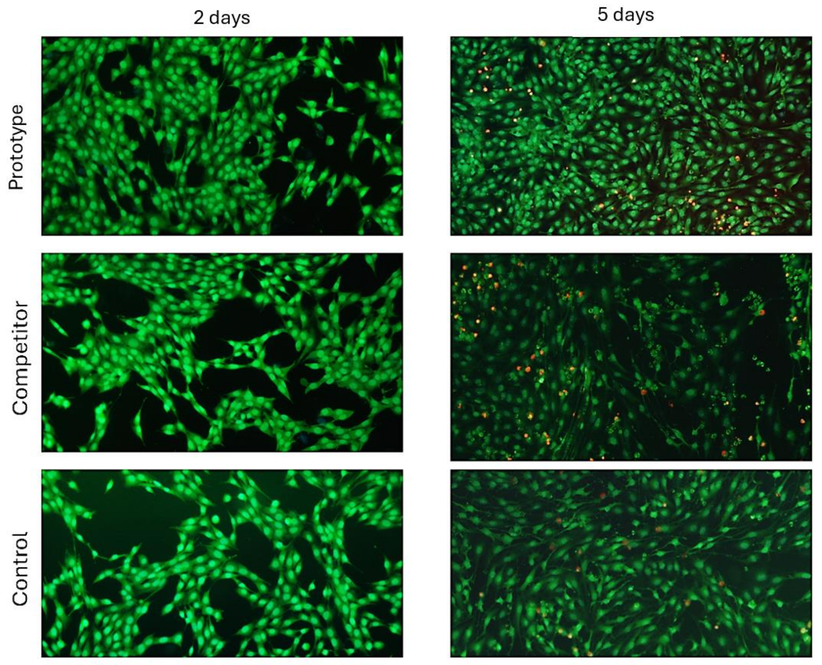
The filter cartridges are tested and guaranteed by the supplier to operate optimally at a maximum pressure of 5 bar. Initially, tests were conducted at an operational pressure of 2 bar, resulting in a product loss of 64%. Operating at 5 bar yielded benefits in terms of filtrate flow (increased) and waste (minimal), totaling 2%.

Sterility test

The test was performed according to F.U. XII ed. 2008 in compliance with GLP and GMP.
The samples tested were HB, AK, AA, LM, AO, TE and HA-CTRL filtered through 0.22 µm filter and all the tested solutions were found to be sterile.

Filter validation
Raw materials and products that cannot undergo terminal sterilization can be sterilized through filtration using a filter validated by a microbial infection test with an appropriate test microorganism, such as a suspension of reduced *Pseudomonas* (ATCC 19146, NCIMB 11091, or CIP 103020). For this process, at least 10^7 CFU per cm^2 of active filtering surface are used, with the suspension prepared in soy-tryptone broth. After filtration, the suspension is aseptically collected and incubated under aerobic conditions at 32°C.

Biological evaluation



The Live/Dead assay, performed on Fibroblast Balb/3T3 clone A31 cell line, is a qualitative method based on esterase activity and membrane integrity.
Specific dyes to distinguish live cells from dead cells are used: the green staining represent viable cells and providing qualitative data on cellular proliferation; while red staining indicate dead cells and apoptotic bodies.
The samples used are:
- Prototype: biostimulator hyaluronic acid product sterilized by NTS® method
- Competitor: biostimulator hyaluronic acid product sterilized by Steam heat sterilization

The assay, conducted at 2 and 5 days, showed a progressive increase in cell numbers, with more viable cells observed at 5 days. Longer incubation periods also increased red spots, indicating natural cell life and death cycles. Prototype supported better cell adhesion and proliferation compared to Competitor at both time points. This suggests that Prototype has potential as a biorevitalization product. Future studies will focus on quantitative cell viability and morphological assessments.

Evaluation and comparation of physical and chemical properties of sterilized solutions

Formulation	Organoleptic variations	pH after sterilization	Viscosity after sterilization*	Release of by-products and/or irritants
HB		Starting pH=7.31 NTS® pH=7.56 Autoclave pH=7.47	Viscosity decreases after autoclave sterilization	✓
LM		Starting pH=7.14 NTS® pH=6.32 Autoclave pH=6.92	Viscosity decreases after autoclave sterilization	✓
TE		Starting pH=7.84 NTS® pH= 7.8 Autoclave pH=7.88	Viscosity decreases after autoclave sterilization	✓
AO		N.A.	Viscosity decreases after autoclave sterilization	✓
AK	No modification	N.A.	Viscosity decreases after autoclave sterilization	✓
AA	No modification	Starting pH=7.30 NTS® pH=8.65 Autoclave pH=8.11	Viscosity decreases after autoclave sterilization	✓

*Demonstrated by autoclave sterilization vs. HA-CTRL

The table on the left summarizes the effects on the formulations between NTS® and autoclave sterilization products.
The pictures reported shows on left side the NTS® products while on the right the products sterilized through autoclave.

To assess the benefits of NTS® method compared to autoclave sterilization, those effects linked to compositional changes were evaluated:
- Color change, due to degradation of thermolabile substances;
- pH shifts beyond tolerance limits from nitrogenous compound release, alcohol oxidation, or intramolecular bond cleavage;
- Decreased viscosity resulting from HA degradation;
- Release of by-products.

Different mesotherapic products were evaluated, but non-visible effects as undesired product interactions, release of irritants like nitrogen compounds, and degradation of thermolabile ingredients were considered.
These factors were crucial to develop the NTS® method to provide safe, performant sterile products, maintaining the physical and chemical features.

The data collected reported in the table supporting that NTS® method do not stress the solutions avoid any chemical-physical changes compared to the starting solution.

Conclusion

The present invention relates to a process for the preparation of sterile products comprising thermolabile and/or bioactive raw materials. The process includes a sterilization phase by filtration. Preferably, the sterile products obtained by the process of this invention are for cosmetic use, more preferably for use in cosmetic mesotherapy, such as anti-aging mesotherapy, or for medical use in aesthetic medicine or dermatology, preferably as injectable products. The process of this invention is particularly preferred for the preparation of sterile hyaluronic acids or their derivatives.

Acknowledgment
This research is supported by the Ministry of University and Research (MUR) as part Innovation Action – DM MUR 1061/2022 of the PON 2014-2020 "Research and Innovation" resources– Green/Innovation Action – DM MUR 1061/2022 and with the collaboration of CMed Aesthetics S.p.A.

References
WO2023169949A1 – C. MICHELINI, A. MALVENTI, L. ZAPPOLINI and A. DAL CANTO
[1] Thermal stability of sodium hyaluronate in aqueous solution; K. Lowry and E. Beavers; Journal of Biomedical Materials Research, Vol. 28, 1239-1244 (1994)

E-mail: noemi.scacciati@phd.unipi.it
Department of Pharmacy, University of Pisa - Via Bonanno, 6 – 56126 Pisa (PI)
CMed Aesthetics S.p.A. - Via Panfilo Castaldi 4 - 56121 Pisa (PI)