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## **Stability analysis on class III medical devices**

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*A mia madre*

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# Abstract

The stability studies of a medical device represent one of the most important analyses within the medical device lifecycle. Their results are unavoidable data that must be included in the technical documentation examined by notified bodies, organs responsible for the conformity assessments to European regulations. Stability is a measure of the device ability to maintain the initial properties and the characteristics owned at the time of manufacture (time 0). Whenever stability is studied on products stored under specified conditions, those studies are termed real-time stability studies. Whereas, if the product is subjected to accelerated aging, achieved through the application of external stresses, they are called accelerated stability studies. A widespread tool to assess the stability of a product through chemical investigations is Fourier Transform Infrared (FT-IR) spectroscopy. Its application guarantees the identification of compounds that are present in a material and the quantitative similarity assessments with other products.

The purpose of this master thesis work conducted at Tiss'you regenerative company has been the stability analysis on class III medical devices through chemical investigations with the aim to certify their stability in technical documentations submitted to notified bodies.

The studied products have been: Collygen, SpherHA, Sterify gel. The first two devices have been already accepted by notified bodies and they have been real-time studied after 2 years of storing. The last device is the new company proposal and must be still approved. Consequently, accelerated aging protocols have been performed by an external certified company to obtain devices equivalent to 2-years stored products to be analysed. Stability has been examined for all the products with FT-IR spectrometer endowed with Universal Attenuated Total Reflectance (UATR) accessory for solid samples acquisition. However, Sterify gel texture have given first indecisions about the better instrumentation to be used to fulfil such analyses. The High-

Performance Liquid Chromatography (HPLC), an analytical technique for the quantification and/or identification of the substances into a solution through a specific chromatogram, have been considered as an alternative to FT-IR spectroscopy. Nevertheless, first trials on HPLC sample preparation soon highlighted the inapplicability of HPLC for Sterify gel stability analyses. The FT-IR acquisitions have been analysed in the instrumentation software where have been obtained correlations between spectra of the aged products (at time 2) and those at time 0. The mean correlations of the aged Collygen and SpherHA with their products at time 0 have been 0.99, while 0.98 for Sterify gel. Therefore, all the devices have been characterized by mean correlations higher than 0.95, threshold to assess stability. Furthermore, a literature-driven peak association testified the presence of characteristic functional groups present in the analysed products.

To conclude, the current work certifies the stability of the analysed class III medical devices at time 2. These results will be included in the technical reports submitted to notified bodies. Moreover, Sterify gel stability outcomes encourage future studies aimed at defining its shelf life through further accelerated stability studies.

# 1. Introduction

It is well known that nowadays one of the primary employments of biomedical engineering regards the design and development of medical devices. However, the legislative aspects concerning the medical devices field constitute an intricate apparatus with which each biomedical engineer must collaborate for the entire lifecycle of the device in order to launch and keep on the market not only newer and more technological products, but overall safer ones with long lasting performances. The selling of new devices in the European Union must be approved by competent authorities designated and notified in accordance with the Medical Device Regulation (MDR) to carry out conformity assessment procedures [1]. For new medical devices, a technical documentation written in accordance with the MDR requests must be furnished to notified bodies for the medical device approval. On the other hand, for already approved medical devices, a Periodic Safety Update Report (PSUR) is requested by notified bodies mainly to demonstrate the maintained agreement with the Conformité Européenne (CE) marking [1]. In both reports, an important section regards the product verification and validation. Clinical data included in this section contains results on stability studies conducted to assess the shelf life of the product as well as its integrity over time [1]. The stability studies can rely on a real-time or an accelerated aging of the product under analysis. The accelerated aging is generally used to estimate a shelf life of a new device when no real-time data is available and rapidity is needed to fasten the product time to market [2]. However, the validity of accelerated aging studies must be confirmed by subsequent real-time ones [1]. Notified bodies ask for stability studies completed with any instrumentation able to guaranty a comparison, with any possible approach such as chemical or physical, between the original product at time zero and the aged one in order to determine the product degradation over time.

In this context, the objective of the current master thesis conducted at Tiss'you regenerative company has been the stability analysis on class III medical devices

through chemical investigations with the aim to certify stability in a PSUR for already approved devices, while encourage certification procedure and shelf life assessment for a new product still under regulatory approval. In particular, the analysed devices already on the market have been Collygen and SpherHA. Whereas, first studies on product stability regarded the new Tiss'you proposal Sterify gel whose technical documentation is still green for the submission to notified bodies. These terminally sterilized medical devices have been studied at time 2, therefore two years aged after real-time storing or accelerated aging protocols.

The accelerated aging has been conducted externally by another certified company over Sterify gel that has been three months-accelerated to correspond to a 2 years-stored product. The chemical investigations to certify the stability of all the above cited devices has been executed recurring to a Fourier Transform Infrared (FT-IR) spectroscopy, a widespread tool for the identification of molecule inside a sample and for a quantitative-comparative analysis between samples [3]. The utilized spectrometer, with the relative software, has been spectrum Two by Perkin Elmer equipped with a UATR accessory, enabling the only acquisition of solid samples. This represented a first obstacle for the Sterify gel spectra acquisition, ultimately solved with a solidification procedure. An alternative to FT-IR utilization for Sterify gel stability studies would have been the High-Performance Liquid Chromatography (HPLC), an analytical technique for the quantification and/or identification of the substances into a solution through a specific chromatogram [4]. Nevertheless, preliminary trials on Sterify samples preparation for HPLC machine demonstrated the inapplicability of this technique to the case.

The comparison through identity indexes computed by the spectrometer software between products spectra at time 0 and aged ones, together with a literature-driven peak detection of the most relevant groups present in the analysed products, have established stability for each of the analysed device. Indeed, Collygen and SpherHA 2 years aged resulted to be equal at 99% to the relative product at time 0. For Sterify gel the accelerated stability studies reported a similarity between the aged product and the one at time 0 equal to 98%. Moreover, characteristic absorbance peaks in the spectra

dealing with the presence of specific compounds have been found, as expected in a non-degraded product, in all the analysed devices. Collygen spectra have given characteristic peaks of Amide I, Amide II and Amide III which are always present in a formulation made up of several amino acid as in the case of Collygen. The calcium phosphate compound SpherHA provided two characteristic absorbance peaks dealing with the presence of phosphate group within the formulation, as expected. Lastly, in Sterify gel spectra different absorbance peaks have been associated to the presence of pure polyvinyl alcohol (PVA) and pure polyvinylpyrrolidone (PVP) which are important carriers included in the formulation to exert a key role in the antibacterial response.

The present work certifies the stability of the discussed medical devices at time 2 providing results that will be included in the PSUR of Collygen and SpherHA devices and which will enrich the clinical data to be introduced in the technical documentation for Sterify gel approval. Future studies will be intended to let proceed the stability studies just begun on Sterify gel and to define its shelf life.

## 1.1 Medical Device and European Regulation

Currently, the Regulation (EU) 2017/745, commonly known as MDR (Medical Device Regulation), rules the production and selling of medical devices in the European Union. This regulation aims to ensure a proper functioning of the internal medical device market having as objectives the protection of the health for patients and users and, also, the small- and medium- sized enterprises capabilities in the medical field. Furthermore, this Regulation establishes standards of quality and safety for medical devices [1]. The MDR repeals Directive 90/385/EEC, which concerns active implantable medical devices and Directive 93/42/EEC, which concerns medical devices [1]. Published on 5 April 2017, it came into force on 25 May 2017 when it was decided that originally approved medical devices would have a transition time of three years (until 26 May 2020) to meet new requirements. However, after the pandemic emergency of COVID-19, the European Committee extended the transition period to 26 May 2021 [5]. It is composed by 10 chapter, 123 articles and 17 attachments that build up a single legislative regulating all the medical devices other than in vitro diagnostic medical devices which are covered by Regulation (EU) 2017/746 [6].

According to MDR [1] a medical device is defined as “any instrument, apparatus, appliance, software, implant, reagent, material or other article intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the following specific medical purposes:

- diagnosis, prevention, monitoring, prediction, prognosis, treatment or alleviation of disease;
- diagnosis, monitoring, treatment, alleviation of, or compensation for, an injury or disability;
- investigation, replacement or modification of the anatomy or of a physiological or pathological process or state;

– providing information by means of in vitro examination of specimens derived from the human body, including organ, blood and tissue donations, and which does not achieve its principal intended action by pharmacological, immunological or metabolic means, in or on the human body, but which may be assisted in its function by such means.”

Also, the current regulation recognizes as medical devices those used for support of conception and products specifically intended for the cleaning, disinfection or sterilisation of devices that were not considered in the previous directive [7].

The subdivision of medical devices according to their associated degree of risk with respect to the vulnerability of human body was maintained as the previous Medical Device Directive [7] but new rules and new terms were added. With respect to previous directive, five more rules have been added to the classification [8]: rule 11 dealing with software taking clinical decision, rule 19 concerning nanomaterials, rule 20 regarding inhalers, rule 21 concerning substances, rule 22 about active devices with a diagnostic function. The 22 classification rules reported in chapter III of the MDR expect to attribute the medical device class in different scenario: non-invasive devices, invasive devices, active devices, special rules. A non-invasive device either do not touch patient or contact only intact skin, while invasive devices penetrate inside the body, whole or in part, either through a body orifice or through the surface of the body [1]. An active device is any device, whose operation depends on a source of energy other than that generated by the human body or by gravity for that purpose, and which acts by changing the density of or converting that energy [1]. The MDR led manufacturers to consult the new classification rules and update their technical documentations. New clinical evaluations with MDR wording have been necessary for class IIa and IIb [9]. An important variation brought by the current medical device regulation deals with the body of clinical evidence needed for class III and implantable medical devices which became more rigorous [9]. According to MDR, an implantable device is any device partially or wholly absorbed, which is intended to be totally introduced into the human body, or to replace an epithelial surface or the surface of

the eye by clinical intervention and which is intended to remain in place after the procedure.

Other important key changes with respect to the Directive 93/42/EEC regard the implementation of a unique device identification (UDI) system that allows the identification and facilitate the traceability of devices and significantly enhances the Post Market Surveillance (PMS) effectiveness whose aim is to monitor and keep the initial safety and performances of the medical devices already on the market [1,10] The UDI system relies on numeric or alphanumeric code used to register the devices in the UDI database. A rigorous post-market surveillance, possible given the above cited UDI database, is applied through unannounced audits, product sample checks and product testing together with annual safety and performance reporting by device manufacturers [1]. These periodical conformity assessment procedures see as protagonists the notified bodies designated by an EU country to guaranty a continuous safety evaluation of the device whose CE marking is under strict control. Notified bodies are also involved in the first approval of the device prior to the selling [1]. For devices of class IIa, IIb and III a certain level of notified bodies involvement is compulsory to obtain the CE marking [1]. For class I, except for devices that are sterile or have a measuring function, manufacturers may self-apply the CE mark after producing a declaration of conformity [1].

The new regulations provided by the MDR ensure a high level of health and safety protection for EU citizens, as well as a free and fair trade of the products throughout the EU and an adaptation to the significant technological and scientific progresses occurring in medical device sector over the last 20 years [9].

## **1.2 Medical Device Lifecycle**

The MDR rules all the medical device phases of life along with the international standard UNI EN ISO 13485: 2016 [11] which specifies the requirements for the quality management system to organizations that are asked to furnish medical devices and

related services in line with client requisites and regulatory requisites (Figure 1.1). Such organizations can be involved in one or more stages of the medical device life cycle, including design and development, production, storage and distribution, installation, or servicing of a medical device [11]. The lifecycle of a medical device can be divided into three phases [12]:

1. Ideation and feasibility;
2. Design and Development (D&D);
3. Device Production and Post Market Surveillance (PMS).

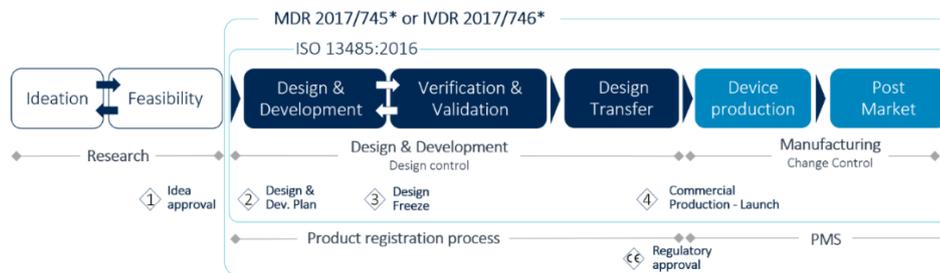


Figure 1.1 Medical device lifecycle [12].

The first phase consists in the creation of an idea that can find concretization after an accurate analysis of the purpose for which the medical device would be intended, the target customers and consequently the user needs. Also, a market analysis about the possible competitors is necessary as a preliminary step for the D&D phase [12]. The second phase, D&D, has as main objective the achievement of a safe device, whose characteristics meet both costumers and regulatory requirements. Once the product realization has been planned and the requisites determined and deeply re-examined according to eventual client complaints received prior to the development, the verification and validation process can occur [12,13]. Verification of the design and development process must be done, in compliance with what planned and documented, to ensure that outputs satisfy the inputs of the process. On the other hand, validation of the design and development process must be performed, in compliance with what planned and documented, to ensure that the resultant product

is able to satisfy the requisites for the specified application and the intended purpose. Verification and validation are mostly supported by tests, analyses, clinical evaluations or clinical investigations, and all is documented within the Quality Management System as part of the design and development files [12]. Stability studies are important data asked by notified bodies for product verification and validation [1]. Finally, the design is transferred into suitable production specifications [12]. It is important to remark that the D&D process does not end with the final transfer of a design to production [12]. It is still open to all possible changes of the device or manufacturing process, including those suggested long after a device has been introduced to the market based on the comeback data from the client, the routine internal inspections and whatever source able to impose a change in the design and development phase [12]. The most important source of useful data for the D&D improvement comes from the Post Market Surveillance (PMS) system which can help the identification of options to enhance the usability, performance and safety of the device. Furthermore, Post-Market Clinical Follow-up (PMCF) plans shall include post market studies to demonstrate the safety and performance of the device [1]. The acquired data must be used by manufacturers to prepare a post-market surveillance report summarising the results and conclusions together with a rationale and description of any preventive and corrective actions taken. For devices belonging to class I, the report has to be updated when necessary and made available to the competent authority upon request. For devices of class IIa, IIb and III, a periodic safety update report (PSUR) is needed for class IIb and III devices at least annually, while for class IIa at least every two years [1]. Thus, throughout the lifetime of the device concerned, that PSUR shall report: the conclusions of the benefit-risk determination, the main findings of the PMCF, the volume of sales of the device and an estimate evaluation of the size and other characteristics of the population using the device and, where practicable, the usage frequency of the device [1]. In parallel with the D&D process and prior releasing the new device to the market, the registration process takes place and consists in the preparation of a technical report that is submitted to the relevant notified bodies designated in accordance with the MDR, responsible for the

demonstration of conformity and the approval of the CE marking that must be placed on the device to market it within the European countries.

### ***1.2.1 Packaging for terminally sterilized medical devices***

The design and the development process regards also the packaging procedure that becomes even more complicated in case of terminally sterilized medical devices. The ISO 11607-1-2019 specifies requirements for the design of sterile barrier systems and packaging systems for terminally sterilized medical devices, the basic attributes required of materials and preformed sterile barrier systems, and design validation requirements [14]. The requirements for the development and validation of processes for packaging medical devices that are terminally sterilized are reported in the standard ISO 11607-2-2019 [15]. For sterile barrier system (SBS) is intended the minimum package that minimizes the risk of ingress of microorganisms and allows aseptic presentation of the sterile contents at the point of use [14]. For terminally sterilized is meant the condition of a product that has been exposed to a sterilization process in its sterile barrier system [14]. The goal of a terminally sterilized medical device packaging system is to allow sterilization, provide physical protection, maintain sterility up to the point of use and allow aseptic presentation. The specific nature of the medical device, the intended sterilization methods, the intended use, expiry date, transport and storage all influence the packaging system design and choice of materials [15]. Today, terminal sterilization technologies for medical devices include Ethylene Oxide and Gamma radiations used in the 95% of the cases and Electronic beam and X-rays in the rest of the cases [16]. The loss of sterile barrier system integrity may occur as a result of physical properties of the materials and adhesive or cohesive bonds degrading over time and by subsequent dynamic events during shipping and handling. Thus, the choice of materials represents an important issue for the fulfilment of the purpose of a terminally sterilized medical device.

Materials for the packaging systems shall be individuated evaluating the following influences [14]:

- temperature range;
- pressure range;
- humidity range;
- maximum rate of change of the above, where necessary;
- exposure to sunlight or UV light;
- cleanliness;
- bioburden;
- electrostatic properties.

Moreover, the source, history and traceability of all materials, especially recycled materials, shall be known and controlled to ensure that the sterile barrier system will consistently agree with the ISO requirements [14]. The documentation for conformity needs to introduce performance data, specifications and test results from validated test methods as well as validation protocols, conclusions with presentation of any necessary actions taken [14]. In this context, of extreme relevance are stability tests that shall demonstrate that the sterile barrier system maintains its integrity over time. Stability testing using accelerated aging protocols shall be regarded as sufficient evidence for claimed expiry date until data from real time aging studies are available [17].

### **1.3 Stability studies**

The stability of a medical device is a measure of its ability to maintain the initial properties and the characteristics owned at the time of manufacture [18]. There are different criteria to evaluate stability [19]:

- Chemical: looking to degradation of chemical compounds present in the product, to interactions between product compounds or between device and packaging

that can alter the safety and the performances of the device, to radioactive decay if the device contains radioactive material, to manufacturing processes that can alter the chemistry of raw materials.

- Physical: investigating in physical characteristics of the device (e.g., appearance, viscosity, elasticity, tensile strength, etc), in manufacturing process, in storage conditions (e.g., temperature, humidity, light, etc)
- Microbiological: concentrating on sterility, environmental control to adjust, for example the microbial load, antimicrobial effectiveness
- Therapeutic: evaluating the ability of the device to perform intended therapeutic or diagnostic purpose
- Toxicological: looking for toxic effects provoked by degradation processes
- Biocompatibility: analysing whether the biocompatibility of the device has changed during storage or use of the device.

The shelf life of a medical device is estimated through stability studies, real-time and accelerated stability studies, performed over products aged real-time and accelerated respectively [20]. It is oftentimes common to find the word 'expiry date' that is the date by which product should be used. In ISO 11607-1-2019 the 'expiry date' is used in case of medical device in a sterile barrier system. According to the international standards, the expression "use by date" is utilized to describe the shelf life of packaging materials and preformed sterile barrier systems prior to assembly into a sterile barrier system [14].

A real-time aging study is conducted to establish long-term product stability and its effective shelf life [20]. It consists in storing the product under specified conditions for an interval of time (usually years) and take it back for the stability test concluded the period in which the product is supposed to be degraded or far from its initial performances [20]. Real-time aging programs provide the best data to assess the expiry date of a product. However, due to market requests and the need to release products in the shortest possible time, real time aging studies are not always feasible [20]. Thus, parallelly, accelerated aging studies are conducted to predict a product's expiration date when there is no real-time data available, or rapidity is anyway needed. Validity

of accelerated aging results must be confirmed by real-time ones. Accelerated aging programs expect to make the system undergo to external stresses in order to simulate the period claimed for product expiration. The stresses required to accelerate the aging process of a product regard temperature and humidity whose values are far from those detectable in everyday life [21]. The standard guide ASTM-F1980, released by the international American Society for Testing and Materials, provides guidelines for developing accelerated aging protocols to assess the effects of the passing of time on the stability of sterilized barrier systems [22]. Accelerated aging techniques assume that the chemical reactions involved in the deterioration of materials follow the Arrhenius reaction rate function [22]. This function states that a 10°C increase or decrease in temperature of a homogeneous process provokes a change of, approximatively, two times or 1/2-time in the rate of a chemical reaction. The aging factor for a 10°C decrease or increase in temperature is called  $Q_{10}$ . The ASTM protocol takes into account only the  $Q_{10}$ , even though it may be introduced also a humidity factor to calculate the accelerated aging time (AAT) considering that high or low relative humidity (RH) can represent an adjunctive load in aging. The AAT able to establish equivalence to real time aging (RT) is determined by dividing the desired (or required) shelf life by the accelerated aging factor (AAF).

Eq. 1  $AAT = \text{Desired } (RT) / AAF$

The AAF is estimated with the computation of the following equation:

Eq. 2  $AAF = Q_{10}^{[(T_{AA}-T_{RT})/10]}$

where:

$T_{AA}$  is the accelerated aging temperature (°C) that should be chosen in order to avoid any material transitions or sterile barrier system distorts and below 60°C unless higher temperature has been demonstrated appropriate, while  $T_{RT}$  is the room or ambient temperature (°C) that represents the actual product storage and use

conditions (usually in between 20 and 25 °C) and  $Q_{10}$  is the aging factor. The higher the  $T_{AA}$ , the higher the AAF and, consequently, the shortest the AAT.

To sum up, the accelerated aging protocol steps provided by the ASTM F1980 are [22]:

1. Selection of the  $Q_{10}$  value. Usually is used a  $Q_{10}$  equal to 2, indeed a more aggressive reaction rate coefficient, for example, 2.2 up to 2.5, may be used if the system under investigation is sufficiently well characterized in the literature;
2. Definition of the desired shelf life of the sterile barrier system according to product and market needs;
3. Definition of aging test time intervals, including the time zero;
4. Definition of aging test conditions and thus, definition of room temperature ( $T_{RT}$ ) and accelerating aging temperature ( $T_{AA}$ );
5. Computation of AAT to know days needed by the accelerated aging protocol to equal the real time aging of the product of interest.

It is important to remark the calculation to estimate time for accelerated aging is based on temperature regardless of the addition of RH. However, Appendix X3 of ASTM protocol gives appropriate concentration (ppm) of  $H_2O$  in air at various temperatures (Figure 1.2). Once concluded the accelerated aging on SBS, a report should be written specifying [22]:

- Accelerated aging conditions (i.e., test temperature, humidity ambient temperature);
- Time frame;
- Sterile barrier system description;
- Documentation about calibrated instruments used for measuring and monitoring the aging condition;
- Documentation of the standard methods used for sterile barrier system evaluation;
- Documentation about post-aging results after evaluation on SBS properties and integrity.

**TABLE X3.1 Relationship of Relative Humidity to Constant Moisture Content and Variable Temperature**

Elevated Temperature (°C)	Relative Humidity (%)	Water Content (ppm)
23	50.0	13 750
40	19.1	13 750
50	11.4	13 750
55	9.0	13 750
60	7.1	13 750

Figure 1.2 Appropriate H<sub>2</sub>O concentration at various temperature (Appendix X3 of ASTM-F1980 protocol)

[22].

## 1.4 Medical devices produced by Tiss'you regenerative company

Tiss'you is a regenerative company placed in San Marino [23]. Its mission is the research and development of medical devices able to help the natural recovery. It produces procedural kits such as Lipocell able to enhance the biological properties of the adipose tissue and Monocytes, a peripheral blood selective filtration system from which it is possible to obtain mononuclear cells. It also offers three kind of food supplements: Formula Fast, Formula Race and Formula Kart. The first is used to reduce pain and swelling and related hematomas after traumatic events. The second improves the neuronal metabolism and reduces the release of pro-inflammatory factors that can lead to a chronicization of the inflammation. The third is intended for articulation problem such as rigidity, swelling, difficulty of movement. In addition, Tiss'you developed biomaterial alternatives, presented here below, that have been the focus of this thesis.

### 1.4.1 Collygen

Collygen [24] is the new proposal of absorbable membranes and fleeces (Figure 1.3) consisting of highly purified type I atelocollagen, of equine derivation, for Guided Tissue Regeneration (GTR) and Guided Bone Regeneration (GBR). At the base of the GBR process there are cells that migrate to the membrane (monocytes / macrophages

CD68 +, osteoblastic precursors) which express and release elements that are essential for bone regeneration. Collygen membranes act like a biological barrier to protect bone grafts and its collagenic structure permits a rapid and optimal adhesion over the bony surface ensuring a good protection at the site of the implant from the tissue infiltration without being manually fixed. Its ease of use and application is accompanied by completely absorbability and a protection of 4-6 weeks. On the other hand, Collygen Fleece is stable to humidity and thanks to its porous structure lead the haemostasis. For this reason, is especially used to reach as soon as possible a stable clot improving the wound healing. Also, the porous structure makes the resorption time shorter. The declared shelf life for the medical device of class III is 5 years.

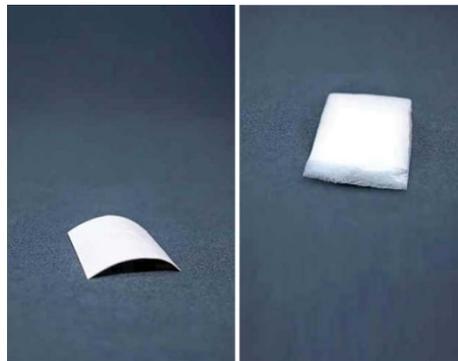


Figure 1.3 Collygen membrane (left) and Collygen fleece (right).

### **1.4.2 SpherHA**

SpherHA [25] is a line of innovative synthetic bone substitutes, based on biomimetic nano-structured hydroxyapatite. SpherHA is available in dense granules, porous chips, injectable paste and moldable crunch in a wide range of sizes, to respond in a practical and functional way to multiple implant requirements in oral-maxillofacial, orthopedics, traumatology and spine surgeries (Figure 1.4). SpherHA hydroxyapatite is a calcium phosphate compound that is remarkably similar to the human mineral bone matrix in composition, structure and size of nano-crystals. The Ca / P ratio is the same of the human bone apatite (1.67). Its high surface/volume ratio makes it an ideal scaffold for osteointegration and regeneration of bone defects. The highly porous and interconnected structure is optimally osteoconductive, promoting

cellular colonization, nutrients exchanges and rapid vascularization. Thanks to specific composition and nano-metric dimension of composing crystals, SpherHA bone substitutes are completely degraded by osteoclastic activity and physiologically remodelled into new vital bone tissue. All the SpherHA solutions belong to class III of medical devices and have a declared shelf life of 3 years.

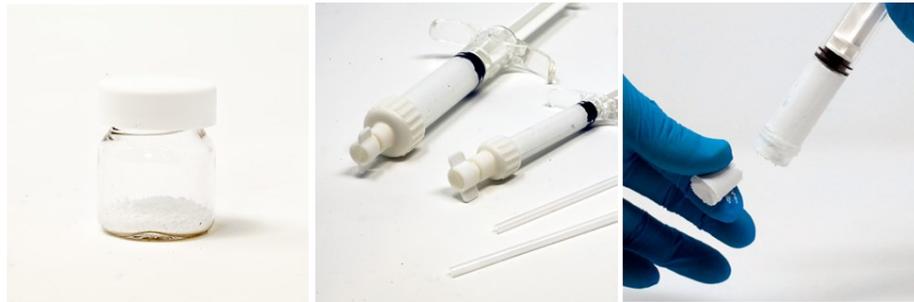


Figure 1.4 SpherHA dense granules (left), SpherHA injectable pase (center), SpherHA mouldable crunch (right)

### **1.4.3 Sterify gel**

Sterify Gel [26] is a mucoadesive device able to fight against bacteria responsible of periodontal diseases (Figure 1.5). Its formulation allows to reduce the pain and to promote the correct activity of gingival fibroblasts, to maintain healthy gums for a long time. Compared to other products, Sterify gel has a broad spectrum of antibacterial action, does not create bacterial resistance, stimulates gum regeneration and it can be stored at room temperature. Its formulation is characterized by polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) that act as carriers for the hydroxytyrosol and nisin Z responsible for the antibacterial response. Even though Sterify gel has lots of potentiality, it is still under regulatory approval. Consequently, its shelf life must be still investigated.



Figure 1.5 Sterify gel.

## 1.5 Infrared spectroscopy

Infrared (IR) spectroscopy is the study of the interaction of infrared light with matter [27]. Infrared light is part of the electromagnetic spectrum (Figure 1.6) and lies in between the ultraviolet light and the microwaves, that correspond in terms of wavelengths, to a range that goes from 0.7  $\mu\text{m}$  to 300  $\mu\text{m}$ . This region can be subdivided into 3 parts [28]:

- Near Infrared (NIRS), from 0.7  $\mu\text{m}$  to 2,5  $\mu\text{m}$ ;
- Mid Infrared (MIRS), from 2,5  $\mu\text{m}$  to 25  $\mu\text{m}$ ;
- Far infrared (FIRS), from 25  $\mu\text{m}$  to 300  $\mu\text{m}$ .

that corresponds in terms of wavenumbers ( $\text{cm}^{-1}$ ) to:

- Near Infrared (NIRS), from 14000 to 4000  $\text{cm}^{-1}$ ;
- Mid Infrared (MIRS), from 4000 to 400  $\text{cm}^{-1}$ ;
- Far infrared (FIRS), from 400 to 10  $\text{cm}^{-1}$ .

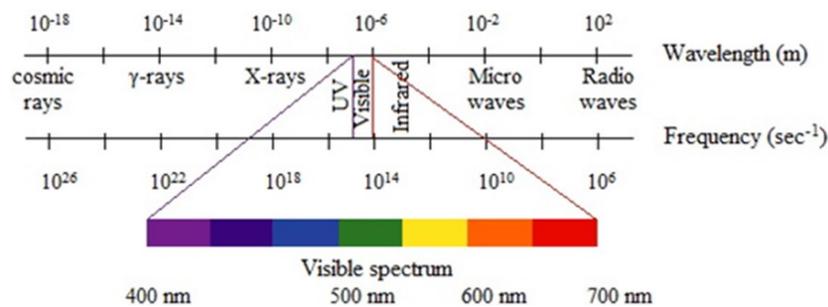


Figure 1.6 Electromagnetic spectrum [28].

The energy coming from the infrared radiation is given by Planck's equation [27]:

$$\text{Eq. 3} \quad E = h\nu$$

where  $E$  is the energy of the photon (J),  $h$  is Max Planck's constant ( $6.63 \times 10^{-34}$  Js) and  $\nu$  is the photon frequency given by the ratio between speed of light  $c$  ( $\sim 3 \times 10^{10}$  cm/second) and wavelength  $\lambda$  (in cm). The energy values associated with the infrared

light can make substances molecules vibrate when they absorb IR beams [27]. A molecule is constituted by N atoms that are all characterized by three degrees of freedom (i.e., movement along each of the axes of a Cartesian coordinate system) [29]. Thus, a molecule has  $3N$  degrees of motional freedom. If we subtract the translational and rotational degrees of freedom from them, which do not provoke a change in atoms distances, there will be left  $3N-6$  degree of freedom. This number corresponds to the motions able to change the distances between atoms and, consequently, the lengths of the chemical bonds and the angles between them. These internal movements are called normal mode of vibration [29]. The ways in which the molecule can vibrate are different; indeed, the molecule can be subjected to [28]:

- Stretching, when there is a change in the length of a bond;
- Bending, when there is a change in the angle between two bonds;
- Rocking, when there is a change in angle between a group of atoms;
- Wagging: when there is a change in angle between the plane of a group of atoms;
- Twisting, when there is a change in the angle between the planes of two groups of atoms;
- Out-of-plane, when there is a change in the angle between anyone of the C-H bonds and the plane defined by the remaining atoms.

The interaction of infrared radiation with the vibrational energy states of the molecules gives the IR spectra in the above mentioned three regions of the IR band [28]. The IR spectra are usually called the “fingerprints” of substances; indeed, they are able to give information about which molecules are present in an analysed sample since there is a correlation between peak positions and molecular structure. The identification of molecules constituting a sample is one important employment of IR spectroscopy [28]. Moreover, another useful utilization is in the comparison between two samples, to see their similarity looking at the position of the peaks, height and width of their spectra [28]. Plots usually report on x-axis the wavenumbers from  $4000\text{ cm}^{-1}$  (on the left) to the  $500\text{ cm}^{-1}$  (on the right). Thus, plots are in the mid infrared region, widely used for the characterization of organic compounds [28]. Also, the spectra are

reported in absorbance units, which measures the amount of light absorbed by a sample. The absorbance spectrum of a sample is calculated from the following equation [27]:

$$\text{Eq. 4} \qquad A = \log(I_0/I)$$

where  $A$  is the absorbance,  $I_0$  and  $I$  are the intensities in the background spectrum and in the sample spectrum respectively. Absorbance is also related to the concentration of molecules in a sample via an equation called Beer's Law [27]:

$$\text{Eq. 5} \qquad A = \epsilon lc$$

where  $A$  is the absorbance,  $\epsilon$  is the absorptivity ( $M^{-1} \cdot cm^{-1}$ ),  $l$  is the pathlength of the light while travelling through the sample (cm) and  $c$  the concentration of the sample solution (M). Beer's Law establish a direct proportionality between absorbance and concentration. The y-axis of an infrared spectrum can also be plotted in units called percent transmittance (%T), which measures the percentage of light transmitted by a sample. %T spectra are calculated as follows [27]:

$$\text{Eq. 6} \qquad \%T = 100 \times (I/I_0)$$

where %T is the percent transmittance,  $I_0$  and  $I$  are the intensity in the background spectrum and in the sample spectrum respectively. It has to be noticed that in the %T spectrum the peaks point down, and the peak bottoms represent wavenumbers where the sample transmitted measurably less than 100% of the incident infrared light. Thus, samples that do not absorb the IR light will record a horizontal line with 100% of transmittance [27].

Defined the theory behind the IR spectroscopy and described the layout of an IR spectrum, it is of fundamental importance shows the regions in which the plot can be subdivided (Figure 1.7, Table 1.1): from higher wavenumbers it is possible to find a single bond stretch region, a triple bond region, a double bond region and a so-called fingerprint region ( $1500 - 400 \text{ cm}^{-1}$ ) [30]. The fingerprint region contains many peaks

constituting an intricate pattern unique for a given compound, therefore, it can be used to make distinction between compounds [31].

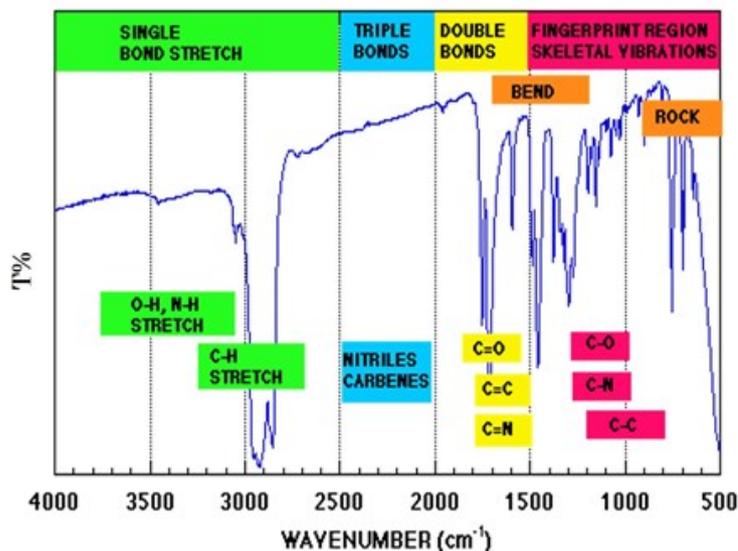


Figure 1.7 IR spectrum [30].

Table 1.1 Regions of IR spectrum.

Wavenumber	Vibration mode
$\geq 3000 \text{ cm}^{-1}$	single bond stretch eg. C-H, O-H, and N-H
2000 - 2500 $\text{cm}^{-1}$	triple bond stretch eg. acetylenes, carbenes and nitriles
1500 - 2000 $\text{cm}^{-1}$	double bond stretch eg. carbonyls (C=O) or alkenes (C=C)
500-1500 $\text{cm}^{-1}$	Fingerprint region

IR spectra are given by the IR spectrometer. We have two types of IR spectrometers: the classical and the modern Fourier Transform IR (FT-IR) spectrometer.

IR classical instrumentation consist of 4 parts [28]:

1. A light source of irradiation
2. A dispersing element, diffraction grating or a prism
3. A detector
4. Optical system of mirror

In Figure 1.8 are represented the basic principles under classical dispersive spectrometer functioning [32]. The infrared radiation from the source is sent to the sample and a reference path. Subsequently, beams reach the chopper that is responsible for moderating the energy conveyed to the detector. Light from the chopper is directed to the dispersing element (grating) which separate the wavelength of light and sent each one, individually, to the detector via a slit to give the spectrum.

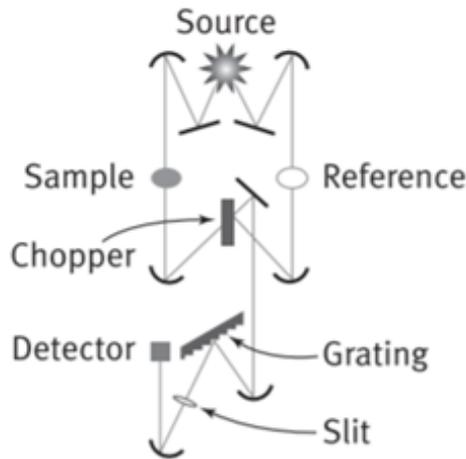


Figure 1.8 Dispersive spectrometer diagram [32].

The main difference between classic and modern spectrometers is the use of Michelson interferometer which enables all the frequencies to reach the detector all at once and not one at the time [28].

The Michelson Interferometer shown in Figure 1.9 [33] has two mutually perpendicular arms intersected by a beam splitter. One arm holds a stationary, plane mirror, while the other arm a moving mirror [33]. The moving mirror moves back and forth with a constant velocity that is timed according to the very precise laser wavelength in the system [32]. On the opposite side of the moving mirror there is a source of radiation whose beam is split into two equal light beams that travels towards the mirrors and are back reflected to the beam splitter and onto the detector (in the opposite side of the stationary mirror). These two beams will interfere constructively or destructively, depending on the relationship between their path difference ( $x$ ) and the wavelengths of light. When the moving mirror and the stationary mirror are positioned the same distance from the beam splitter, the paths of the light beams are

identical ( $x = 0$ ). Under these conditions all wavelengths of the radiation striking the beam splitter after reflection add coherently to produce a maximum flux at the detector and generate what is known as the "center burst" (Figure 1.10). When the moving mirror change position causing a path difference different from zero, each wavelength of the source radiation destructively interferes with itself at the level of the beam splitter [33]. From the beam splitter the interference patterns are transferred to the sample where some energy is absorbed and some transmitted [32]. The transmitted portion strikes the detector which collects information about every wavelength in the infrared range simultaneously [32]. The received beam is equal to the sum of all the beams for each of individual wavelength that rapidly decrease with the moving mirror displacement [33].

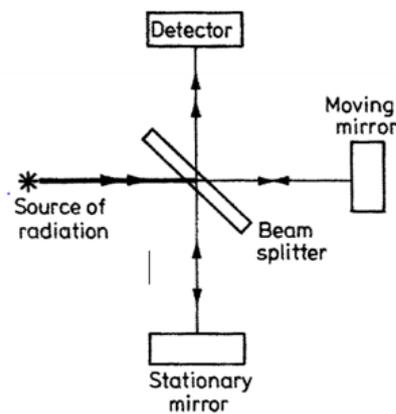


Figure 1.9 Optical diagram of the Michelson interferometer [33].

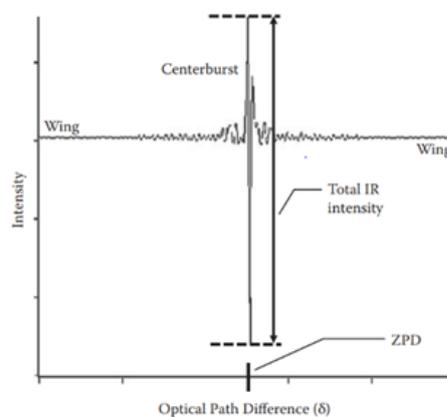


Figure 1.10 A real-world interferogram obtained when many wavenumbers of light pass through the interferometer together [27].

The output of Michelson interferometer is the interferogram. For a monochromatic source of frequency  $\nu$ , the interferogram is obtained through the following expression [33]:

$$\text{Eq. 7} \quad I(x) = 2RTI(\nu) (1 + \cos 2\pi \nu x)$$

where  $R$  is the reflectance of the beam splitter,  $T$  is the transmittance of the beam splitter,  $I(\nu)$  is the input energy at frequency  $\nu$  and  $x$  is the path difference. Thus, it consists of two parts: one is represented by the constant first term, the other one is a modulated component represented by the second trigonometric term.

The interferogram for a polychromatic source  $A(\nu)$  is given by [33]:

$$\text{Eq. 8} \quad I(x) \int_0^{\infty} A(\nu) (1 + \cos 2\pi \nu x) d\nu$$

Where the path difference varies from 0 to infinite values. From this formula, after some considerations [33], the actual interferogram  $F(x)$  is:

$$\text{Eq. 9} \quad F(x) = I(x) - I(\infty) = \int_0^{\infty} A(\nu) \cos (2\pi \nu x) d\nu$$

To obtain the infrared spectrum, the signal acquired by the detector is sent to the computer which applies the FFT converting the interferogram in an IR spectrum as shown in Figure 1.11 [32,33]. Applying the Fourier transform and using as approximation to infinite extremities of the integral the maximum distance of the mirror drive ( $L$ ) it is mathematically obtained [33]:

$$\text{Eq. 10} \quad S(\nu) = 2 \int_{-L}^{+L} F(x) \cos (2\pi \nu x) dx$$

Where  $S(\nu)$  is used to represent an approximation of Fourier transform.

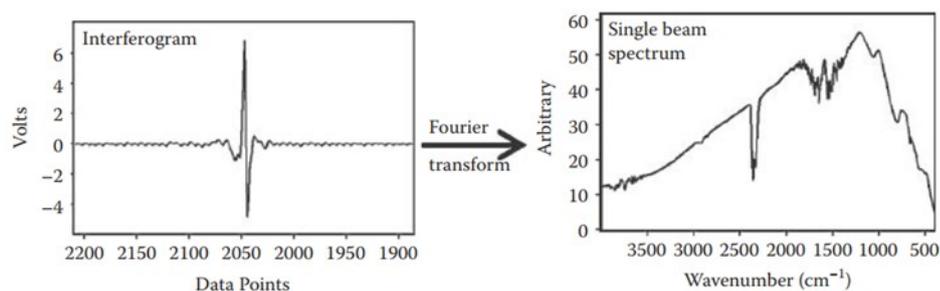


Figure 1.11 Interferogram (on the left) which is Fourier transformed giving a single beam IR spectrum (on the right) [27].

The diagram resuming the basic elements and functioning of modern FT-IR spectroscopy is given in Figure 1.12.

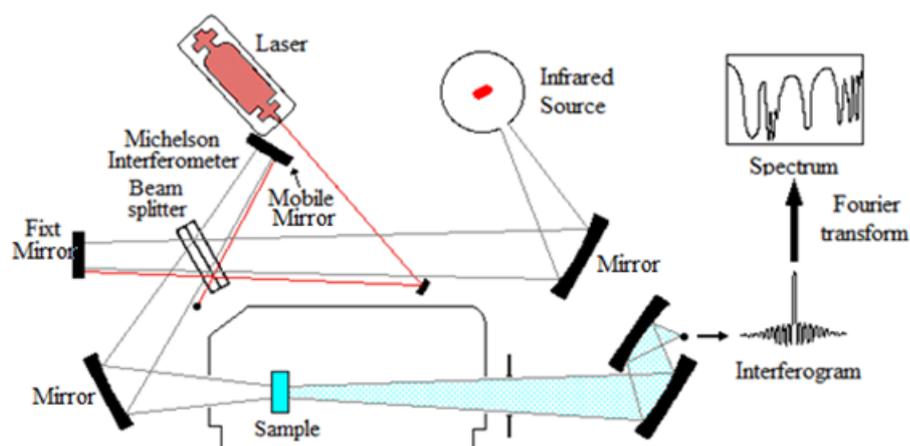


Figure 1.12 Diagram of FT-IR spectrometer [28].

## 1.6 High-Performance Liquid Chromatography

High-Performance liquid chromatography or High-Pressure liquid Chromatography (HPLC) is an analytical technique that is used to separate a mixture in solution into its individual components. The injection of the sample contained in a vial is pumped through a liquid mobile phase through a column that contains a stationary phase where sample molecules get separated for their different affinity to the stationary phase [34]. Solutes with stronger interactions with the stationary phase move slower than others that are characterized by lower interaction with the stationary

phase. Therefore, compounds elute from the column and reach the detector at different time. The time required for an analyte to travel through the column after injection until the analyte reaches the detector is called the retention time ( $t_R$ ). If the sample contains un-retained species, such species travel with the mobile phase where the time spent by that species to exit the column is called column dead time or hold-up time ( $t_0$ ). The time the molecules spent in the stationary phase is called corrected retention time ( $t'_R$ ), which is calculated as the difference between the retention time and the dead time:

$$\text{Eq. 11} \quad t'_R = t_R - t_0$$

When substances elute, the detector send inputs to the dedicated software which provide chromatograms (Figure 1.14) with bigger or smaller peaks depending on the concentration of the substance in the solution. Therefore, the area under the curve peaks can be used to quantify the concentration of substances. In addition, the corrected retention time can be used for a qualitative analysis aimed at identifying solutes present in the analysed solution.

Therefore, the HPLC is able to provide a quantification and/or identification of the substances into a solution through a specific chromatogram. This makes this technique useful for quality control on products stability [35] .

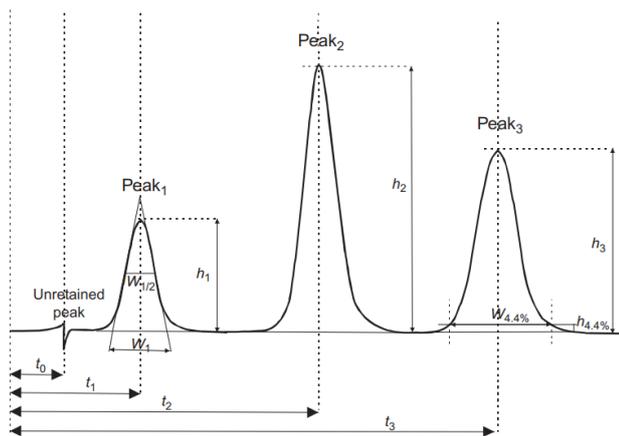


Figure 1.13 Chromatographic peaks and their attributes.  $t_0$ , hold-up time or column dead time;  $t_{1-3}$ , retention time;  $h_{1-3}$ , peak height;  $w$ , peak width;  $w_{1/2}$ , peak width measured at half peak height;  $w_{4.4\%}$ , peak width measured at 4.4% peak height ( $h_{4.4\%}$ ) [36].

A modern HPLC system, shown in Figure 1.15, is equipped with a solvent delivery system, sample injector, column, detection system, and computer data station [36]:

- The solvent delivery system in liquid chromatography consists of solvent reservoirs, high-pressure pumps, check valves, flow controllers, mixing chamber, pulse dampener, and pressure transducers. Oftentimes solvents are characterized by dissolved solid particles and gasses which can reduce the resolution of the instrumentation as well as the operation of the pump system and the lifetime of column. For this reason, glass or stainless-steel reservoirs have filters to remove the solid particles in suspension of the mobile phase. Moreover, solvent delivery systems are equipped with degassers, which may consist of vacuum pumping system, distillation system, device for heating and stirring, or system of sparging, in which the dissolved gases are swept out of solution by fine bubbles of an inert gas that is not soluble in the mobile phase.
- The sample injection system introduces the liquid sample contained in vials introduced in the machine into the flow stream of the mobile phase. This system requires to maintain the characteristic of the sample and to withstand the high pressures to which it has to be subjected.
- The liquid-chromatographic columns are usually constituted by smooth-bore stainless steel tubing, although heavy-walled glass tubing can occasionally be found. Small dimensions and particle size of the packing materials are preferred to improve the peak resolution and the sensibility and to reduce the amount of solvent needed. The diameter of a column in analytical separation is about 2–5mm; its length is about 10–30 cm. Sometimes the temperature is controlled by a column oven.
- The detector is the component that emits a response generated by the eluting sample compound and that send signals to the computer data station where detected signals are visualized into peaks on the chromatogram. It is positioned immediately posterior to the stationary phase. Today, there are different kinds of

detectors available [36]. Very common are absorbance detectors which only respond to substances that absorb radiation at the wavelength of the light source. Detectors that measure only in the range 190-350 nm are ultraviolet (UV) absorbance detectors, whereas those that measure in the region 350-700 nm are visible (Vis) detectors. Detectors that span the range 190-700 nm are known as UV/Vis detectors [34]. Another example of detector may be fluorescence detector that is based on the principle that some compounds fluoresce when bombarded with UV light. Other detectors used in HPLC are electrochemical detectors, conductivity detector, refractive index detectors, massive spectrometry detectors [36].

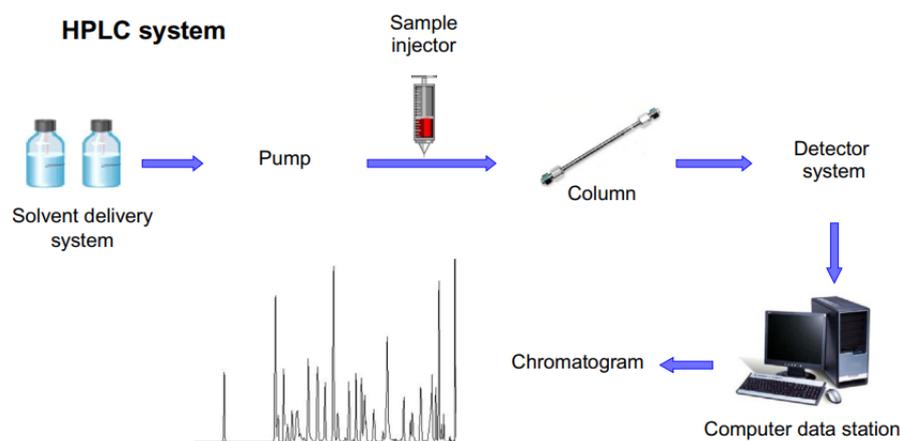


Figure 1.14 High-performance liquid chromatography (HPLC) instrument [36].

## 1.7 The Aim

The aim of the current work has been the stability studies on three class III medical devices produced by Tiss'you regenerative company, with the purpose to acquire clinical data to be included in technical documentations examined by notified bodies.

## 2. Material and Methods

### 2.1 Medical devices under study

The stability studies have been conducted on three medical devices produced by Tiss'you company. The already approved devices have been analysed through real-time stability studies, while the still uncertified device has been analysed through accelerated stability studies. For each product have been considered three lots and, for each lot, three samples have been submitted to stability studies. All the reagents used in the studies have been acquired by VWR.

#### 2.1.1 *Collygen*

The analysed version of Collygen proposal has been Collygen fleece. The product underwent to real-time storing under the following controlled conditions:

- Constant temperature: 15-25 °C;
- Constant humidity: 40-60%;
- Storing time: 2 years.

The analysed lots have been: 21086, 21087, 21088.

#### 2.1.2 *SpherHA*

SpherHA products have been analysed in granular and injectable paste versions. They both have been evaluated after a real-time storing under the following controlled conditions:

- Constant temperature: 15-25 °C;
- Constant humidity: 40-60 %;
- Storing time: 2 years.

### ***2.1.3 Sterify gel***

Sterify gel, still in the certification phase, has been analysed through accelerated stability studies. The accelerated aging was performed by an external company in accordance with protocol released by standard ASTM-F1980 imposing the following parameters:

- Temperature: 55°C;
- Humidity: 75%
- Accelerating time: 3 months.

Under the above conditions, the resultant product is equivalent to 2 years real-time stored product. The considered lots have been: 210002, 210003, 210004. Sterify gel texture has given initial doubts on the kind of technique to be used for its stability studies.

## **2.2 FT-IR spectroscopy and adjunctive tools**

Stability studies have been executed from a chemical point of view through FT-IR spectroscopy for all the above cited devices. The spectrometer available at Tiss'you laboratories expects to have the Universal Attenuated Total Reflectance (UATR) accessory, which can be used to analyse homogenous solid samples, solid surfaces, or coatings on solid samples. Among the devices studied, Sterify gel has been the only one necessitating preliminary procedures to make the product available for the acquisition given its gelatinous texture. Acquisition and visualization of spectra have been realized in the software connected to the spectrometer with a precise flowchart and some precautions further described. Subsequently, acquired spectra have been analysed with the aid of software options summarized in sub-chapter 2.2.4. Computations on data retrieved from the software have been executed in Microsoft Excel to have conclusive results on products stability. Additional observations on spectra have been carried out consulting literature.

### ***2.2.1 FT-IR spectrometer***

The FT-IR analysis has been conducted by means of FT-IR spectrometer Spectrum Two by Perkin Elmer (Figure 2.1) [37]. The machine is characterized by high compactness and robustness which enable users to acquire data anywhere and anytime, also considered the wireless option and the multi-user site licenses availability. Its advanced electronic guaranties consistent performances in term of high signal-to-noise ratio and good sensitivity (Table 2.1). The major source of innovation regards the introduction of the lithium tantalate detector ( $\text{LiTaO}_3$ ), an alternative to the deuterated triglycine sulphate (DTGS) detector used in standard FT-IR instruments [38]. The  $\text{LiTaO}_3$  advantages include lower manufacturing cost and the elimination of the DTGS Curie point very close to room temperature [38]. However, spectra measured with  $\text{LiTaO}_3$  detector present slightly higher noise levels in short wavelength range compared to DTGS spectra. This difference is anyway negligible and an unambiguous identification of material is for sure guaranteed [38]. The optical system is characterized by a calcium fluoride ( $\text{CaF}_2$ ) window, an excellent IR window material. Perkin Elmer released different versions of Spectrum Two, depending on the different kind of accessories owned by the machine. The spectrometer used in this study is equipped with the UATR accessory (Figure 2.1), that is an internal reflection accessory for simplifying the analysis of solids, powders or pastes. The UATR working principle shown in Figure 2.2 involves the use of a crystal with a high refractive index placed above the sample plate. The crystal used in Spectrum Two UATR is a diamond crystal. It has been chosen by Perking Elmer because it is hard, not easily scratchable, resistant to strong acids and bases, and can withstand high pressures [37]. The diamond UATR has an effective scanning range that matches the full range of the instrument ( $4000\text{-}400\text{ cm}^{-1}$ ), although sensitivity is slightly reduced in the approximate range  $1900\text{-}2700\text{ cm}^{-1}$  [37]. When the infrared beam is sent from the instrument to the accessory, it goes up into the crystal where it is reflected within the crystal provoking the sample penetration by few microns (Figure 2.3). Reflections are finally back propagated to the detector housed in the machine [37]. The UATR technology expects

to acquire solid samples and surfaces kept on the sample plate throughout a proper pressing action exerted by a mechanical arm which ensures a good contact between the sample and the crystal.



Figure 2.1 Spectrum Two by Perkin Elmer (on the left), Universal Attenuated Total Reflectance (UATR) accessory (on the right) [37].

Table 2.1 FT-IR Spectrum Two specifications [37].

Specification	Value
Dimension	450 x 300 x 210 mm
Weight	~13 kg
Power input	100-230 V, 50/60 Hz, Max 65 VA
Detector	LiTaO <sub>3</sub>
Sensitivity range	1900-2700 cm <sup>-1</sup>
Optical system	CaF <sub>2</sub> window
Operating temperature range	5 - 45 °C
Storage temperature range	-20 - 60 °C
Maximum relative humidity	80% (non-condensing) with CaF <sub>2</sub> window
Accessories	UATR accessory

The sample spectrum from Spectrum Two endowed with UATR can be obtained running Spectrum software. Once the instrumentation is switched on and connected to the software, firstly, must be performed an acquisition of the background spectrum to avoid molecules entrapped in the air (e.g., CO<sub>2</sub>) will affect and modify the measurements. Secondly, the user can proceed with the acquisitions of the samples under study. Each acquisition, background one included, must be preceded by a plate cleaning procedure with a volatile organic solvent that must completely evaporate before the acquisition otherwise signals would be corrupted by vibrations of bonds concerning the acquired solvent. If the crystal is not properly cleaned, negative bands in the absorbance spectra may be observed [37].

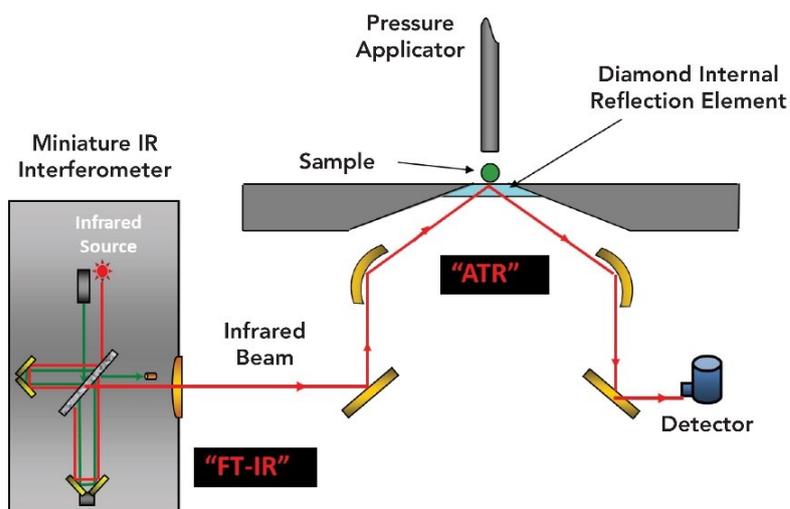


Figure 2.2 Spectrometer with ATR accessory [39].

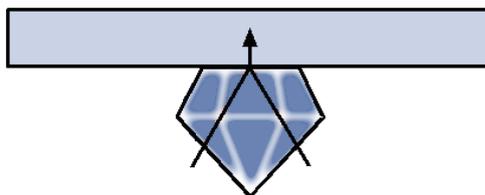


Figure 2.3 Crystal reflection and sample penetration [37].

## 2.2.2 FT-IR spectrometer software

The instrument is connected to a computer, either USB 2.0 or Ethernet cable, or via a wireless network using the optional wireless router. The provided software to acquire, visualize and analyse the spectra is Perkin Elmer Spectrum IR (Figure 2.4) which controls one instrument at time [40]. Acquisitions are generally performed with the option Scan and Preview to control the force of the pressing arm on the plate and check the quality of the spectrum prior to its effective acquisition. Visualization occurs in the Viewing area immediately after the acquisition procedure. Superimposition of acquired spectra is possible selecting the data in the “Data Explorer” pan. Analysis and processing of spectra is accomplished through several options available in the process menu (Figure 2.5A).

Among the options at disposal, three options have been fundamental for the stability studies. The most important one has been the “Comparing” process that estimates similarity between acquired spectra and reference spectra saved on Spectrum database. The degree of similarity between two spectra is expressed with the correlation value which varies from 0 for unrelated samples to 1 for identity cases [40]. Whenever the comparison between a sample and a spectrum found in the software database gives a certain identity index, the acquired sample quantitatively correlates with the software spectrum with that value. In the displayed comparing table (Figure 2.5 B) achieved from the Setup menu, together with the identity index, is shown the Fail/Pass correlation result determined from an imposed correlation threshold that has been set to 0.95 for the purpose of the current work. The spectra comparison is performed by the software throughout difference processes: the analysed spectrum is subtracted to spectra available in the software library [40]. Furthermore, the identity index considers the spectra wavenumbers and the ratios between subsequent peaks areas. The identity indexes are representative of spectra molecular content. From the Setup Compare Parameters tab, shown in Figure 2.7 C, has been defined as wavelength range for the comparison the overlapping region. In addition, some filters to eliminate

differences in spectra that are not related with differences in sample were applied: Resolution weighting, Intensity weighting, Noise weighting, CO<sub>2</sub> blanking.

Other two useful options during the analysis of acquired spectra for stability assessments have been “Peak Table” option from the Process menu and “Label Peaks” in the View menu. The first provide peaks tables reporting the wavenumber and the area associated to each peak, the second gives the visualization of the wavenumbers onto the labelled spectrum peaks.

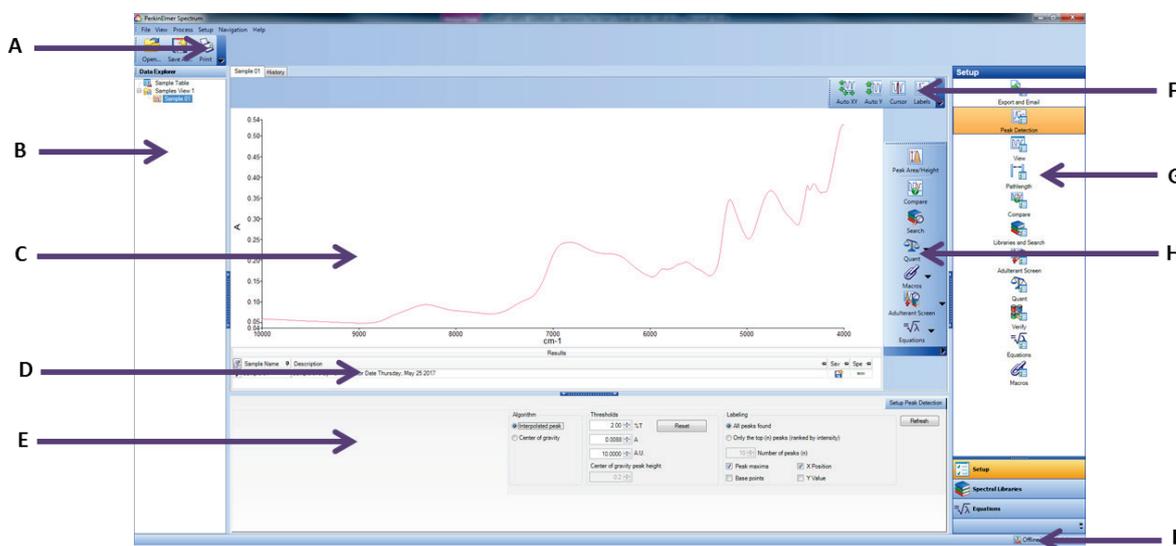


Figure 2.4 PerkinElmer Spectrum IR software window:

Global Toolbars (A), Data Explorer (B), Viewing Area (C), Information Pane (D), Dialog Pane (E), Graph bar (F), Navigation pane (G), Process Bar (H), Status Bar (I) [40].

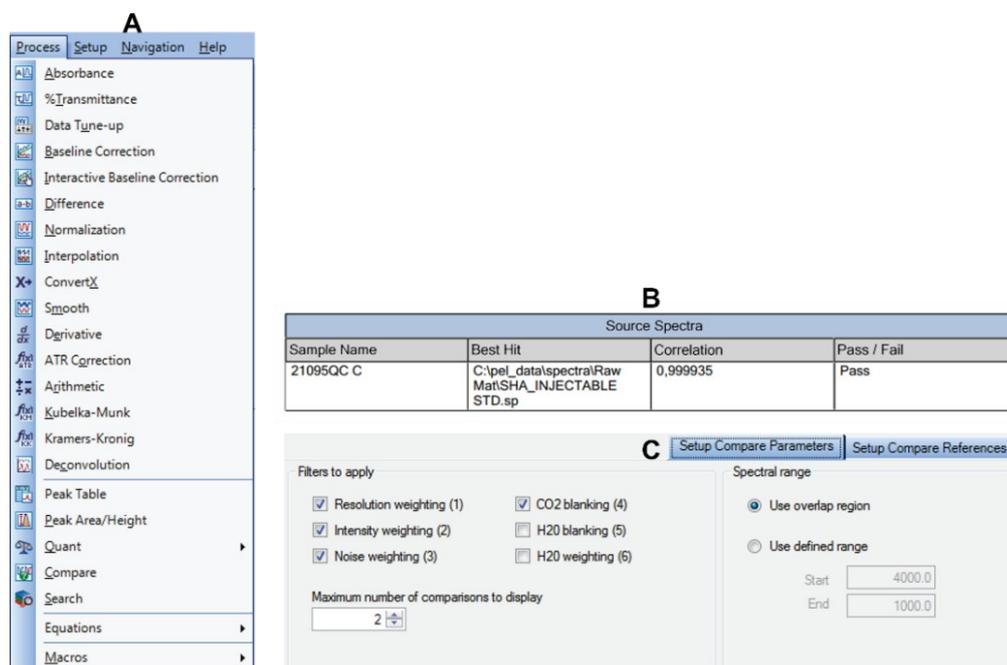


Figure 2.5 Spectrum IR software: Process menu (A) [40], Comparing Table (B), Setup Compare Parameters tab (C) [40].

### 2.2.3 Acquisition procedure

The adopted flowchart of spectra acquisition procedure is reported in Figure 2.6

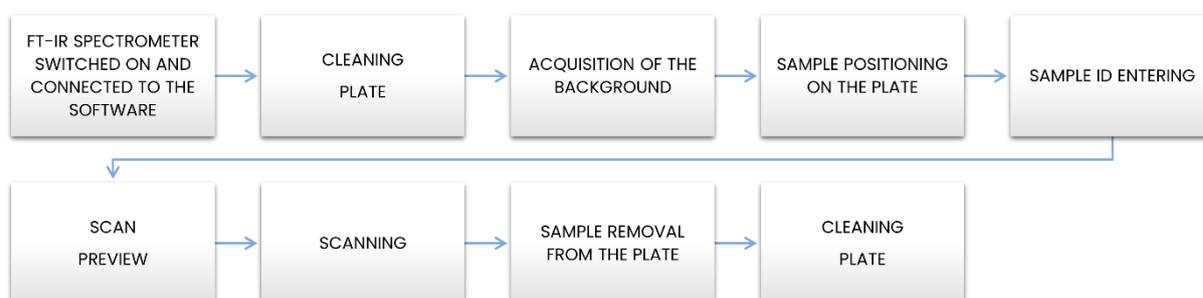


Figure 2.6 Acquisition procedure flowchart.

The FT-IR acquisition begins with the starting of the FT-IR Spectrometer. The cable is simply inserted into the socket and the instrument is switched on. To acquire a sample spectrum, the machine must be connected to the related Perkin Elmer Software. Connection established, the first important passage is the cleaning of the plate and acquisition of the background. On the monitor the signal received is nothing more than the energy of the lamp that must be always in the proper range; any problem or need to change the lamp will be signalled by the software. The next passage consists in positioning the sample on the plate in correspondence of the crystal as shown in Figure 2.7. A thin surface of the sample is required and it must be pressed over the plate through the pressing arm with a force in between 135 and 145 Gauge. This value must be always controlled through the software feedback during the Preview.



*Figure 2.7 Acquisition of SpherHA injectable paste.*

For Collygen and SpheraHA no special preparing procedure of the sample have been performed, indeed the solid samples have been easily put over the plate with the eventual aid of pins. For Sterify gel was needed a solidification process to acquire best spectra. Specifically, for each lot, Sterify gel was spotted 3 times over an aluminium sample disk and put into Memmert oven at 70°C for 24h (Figure 2.8).



Figure 2.8 Spotted Sterify gel (on the left). Aluminium plate in Memmert oven (on the right).

After positioning the sample onto the crystal, before pressing the “Scan Preview option”, the name of the sample is entered in a specific manner. First numerical part of the Sample ID regards the analysis code corresponding to a specific lot of the product. The second part of the ID is for all the analysed samples “QC” that stands for “Quality Control”, while the remaining part is an uppercase letter going from “A” to “C” for the representation of each of the 3 samples for just one lot. In Figure 2.5 B is reported an example of the sample name format. In case of unsatisfying spectrum, the reacquired sample is automatically renamed by the software adding a “\_00n” depending on the n<sup>th</sup> fulfilled acquisition. Successively, the identified samples undergo to a scanner preview and in case of satisfying spectra preview, the acquisition of each sample can be completed. Visualized the obtained spectrum, samples are removed from the plate which is accurately cleaned with an organic solvent. In the current work it has been used acetone solvent to execute the cleaning procedure.

The goal of the above described acquisition procedure has been the achievement of clean and reliable spectra, 9 for each product.

#### ***2.2.4 Analysis of FT-IR spectra***

The analysis of the obtained spectra has been completed mainly in the Spectrum Two software. Firstly, the acquisitions have been labelled with the option “Label Peaks” in the software to check the eventual occurrence of absorption peaks related to acetone presence on the sample plate in case of still wet surface. In the event of satisfying cleaning procedure, and so of not corrupted signal, all the obtained spectra for a particular product have been superimposed to see the general trend of the samples. Verified that all the spectra followed the same trend, and visually checked that ratios between peaks have been approximately maintained in every spectrum, peak wavenumbers have been the focus of the attention. Indeed, for a more accurate analysis on the presence of some characteristic peak associated to the vibration of specific functional groups, has been arbitrary chosen a sample for each product. For Collygen has been chosen sample A of lot 21087, for granular version of SpherHA the sample C of lot 21090, for SpherHA injectable paste the sample C of lot 21095, for Sterify gel the sample A of lot 210002. Considerations about chosen samples and related detected peaks have been guided by literature findings.

Furthermore, assessments on product degradation and observations on product stability have been significantly achieved through the “Comparing” option of the software giving the correlation value between the acquired sample spectra and those present in the database. The comparing process regarded all the acquisitions. Thus, the 9 acquisitions at disposal for each product at time 2 have been compared with the standard spectra present in the database (products at time 0). The correlations obtained for each comparing have been registered on a Microsoft Excel file. Here, the mean of all the 9 correlations for a product has been computed in order to find the mean percentage of similarity between the product at time 2 and the same at time 0 whose spectrum is saved in the software database.

## 2.3 HPLC: preliminary trials on Sterify gel samples preparation

In the preliminary evaluations regarding the most suitable technique to be adopted for Sterify gel stability studies, the additional option to FT-IR spectroscopy has been the HPLC. This technique requires a liquid sample to be introduced in vials for the chromatographic analysis.

The considered samples preparation for the HPLC analysis expects an extraction procedure to pick a quantity of supernatant from one lot able to fill at least one of the HPLC vials. Lots 210002, 210003 and 210004 underwent to the same sample preparation procedure described in Figure 2.9

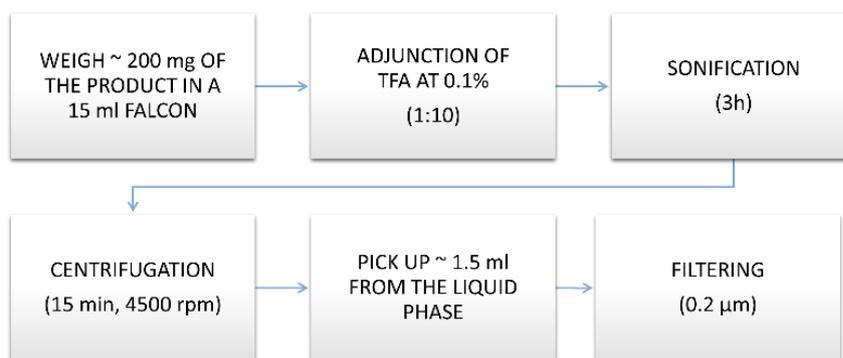


Figure 2.9 Sample preparation procedure for an HPLC vial.

In the first step, for each lot, have been weighted ~200 mg of the product in falcon of 15 ml. In particular, for the lot 210002 has been taken a quantity of 188 mg, for 210003 of 196 mg and for 210004 of 313 mg. Weights have been measured with the high-precision Balance Adventurer AX124 (Ohaus) shown in Figure 2.10 . Specifications are reported in Table 2.2.



Figure 2.10 High- precision Balance Adventurer AX124 (Ohaus) [41].

Table 2.2 High- precision Balance Adventurer AX124 specifications [41].

Specification	Value
Max. Capacity	120 g
Resolution	0.1 mg
Pan size	90 mm
Display	Touchscreen
Linearity	$\pm 0.2$ mg
Repeatability	0.1 mg
Stabilization time	3 s

The subsequent dilution with a solution of trifluoroacetic acid (TFA) at 0.1% has provided the adjunction in the 3 falcons of a certain quantity of the solution following the proportion 1:10. Therefore, with a 1000  $\mu$ l pipette, the samples have received 1.69 ml, 1.76 ml and 2.82 ml for lot 210002, 210003 and 210004 respectively.

Filled the three falcons, a sonification procedure to break up the gel and its bonds have been executed for 3 hours with the Ultrasonic Cleaner DK-80 (DK sonic) shown in Figure 2.11 whose specifications are found in Table 2.3. The three falcons have been kept inside the tank, under the ultrasonic cleaner water, with 2 additional weights to avoid the samples flotations.



Figure 2.11 Ultrasonic Cleaner DK-80 (DK sonic) [42].

Table 2.3 Ultrasonic Cleaner DK-80 specifications [42].

Specification	Value
Tank dimension	155 x 90 x 65 mm
Global dimension	176 x 115 x 125 mm
Capacity	0.7 ml
Frequency	42 KHz
Ultrasound power	35 W
Timing	90-18000 s

Successively, a centrifugation with Refrigerated Centrifuge FC5816R (Ohaus) shown in Figure 2.12 has been performed to separate liquid from solid phase. Centrifugation lasted 15 minutes at 4500 rpm with a temperature of 22°C .

Specification of the used instrumentation are reported in Table 2.4.



Figure 2.12 Refrigerated Centrifuge FC5816R (Ohaus)[43].

Table 2.4 Refrigerated Centrifuge FC5816R specifications [43].

Specification	Value
Dimension	538 x 354 x 723 mm
Weight	77 kg
Velocity range	200 - 16000 rpm
Max. relative centrifugal force	24.325 g
Max. capacity (rotor)	6 x 250 ml
Refrigeration interval	-20 - 40 °C

Finally, the purpose has been to pick up from the 3 falcons ~ 1.5 ml of the separated liquid phase and filtering this volume with a 2  $\mu$ m syringe filter (Figure 2.13) during the introduction into the vial. The filtering procedure has been intended for the finer removal of solute particles that could affect the quality of HPLC analysis and destroy the chromatographic column.



Figure 2.13 Syringe filter [44].

# 3. Results

## 3.1 Collygen

The spectra obtained for the three lots of Collygen are shown in Figures 3.1, 3.2 and 3.3. For each lot are displayed, in different colours, the three samples acquired in transmittance. The overall trend of Collygen spectra is appreciable in Figure 3.4. Through the “Comparing” option, the most similar product to the acquired samples found in the library was Collagen Standard Euroresearch with which a mean correlation of 0.99 has been detected (Table 3.1). In Figures 3.5, 3.6 and 3.7 are reported, respectively, arbitrary spectra for lots 21086, 21087 and 21088 with the superimposed Collagen Standard Euroresearch.

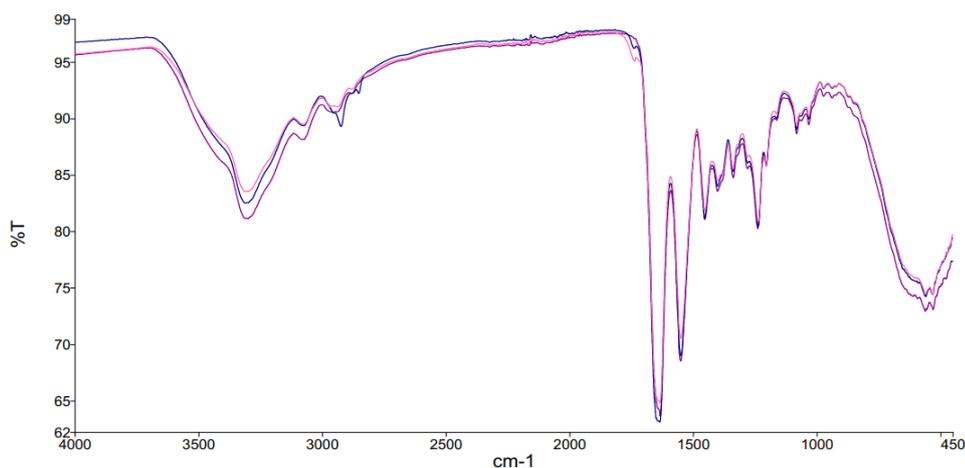


Figure 3.1 Collygen lot 21086: sample A (purple), sample B (blue), sample C (pink).

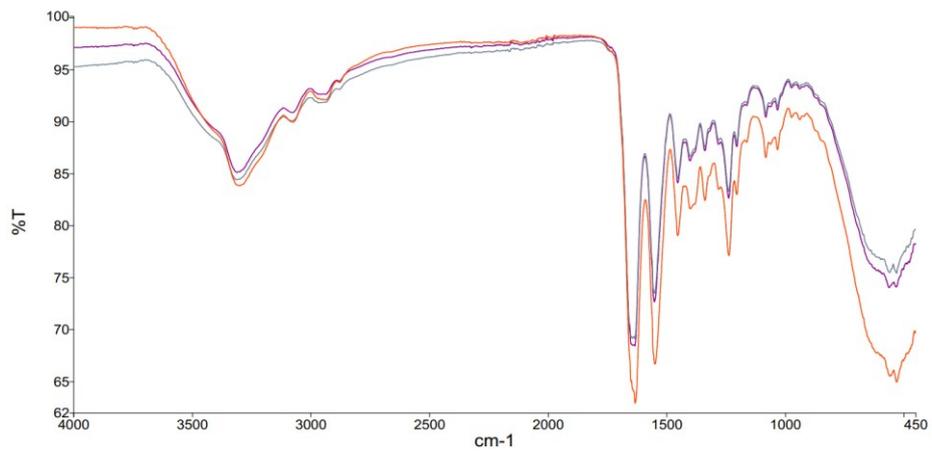


Figure 3.2 Collygen lot 21087: sample A (purple), sample B (grey), sample C (red).

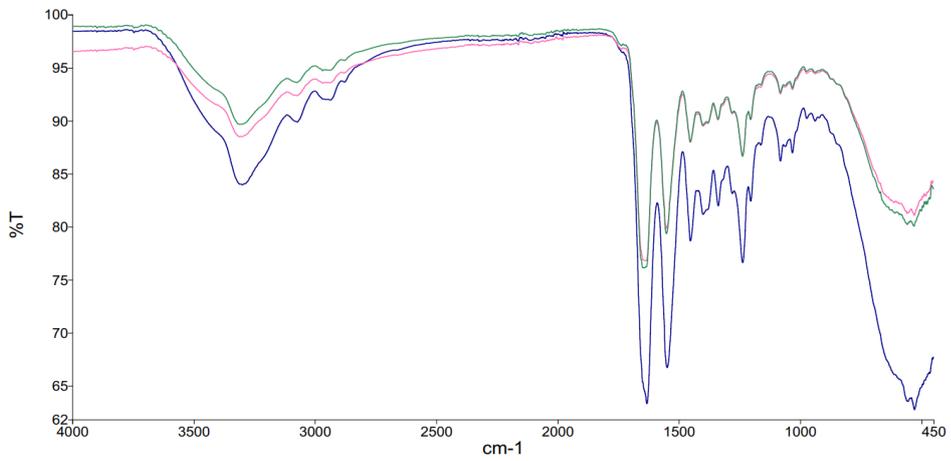


Figure 3.3 Collygen lot 21088: sample A (blue), sample B (pink), sample C (green).

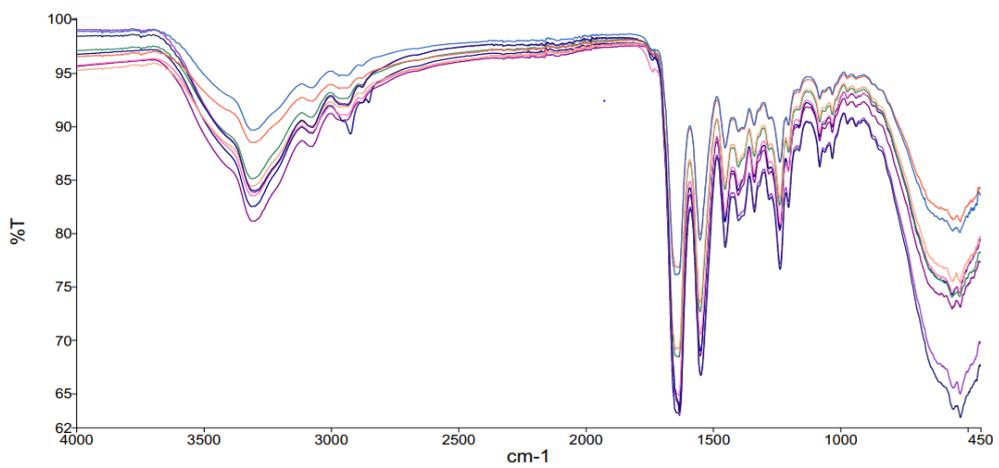


Figure 3.4 Collygen superimposed spectra.

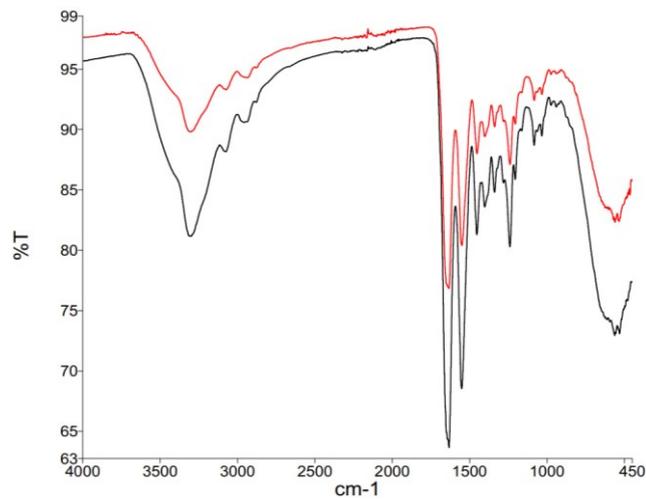


Figure 3.5 Collygen 21086 A (black) and Collagen Standard Euroresearch (red) spectra.

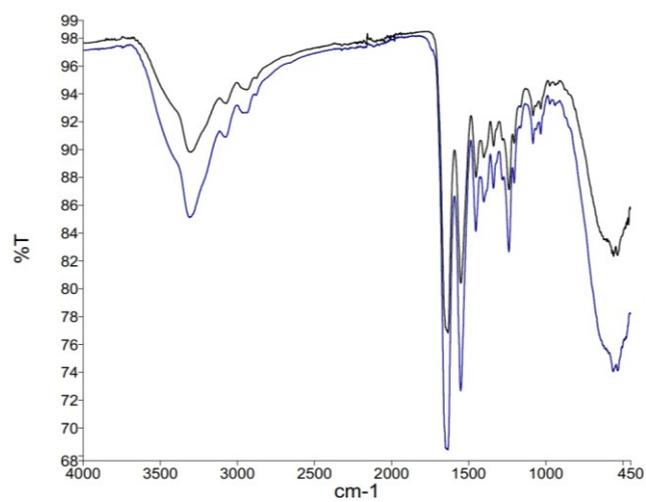


Figure 3.6 Collygen 21087 A (blue) and Collagen Standard Euroresearch (black) spectra.

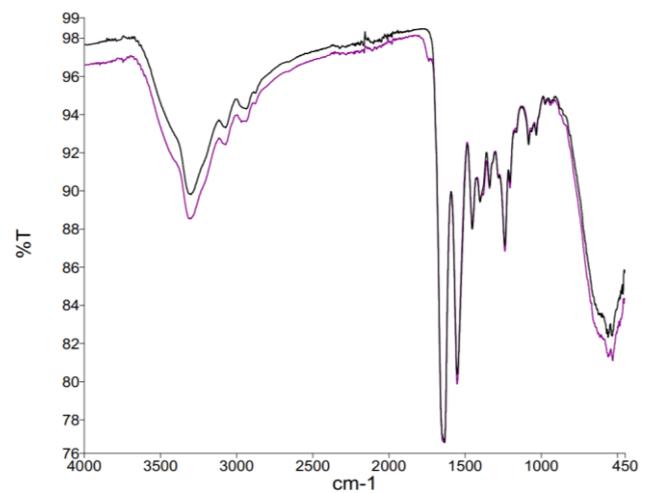


Figure 3.7 Collygen 21088 B (violet) and Collagen Standard Euroresearch (black) spectra.

Table 3.1 Collygen correlations with Collagen Standard Euroresearch.

Sample	Correlation
21086 A	0.9908
21086 B	0.9874
21086 C	0.9913
21087 A	0.9915
21087 B	0.9893
21087 C	0.9875
21088 A	0.9921
21088 B	0.9892
21088 C	0.9935
<b>Mean</b>	0.9903

## 3.2 SpherHA

### 3.2.1 SpherHA dense granules

The spectra obtained for the three lots of the granular version of SpherHA are reported in Figures 3.8, 3.9 and 3.10. For each lot are displayed, in different colours, the three samples acquired in transmittance. The overall trend in transmittance of granular SpherHA spectra is shown in Figure 3.11. As for regard the correlation found with the spectra in the software database, a mean correlation of 0,99 has been obtained between SpherHA dense granules samples and nanoXIMG1000 as reported in Table 3.2. Figures 3.12, 3.13, 3.14 show, respectively, the spectra of arbitrary samples for lots 21089, 21090, 21091 with the superimposed standard nanoXIMG1000 spectrum.

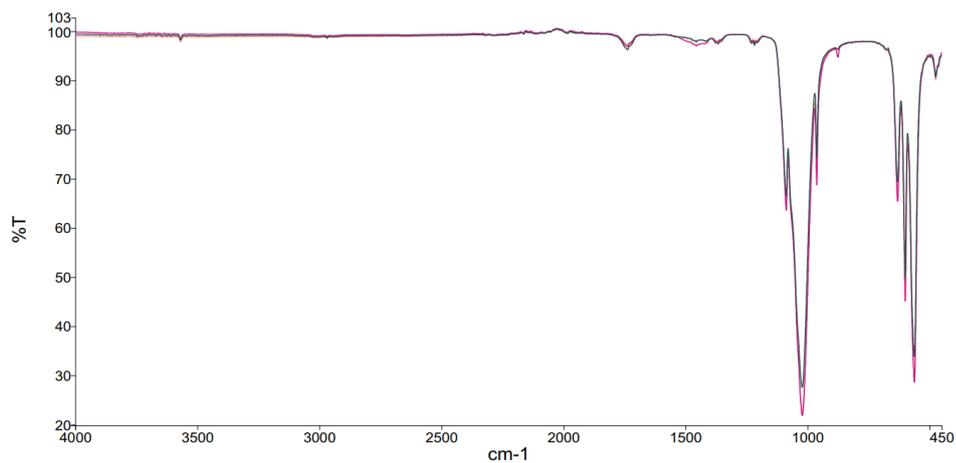


Figure 3.8 Granular SpherHA lot 21089: sample A (yellow), sample B (purple), sample C (black).

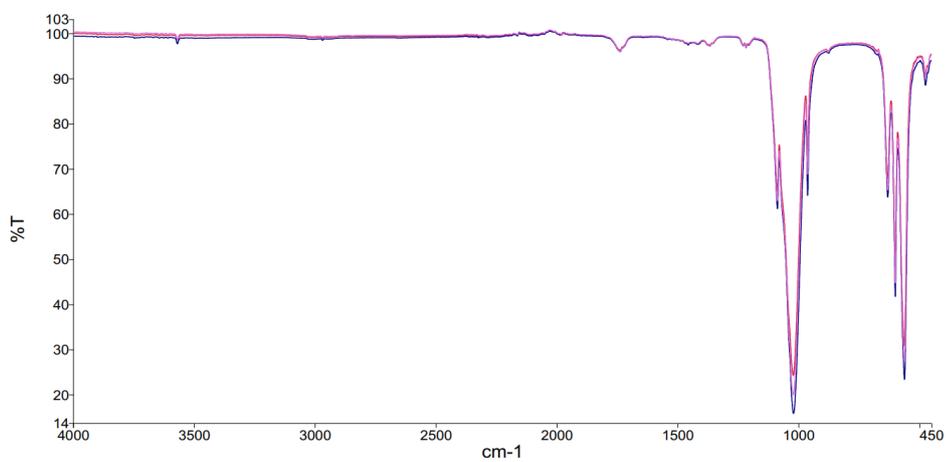


Figure 3.9 Granular SpherHA lot 21090: sample A (red), sample B (blue), sample C (pink).

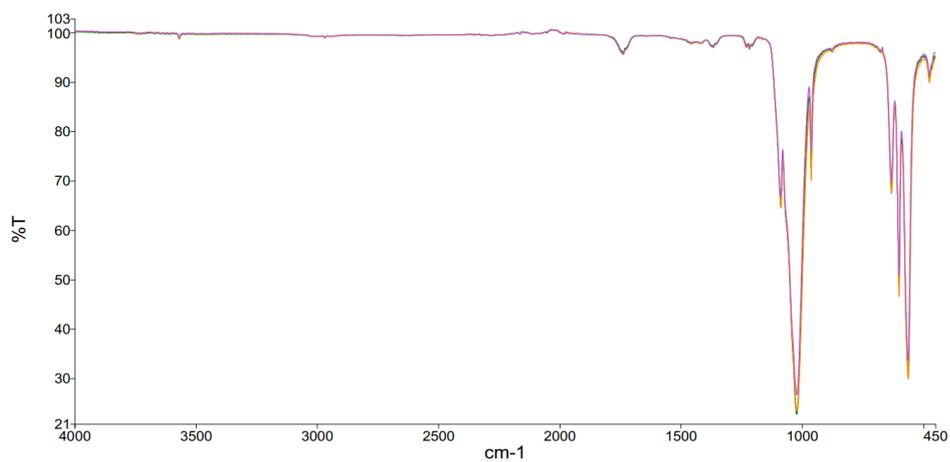


Figure 3.10 Granular SpherHA lot 21091: sample A (green), sample B (orange), sample C (purple).

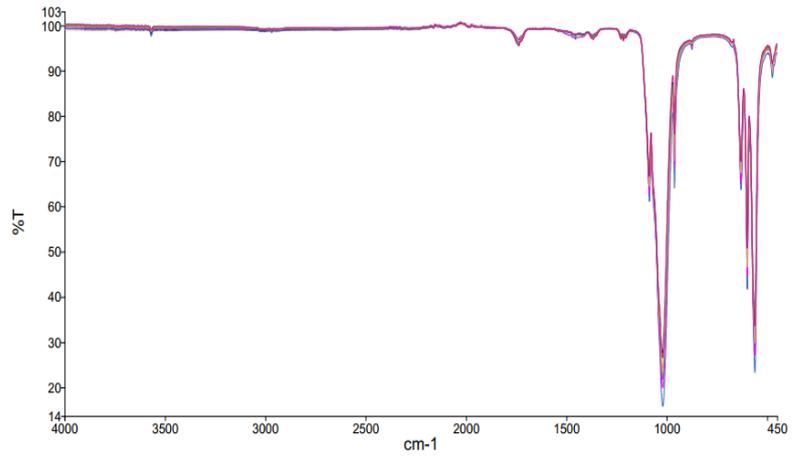


Figure 3.11 *Superimposed spectra of SpherHA granules.*

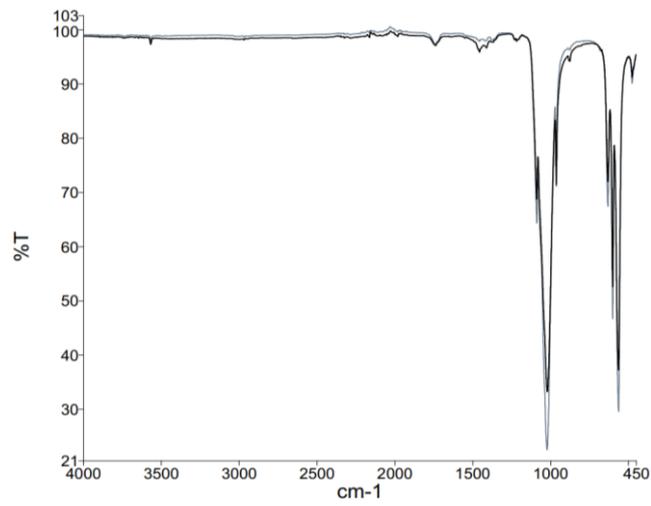


Figure 3.12 *SpherHA granules 21089 A (grey) and nanoXIMG1000 (black) spectra*

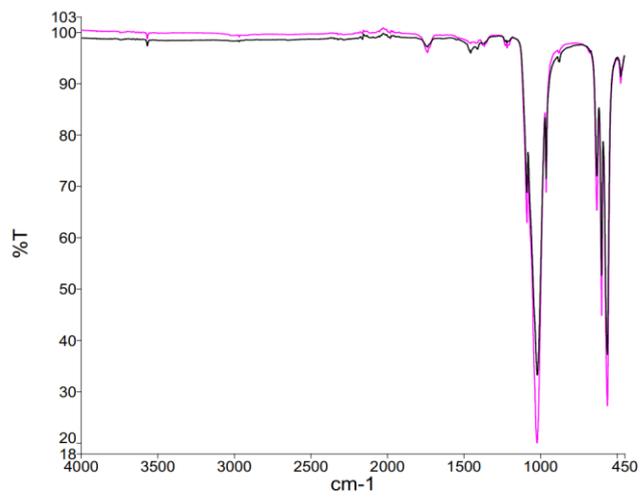


Figure 3.13 *SpherHA granules 21090 C (pink) and nanoXIMG1000 (black) spectra.*

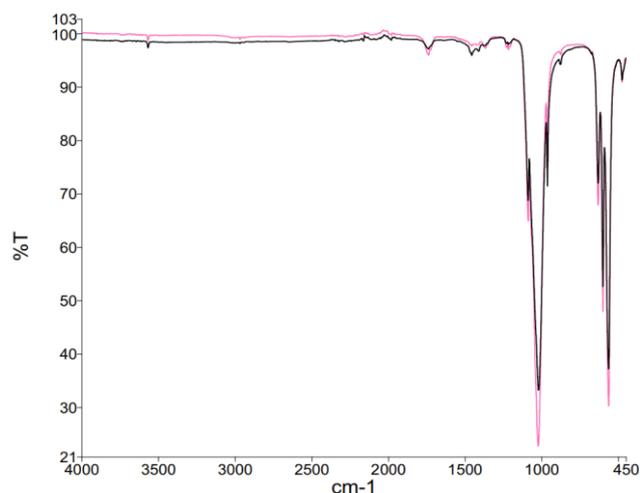


Figure 3.14 SpherHA granules 21091 A (pink) and nanoXIMG1000 (black) spectra

Table 3.2 SpherHA granules correlations with nanoXIMG1000

Sample	Correlation
21089 A	0.9888
21089 B	0.9873
21089 C	0.9867
21090 A	0.9886
21090 B	0.9852
21090 C	0.9862
21091 A	0.9854
21091 B	0.9869
21091 C	0.9892
<b>Mean</b>	<b>0.9871</b>

### 3.2.2 SpherHA injectable paste

As for regard the injectable paste version of SpherHA, the obtained spectra concerning the three lots are reported in Figures 3.15, 3.16 and 3.17. For each lot are shown, in different colours, the three samples acquired in transmittance. Figure 3.18 shows superimposition of all the obtained spectra for the injectable paste samples. As for regard the correlation found with products in the software library, a mean correlation of 0.99 has been obtained between injectable paste version of SpherHA and the standard SpherHA injectable paste (Table 3.3). The comparing between an

arbitrary sample of each lot with standard spectrum of SpherHA injectable paste is shown in Figures 3.19, 3.20, 3.21

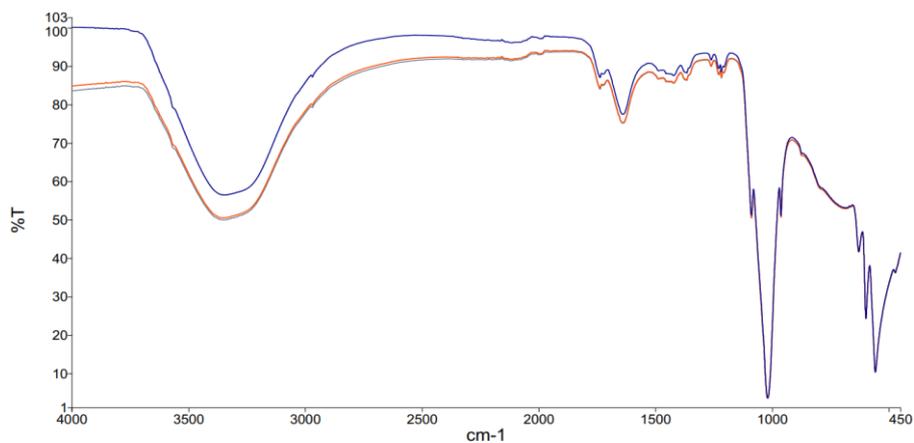


Figure 3.15 Injectable paste SpherHA lot 21095: sample A (gray), sample B (red), sample C (blue).

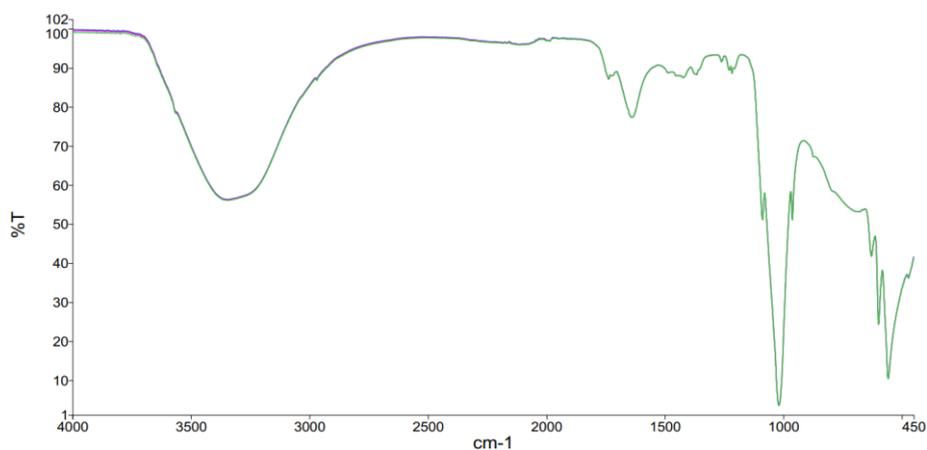


Figure 3.16 Injectable paste SpherHA lot 21096: sample A (blue), sample B (pink), sample C (green)

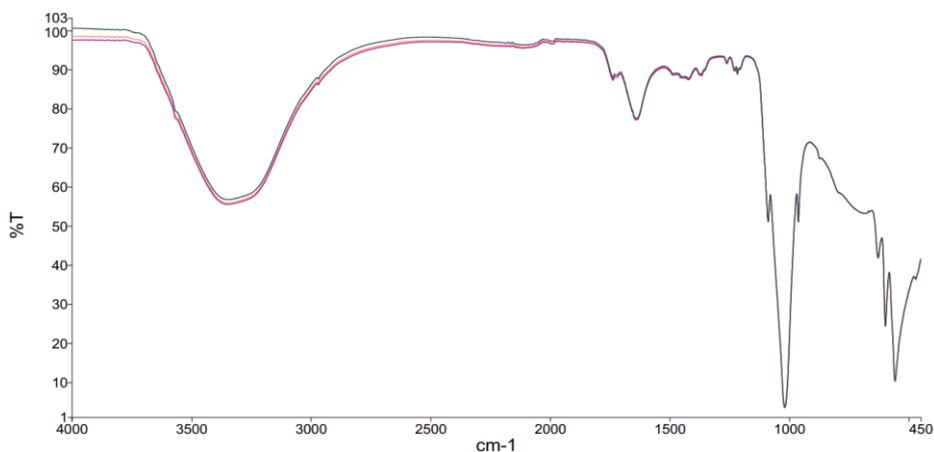


Figure 3.17 Injectable paste SpherHA lot 21097: sample A (orange), sample B (purple), sample C (grey).

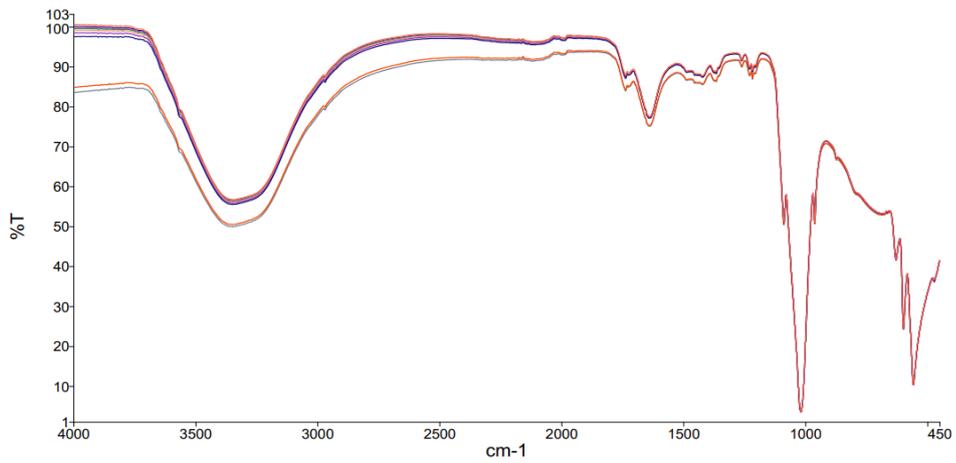


Figure 3.18 Superimposed spectra of SpherHA injectable paste.

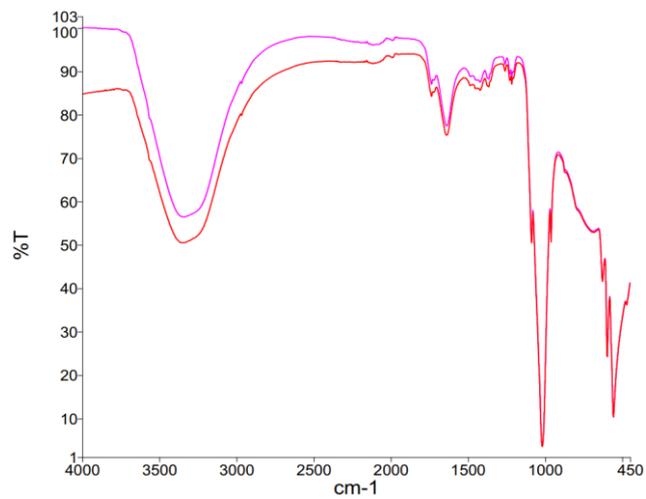


Figure 3.19 SpherHA injectable paste 21095 C (pink) and standard SpherHA injectable paste (red) spectra.

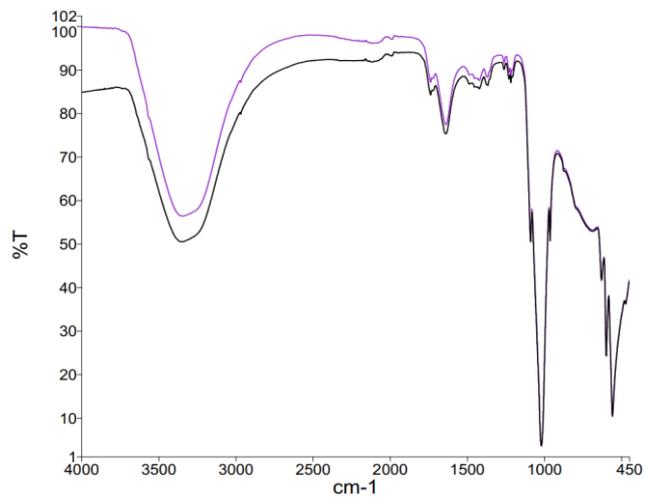


Figure 3.20 SpherHA injectable paste 21096 A (violet) and standard SpherHA injectable paste (black) spectra.

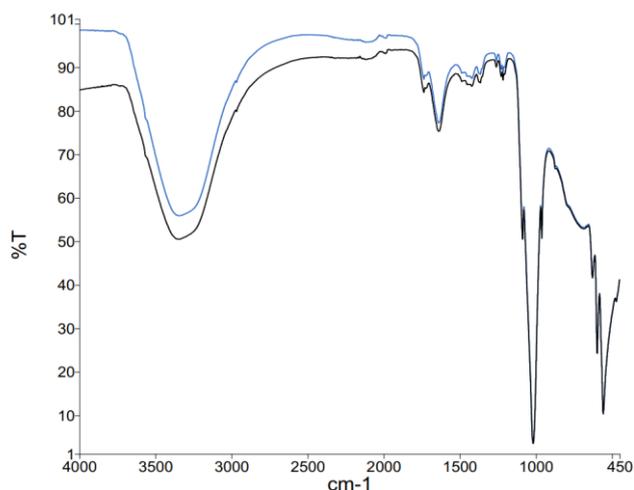


Figure 3.21 SpherHA injectable paste 21097 A (blue) and standard SpherHA injectable paste (black) spectra.

Table 3.3 SpherHA injectable paste correlations with standard SpherHA injectable paste.

Sample	Correlation
21095 A	0.9978
21095 B	0.9998
21095 C	0.9999
21096 A	0.9999
21096 B	0.9996
21096 C	0.9998
21097 A	0.9999
21097 B	0.9994
21097 C	0.9999
<b>Mean</b>	<b>0.9996</b>

### 3.3 Sterify gel

Spectra of the three lots of Sterify gel are reported in Figures 3.22, 3.23 and 3.24. Graphs are shown in transmittance, with three different colours, for the three samples of each analysed lot. The overall trend of Sterify gel spectra is shown in transmittance in Figure 3.25. As for regard the correlation found with products in the software library, a mean correlation of 0,98 has been obtained between acquired samples and

the standard Sterify gel (Table 3.4). The comparing between an arbitrary sample of each lot with standard spectrum of Sterify gel is shown in Figures 3.26, 3.27, 3.28.

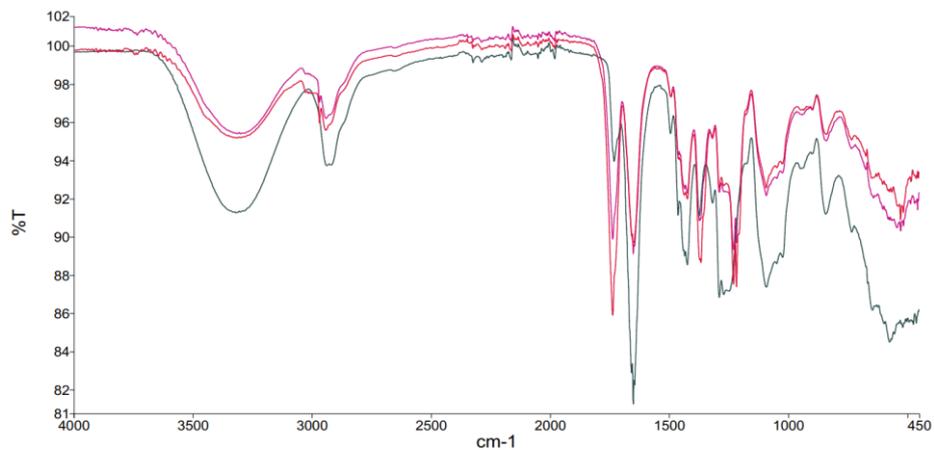


Figure 3.22 Sterify gel lot 210002: sample A (purple), sample B (black), sample C (red).

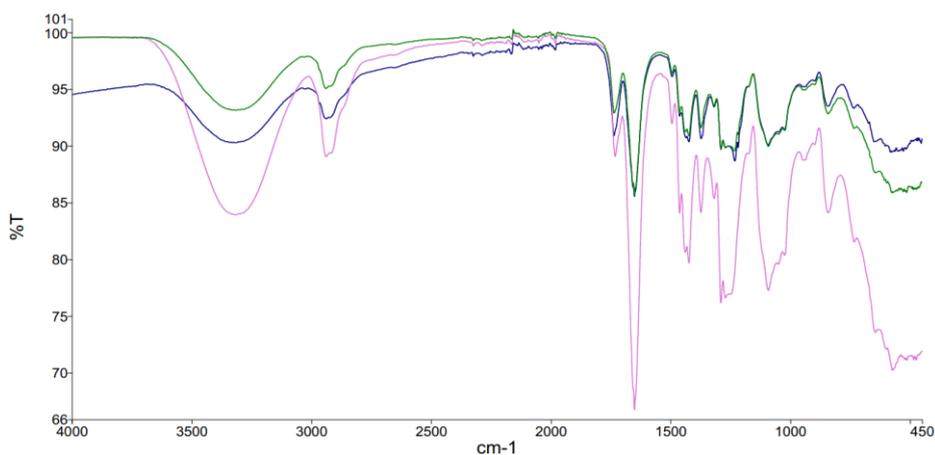


Figure 3.23 Sterify gel lot 210003: sample A (blue), sample B (pink), sample C (green).

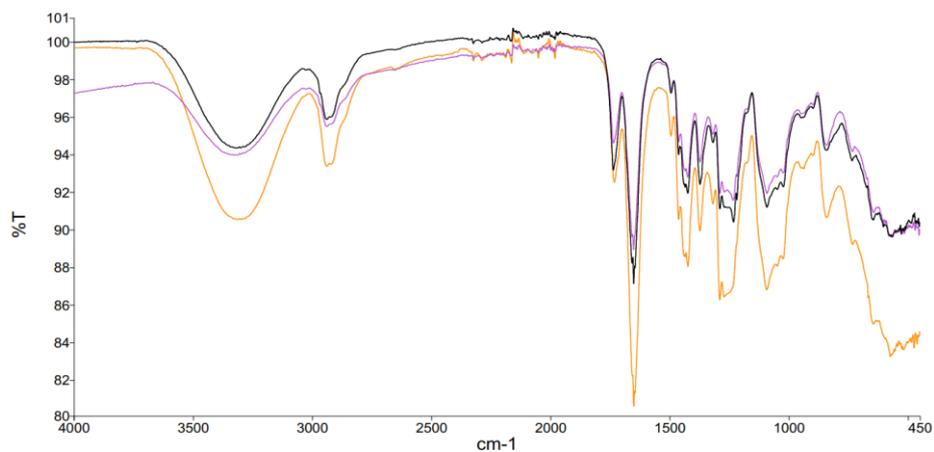


Figure 3.24 Sterify gel lot 210004: sample A (orange), sample B (pink), sample C (black).

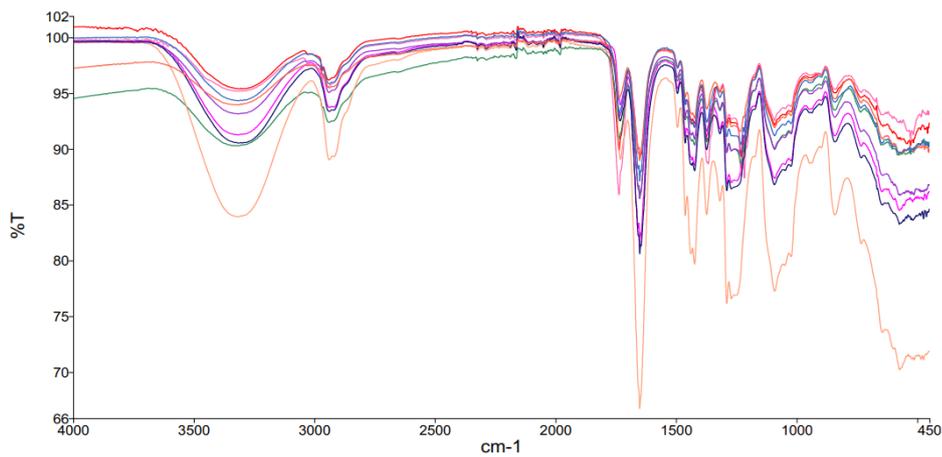


Figure 3.25 Sterify gel superimposed spectra.

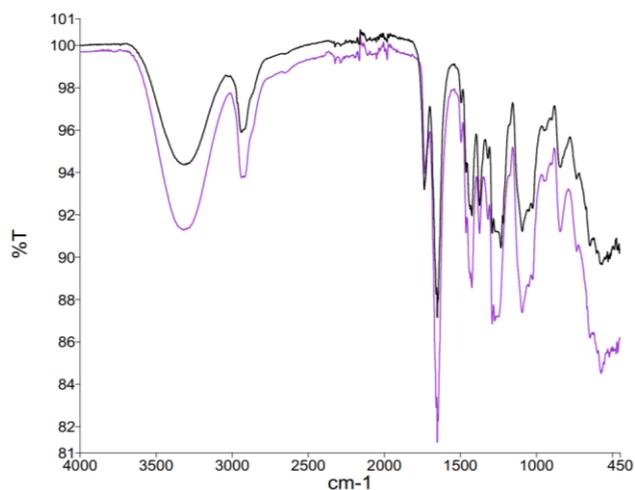


Figure 3.26 Sterify gel 210002 B (violet) and standard Sterify gel (black) spectra.

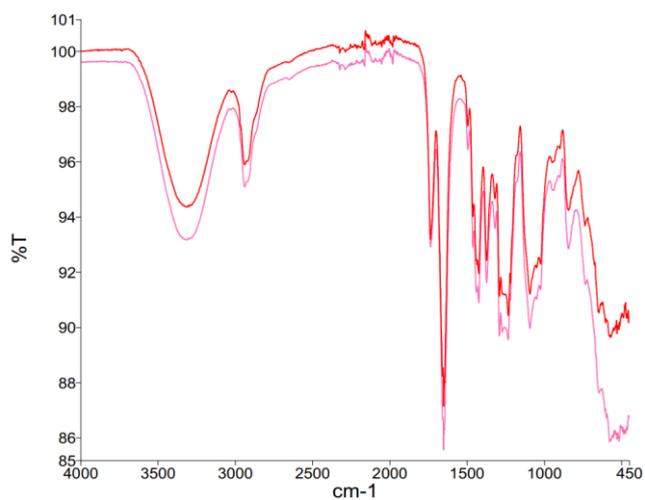


Figure 3.27 Sterify gel 210003 C (pink) and standard Sterify gel (red) spectra.

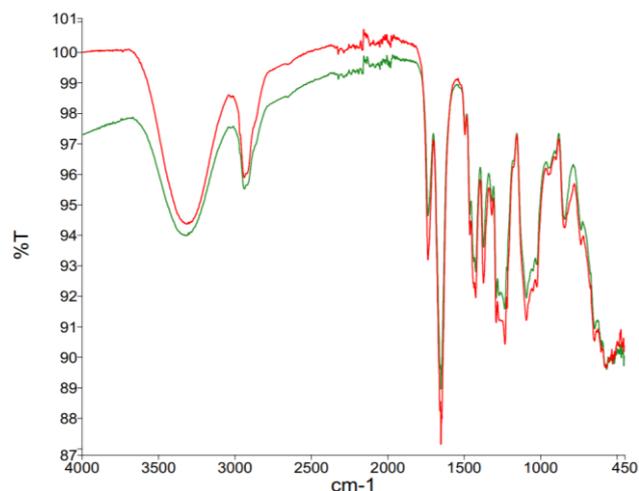


Figure 3.28 Sterify gel 210004 B (green) and standard Sterify gel (red) spectra.

Table 3.4 Sterify gel correlations with standard Sterify gel.

Samples	Correlation
210002 A	0.9674
210002 B	0.9523
210002 C	0.9751
210003 A	0.9849
210003 B	0.9889
210003 C	0.9876
210004 A	0.9861
210004 B	0.9899
210004 C	0.9884
<b>Mean</b>	<b>0.9801</b>

### 3.3.1 Preliminary results on Sterify gel samples preparation for HPLC analysis

After the filtration procedure, the quantity of liquid phase extracted from one lot has been too low even to fill a third of the vial whose capacity is  $\sim 1.5$  ml.

Most of the solution injected into the filter during the vial filling has been retained within it.

## 4. Discussion

The stability studies of a medical device represent one of the most important analyses within the medical device lifecycle. Stability studies results must be included in the technical documentation examined by notified bodies for the CE marking obtaining and application. The maintenance of all the chemical and physical properties must be guaranteed until the declared shelf life. Also, the storing conditions needed to preserve product stability must be confirmed in case of on-market product or established in case of under regulatory approval devices. Notified bodies ask for confirmations of product stability with whatever instrumentation and methodology is able to ensure maintained physical or chemical properties by the stored product through the comparison with the original product at time 0.

A widespread tool to assess stability of a product through chemical investigations is FT-IR spectroscopy able to identify compounds that are present in a material and give quantitative similarities with other products by means of identity indexes. Molecules are recognized in IR spectra because they own a particular and characteristic fingerprint [28]. Each molecule vibrates at a characteristic wavenumber when absorbing an IR beam, vibration that is visible in absorbance/ transmittance spectrum plots [27]. Furthermore, given two spectra, ratios between two subsequent peaks areas are equal in case the two acquired samples have the same substances concentrations [28]. Thus, peaks ratios are representative of molecular content. This principle is at the basis of several algorithms used by dedicated software to determine the quantitative similarity between two spectra [28].

These premises accompanied the real-time stability assessments on Collygen and SperHA and accelerated stability ones on Sterify gel still under regulatory approval.

The first product under FT-IR analysis, Collygen, is made up of equine collagen which is, in turn, composed by several amino acids. Two amino acids form an amide

group. The Amide group has different ways of vibrating that give rise to different peaks at specific wavenumber. Amide I is the most intense absorption due to the stretching vibrations of the C=O (70-85%) and C-N groups (10-20%). Its associated absorbance peak is found between 1600 and 1700  $\text{cm}^{-1}$ . Amide II related peak is found in the 1510 and 1580  $\text{cm}^{-1}$  region with an intermediate absorbance value. Its vibration derives from C-N stretch strongly coupled with N-H bending. Lastly, the less intense absorption value, indicated as Amide III, is detected in between 1200 and 1350  $\text{cm}^{-1}$ . Amide III vibrations see as protagonists N-H in plane bending coupled with C-N stretching [45]. As noticeable in Figure 4.1, taking as Collygen reference example the sample A of lot 21087, the amide groups peaks are all detectable. In particular, from the transmittance spectrum, the peak associated to Amide I is found at 1634.69  $\text{cm}^{-1}$ , Amide II at 1551.56  $\text{cm}^{-1}$ , Amide III at 1238.37  $\text{cm}^{-1}$ . This also regards all the other acquired Collygen samples as appreciable from Figure 3.4 displaying the superimposed spectra.

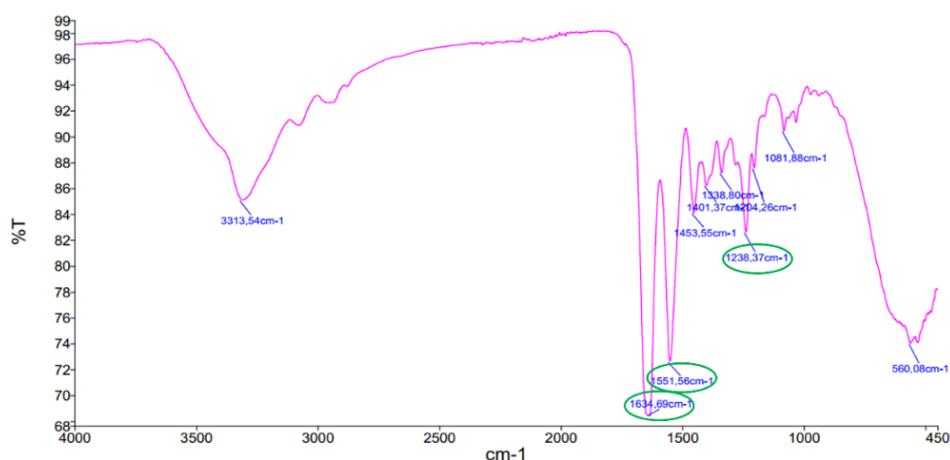


Figure 4.1 Collygen 21087 A with Amide groups wavenumbers in green circles:  
 Amide I (1634.69  $\text{cm}^{-1}$ ), Amide II (1551.56  $\text{cm}^{-1}$ ), Amide III (1238.37  $\text{cm}^{-1}$ ).

The presence of the peaks related to the vibrations of the Amide group can be an indicator of a product that has not been subjected to substantial degradation processes. However, the most important check on the product degradation relies on the comparison between Collygen and other products saved in the software database. The most similar product found in the library for all the acquired samples has been

Collagen Standard Euroresearch with which a mean correlation of 0.99 has been detected. Collagen standard Euroresearch is the product acquired by Tiss'you company as raw material to produce Collygen. The obtained high identity index is a quantitative-comparative result that takes into account the wavenumbers of the two spectra and the areas of the peaks. The higher the identity index, the higher the similarity found between the two products. This means that Collygen 2 years stored resulted to be in mean 99% equal to original raw material at time 0. Since a 100% similarity is practically impossible, for the product there was no consistent degradation after 2 years of storing. This expected result is in accordance with the declared shelf life of 5 years.

The second product analysed by FT-IR spectrometer is SpherHA hydroxyapatite in both dense granules and injectable paste versions. The hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) has a typical spectrum with two intense peaks given by the vibration of phosphate group ( $\text{PO}_4^{3-}$ ) at the band of 560 - 600  $\text{cm}^{-1}$  and at 1000 - 1100  $\text{cm}^{-1}$  [46].

The SpherHA dense granule analysis has given spectra with easily detectable phosphate peaks as highlighted in Figure 4.2 reporting an arbitrary spectrum of the product. Indeed, for the sample C of lot 21090 the two peaks are placed at 561.15  $\text{cm}^{-1}$  and at 1021.49  $\text{cm}^{-1}$ . These wavenumbers agree with the above cited ranges reported in literature. This also stands for all the other samples as appreciable from Figure 3.11. As for regard the correlation found with the spectra in the software database, a mean correlation of 0.99 has been identified with nanoXIMG1000. This is a commercial formulation of hydroxyapatite nanoparticle used as raw material to produce dense granule version of SpherHA. Therefore, the high identity index with the product at time 0 confirms the stability of ShperHA granules 2 years stored, as predicted by the observations achieved with the phosphate peaks detection.

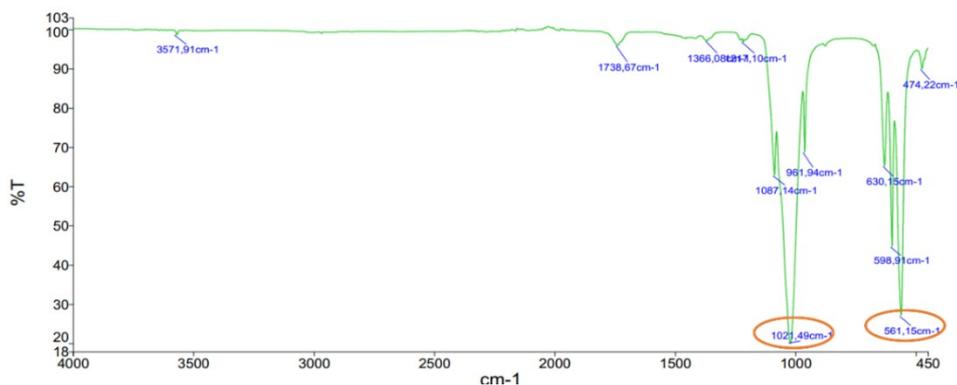


Figure 4.2 SpherHA granules 21090 C with phosphate peaks highlighted by red circles.

For the SpherHA injectable paste version, equal observations can be made. Hydroxyapatite phosphate peaks for the arbitrary chosen sample C of lot 21095 are in the range indicated by literature (Figure 4.3). The phosphate peaks can be detected at  $557.33\text{ cm}^{-1}$  and at  $1019.01\text{ cm}^{-1}$  and this also pertains to all other injectable paste samples as shown in Figure 3.18. As for regard the correlation found with product in the software library, a mean correlation of 0.99 has been obtained with the standard SpherHA injectable paste that is the spectrum of SpherHA injectable paste at time 0.

For the same observations and results obtained with SpherHA granules, also the injectable past version of SpherHA resulted to be stable after 2 years of storing.

This is in accordance with the declared SpherHA shelf life that is equal to 2 years.

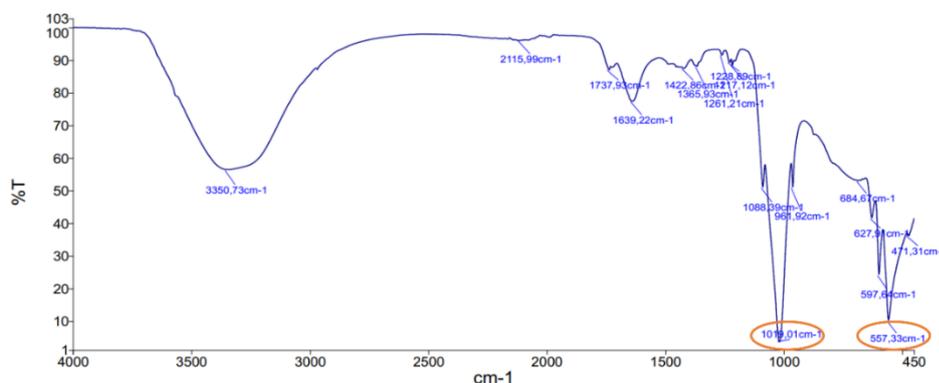


Figure 4.3 SpherHA injectable paste 21095 C with phosphate peaks highlighted by red circles.

As for regard the last device, the detection of characteristic peaks for the Sterify gel spectra has been more intricated since the product is constituted by more compounds. The attention has been posed on the two carriers playing a fundamental role in the antibacterial action: polyvinyl alcohol and polyvinylpyrrolidone. Since the spectra follow the same trend as noticeable from Figure 3.25, the study on absorbance peaks has been performed on Sterify sample A of lot 210002 (Figure 4.4). The molecular vibrations associated to the presence of polyvinyl alcohol are reported in Table 4.1 with the respective wavenumbers for both chosen spectrum and reference pure PVA retrieved from literature [47]. The same approach is maintained in Table 4.2 for polyvinylpyrrolidone [48].

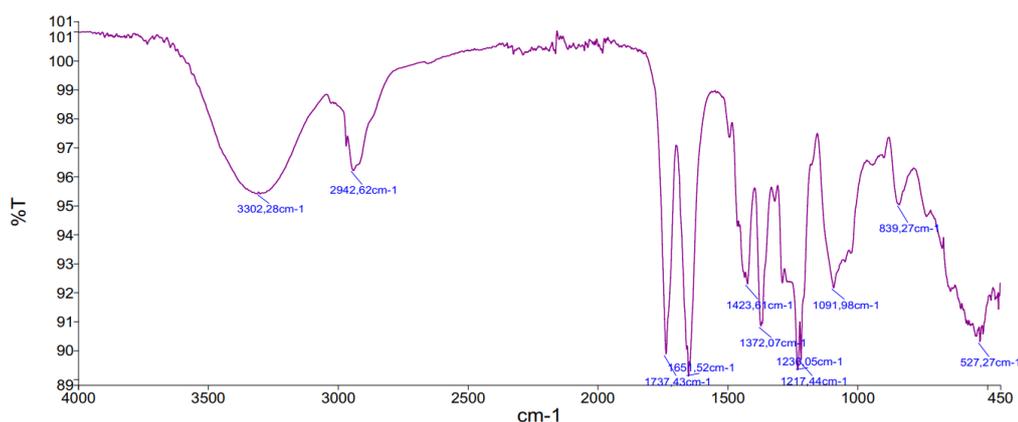


Figure 4.4 Sterify gel lot 210002 sample A in trasmittance.

Table 4.1 Comparison of absorbance peaks between literature pure PVA [47] and Sterify gel sample A of lot 210002 .

Pure polyvinyl alcohol	Sterify gel	Vibrational mode
2980 cm <sup>-1</sup>	2970 cm <sup>-1</sup>	CH <sub>2</sub> asymmetric stretching
1736 cm <sup>-1</sup>	1737 cm <sup>-1</sup>	C=O
1655 cm <sup>-1</sup>	1652 cm <sup>-1</sup>	C=C
1373 cm <sup>-1</sup>	1372 cm <sup>-1</sup>	CH <sub>2</sub> wagging
1330 cm <sup>-1</sup>	1320 cm <sup>-1</sup>	CH-OH bending
1241 cm <sup>-1</sup>	1230 cm <sup>-1</sup>	CH wagging
1096 cm <sup>-1</sup>	1092 cm <sup>-1</sup>	C=O stretching
850 cm <sup>-1</sup>	839 cm <sup>-1</sup>	C-C stretching

Table 4.2 Comparison of absorbance peaks between literature pure PVP [48] and Sterify gel sample A of lot 210002

Pure polyvinylpyrrolidone	Sterify gel	Vibrational mode
2950 cm <sup>-1</sup>	2943 cm <sup>-1</sup>	CH <sub>2</sub> asymmetric stretching
1646 cm <sup>-1</sup>	1646 cm <sup>-1</sup>	C=O stretching
1373 cm <sup>-1</sup>	1372 cm <sup>-1</sup>	C-H stretching
1286 cm <sup>-1</sup>	1289 cm <sup>-1</sup>	CH <sub>2</sub> wagging and C-N stretching
843 cm <sup>-1</sup>	839 cm <sup>-1</sup>	CH <sub>2</sub> stretching

For both carriers the wavenumbers values are not far from those found in literature, indeed the mean difference between reference and Sterify spectra wavenumbers is 6,1 for the PVA and 1,8 for PVP. These differences are very small if we consider that the PVA and PVP in Sterify gel are bound with other compounds. Also, the product during packaging procedure has been subjected to Gamma rays for the sterilization whose energetic power can be responsible for change in molecule vibrations wavenumbers [49].

Finally, the comparing between Sterify spectra at time 2 with the related spectrum at time 0 has given a mean identity index of 0.98. This, together with observations from the PVA and PVP peak detection, testifies the stability of the accelerated aged product.

In addition, a result that should not be ignored for Sterify gel is the efficacy of the method used to study its stability. The owned texture has given preliminary indecision regarding the instrumentation to be used for stability investigations. The spectrometer with UATR accessory has been the first candidate to accomplish the purpose. FT-IR spectroscopy with the necessary solidification procedure, even though requiring 24 h to be completed, has been the cleverest and fastest solution. Indeed, an alternative to FT-IR could have been the HPLC requiring a liquid sample to be inserted into the vial for the autosampler of the instrument. This represented an important limit for the fulfilment of the stability analysis with the HPLC technique. First experiments conducted at Tiss'you laboratories during the internship demonstrated that the liquid quantity that can be extracted from each lot, with a long and complex procedure, is too low to properly fill an HPLC vial. Surnatant liquid from different lots is needed to fill

a unique vial and this surely refuse the occurrence of a correct quality control analysis that is usually performed individually on different lots taking at least three samples. In case of successful extraction procedure, the aim of the stability study would have been the quantification of nisin Z and hydroxytyrosol present in Sterify gel formulation at time 2 through the chromatograms. Nevertheless, HPLC utilization for Sterify gel stability studies blocked still before the beginning leaving the place to the most convenient and satisfying FT-IR alternative. The precise concentration of compounds is not necessary and beyond the scope of the stability studies. Indeed, stability of a device is studied relatively to the same product at time 0. FT-IR spectroscopy, through spectra identity indexes, can quantitatively compare aged devices with those at time 0 with rapidity and efficacy.

To summarize, all the products proved to be not subjected to degradation processes through a quantitative-comparative analysis conducted by means of FT-IR spectroscopy in the software environment at disposal with the Spectrum Two instrumentation. High identity indexes have been found between products at time 2 with the relative one at time 0, indeed all the correlations have been higher than 0.95 which is the threshold to assess stability. Also, in the obtained spectra a literature-driven association of the peaks has highlighted, in all the devices, the presence of characteristic functional groups.

Ultimately, the exposed work certifies the stability of Collygen and SpherHA at time 2, result in accordance with the declared shelf life of the devices. In addition, the stability of the device at time 2 is certified also for the still under regulatory approval Sterify gel.

## 5. Conclusion

The objective of the proposed work regarded the stability studies on medical devices of class III produced at Tiss'you company in the optic of feeding the clinical data section of product validation in a PSUR or in a first technical documentation for the device approval.

For the analysed already approved devices, Collygen and SpherHA, real-time stability studies at time 2 have been conducted through FT-IR spectrometer Spectrum Two by Perkin Elmer endowed with the UATR accessory. No preliminary preparation of the samples has been required for Collygen and SpherHA, whose solid state made them immediately available for the acquisition with the UATR accessory. Each acquired sample spectrum at time 2 has been compared with the relative spectrum at time 0 in the spectrometer software, providing the detected correlation between the two. The mean correlation computed in Microsoft Excel has been 0.99 for both devices, consequently they proved to be 99% quantitatively similar to their products at time 0.

For the new Tiss'you proposal Sterify gel, accelerated aging protocols executed by an external certified company furnished 3-month accelerated aged products correspondent to a 2-years stored ones. The gelatinous texture of the device has given first indecisions regarding the most convenient instrumentation to be used for stability studies. The pathway of FT-IR spectroscopy with UATR accessory seemed to be right away the better one, although a solidification procedure has been necessary prior to the acquisition. First trials on supernatant extraction from the gel formulation have immediately revealed the impossibility to perform stability analyses through HPLC instrumentation given the poor liquid quantity that could be extracted from a lot. The applied method involving the FT-IR acquisitions has given good results for Sterify gel stability at time 2, indeed from the comparison with the product at time 0 the mean identity index has been equal to 0.98. All the obtained identity indexes have been above 0.95, threshold assigned to confirm the stability of the products.

The literature-driven peak associations have highlighted the presence of characteristic peaks of Amide I, Amide II and Amide III in Collygen spectra as expected from its amino acid formulation. In addition, in SpherHA spectra have been detected peaks associated to the presence of the phosphate group in accordance with the device calcium phosphate composition. Lastly, in Sterify gel spectra different absorbance peaks have been associated to the presence of carriers PVA and PVP included in the gel formulation.

To conclude, Collygen and SpherHA stability studies certify that the products are stable after 2 years of controlled storing. This is in accordance with the declared shelf lives: 5 years for Collygen and 3 for SpherHA. Stability is certified also for Sterify gel 2 years aged with accelerated aging protocols. Real-time stability studies will confirm or refuse the validity of the exposed accelerated aging study, as ruled by European regulations. The accelerated aging results give significant contribution in the technical report preparation which is mandatory for the device regulatory approval. Also, first steps have been walked for the device shelf life assessment. Future studies aim at continuing the stability studies at further times with the purpose to ultimately define Sterify gel shelf life and make the technical documentation available for submission to notified bodies.

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