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Training Placement Report presented to *Escola Superior de Biotecnologia* of the *Universidade Católica Portuguesa* to fulfil the requirements of Master of Science degree in Biomedical engineering

by

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Resumo

Considerando a evolução tecnológica e as limitações relacionadas com os materiais de pensos existentes, é essencial o desenvolvimento de novos dispositivos que ofereçam uma resposta efetiva e acessível no cuidado de feridas. Características como o controlo da humidade, a extração de exsudato, uma aplicação de longa duração e atividade antibacteriana são muito desejadas num curativo. Com isso, o objetivo deste projeto foi incorporar aditivos num têxtil, que pudesse ser posteriormente incluído num penso de alta absorção com multicamadas para o tratamento de feridas.

Aloe vera e óleo de girassol são aditivos naturais com atividades hidratantes, anti-inflamatórias e antimicrobianas. Estes foram os aditivos utilizados para este trabalho e, de forma a obter uma libertação controlada dos mesmos, estes foram encapsulados através do método de gelificação iónica externa. Relativamente à parede das cápsulas, foram testados diferentes materiais: shellac, eudragit, alginato e quitosana. Para uma libertação controlada e específica, foram então realizados estudos de libertação, dependente do pH, nas cápsulas, para avaliar quais as mais adequadas para o microambiente da ferida. Métodos de caracterização adicionais foram usados e as cápsulas foram incorporadas no têxtil, utilizando diferentes soluções de impregnação.

Com este trabalho, foram realizados diversos progressos de interesse. Foram produzidas, com sucesso, cápsulas homogéneas e esféricas, com diâmetros abaixo de 1000 µm, após a incorporação de ar comprimido no seu processo de formação. As cápsulas de alginato-quitosana, formadas através do método de camada por camada, demonstraram uma libertação dependente do pH, adequada para a cicatrização de feridas. Foi alcançada uma maior e mais rápida libertação a pH 7,5, seguido do pH 9 e por último pH 6, adequando-se, pois o pH de uma ferida encontra-se entre 6 e 7,5, aumentando para um intervalo de pH entre 7 e 9 quando infetada. Além disso, um maior número de camadas mostrou-se associado a uma libertação, no geral, mais lenta, presenteando um efeito mais duradouro. Estas cápsulas mostraram-se superiores às restantes desenvolvidas com shellac-alginato, alginato revestido com Eudragit e de apenas alginato. Relativamente à impregnação, que foi realizada através do despejo das dispersões no têxtil, todas as cápsulas confecionadas com ar comprimido encontravam-se fixas no têxtil. Com isso, glutaraldeído e ácido cítrico mostraram ser os agentes reticulantes mais adequados, em comparação com o glioxal, que aparentou destruir ligeiramente as cápsulas.

No futuro, teriam de ser usados outros métodos de caracterização para a deteção do óleo de girassol e *Aloe vera* nas cápsulas, como a cromatografia gasosa e líquida. São também necessárias melhorias na consistência da dispersão da impregnação para uma distribuição mais homogénea das cápsulas.

Palavras-chave: Encapsulação, pensos, aditivos naturais, impregnação têxtil

Abstract

Considering the technological evolution and the limitations related to the existing dressing materials, the development of new and advanced devices that offer a successful and cost-effective response in wound care is essential. Characteristics such as moisture control, exudate extraction, long-lasting application and antibacterial activity are very desired in a dressing. With this, the aim of this project was to incorporate additives in a textile structure that would enhance its properties, so that it could be posteriorly included in a multi-layered high-absorption bandage for wound treatment.

Aloe vera and sunflower oil are natural additives with moisturizing, anti-inflammatory and antimicrobial activities. They were selected for this work and, in order to achieve a controlled release of the active agents, were encapsulated through external ionic gelation. Regarding the wall of the capsules, different materials were tested: shellac, eudragit, alginate and chitosan. For a controlled and specific release, pH-dependent release studies were then performed on the capsules to evaluate which capsules were more suitable for the wound microenvironment. Additional characterization methods were used, and the capsules were incorporated in the textile, using different impregnation solutions.

With this work, several interesting developments were made. As results, homogeneous and spherical capsules, with diameters bellow 1000 µm, were successfully produced after the incorporation of compressed air in their formation procedure. The alginate-chitosan capsules formed through the layer-by-layer method have proven to provide a suitable pH-dependent release suitable for wound healing. A greater and faster release was achieved at pH 7.5, followed by pH 9 and lastly pH 6, which is appropriate since the pH of a wound is around 6-7.5, increasing to a pH range between 7 and 9 when infected. Additionally, a higher number of layers was able to deliver a slower release in general, offering a more long-lasting effect. In these aspects, these capsules were proven to be superior to other capsules: shellac-alginate, Eudragit-coated alginate and alginate only capsules. Regarding impregnation, through the pouring of the dispersions in the textile, all the capsules made with compressed air were fixated to the textile structure. With this, glutaraldehyde and citric acid appeared to be the most fitting crosslinkers, compared to glyoxal, which might destroy slightly the capsules.

In the future, further characterization methods would have to be used for the detection of sunflower oil and *Aloe vera* in the capsules, such as gas and liquid chromatography. Improvements in the impregnation dispersion consistency is also necessary for a more homogenized distribution of the capsules.

Keywords: Encapsulation, wound dressings, natural additives, textile impregnation

Contents

Figure list

Table list

List of abbreviations

ATR	Attenuated Total Reflectance
BTCA	1,2,3,4-butanetetracarboxylic acid
DSC	Differential scanning calorimetry
EOs	Essential oils
FTIR	Fourier transform infrared
G	α-L-guluronic acid
LBL	Layer-by-layer
M	β-D-mannuronic acid
PLA2	Phospholipase A2
PNIPA	Poly(N-isopropyl acrylamide)
PPADT	Poly-(1,4-phenyleneacetone dimethylene thioketal)
PSA	Particle size analyser
PVA	Polyvinyl alcohol
ROS	Reactive Oxygen Species
rpm	Rotations per minute
SDF-1α	Stromal cell-derived factor-1α
SEM	Scanning electron microscopy
SHP	Sodium hypophosphite
TEM	Transmission electron microscopy
TG	Thermogravimetric

1. Introduction

1.1. Wound environment and the healing process

The skin is a fundamental organ of protection against the environmental elements. It is composed by many layers: the epidermis that, as the outer layer, is the main source of protection against UV radiation and pathogens; the middle layer, the dermis, is the widest layer rich in hair follicles, nerve endings, glands, vessels, hair follicles and connective tissues. It provides a great release of collagen and elastin related to the abundancy of fibroblasts, responsible for these components, having a significant structural role. At the innermost level there is a layer named subcutaneous or hypodermis, that is associated with thermal isolation and shielding of other organs (Moeini *et al.*, 2020).

With this, wounds can be described as disruptions that can happen at any moment due to trauma, burns, surgery, genetic disorders and others, which affects the natural function and structure of the skin. In one way, wounds can primarily be categorized as close (for example hematomas and contusions) or open (such as laceration and puncture), depending on the exposure of the underlying tissues. Another relevant classification distinguishes acute and chronic wounds, and this is based on the persistence of the inflammation and on whether the healing process is taking its normal course. In acute wounds the recovery usually takes approximately 8 to 12 weeks and in chronic ones it can take various months to fully repair (Moeini *et al.*, 2020).

Infection is associated with the disbalance of the tissues' microflora that leads to the invasion of the wound by some opportunistic pathogens. This pathogenic attack could also occur due to external exposure through contact with others, the air, or even contaminated surfaces and it is followed by the expansion and colonization of these organisms, resulting in the formation of a biofilm. For this reason, it is key to eradicate these bacterial pathogens at the initial stage, since the development could lead to the inability of the patient's immune system to eliminate them, increasing the risk of infection and the possibility of the wound aggravating into chronic (Low *et al.*, 2017).

Bacteria are also known to consume nutrients and oxygen from the wound site, which, with critical bacterial contamination, present in chronic wound, leads to acute hypoxia that can be harmful since it interrupts neovascularization and consequent tissue death at the wound site (Farahani & Shafiee, 2021).

In the case of wound infections, the most commonly present bacteria in the early phase of infection are gram-positive bacteria, mainly *Staphylococcus aureus* and *Streptococcus pyogenes*. As the wound develops more gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa*, can be found (Moeini *et al.*, 2020).

The increase of the inflammatory responses, related to infections and the consequent release of components such as toxins and pro-inflammatory molecules, results in the delay of the normal healing process. The reparation course of action could also be influenced by factors such as wound size and the extension of the tissues' damages, age and some medical debilitations. This healing process is very dynamic and involves many elements, enabling the division of this cicatrisation action into mainly four stages, represented in figure 1.1. It starts with haemostasis, as the immediate response to the damage, in order to cess the bleeding. This stage is called exudative and is related with the development of clots and the release of inflammatory intermediaries. Next, there is the inflammatory stage, in which redness

starts to appear, pain and even swallowing and normally lasts between 24 hours to 6 days. This phase is characterized by the secretion of proteolytic enzymes and pro-inflammatory cytokines, to facilitate the growing of new blood vessels and eradicate the pathogens. The exudate of the wound is also crucial to keep the environment moisturized for proper healing. There is also a higher abundance of reactive oxygen species (ROS), such as hydrogen peroxide, superoxide and peroxynitrite, that develop from the inflammatory cells that are released into the wound cite. These work as mediators for many pathological events, but in greater amounts, as in chronic wounds or burns, can cause oxidative stress to the host. The third stage is the proliferative phase and is associated with the accumulation of granulation tissue and consequent formation of extracellular matrix, starting the wound's closing process. Allied to the last stage, the regenerative phase, is the resurface of the injury site with new epithelium, scar formation and tissue remodelling to restore functionality and strength (Low *et al.*, 2017; Moeini *et al.*, 2020).

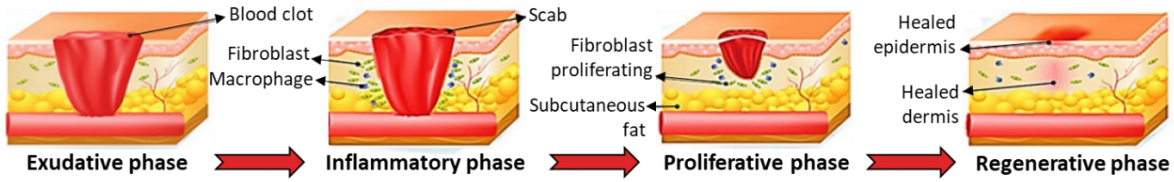


Figure 1.1- Wound healing process divided in 4 phases, from the moment the injury takes place to complete wound recovery. Adapted from Moeini *et al.* (2020).

During this process, another change that happens in the wound microenvironment is regarding the pH, that shifts from acidic to basic. The pH of a healthy skin is slightly acidic, varying between pH 4 and 6, which is important to prevent infections, regulates the establishment of microorganisms, and increase fibroblast production. However, a wound's pH is proven to become more alkaline, since the skins' acidic environment is compromised. The pH changes with the unveiling of the underlying tissues and is also related with its complex microenvironment associated with bacterial colonization, malfunction of the extracellular matrix and overabundance of proteases. Therefore, the pH increases with infection, meaning that the range in acute wounds is pH around 6-7.5 and in chronic wounds varies between pH 7 and pH 9 (Farahani & Shafiee, 2021; Jones *et al.*, 2015).

The changes in the wound microenvironment, derived from the biochemical and cellular activities that happen during the healing process described, and possible infection of the injury, may be used to achieve a controlled release from drug-delivery systems and are going to be explored further on. Meanwhile, these are some of the reasons that summarize the importance of the antimicrobial healing dressings to inhibit the formation of infections while it is still manageable (Farahani & Shafiee, 2021).

1.2. Wound dressings

The best route for a proper wound healing is to accurately chose the dressing that can provide specific treatment for that wound. Although there are different dressings for distinct purposes, there are

some qualities that are essential for all wound dressings so that they can promote healing and reduce infections.

Essentially, they should have properties such as the ability to prevent microbial infiltration and growth and remove excessive exudate, which is very important since certain wounds are described as extremely exuding and the composition of the exudate varies with the type of wound in question. Acute wounds' exudate has components that can stimulate the production of fibroblasts and endothelial cells, promoting healing. On the other hand, the elements in chronic wounds' exudate, such as large quantities of proteolytic enzymes and proinflammatory cytokines, restrain the production of keratinocytes, fibroblasts and endothelial cells, enabling the healing process (Farahani & Shafiee, 2021). It should be also able to keep a correct moister ratio at the wound site, since an overly moisturized environment can promote wound maceration, known as the softening and fragilization, increasing the risk of infection if it is for long periods of time. Dressings that are designed to retain and manage moisture should be in the right direction to encourage epithelialization. A dry environment decreases the hydration of the tissue within the wound bed, delaying the healing process and can increase scaring. Therefore, a balanced fluid level at the wound site is essential (Bishop *et al.*, 2003; Coulombe, 2003).

Regarding the material properties, it should be biocompatible, non-toxic, malleable, stable and have gas exchange permeability to promote healing with fewer chances of infection. Dressings should also be adhesive, to stay in place during patient movements, have appropriate mechanical strength, and be effortlessly removable, essential to not cause any more trauma to the patient, that being one of the main challenges of traditional dressings (Cutting, 2010).

There are many types of dressings in the market, and regarding the type of materials they can all be distributed into three categories, those being the traditional, biomaterial-based, and artificial dressings, as represented in figure 1.2 (Stoica *et al.*, 2020). Traditional dressings, such as gauze and gauze/cotton composites, are especially absorbent, for which they are mostly used for the first segment of the treatment, to clean, debride, and dry the wound site. However, the retained exudate can cause infections and, additionally, the removal of this type of dressings is often damaging to the newly formed tissue. Biomaterial-based dressings are basically skin substitutes, for example, allografts (which are tissue parts from donors), collagen derivatives (mostly used for ligament tissue engineering applications) and xenografts (a fragment of tissue from an individual of another species). They are very successful in restoring the functionality of the tissue, although they are highly priced, and vulnerable to inflammation and transmittable diseases (Aderibigbe & Buyana, 2018).

Artificial dressings usually have a lower cost and last longer and include several materials such as films or membranes, which are polymeric networks that shield the injury from outer factors and therefore allow their recuperations (Aderibigbe & Buyana, 2018). Moreover, it includes sprays and foams, made by a drug and polymer solution, used to protect and help heal the wound site. Gels, such as hydrogels, are also included, and can be defined as crosslinked hydrophilic polymers that are effective, can be effortlessly applied and present a high oxygen permeation, suitable for skin irritation. Lastly, composites are also artificial dressings made from the combination of different components, generally structured in layers, a resistant and flexible external layer that keeps a moist microenvironment for the wound and an adhesive internal layer, promoting the realisation of multiple functions (Moeini *et al.*, 2020).

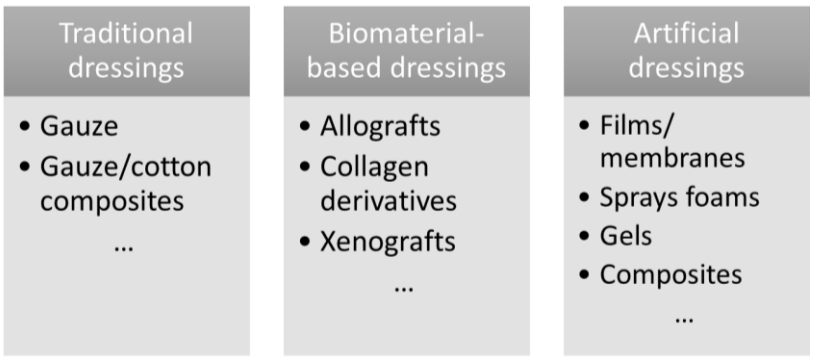


Figure 1.2- Three categories of dressings based on the type of materials and respective examples.

Bandages can also be categorized regarding their interaction with the tissue, dividing them in passive or bioactive dressings (Stoica *et al.*, 2020). Passive dressings mainly consist of the traditional ones, being non-occlusive and primarily used to cover the injury to re-establish its function. The bioactive dressings are prepared with biomaterials, such as alginate, chitosan, or gelatin, and can be semi-occlusive or occlusive, possibly containing additionally growth factors and antimicrobials, promoting a faster healing process (Aderibigbe & Buyana, 2018). The hypoxic circumstances that are generated with these types of bandages improve the angiogenesis, and, therefore, re-epithelialization, the development of granulation tissue and the production of collagen (Farahani and Shafiee, 2021).

Throughout the years, many advancements have been made regarding wound dressings and several researchers have explored the incorporation of antibacterial agents into the dressings' material. In addition to the intrinsic antibacterial activity of some polymers, such as chitosan and polyethyleneimine, antibacterial agents, such as gentamycin and benzylpenicillin, nanoparticles (most commonly made with metals), and essential oils have been successfully integrated in them (Bal-Öztürk *et al.*, 2021). More recently, diverse studies have also been made in the field of sensor-integrated dressings, that identify and transform data from their biomarkers into signals, monitoring the wound site and acting according to it (Farahani and Shafiee, 2021).

1.3. High absorption wound dressings in the market

Cutaneous wounds are often a challenge to treat and require a complex environment involving them to heal properly. In the recent years, a great variety of wound dressings have been developed, each providing multiple benefits and accelerating the healing process, which is essential especially regarding chronic wounds (Farahani & Shafiee, 2021). The numerous commercially available wound dressings have different physical forms such as hydrogels, sponges, nanofibers, foams, films and membranes (Aderibigbe & Buyana, 2018).

Additionally, a deeper understanding of the parameters associated with the healing process of a wound has incentivized the development of advanced dressings that aim to overcome the limitations of the existing ones. Multi-layered composite dressings, as a combination of different types of materials exposed in layers, have also appeared as a promising alternative. They are generally a combination of a non-adherent or semi-adherent layer in conjunction with a layer characterized by its absorption

capacity (Stoica *et al.*, 2020). With this, some of the most relevant commercially available wound dressings of different types are represented in table 1.1.

Table 1.1- Examples of commercially available wound dressings

Dressing category	Commercial name	Manufacturer	Composition	Characteristics	Reference
Hydrofibre	Aquacel® Ag	ConvaTec	Microfibers of Sodium carboxymethylcellulose with silver ions embedded	High absorption, protection against maceration, adaptable and easily removable	(*Convatec*, 2022)
Alginate	Kaltostat®	ConvaTec	Alginate fibres	High absorption, bleeding control	(*Convatec*, 2022)
Semi-permeable foam	Lyofoam® Max	Mölnlycke	Polyurethane	High absorption, optimal moisture level	(*Mölnlycke*, 2021)
Composite	Allevyn Life	Smith&Nephew	Silicone contact layer, a polyurethane foam, a cellulose polyacrylate interior and a polyurethane film	Good pressure distribution, easily removable	(*Smith&Nephew*, 2018)
Composite	PolyMem®	PolyMem®	Polyurethane foam with a starch copolymer, a cleaning agent (F-68 surfactant), a moisturizer (glycerol) and a semi-permeable film	Pain relief, inflammation reduction, cleansing of the wound site, exudate management and absorption	(*PolyMem® Dressings*, 2019)

Although there are numerous high absorption wound dressings in the market, only a few are able to conjugate all the desired properties for a bandage designed for chronic wounds, such as maintaining a moist environment, reduce bacterial infections, extract the excess exudate, but without increasing the material volume, and allow a long-lasting and easy application and removal (Stoica *et al.*, 2020).

Many wound dressings, such as Aquacel® Ag and Kaltostat® for example, require the use of secondary material for adhesion and protection which is not ideal (Minsart *et al.*, 2022). In addition, many of the dressings with antimicrobial activity use silver ions, which are currently associated with possible toxicity and the impairment of wound healing, therefore, the exploration of new materials for this purpose is relevant (Haidari *et al.*, 2020). Moreover, several of the multi-layered composite dressings commercially available, known for retaining all the essential properties for the treatment of chronic wounds, do not possess a very high absorption capacity, requiring a regular replacement.

Hence, there is a need to develop a novel layered dressing that meets all the necessary criteria while still being financially accessible.

1.4. Textile structures functionalization

1.4.1. Natural additives for textiles structures

There are many elements that can be added to the textiles in order to improve their properties so that the intended activity of the final product is achieved. A commonly type of additives used to confer antimicrobial activity are synthetic agents, such as quaternary ammonium composites, triclosan, silver and other metals. These agents are known for being versatile, efficient and durable, however, synthetic additives are usually associated with adverse effects on the users, such as increasing host tissue toxicity, and have a bigger environmental effect related with their production and disposal. Taking these aspects into consideration, natural additives, such as clove oil, *Aloe vera*, eucalyptus, chitosan, basil and several more, are being progressively more explored in textiles since they are considered a safer option for the human health and the ecosystem (Reshma *et al.*, 2018).

Regarding textiles and especially in the context of wound dressing fabrication that is going to be explored in this work, there are several properties that are desired and expected from the active agents that are produced and incorporated into the fabric. To achieve better results in the wound healing process and focusing more in controlling possible infections, properties such as anti-inflammatory, antibacterial, antifungal, antioxidant and healing are frequently analysed and looked for (Moeini *et al.*, 2020).

The desired healing activity is based on the interaction of these active agents with growth factors and their production processes. This action stimulates skin regeneration by promoting connective tissue accumulation, cell migration and generating immune responsive compounds, such as cytokinins, related to the anti-inflammatory process. The antioxidant activity has also major relevance due to the consequent reduction in the production of reactive oxygen species inside the cell, by interacting and neutralizing them, thus preventing the formation of free radicals that cause tissue damage (Moeini *et al.*, 2020).

Concerning the necessity of the antimicrobial activity in active agents, it is important to acknowledge that microorganisms develop easily in natural environments, varying their growth according to parameters such as temperature, nutrient availability and humidity. Thus, textiles are very susceptible to contamination, contributing to the adherence and growth of microorganisms, since they provide a large and a moist surface for their expansion (Morais *et al.*, 2016). Consequently, contamination could lead to undesirable odours, fibre deterioration, discoloration, decrease of functionality and could even compromise the user's health and hygiene, adding to the importance of antimicrobial agents in the industry. A fundamental aspect of antimicrobial agents is that they should be effective only on the pathogenic microorganisms in question, maintaining the properties and other components of the material intact. It should also be non-toxic and eco-friendly, which also brings more attention to the natural agents when comparing to synthetic ones (Reshma *et al.*, 2018). Antimicrobial properties can be added to textiles by physical modification of the structure or by the incorporation of agents through

inclusion of fibres in the polymeric matrix or even by the implementation of agents to the polymer's surface (Morais *et al.*, 2016).

Antimicrobial agents are also classified based on their mode of action towards the microbes, such as bacteria and fungi, producing either a biocidal impact, if they kill the microorganism, which is the most frequent in the textile industry, or a biostatic impact if they only prevent their expansion. There are several mechanisms that the antimicrobial agents could use in order to incite these effects, such as provoking damages to the cell wall or inhibiting its synthesis, since it is a vital structure of the organism responsible for maintaining the integrity and protecting the cell. Another approach from the antimicrobial agents could also be affecting the function of the semi-permeable cell membrane, impacting essential exchanges of compounds with the medium. They could also influence processes such as the synthesis of nucleic acids, proteins or other metabolic processes by damaging components that have important roles in these, harming the development, proliferation and survival of the microorganisms (Morais *et al.*, 2016).

In the textile industry there are many active compounds that are being used for their antimicrobial properties and a lot of the research in the subject is based in organic and inorganic components. One example of a highly used organic compound is triclosan, that corrupts the target's wall by an electrochemical mechanism. Other compounds use their connection to the microorganisms to affect their structure and functionality, such as quaternary ammonium compounds, amines and others. On the other hand, metallic compounds are very well known in this context, as effective inorganic complexes that block the enzyme's sites affecting the metabolic process (Murugesh Babu & Ravindra, 2015). There is a particular interest in silver, that has been proven to being effective against virus, bacteria and other microorganisms, which are very important aspects to consider when using metallic compounds, although it has been recently associated with toxicity regarding the host and the environment (Haidari *et al.*, 2020; Rivero *et al.*, 2015). Additionally, as emphasised before, natural additives have gained significant importance in research, possibly deviated from animals, like chitosan, minerals and commonly from extracts of plants such as *Aloe vera*, eucalyptus, and tee tree. This last category incorporates, for example, natural dyes, essential oils (which will be addressed more in detail further on), phenolics, terpenoids and others (Murugesh Babu & Ravindra, 2015).

1.4.1.1. Essential oils and plant extracts

Essential oils (EOs) have gained a lot of attention as active agents mostly because of their great and concentrated properties, from which the antibacterial activity of many EOs can be mentioned. Since Gram-positive and Gram-negative bacteria resistance to antibiotics has been increasing, many industries have been forced to explore alternative, among which, more natural options for antimicrobial additives, and also for other functions. These oils are known to have anti-inflammatory, antibacterial, antiviral, antifungal, antioxidant and many other properties, that are being explored for many purposes, including wound healing. For this end, many oils are being studied, for example, oregano, mentha, eucalyptus, peppermint, lemongrass, rosemary, lemon, clove, thyme, sunflower-seed and tea tree oils

as well as other plant extracts such as *Aloe vera*, manuka honey and many more (Low *et al.*, 2017; Moeini *et al.*, 2020).

Essential oils are mixtures of aromatic secondary metabolites of plants, which are produced as a defence mechanism in order to avoid occupation by pathogens. The extraction method of these compounds is usually distillation, which allows the collection of volatile liquids (Low *et al.*, 2017).

The properties of the essential oils are directly associated with the structure of the main types of compounds present in them. Plant-derived polyphenolic components, such as flavonoids and phenolic acids, are associated with a lot of activities, especially antibacterial. Groups such as phenols, usually very prevalent in these oils' composition, present groups like hydroxyl (hydrogen and oxygen atoms covalently bonded) that are very reactive and are related with the inhibition of microbial enzymes, associated with metabolic alterations, binding to the active sites of enzymes (Gyawali & Ibrahim, 2014).

The antimicrobial property is also attributed to the interactions that these compounds have with the cell membrane. Basically, the hydroxyl groups in phenolic compounds stimulate the electron's delocalization, propagating electric charge over more than one atom, reducing the gradient of the bacterial membrane, which affects the proton motion force and creates a decrease of ATP production. This process leads to the disruption of the bacteria membrane, consequent increase of its permeability and subsequent release of certain molecules, contributing to cell death (Gyawali & Ibrahim, 2014).

Thus, the antioxidant activity of these compounds is related to how easily the free hydroxyl groups react with free radicals, exchanging electrons. This results in the neutralization of free radicals and diminishes the creation of reactive oxygen species, decreasing the redox potential and therefore protecting the structure from microbes (Gyawali & Ibrahim, 2014).

In essential oils, it is very common to find in abundance simple phenols such as eugenol, carvacrol and thymol and their antimicrobial effect is also dependent of the position of the mentioned hydroxyl groups. This phenomenon is easily noticed by comparing, for example, the last two mentioned compounds, carvacrol and thymol, since the different location of this reactive group provides thymol a considerably higher impact against microorganisms. The number of double bonds in these types of compounds is also believed to have an affect regarding antimicrobial properties, associating a greater amount of double bonds to a superior efficiency against microbes (Gyawali & Ibrahim, 2014).

1.4.1.1.1. *Aloe vera*

Aloe vera is a plant that grows in tropical climates and is often used in applications, especially in the cosmetic industry and regarding medicinal wound healing treatments (Moeini *et al.*, 2020). It is known for having healing, moisturizing, anti-inflammatory and antimicrobial properties. *Aloe vera* is an interesting plant that possesses a mucilage-like gel in its foliage, which contains a great variety of compounds, including carbohydrates, minerals, and vitamins (Badke *et al.*, 2019). It has been proven that part of the skin soothing characteristics of this plant is related to the presence of magnesium lactate, a mineral that inhibits the manufacture of histamine, an organic compound related with immune responses, often causing skin irritation. Additionally, it stimulates the production of cytokines, increases the amount of collagen, and the glucomannan present in its composition, as a carbohydrate that influences the fibroblast growth factor, resulting in the enhancement of wound healing (Hekmatpou *et*

al., 2019). Another carbohydrate present in *Aloe vera* that promotes antibacterial and antitumor effects is acemannan, and enzymes such as bradykinase which promotes anti-inflammatory responses (Murugesh Babu & Ravindra, 2015). These are only a few of the several beneficial mechanisms related to the potential of *Aloe vera* in wound treatment applications.

1.4.1.1.2. Sunflower oil

Sunflower oil is extracted from the seeds of the sunflower plant (*Helianthus annuus*), which has been used as a therapeutic plant for a very long time. As a vegetable oil, it is mainly composed by triacylglycerols, also possessing linoleic acid and oleic acid, along with other less abundant components, such as stearic and palmitic acids (Grompone, 2011; L. Oliveira *et al.*, 2012). The concentration of the two primary acids, oleic and linoleic acids, can vary from between sunflower oils, resulting in different types of oils (Grompone, 2011).

Linoleic acid, as a prominent unsaturated fatty acid, functions as a precursor to arachidonic acid, which has a role in the biosynthesis of prostaglandins, thromboxane, and leukotrienes. These components promote inflammatory responses, stimulating cellular migration, blood vessels formation, fibroblasts proliferation, consequently resulting in the deposition of collagen and extracellular matrix synthesis, very useful in wound healing treatments (L. Oliveira *et al.*, 2012).

1.4.2. Microencapsulation of additives

Multiparticulates, such as microspheres, microcapsules, microgranules and more, are drug-containing particles that are widely used as drug-delivery systems in the textile, cosmetic and food industries, as well as in the medical field. These functional structures can be designed to have specific and enhanced properties, possibly improving the characteristics of the material intended for their incorporation. Many active agents with interesting attributes, such as essential oils and plant extracts are often encapsulated to preserve their natural and functional traits, remaining a stable and viable form for their application (Stan *et al.*, 2019).

The innumerous advantages regarding microencapsulation makes them a remarkably interesting and versatile technique, applicable in multiple areas. Besides the already mentioned protection of the core substances, it allows to carry toxic substances, a more consistent and reliable dosage of the substance, reducing variability regarding the release kinetics, a targeted and specific release, and many more. On the other hand, microencapsulation is a technique that is usually associated with a narrower quality control and with high-cost excipients and equipment (Lengyel *et al.*, 2019).

Microencapsulation is a method in which active agents, in a solid, liquid, or gaseous state, are constrained within a coating material, or simply incorporated in the matrix. These capsules can be structurally divided into a core, corresponding to the internal substance and a wall/shell part, which surrounds the active agent (Bakry *et al.*, 2016). Regarding the size range, microcapsules typically have a diameter between 1 and 1000 μm. In terms of shape, microcapsules are usually spherical but can also present an irregular shape (Cheng *et al.*, 2009). They can also be characterized by having a single or a

multicore and, concerning the shell, they can also present a single or multiwall (figure 1.3) (Bakry *et al.*, 2016).

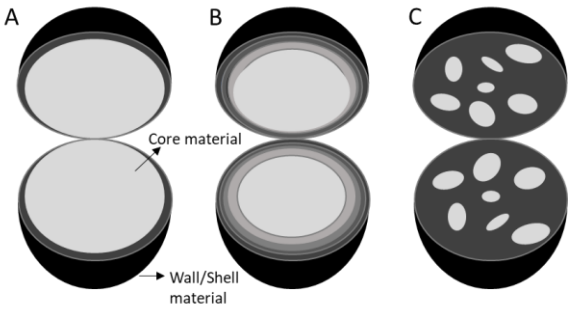


Figure 1.3- Capsule structural categorization: A) single core capsules; B) multiwall capsule; C) multicore capsule. Adapted from Lengyel *et al.* (2019).

The application of microcapsules can also be performed in any textile, such as silk, cellulose-based or synthetic fibers. The wall material and its reticulation method, as well as the process used for the encapsulation, all influence characteristics such as the entrapment efficiency, stability, functionality and even morphology of the produced capsules (Bakry *et al.*, 2016). The physical nature of the core substance is also known to have an influence on the shape of the capsule (Cheng *et al.*, 2009).

The release of the active agent can occur in many circumstances, including though dissolution or diffusion and in some cases, the erosion of the polymer, as illustrated in figure 1.4. Osmotically motivated release is also one of the causes, since the water that enters the capsule results in the swelling of the polymer structure and, consequently, the development of new pores and the pressure created allows the active agent to be easily released (Lengyel *et al.*, 2019).

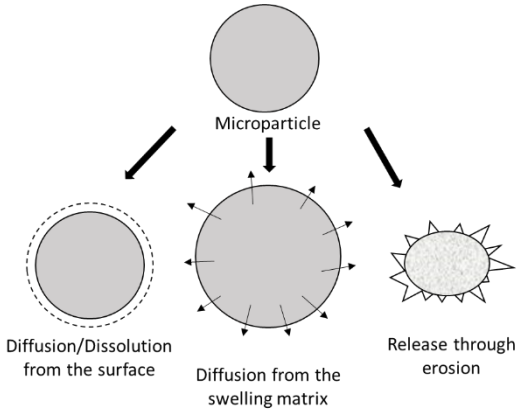


Figure 1.4- Microcapsules release mechanisms. Adapted from Agnihotri *et al.* (2004).

The swelling, the change of the polymer' state from glassy to rubbery and the final dissolution of the core substance, creates a characteristic slow initial release, followed by a fast one, which is typically seen in hydrogels (Agnihotri *et al.*, 2004). Furthermore, internal, external, physical, chemical and microbiological stimuli are often used to promote the additives' release. These stimuli can occur simultaneously and some examples of them are friction, pressure, temperature and pH changes (Cheng *et al.*, 2009; Lengyel *et al.*, 2019).

1.4.2.1. Microencapsulation techniques

There are various techniques that can be used for the production of microcapsules, and they can be divided in physical, chemical and physical-chemical processes, as demonstrated in figure 1.5.

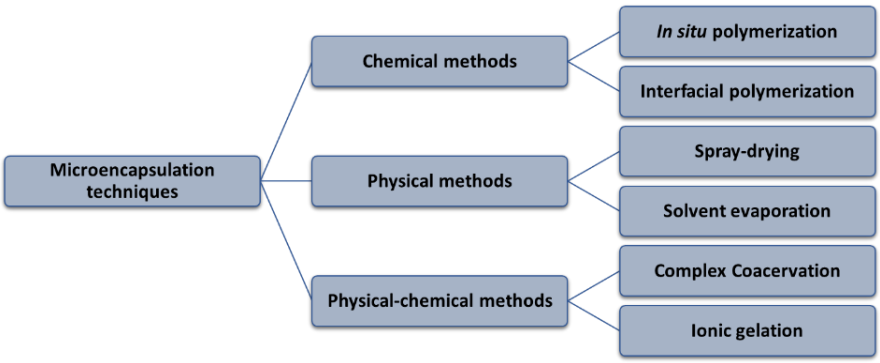


Figure 1.5- Scheme of the types of microencapsulation methods and some examples of their techniques.

The chemical methods involve the polymerization and condensation of monomers, oligomers or prepolymers, as the initial materials, and through these chemical reactions the capsules are produced (Peng *et al.*, 2020). With this, the most commonly used chemical microencapsulation techniques are *in situ* polymerization and interfacial polymerization (Cheng *et al.*, 2009; Peng *et al.*, 2020). Interfacial polymerization is a process that involves the addition of two reactive monomers, in the oil phase and in the aqueous phase. This method is based on the interaction between an oil-water emulsion and hydrophilic and lipophilic monomers, which, through the polymerization of these two immiscible phases, creates a membrane at the surface of the droplet (Ozkan *et al.*, 2019; Peng *et al.*, 2020). *In situ* polymerization has a similar procedure except there is no addition of reactants and the polymerization takes place in the continuous phase (Bakry *et al.*, 2016).

Regarding physical processes, they only involve methods such as drying, adhesion and dehydration. The main techniques used in this category are spray-drying and solvent evaporation (Ozkan *et al.*, 2019; Peng *et al.*, 2020). Spray-drying is based on the atomization of the emulsions and posterior fast drying at a high temperature, evaporating the water and creating the capsules with the core material (Bakry *et al.*, 2016). Solvent evaporation is a technique that involves the dissolution of the polymer and active agent in an organic solvent, creating the dispersed phase. Then, this phase is added dropwise into an aqueous solution containing an appropriate stabilizer, corresponding to the continuous phase. Due to an employed low pressure or high temperature, the solvent evaporates and capsules are formed (Dubey *et al.*, 2009; Ozkan *et al.*, 2019).

Physical-chemical methods, as implied, combine the two types of categories previously described, and two of the most used ones are coacervation and ionic gelation. The coacervation method can be separated as single, if it only requires one type of wall material, or complex, when it uses two materials with opposite charges for the shell (Peng *et al.*, 2020). In complex coacervation, pH is an extremely significant parameter since the process involves the isoelectric point of the polymers. This method starts with the emulsification of oil with an aqueous solution containing the two polymers and a posterior change in pH, temperature or even the addition of salt, until a narrow range in which the second polymer

acquires an opposite charge to the other polymer. The interaction between them forms a deposition around the active agent that, with crosslinking, desolvation or thermal treatment, hardens and forms the capsules (Bakry *et al.*, 2016; Cheng *et al.*, 2009). Ionic gelation is based on the electrostatic interactions between two opposite charge species, using multivalent ions, in most cases Ca^{2+}, which results in the crosslinking of the polyelectrolytes, forming capsules (Ozkan *et al.*, 2019). This technique is going to be further described in the next segment, as it was the chosen method for the practical part of this project.

1.4.2.1.1. Ionic gelation

Ionic gelation is a procedure commonly used for the formation of capsules. It revolves around the electrostatic interactions between two ionic species and is mainly accomplished using a syringe needle. Encapsulation through the gelation method can be performed externally or internally. In external gelation, as illustrated in figure 1.6, a polymeric solution with the active agent is added, dropwise, into the aqueous solution containing polyvalent cations, such as Ca^{2+}, Ba^{2+} or Al^{3+}. As implied, in this technique these ions diffuse from an external source (Ozkan *et al.*, 2019; Pedroso-Santana & Fleitas-Salazar, 2020). A widely used compound to prepare the gelling bath is calcium chloride. In internal gelation, the ionic solution is added to the one containing the polymer and active agent. The capsules start to instantly form when the drop reaches the solution, creating spherical gel constructs (Pedroso-Santana and Fleitas-Salazar, 2020).

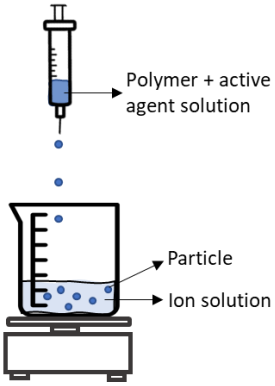


Figure 1.6- Schematic representation of the external ionic gelation method for the encapsulation of active agents. Adapted from Agnihotri *et al.* (2004).

With this, there are various aspects throughout these techniques that have an influence on the characteristics of the created capsules, such as morphology, strength, and porosity. Parameters like the diameter of the needle, the flow rate, the concentration and compound used for the ionic solution, and the surface tension of the polymer solution are known to alter the shape, size and physical properties of the gel particles (Lengyel *et al.*, 2019). Numerous researches are conducted to study the way that these factors are influenced, such as the ones conducted by Moghaddam *et al.*, (2015). It was settled that a reduced distance between the needle and the solution can result in smaller and more spherical shaped capsules. Additionally, an increased flow rate is related to a bigger capsule size and a lower surface charge density (Moghaddam *et al.*, 2015). Moreover, the interfacial tension of the particle can be altered by modifying the pH of the gelling solution, which has been proven to have a consequential influence

on the shape of the capsule. For example, Chuang *et al.* (2017) reported that, regarding alginate particles, a lower pH gelation solution originated a lower interfacial tension. These capsules also showed a more significant change in shapes when associated with a lower alginate and Ca^{2+} concentration, showing a less spherical shape. Consequently, the shape of the particles also influences the release rate. In this case, an oblate capsule revealed a higher release rate in comparison to more spherical ones, which is related to a greater surface area (Chuang *et al.*, 2017).

Furthermore, ionic gelation is generally known for its advantages. It's a low-cost technique, with simple equipment, an uncomplicated process and its protocol is bio-safe, using water-based solutions and not requiring high temperatures or organic solvents. It is also time efficient, the conditions of production are easily adjustable and, when under optimal conditions, is related to a high encapsulation efficiency. On the other hand, there are some limitations to this method, the main one being related to a wider particle size distribution, compared to other procedures, correspondent to a more heterogeneous gelation (Pedroso-Santana and Fleitas-Salazar, 2020).

1.4.2.2. Polymers used for encapsulation

The wall material of a particle has a significant role in the release of the encapsulated active agent, influencing its release properties. Properties such as the stabilization of the final product and the interaction with the core substance have a high importance in the selection of the shell material. With this, a great variety of polymers are frequently used for encapsulation (Singh *et al.*, 2010).

1.4.2.2.1. Alginate

Alginate is a natural polyanionic polymer collected from brown algae, generally *Laminaria hyperborean*, *Macrocystis pyrifera* and *Ascophyllum nodosum*. For this, an alkaline solution is used in order to free the alginic acid, that is afterwards transformed into a salt, typically sodium alginate, although it can also be, for example, potassium or ammonium salts. Even though it is not common or available in the market, it can also be obtained from bacteria such as *Azotobacter vinelandii* and *Pseudomonas aeruginosa*, and distinct sources frequently are associated with different properties, such as higher molecular weight, unique structural organization, and the presence of O-acetyl groups (Puscaselu *et al.*, 2020; Tønnesen & Karlsen, 2002).

It is known for its non-toxicity, biodegradability, biocompatibility, great swelling properties, water solubility and low commercial price. These properties make this polymer very appealing for various applications in fields such as the food industry, as a coating material, biomedical, pharmaceutical, agriculture and many more, functioning as a drug-delivery system, thickener or even gelling agent (Puscaselu *et al.*, 2020).

Between all these advantages and interesting characteristics of alginate, it also has some limitations including its low stability, which can make storing difficult, poor tear resistance, incompatibility with heavy metals, precipitation at low pHs and depolymerization when exposed to heat treatments, possibly resulting in a loss of viscosity. These disadvantages opened a new exploration between researchers on the correlation of alginate with other biopolymers in order to improve its properties and allow a broader

use in the described industries. It is very often associated with natural polymers, especially chitosan, whose connection with alginate will be described in this paper, but also pectin, guar gum and protein-based polymers or even with synthetic ones such as polyvinyl alcohol (PVA) (Puscaselu *et al.*, 2020).

Regarding the chemical structure of alginate, it is composed mainly by two monomeric units (figure 1.7), the β-D-mannuronic (M) and the α-L-guluronic (G) acid residues, linked by (1–4) glycosidic bonds (Ching *et al.*, 2017).

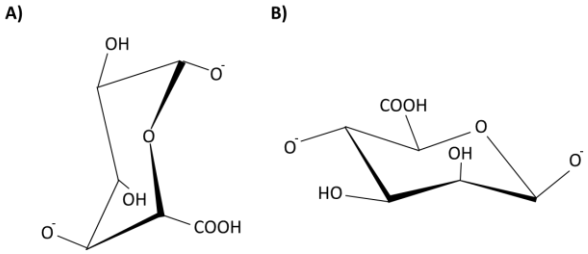


Figure 1.7- Chemical structure of the alginate monomeric units: A) L-guluronic acid (G) and B) D-mannuronic acid (M). Adapted from Ching *et al.* (2017).

These residues can be found separately in blocks or structured as alternating sequences blocks (MG blocks) (figure 1.8). One of the aspects that distinguish the various seaweed extracted alginates is the ratio between these M and G residues and their sequences, since they are responsible for different properties and molecular weights (Ching *et al.*, 2017).

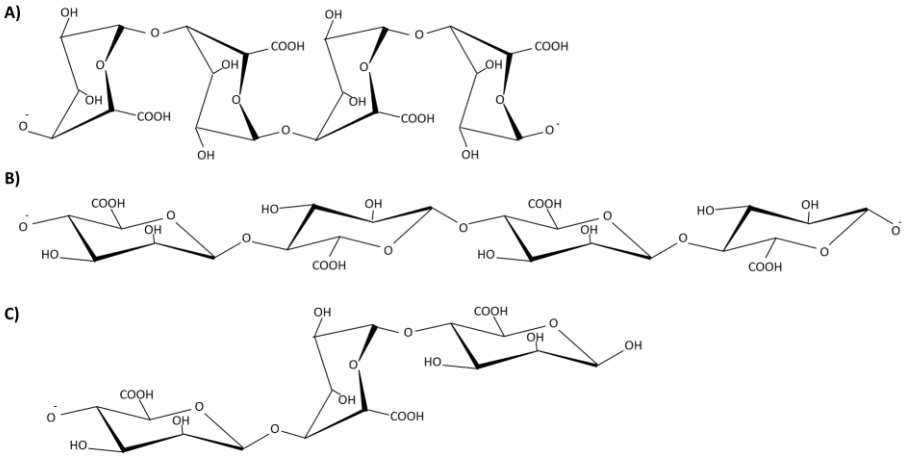


Figure 1.8- Chemical structure of blocks found in alginate, formed by the monomeric units: A) G blocks; B) M blocks and C) MG blocks. Adapted from Ching *et al.* (2017).

One ability that characterizes alginate and that is valuable, is its capability to form gels by ionic crosslinking, creating ionic gels and by acid precipitation, originating acid gels, although less explored since they have low applicability. Ionic gel formation occurs when divalent cations bind to the monomeric units of the carboxyl groups present in its structure. Usually, the divalent cations used are Ca^{2+}, although there are others that could be used in this method, with a higher affinity, such as lead or copper, but calcium has the benefit of being non-toxic while still having good affinity. With this, calcium chloride ($CaCl_2$) has been widely utilized to perform ionic gelation, also having the advantage of being versatile, as it can be used in aqueous solutions at low temperatures and allows the encapsulation of both high and low molecular weight additives. On the other hand, one disadvantage of using this metal ion is that

it can become instable when in the presence of calcium chelators, since their affinity to calcium is very high (Ching *et al.*, 2017; Szekalska *et al.*, 2018).

The calcium is able to bind to the G-blocks of the alginate structure, which links the crosslinking capacity to the number of G-blocks, although some researchers mentioned that it can also attach to MG-blocks. The dimerization of the G residues results in the connection of the G monomeric units in opposite sides, forming a hydrophilic space that allows the connection between the calcium ions through the oxygen atoms of the free carboxyl groups in the alginate molecules, originating what is known as the "egg-shaped model", as illustrated in figure 1.9 (Ching *et al.*, 2017; Szekalska *et al.*, 2018).

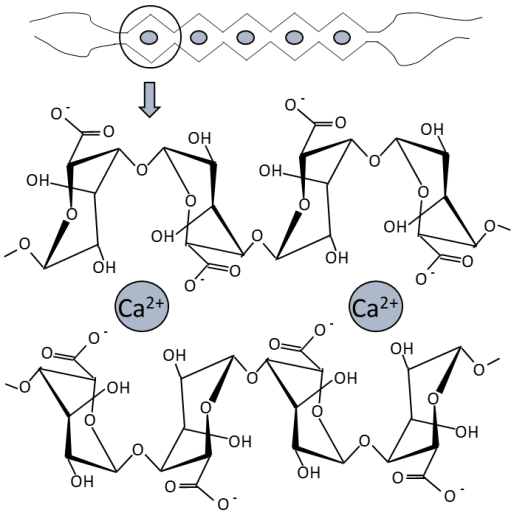


Figure 1.9- Representation of the chemical structure of alginate crosslinked with $CaCl_2$, forming the "egg-box structure". Adapted from Szekalska *et al.* (2018).

To obtain this crosslinked alginate gel there are two well-known methods, internal and external gelation. Regarding the internal gelation, an insoluble calcium salt, such as $CaCO_3$, is added to the alginate-additive solution and extruded into oil, requiring a posterior acidification of the solution in order to release the calcium from the compounds. This process results in a possibly better solute exchange but lower encapsulation efficiency, when compared with external gelation. The external gelation is based on the extrusion of the alginate-additive solution into the calcium solution, meaning that the cations diffuse into the solution that is being dropped, allowing the entrapment of the active agent, a quicker gel formation process and a more consistent particle morphology. Additionally, other capsule formation methodologies will be described further on (Ching *et al.*, 2017).

Furthermore, besides adding other polymers to the alginate as already mentioned, some researchers, such as Morales *et al.* (2017), have also proved that strategies such as altering the concentration of calcium can improve the encapsulation efficiency of the particles and, therefore influence their physicochemical stability and permeability (Morales *et al.*, 2017).

1.4.2.2.2. Chitosan

Chitosan is a linear polysaccharide that derivates from chitin, which is one of the most widely found organic compounds in nature. It is present in the exoskeleton of certain marine animals, in insects, as well as in the cell wall of some fungi, and can also be obtained from residues originated from the

industrialization of seafood (Croisier and Jérôme, 2013). Chitosan has various attributes that make it appealing as a biomaterial, besides the fact that it has a low commercial price. These properties include biodegradability, biocompatibility, gelling attributes, high antimicrobial activity, is prone to easily suffer chemical modifications and low immunogenicity (Kim *et al.*, 2008).

Regarding its structure, chitosan is composed by glucosamine and N-acetyl glucosamine units connected through β (1-4) glycosidic bonds. It is obtained by the deacetylation of chitin (figure 1.10), which is performed by chemical or enzymatic hydrolysis and results in a greater ratio of glucosamine. This allows comparison with the original quantity, granting the determination of the deacetylation degree, that usually stands between 60 and 100% (Kim *et al.*, 2008; Valle *et al.*, 2021).

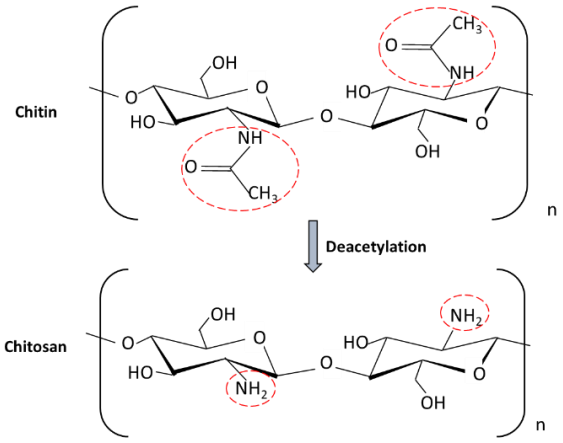


Figure 1.10- Chemical structure of chitin and chitosan. Adapted from Tanha *et al.* (2017).

Besides the deacetylation degree, other characteristics differ from the distinct types of chitosan, derived from different sources or methodologies, such as the deacetylated groups' distribution, molecular mass distribution and molecular weight, which usually varies between 300 and 1000 kDA (Kim *et al.*, 2008).

Additionally, chitosan possesses a characteristic that can be interesting as a trigger mechanism, which is its low solubility in neutral or alkaline means, because of its primary amino group. For this reason, it does not dissolve in environments that are not acidic, for which it is mainly used acetic acid (Moeini *et al.*, 2020). Another very appealing property for the biomedical industry, and most likely the main one, is the fact that chitosan is a positively charged polysaccharide, which makes it appealing for the creation of multilayer films, since there are many other biopolymers, negatively charged, that can interact with it. The protonated amino groups of chitosan used in conjunction with, for example, poly(acrylic acid), gum Arabic, gelatine and alginate, are able to establish ionic complexes with these oppositely charged compounds (Kim *et al.*, 2008; Valle *et al.*, 2021).

With this, besides all the advantages that come with the use of chitosan, the low points of it are its poor mechanical properties and solubility. Many studies have been searching for these connections with different polymers and even for ways to modify chitosan's structure in order to improve its microbial resistance and solubility, as well as maintaining the characteristic biosafety associated with it (Valle *et al.*, 2021).

There are many studies that further explore these types of interactions, between chitosan and other components, such as the research done by Kofuji *et al.* (2010), that investigated the development of a

transparent wound dressing sheet made out of a β-glucan-chitosan complex. This dressing sheet was proven to be more efficient than dressings made by only chitosan and, by altering the properties of chitosan, it enabled a control over the biodegradation of the sheet and, consequently, control over the release rate of the compounds (Kofuji *et al.*, 2010).

Moreover, the main example of application of chitosan is in wound healing and drug-delivery systems, studying its use as a microcapsule material, specially associated with other compounds. It is mentioned a lot of the times alongside alginate, as described in the research made by Hui *et al.* (2013), that used Traditional Chinese Herbs as the bioactive compound in chitosan-alginate capsules, utilizing the emulsion-chemical cross-linking method. The capsules were successfully made and provided a release of the drug in a controlled manner, unveiling a promising path for the goal of this paper, the treatment of atopic dermatitis (Hui *et al.*, 2013).

Radhakumary *et al.* (2011) also presented an interesting study about a thermoresponsive chitosan-based hydrogel. The hydrogel was prepared with thiolated chitosan and poly(N-isopropyl acrylamide) (PNIPA), a thermosensitive polymer that can be used to form a hydrogel. This dressing was loaded with ciprofloxacin, and it proved to be a potential new formulation for wound healing. It revealed good mechanical properties an adequate response to the decreasing of the temperature, facilitating the removal of the material according to it (Radhakumary *et al.*, 2011).

One more highly regarded topic is the polymer combination of chitosan with alginate. Benefiting from the non-toxicity and biodegradability of these natural polymers, Cahyaningrum *et al.* (2015) used the most conventional method to produce the chitosan-coated alginate capsules, the external gelation, with $CaCl_2$ as the crosslinker. The structural chemistry behind this technique and the mechanism of interaction between these components can be observed in figure 1.11 (Cahyaningrum *et al.*, 2015)

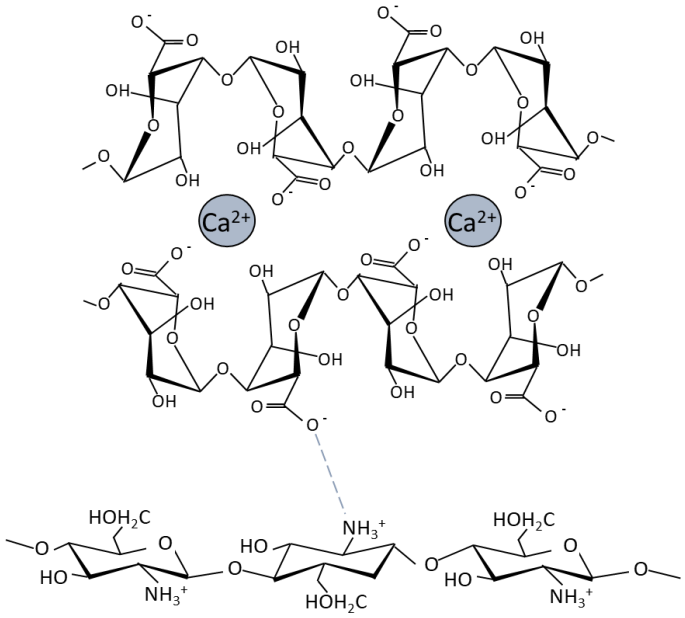


Figure 1.11- Structural representation of the interaction between the alginate crosslinked with $CaCl_2$ and the chitosan, diluted in acetic acid, used as an external layer of the capsules. Adapted from Cahyaningrum *et al.* (2015).

The results revealed an enhanced microparticle's stability in acidic medium (Cahyaningrum *et al.*, 2015). Additionally, Tan *et al.* (2021) , used chitosan-coated alginate microcapsules with palm tocotrienols as active compounds encapsulated. This research studied many parameters in order to improve and optimize the final product, one of them being encapsulation efficiency, comparing alginate capsules with the composite capsules. The results showed that the addition of a higher concentration of chitosan provided a firmer and denser capsule wall. It also concluded that the encapsulation efficiency was significantly higher in the coated capsules, reaffirming that the alginate can be of greater use when it comes to complementing chitosan and improving its properties (Tan *et al.*, 2021).

Furthermore, since the conjugation of chitosan and alginate has demonstrated plenty advantages, various other researches explored the interaction between these two polymers a step further, by adding several layers to the capsules in formation. The polycationic nature of chitosan allowed the use of a very well-known technique, the layer-by-layer (LBL) method, which ended up being widely explored by the scientific community. Zhang *et al.* (2019) , for example, studied the release and antibacterial influence of layer-by-layer self-assembly alginate-chitosan microcapsules, loaded with thyme oil. It's an interesting technique because it allows control over the capsule's parameters, such as morphology, thickness of the wall and roughness. Regarding the methodology, as the technique describes, the chitosan solution made with acetic acid, was dripped into the thyme oil nano emulsion and the layers were made by adding the alginate solution in the same way, and then alternating between these two. A calcium solution was then used to work as a crosslinker, after the electrostatic self-assembly of the desired number of layers. At the end, the capsules showed a slower and more controlled release when they had more alginate-chitosan layers and also revealed a useful pH sensitivity between the pH of 4 and 10, which could be appropriate for wound healing (Zhang *et al.*, 2019).

1.4.2.2.3. Shellac

Shellac is a natural, biodegradable, non-toxic and biocompatible polymer, more specifically a resin, that is secreted by an insect, Laccifer lacca, mostly found in South-East Asia. It has low molecular weight, and it is mainly constituted of anhydrides, polyhydroxy polycarboxylic esters and lactones. Shellac's composition is associated with great amphiphilicity, since it contains aleuritic acids and cyclic terpene acids, which are respectively non-polar and polar components responsible for polymerization (figure 1.12). Additionally, the esters and carboxyl groups in it are associated with shellac´s pka between 5.6 and 7 and, accordingly, with it being insoluble at neutral and acidic aqueous medium, meaning that shellac cannot be ionized at these pHs and maintains a solid arrangement (Messaoud *et al.*, 2016; Yuan, He, Dong, *et al.*, 2021).

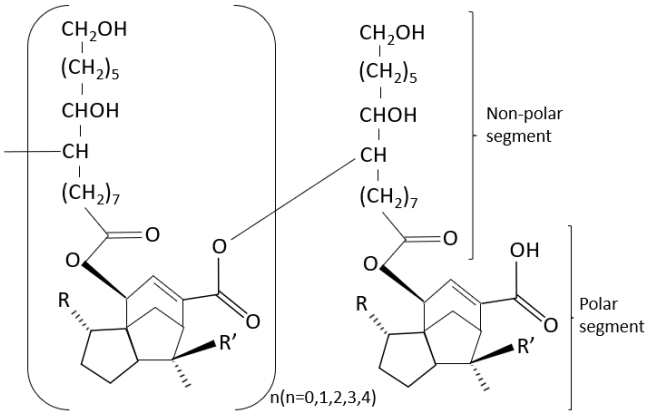


Figure 1.12- Shellac's molecular structure. Adapted from Yuan, He, Dong, *et al.* (2021).

Shellac is widely used in the pharmaceutical, medical and food industries for example, also being extensively explored for drug delivery systems, as composite beads and particularly as an external layer for coated capsules. This is possible since in acidic environments, the carboxyl groups allow the formation of strong bonds between hydrogen molecules and, therefore, a tight display when it comes to shellac (Messaoud *et al.*, 2016; Yuan, He, Dong, *et al.*, 2021).

For this end, the combination between alginate and shellac has been studied by some researchers, many times related to its pH-dependent aqueous solubility. The main application related to encapsulated systems has been linked to gastro-resistance (Yuan, He, Xue, *et al.*, 2021). Many studies refer that these capsules with the shellac external layer were able to establish a shielding effect in the gastric acid environment and only release the active substance in the intestine, where the pH is slightly alkaline, around 7.5. In this type of solutions, the ionization of H^+ starts and shellac salts begin to form from the dissolution. An excessively alkaline medium might provoke hydrolysis (Patrícia & Leopoldo, 2021; Yuan, He, Dong, *et al.*, 2021).

1.4.2.2.4. Eudragit

Eudragit is a manufactural name of a synthetic poly(meth)acrylate polymer with different variations regarding the composition. They are acquired through the polymerization of methacrylic acids, acrylic acid, and their esters (Santos *et al.*, 2021). Regarding applications, the great chemical stability and pore structure of these polymers makes them suitable for drug-delivery systems (Yus *et al.*, 2020).

The different types of Eudragit can differ in solubility, molecular weight, glass transition temperature, product form available and in charge, varying between cationic, anionic and neutral (Santos *et al.*, 2021). The physical properties of this polymer can differ significantly, based on their chemical structure, which allows their use for numerous purposes. The main structure of this material is present in all of Eudragit polymers, as demonstrated in figure 1.13, only differing between each other on the substituents, that is, in the composition of the monomers (Hajba-Horváth *et al.*, 2021).

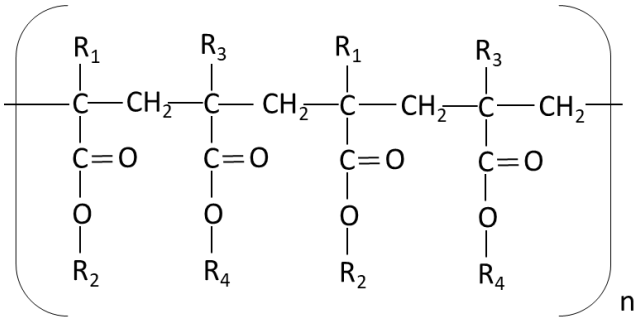


Figure 1.13- Main chemical structure of Eudragit polymers. The radicals vary between the different types of Eudragit (Santos *et al.*, 2021).

With this, the most frequent monomers are methacrylic acid esters, methacrylic acid and dimethylaminoethyl methacrylate (Hajba-Horváth *et al.*, 2021). These differences allow the classification of these polymers in groups based on their characteristics, for example, the pH independent Eudragit polymers can be divided in Eudragit RS, Eudragit RL, Eudragit NM and Eudragit NE (Yus *et al.*, 2020).

Eudragit RLPO is a cationic polymer composed of ethyl acrylate, methyl methacrylate, as well as a small portion of methacrylic acid ester containing quaternary ammonium groups. Eudragit RLPO and RSPO are similar between each other, although the permeability of Eudragit RLPO is much higher, associated with a higher quantity of ammonium functional groups (Santos *et al.*, 2021). These polymers are impermeable to water but possess a good swelling capacity that facilitates the release of the active substances when applied in drug-delivery systems (Hasan *et al.*, 2013). The combination of these polymers is frequently used in different concentrations in order to achieve the desired permeability. Additionally, there are some pH sensitive polymers such as Eudragit S100 (ES100) and Eudragit L100 (EL100), respectively soluble above the pHs of 7 and 6. These are anionic polymers and differ in their active carboxylic group (Santos *et al.*, 2021).

2.4.2.3. Controlled release capsules (trigger) for wounds

Derived from the wound healing process described and associated with infections, the wound microenvironment undergoes modifications that can potentially be used as triggers for drug-delivery systems in wound dressings, allowing a controlled and specific release. Stimuli such as the amount of reactive oxygen species (ROS), enzymes present, temperature, pH or even other toxins released are being very much explored for medical applications. (Farahani & Shafiee, 2021).

ROS are substances, such as hydrogen superoxide, peroxide and peroxynitrite, that are produced during the cellular oxidative metabolism and function as intermediates in many pathways (Farahani & Shafiee, 2021). These radical derivatives of oxygen are produced by the process of mitochondrial oxidative phosphorylation and through enzymes called oxidoreductases. Phagocytes increase the amount of these species in the wound site, as a defense mechanism against pathogens since they are able to regulate angiogenesis and adjust the quantity of lymphoid cells in order to stimulate wound healing. However, when the wound is chronic and the inflammatory state is constant, these species exist in excess and capture electrons from essential molecules, creating an oxidative stress at the wound

site, which results frequently in cell death or necrosis (Dunnill *et al.*, 2017). Therefore, several researchers explored drug-delivery systems that involved these species for the targeted release of the encapsulated drugs. One example is the study developed by Tang *et al.*, (2015), that developed ROS-sensitive nanoparticles containing stromal cell-derived factor-1α (SDF-1α). The release mechanism was related to the type of wall material used for the particles, which was poly-(1,4-phenyleneacetone dimethylene thioketal) (PPADT). This polymer possesses ROS-reactive thioketals, therefore, when in the presence of ROS, there is a breakage of the thioketal bonds, resulting in the capsules' release. The capsules were successfully produced, achieving their purpose of promoting wound healing by releasing SDF-1α at the desired zone, which stimulated chemotaxis and the presence of bone marrow mesenchymal stem cells at the wound site (Tang *et al.*, 2015).

Regarding the pH, it increases with infection, due to the complex microenvironment associated with bacterial colonization, excess of proteases and more. It varies between the pH of 4 and 5 in healthy skin, rising to the range of 5-6 in acute wounds and then 7-9, approximately, in chronic wounds (Farahani & Shafiee, 2021). Therefore, this parameter is also widely used as a trigger mechanism for drug-delivery systems. A study performed by Haidari *et al.* (2021) explored this topic though the creation of a pH-responsive hydrogel with silver nanoparticles. This hydrogel was made with methacrylic acid and acrylamide, crosslinked with N, N'-Methylenebisacrylamide and loaded with the nanoparticles. Methacrylic acid possesses an ionizable anionic acid group, which, due to the deprotonation of the carboxyl group, enables a phase change above the pH of 5. As a result, the hydrogel switched to a more hydrated state at a more alkaline pH, consequently causing a greater release of the silver nanoparticles at the pHs of 7.4 and 10, in comparison to the pH of 4, which is relevant for the treatment of chronically infected wounds (Haidari *et al.*, 2021).

Enzyme triggered release is another possible mechanism for infected wounds drug-delivery systems. The infection of the wound site happens upon the invasion of pathogenic organisms, which generally secrete lipolytic enzymes in order to keep developing (Park *et al.*, 2013). One of the main types of lipolytic enzymes is Phospholipase A2 (PLA2), with the function of hydrolyzing ester bonds in glycerophospholipids, releasing free fatty acids. This enzyme is prominent in infected wound exudates and its activity was investigated in the research performed by D. Li *et al.* (2019). In this study, a liposomal hydrogel was created through the encapsulation of the liposomes in a material composed by chitosan and gelatin and loaded with curcumin. This research is an example of an enzyme triggered release in which the PLA2 in the wound site hydrolyzed the phospholipids in the liposomes, facilitating the release of the curcumin (D. Li *et al.*, 2019). These are only a few examples of controlled release mechanisms related with wound treatment since it is an appealing topic in constant development.

1.4.3. Capsule incorporation in textiles

The functionalization of textile structures is a widely explored topic, in a way of innovating and improving their characteristics. The addition of insect repellents and skin softeners is becoming more relevant, as well as the incorporation of antimicrobial agents for medical applications (Teixeira *et al.*, 2012). Moreover, for several of these textiles' functionalization, active agents are encapsulated, as in the case of textiles fabrics with durable fragrance release capacity (Azizi *et al.*, 2019).

Textile structures, such as cotton, wool, or even synthetic fibers, like polyesters, can be functionalized with capsules, either through a direct application on the fibers during the fabric making process, or as a post-treatment of the textile. Different techniques can be used for the incorporation of the capsules into the textiles, such as padding, exhaustion, coating or spraying. The binding of the capsules is preferably attained through covalent or ionic connection to the fibers or through the connection made with the used binder/crosslinker (Silva *et al.*, 2017). The connection can also be attained only by adsorption of the capsules in the textile structure, but this leads to a less resistant functionalization (e.g., the capsules are easily removed upon a simple washing process). There are different types of binders that can be used in these techniques, such as polymeric resins and polyfunctional cross-linking agents. Consequently, the type and quantity of binder utilized can directly affect the release mechanism of the capsules (Stan *et al.*, 2019).

1.4.3.1. Impregnation technique

The most common process in the textile industry, known for its simplicity, is the padding or impregnation method, which uses a *Foulard* system capable of performing several finishing treatments, such as the application of dyes and microcapsules (Teixeira *et al.*, 2012).

For the incorporation of microcapsules, the impregnation technique requires the preparation of a finishing bath, usually containing a softener, the microcapsules and a crosslinking agent. The concentration of each of these components can significantly influence the performance of the final product, therefore being essential to adjust the formulation to the desired final activity. As demonstrated in figure 1.14, the textile structure is evenly immersed in the bath and then goes through the *Foulard*, a set of rollers that allow the desired impregnation thickness to be reached. Then the textile goes through a drying process, usually done through an oven, and is later exposed to a curing process, all settled to a specific temperature and time period (Silva *et al.*, 2017).

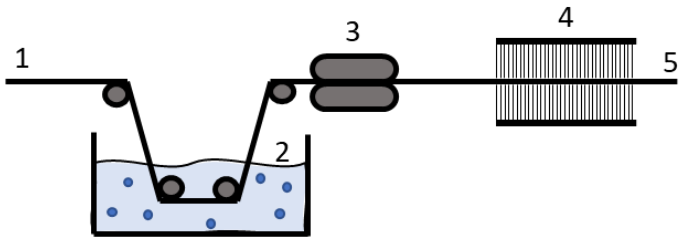


Figure 1.14- Schematic representation of the impregnation process, divided in stages: 1) Untreated textile; 2) Impregnation bath with microcapsules; 3) *Foulard* rollers; 4) Drying and curing; 5) Textile with microcapsules incorporated. Adapted from Rodrigues *et al.* (2009).

1.4.3.2. Crosslinkers and surfactants

Crosslinking is the process of bonding polymer chains by connecting with certain reactive sites, altering their structure. It is widely used to promote a modification on the polymers' properties such as

mechanical resistance, swelling, stability and the drug loading capacity, influencing the release rate in drug delivery systems, improving their performance (Moeini *et al.*, 2020).

This bonding between the polymer and the crosslinking agent can occur physically or chemically, mainly through ionic or covalent bonds, respectively. The type of interactions depends on the nature of the crosslinking agent used (Valle *et al.*, 2021). Ionic bonds are based on the electrostatic attraction between oppositely charged ions and covalent bonds share electrons between two or more atoms. Covalent interactions are generally irreversible and have a higher strength when compared to non-covalent ones (Liu *et al.*, 2019). Covalent reactions result in the permanent bonding of the polymers' reactive sites through intermolecular bonds. Concerning microcapsules, this form of connection often results in easier release of the active compound through diffusion, preventing the dissolution of the wall material. In chemical crosslinking, the reaction time and the quantity of crosslinking agent used are known to significantly influence the properties of the final product. Moreover, there are several crosslinkers that can be utilized for the pretended application, depending on aspects such as reactivity. To achieve the covalent bonding, the ones with multiple reactive functional groups are used in order to bond the polymeric chains of the polymer coating the capsule with the textile fibres (Valle *et al.*, 2021).

With this, crosslinking agents have a role in the fixation of the capsules in textile structures. There are numerous crosslinkers that can be utilized for this purpose, such as glutaraldehyde, glyoxal, citric acid and 1,2,3,4-butanetetracarboxylic acid (BTCA), which some of the chemical structures are represented in figure 1.15. (Dapson, 2007; Kumar & Kumar, 2017; Valle *et al.*, 2021).

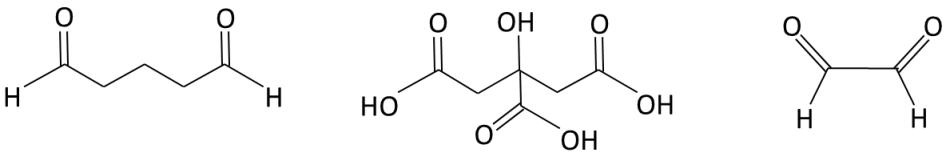


Figure 1.15- Chemical structure of some common crosslinkers. Adapted from Dehabadi *et al.* (2013).

Glutaraldehyde is one of the most commonly used crosslinkers for various applications, since it is an di-aldehyde organic compound of low cost, with easy access and is water soluble (Valle *et al.*, 2021). This crosslinking agent, due to its aldehydic groups, is highly reactive with different groups, such as amines, hydroxyl, phenols, thiols and imidazole, developing covalent bonds. Over the years, the connection between glutaraldehyde and chitosan has been very explored, as a polymeric material with amino groups (Pal *et al.*, 2009). The aldehyde groups of this crosslinker interact with the amino groups of the polymer, producing covalent Schiff bases, a sub-category of imines, as well as reacting with the hydroxyl groups (present e.g., in cellulose-based or polyester based textiles), originating acetal bonds (Valle *et al.*, 2021). Additionally, this connection with the hydroxyl groups is favorable at an acidic pH, in order to catalyze the acetalization between these groups, although at this pH the linkage with the amines is slower (Pal *et al.*, 2009). However, a few discoveries regarding glutaraldehyde have been made, confirming that this compound can be toxic and it has also been proven to hasten the calcification of certain implants. Therefore, glyoxal has been used as an alternative in some applications (Kumar & Kumar, 2017), and glutaraldehyde used only for comparison.

Glyoxal is a dialdehyde that can work as an effective crosslinker for polysaccharide polymers. It is established that it improves their physicochemical and mechanical properties. This compound is known for its non-toxicity, biodegradability and low cost and, equally to glutaraldehyde, glyoxal has two reactive ends. The crosslinking process involves the developing of acetal bonds at the hydroxyl groups of polysaccharides, connecting the polymers' chains, showing specially interesting crosslinking results when used with chitosan-based composite polymers, through the Schiff-base reaction (Ni *et al.*, 2019).

Furthermore, citric acid is another non-toxic and economical compound used in the textile industry as a crosslinking agent and can easily react with polysaccharides. The polymers' hydroxyl groups connect with the three carboxylic acid groups present in citric acid and these groups react by the process of esterification, resulting in the formation of esters (Valle *et al.*, 2021). Generally, this mechanism is enhanced through the addition of a catalyst and, for cellulosic textiles with citric acid, sodium hypophosphite has been recognized as being the most efficient. BTCA is another widely used polycarboxylic acid that functions through a very similar process to citric acid, although the higher quantity of carboxyl groups is BTCA increases its effectiveness as a crosslinking agent of cellulosic textiles. On the other hand, it is associated with a higher cost (Feng *et al.*, 2014).

In addition, the use of surfactants can also be implemented as a form to stabilize the solution and to increase the solubility of some constituents, creating a more even distribution. Surfactants are amphiphilic molecules, possessing a hydrophilic polar part and a lipophilic nonpolar part. The size and strength of these two parts determine the hydrophilic-lipophilic balance, which is a relevant parameter to determine the suitability of a certain surfactant for the procedure (Valle *et al.*, 2021). Surfactants reduce the surface tension and are able to form self-assembled micelles in a solution. These present a hydrophobic centre and a hydrophilic surrounding and are created by the interactions between the water and the surfactants' lipophilic part. Commonly, concerning microencapsulation, surfactants such as Span 20 and 80, Tween 40 and 80 and sodium dodecyl sulphate are widely used (Moeini *et al.*, 2020).

1.4.4. Characterization techniques

There are various methods that can be used in order to characterize particles that allow the evaluation of specific properties, that can be either physical, chemical, mechanical and many more. Regarding their morphology, the size and shape of the particle is extremely relevant, which is often analysed through a particle size analyser (PSA), that utilises laser diffraction technology to obtain the size distribution of the capsules. Other commonly utilized techniques for this purpose are optical microscopy as well as scanning electron microscopy (SEM), which obtains a surface image through a high-energy electron beam that perceives the variation of the signal by the electron scattering (Peng *et al.*, 2020). Transmission electron microscopy (TEM) can also be used concerning the capsules' morphology, using an electron beam to produce an image through the electrons that are transmitted across the sample, also providing information about the internal structure of small samples. Staining agents are frequently added in this method to improve the quality of the characterization (Pedroso-Santana & Fleitas-Salazar, 2020; Peng *et al.*, 2020).

Concerning the chemical structure and composition of the capsules, a Fourier transform infrared spectroscopy (FTIR) analysis is widely used, which is also useful to understand some of the interactions that occur throughout the encapsulation procedure. The infrared spectrum is achieved through the absorption of infrared light with a certain wavelength, associated with the vibration of an atom in a molecule. The interactions between compounds can be observed by the comparison of the spectra of pure reagents and the capsules spectra. Further methods such as thermogravimetric analysis (TG) and differential scanning calorimetry (DSC) can be done to analyse the polymers behaviour and properties (Pedroso-Santana & Fleitas-Salazar, 2020; Peng *et al.*, 2020).

Other studies can be performed on the capsules to achieve, for example, the release properties of the active agent. The release kinetics are generally studied through the measurement of the core substance' concentration in the medium where the capsules are inserted. This is often obtained by performing fluorescent resonance energy transfer, liquid chromatography, radioactive assays and UV-Visible spectroscopy, utilized for the quantification of chemical substances, frequently providing results for time-dependent release studies (Pawley *et al.*, 2017).

2. Work outline

The work was outlined primarily to research different additives already approved for human application, even if not in wounds and even active agents already being researched. This was followed by the experiment and development of a method for the encapsulation of the additives with the goal of obtaining high absorption dressing materials with a controlled release of active agents. Various methodologies were explored with different materials and active agents, in order to achieve the desired results and optimize the natural additives' incorporation in textiles.

The next step was the impregnation of the additives in the textile with the addition of different impregnation solutions. This phase was followed by the research of approaches to detect and test the release of the additives incorporated. The characterization techniques developed were then executed in order to evaluate and improve the final design of the samples.

The aim of this project was to start the development of additive incorporation in textile, that could in the future be employed as a first layer, the interface layer between the material and the wound site, of a multi-layered novel high-absorption bandage with enhanced properties.

3. Materials

Primarily, the research started with the finding of additives with interesting properties that would be appropriate for the wound environment. Between many suitable natural options already found, in preliminary studies for this exact purpose, such as manuka honey, oregano oil, curcumin and thyme oil, two extracts, all with similar and appropriate properties, were kindly provided by the company FOURMAG for the project: sunflower oil and *Aloe vera* extract (figure 3.1). In some primary studies, food colouring was used as an additive to facilitate the visualization of the capsules and their behaviour before adding the plant extracts.

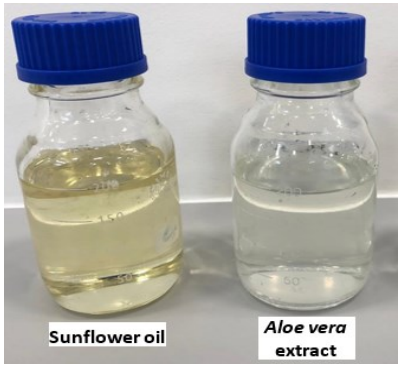


Figure 3.1- Natural sunflower oil *Aloe vera* extract provided to work as additives for the functionalization of the textile structures.

Regarding the materials for the encapsulation, the following substances were used independently and combined, represented in table 3.1.

Table 3.1- Polymers used for encapsulation and respective provider

Polymer	Supplier
Alginate (SMT)	CHT (Germany)
Chitosan (Chitoclear cg1600)	Primex (Iceland)
Eudragit RLPO	Evonik (Germany)
Shellac (aqueous solution)	A.F. Sutter (United Kingdom)

The functionalization was achieved with the produced capsules, and was performed on knitted textile substrate, kindly provided by the company SOMANI, whose composition was polyester (35 %), cotton (30 %), modal (28 %) and polyamide (7 %). The textile went through a washing process, at 40 ˚C with Diadavin® UN (Tanatex Chemicals – The Netherlands), a non-ionic detergent. After the washing, the textile measured approximately 330 g/m^2. Afterwards, a bleaching procedure was implemented using hydrogen peroxide and caustic soda (50%) and oxygenated water (200 vol., 50 %) (João Manuel Lopes de Barros Lda. - Portugal), at the temperature of 98 ˚C, 30 minutes with Diadavin® UN, TANNEX® GEO (Tanatex Chemicals – The Netherlands) as a balanced bleaching auxiliary.

During the development of the project, for the experimental trials and respective testing of the products, several reagents were used and are described in table 3.2.

Table 3.2- Reagents used in experimental trials and respective provider

Reagent	Supplier
Calcium chloride ($CaCl_2$)	Panreac (Spain)
Acetic acid (CH_3COOH)	Fisher (USA)
Sodium hydroxide (NaOH)	Fisher (USA)
Potassium di-Hydrogen Phosphate (KH_2PO_4)	Sigma (Germany)
Sunflower lecithin fluid	Escotex (Belgium)
Ethanol	Carlo Erba (France)
Sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$)	Merck (Germany)
Sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$)	Merck (Germany)
Red Remazol RGB	DyStar (Spain)
Glutaraldehyde solution (25%)	Sigma (Germany)
Citric acid, monohydrated	Sigma (Germany)
Sodium hypophosphite (SHP), monohydrated	Sigma (Germany)
Glyoxal solution (40%wt)	Sigma (Germany)
Tween 80	Croda (Spain)
Span 80	Croda (Spain)
Imerol JET-B conc.	Archroma (Switzerland)
Sodium carbonate (Na_2CO_3)	Solvay (Portugal)

The equipment used in the protocols are also described in table 3.3, as well as the brand.

Table 3.3- Equipment used in experimental trials and respective provider

Equipment	Supplier
AG245 Analytical Balance	Mettler Toledo
Binocular microscope BL224	VWR
Optical stereo microscope MSC-ST45T	Bioevopeak
UV-2600i Spectrophotometer	Shimadzu
Spectrum 100 FTIR	Perkin Elmer
Peristaltic pump 101F/R	Watson Marlow®
pH meter 7110	WTW inoLab®
Horizontal, vertical padder type «HVF»	Werner Mathis
Dryer/ Vaporizer Type DHE 51991	Werner Mathis
Drying and heating chamber B53	Binder
Magnetic stirrers, with heating, M21 and M6.1	CAT

4. Methodology

4.1. Preparation of capsules

The first approach was to obtain capsules with a well-known polymer, employing a simple method of ionic gelation, using food colouring in order to analyse better the encapsulation and analysing the shape, size and content distribution.

Alginate-dye capsules dry:

An alginate solution was prepared with the concentration of 2% (w/v) in distilled water with the addition of food colouring 2% (v/v). For the alginate to completely dissolve, slight heating (~40 ˚C with stirring for 2 hours was required).

A calcium chloride ($CaCl_2$) solution 5% (w/v) was also prepared in an acetic buffer (to a solution of glacial acetic acid in distilled water (0.1_M) was carefully added sodium hydroxide (NaOH) (1_M) until reaching a pH of 4.5, and the final volume was just adjusted till 1 L with distilled water). Similarly, a 1% (w/v) calcium chloride solution was prepared.

Microencapsulation was accomplished by ionic gelation. Drops of the alginate solution were added, with a regular bevel 21G syringe, to the $CaCl_2$ solution, which was under stirring, with the magnetic stirrer settled to minimal agitation. After approximately 15 minutes the capsules were washed in the previously prepared solution of 1% (w/v) of $CaCl_2$ in the acetic acid solution with pH 4.5. The samples were then filtered and placed in the oven at 40 ˚C for ~24h, as demonstrated in figure 4.1.

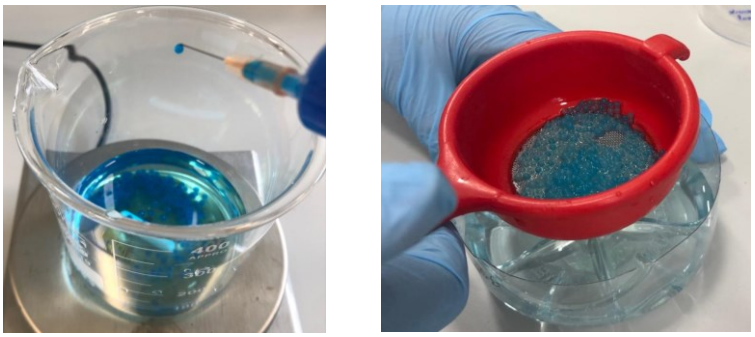


Figure 4.1- Images from the external gelation process, displaying the syringe dropping the alginate solution into the $CaCl_2$ solution and the filtration part of the procedure.

Chitosan-Alginate Multilayer Alginate Capsules with dye:

This procedure was based on the one developed by Cook *et al.* (2013). An alginate solution was prepared with the concentration of 2% (w/v) in water with the addition of food colouring 2% (v/v). A $CaCl_2$ solution (0.05_M) was also prepared with distilled water. Afterwards, drops of the alginate solution were added, with a regular bevel 25G syringe, to the $CaCl_2$ solution, which was under stirring, with the magnetic stirrer settled to minimal agitation. Then, approximately 30 minutes later, the capsules were filtered. In this trial, 3 samples were created, as described in table 4.1. Therefore, two of the samples followed the layering process (samples 2 and 3), with chitosan and alginate solutions.

Table 4.1- Description of the samples and respective layers

Sample no.	Description	Parameters
1	Alginate capsules	Alginate 2% in water and food colouring 2%;
2	Alginate capsules with 4 layers	Alginate 2% in water and food colouring 2%; Alternating layers: • 2 of chitosan 0.4% in acetate solution of pH 4; • 2 of alginate 0.04% in water
3	Alginate capsules with 8 layers	Alginate 2% in water and food colouring 2%; Alternating layers: • 4 of chitosan 0.4% in acetate solution of pH 4; • 4 of alginate 0.04% in water

For these layers, two solutions were prepared: a chitosan 0.4% (w/v) solution in a buffer of acetic acid (same procedure as above explained, but the addition of NaOH was only until reaching pH 4), and an alginate solution with the concentration of 0.04% (w/v) in distilled water. The capsules were placed in the chitosan solution for 10 minutes and followed by 10 minutes in the alginate solution, repeating this process for samples 2 and 3 according to the number of layers pretended. The samples were then filtered and stored for a posterior analysis in the UV-Vis spectrophotometer.

Alginate Capsules with and without *Aloe vera* extract:

Two types of capsules were prepared, ones with *Aloe vera* extract and ones without, for comparison. Two alginate solutions were prepared with the concentration of 2% (w/v) in distilled water, with the addition of 2% of *Aloe vera* extract (v/v), in one of the solutions. A $CaCl_2$ solution (0.05_M) was also prepared in distilled water. Afterwards, each alginate solution was added, by spraying (using a spray bottle) to the $CaCl_2$ solution, which was under stirring, with the magnetic stirrer settled to minimal agitation. Then, approximately 30 minutes later the capsules were filtered and stored.

Eudragit-coated alginate capsules:

An alginate solution was prepared with the concentration of 2% (w/v) in distilled water. A $CaCl_2$ solution (0.05_M) was also prepared in distilled water. Afterwards, the alginate solution was added, by spraying (using a spray bottle) to the $CaCl_2$ solution, which was under stirring, with the magnetic stirrer settled to minimal agitation. Then, approximately 30 minutes later the capsules were filtered and submersed, for another 30 minutes, in a previously prepared emulsion of Eudragit RLPO 0.5% (w/v) in distilled water. The capsules were then filtered and stored.

Shellac-Alginate and alginate capsules with sunflower oil:

The method used to form the capsules was based on the procedure described by Morales *et al.* (2017), regarding the capsules that reflected a better encapsulation efficiency. Firstly, the shellac-alginate capsules with sunflower oil were prepared. For this, sunflower lecithin fluid solution was made with the concentration of 3% (w/v) in distilled water, as a natural emulsifier, in order to form the aqueous phase of the emulsion. Afterwards, this solution and the sunflower oil were mixed in the proportion of 30-70% (v/v), respectively, for 10 minutes at 1600 rpm in a magnetic stirrer. To produce the wall of the capsules, an alginate solution was prepared (2% w/v) in distilled water, stirred for 1h at 600 rpm, plus

30 minutes in minimal agitation, to complete hydration and 30 mins more setting still for deaeration. Afterwards, a shellac solution (Swanlac ASL10), after a pH adjustment with NaOH (1_M) to 7.5 was done, was added to the alginate solution (50/50% v/v). Subsequently, the emulsion was mixed with the wall material solution, in the proportion of 20-80% (v/v), respectively. This mix was then added drop-by-drop, with a regular bevel 25G syringe and the magnetic stirrer settled to minimal agitation, to a $CaCl_2$ solution (1.5% w/v) made with ethanol and deionized water (1:1 v/v). After 30 minutes, the capsules were filtered and stored separately.

The alginate capsules with sunflower oil were produced using the same protocol as described, although without the addition of the shellac solution to the alginate solution, in the wall material solution preparation step.

Chitosan-alginate 8-layered alginate capsules with and without dye:

The production of these capsules was based on the same procedure used previously by Cook *et al.* (2013), although a different component for the colouring was used and the alginate capsules were prepared with and without it, for comparison. An alginate aqueous solution was prepared with the concentration of 2% (w/v) with the addition of, in the case of the capsules with dye, red Remazol RGB 2% (w/v) and distilled water. A $CaCl_2$ solution (0.05_M) was also prepared in distilled water. Afterwards, drops of the alginate solution were added, with a regular bevel 25G syringe, to the $CaCl_2$ solution, which was under stirring, with the magnetic stirrer settled to minimal agitation. Then, approximately 30 minutes later the capsules were filtered. Afterwards, the capsules went through the layering process, with chitosan and alginate, in order to achieve 8 layers.

For this procedure, two solutions were prepared: a chitosan 0.4% (w/v) solution in a buffer of acetic acid (same procedure as above explained, but the addition of NaOH was only until reaching pH 4), and an alginate solution with the concentration of 0.04% (w/v) in distilled water. The capsules were placed in the chitosan solution for 10 minutes and followed by 10 minutes in the alginate solution, repeating this process until achieving eight alternating layers, four of chitosan and four of alginate. The samples were then filtered and stored.

Alginate-dye capsules made with compressed air:

An alginate solution was prepared with the concentration of 2% (w/v) in distilled water with the addition of red Remazol RGB 2% (w/v). A $CaCl_2$ solution (0.05_M) was also prepared in distilled water. Afterwards, the setting for the capsules' formation was prepared, with the goal to obtain micro-sized capsules. The alginate solution was placed alongside the peristaltic pump (4 rpm) with the tube working as the supplier, and a syringe needle (regular bevel 21 G) was placed at the other end of the tube, which was additionally attached to the compressed air hose (figure 4.2).

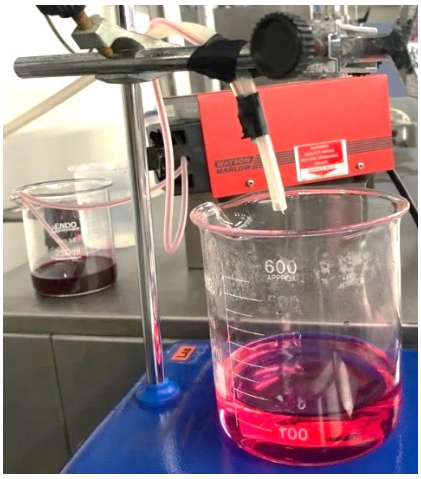


Figure 4.2- Assembled system for the formation of alginate capsules with dye using compressed air. The alginate aqueous solution is being fed to the peristaltic pump, which is connected to a syringe attached to the compressed air hose. Smaller capsules are then formed when the droplets come into contact with the calcium chloride solution from the beaker below it.

With the desired pressure of compressed air settled (try and error method, as the system did not allow a precise control of the pressure of the air), very small drops of the alginate solution were added to the $CaCl_2$ solution through the syringe, which was fixated to a support, and microcapsules immediately started to form, by ionic gelation. Then, approximately 30 minutes later the capsules were washed with a $CaCl_2$ aqueous solution (1% w/v), filtered, and stored.

Alginate capsules with and without *Aloe vera* extract made with compressed air:

Two types of capsules were prepared: alginate capsules and alginate capsules with *Aloe vera* extract, for comparison. An alginate aqueous solution was prepared with the concentration of 2% (w/v) and, separately, for the formulation of the capsules with the additive, the *Aloe vera* 2% (v/v) extract was mixed into the solution. A $CaCl_2$ solution (0.05_M) was also prepared in distilled water. Afterwards, each alginate solution (with and without the *Aloe vera* extract) was added using the same system as described earlier. The alginate solution with and later the one without the extract, was placed alongside the peristaltic pump (4 rpm) with the tube working as the supplier, and a syringe needle (regular bevel 21 G) was placed at the other end of the tube, which was additionally attached to the compressed air hose.

Small drops of the alginate solution were added to the $CaCl_2$ solution through the syringe fixated to a support, and, with the correct air pressure on, microcapsules started to form. Approximately after 30 minutes both types of capsules were filtered and stored separately.

Alginate capsules with and without sunflower oil made with compressed air:

This process was based on the same method as the previously used to produce the shellac-alginate capsules with sunflower oil, described by Morales *et al.* (2017).

For the alginate-oil capsules the procedure is described as it follows: a sunflower lecithin fluid solution was prepared with the concentration of 3% (w/v) in water, as a natural emulsifier, in order to form the aqueous phase of the emulsion. Afterwards, this solution and the sunflower oil were mixed in the proportion of 30-70% (v/v), respectively, for 10 minutes at 1600 rpm in a magnetic stirrer. To produce

the wall of the capsules, an alginate solution was prepared (2% w/v) in distilled water, and stirred for 2h at 600 rpm. Subsequently, the emulsion was mixed with the alginate solution, in the proportion of 20-80% (v/v), respectively, originating an emulsion with approximately 14% of sunflower oil. A $CaCl_2$ solution was then prepared (1.5% w/v) in ethanol and deionized water (50-50% v/v).

In order to have a sample that would work as a control, the alginate capsules were made using the exact same method, although the sunflower oil was not added, meaning that only the aqueous phase of the emulsion described above (with sunflower lecithin fluid), was added to the alginate solution. With this, a proportion ratio error was made and, instead of diminishing the quantity of the lecithin aqueous solution added to the alginate solution, since without the sunflower oil it was more concentrated in lecithin, this solution was still added to the alginate in the proportion of 20-80% v/v, respectively. This means that the capsules without the sunflower oil were composed by *circa* 0.42% (v/v) more lecithin, but since it should not affect much the results of future analysis, the method was not repeated.

The same peristaltic pump and compressed air system was used for the formulation of the capsules. The prepared solutions were, one by one, placed alongside the peristaltic pump (4 rpm) with the tube working as the supplier. A syringe needle (regular bevel 21 G) was placed at the other end of the tube, which was additionally attached to the compressed air hose. With this, small drops of the alginate solution were added to the $CaCl_2$ solution through the syringe fixated to a support, and, with the correct air pressure on, microcapsules started to form. Approximately after 30 minutes both types of capsules were filtered and stored separately.

4.2. Release studies

The samples were analysed in a UV–Vis-NIR spectrophotometer with double beam (Shimadzu 2600i). To analyse the absorbance of solutions, the equipment has an accessory for the cuvettes and allows readings in a spectral range between 185 and 900 nm. To analyse the reflectance (total or diffuse) or transmittance od dispersions or solid samples, the equipment has an integrating sphere as accessory (ISR-2600Plus), which allows readings in a spectral range between 220 and 1400 nm.

Analysis of the UV-Vis spectra of the additives and the alginate:

The absorbance spectra of the sunflower oil and *Aloe vera* extract were acquired in the UV-Vis spectrophotometer with a wavelength range between 185 and 900 nm, and a spectral resolution of 1 nm. Regarding dilutions, necessary for the measurements intended, the sunflower oil was diluted in acetone and the *Aloe vera* extract was diluted in distilled water, since it was the only additive that is water-soluble. The solvents used for the dilutions were used as background. For comparison of the spectra, an alginate aqueous solution (0.5% w/v) was also analysed in the same manner, using water as background.

Effect of pH on the multi-layered capsule release:

Three acetic acid buffer solutions with the pHs of 5, 7 and 9 were prepared, using the already described protocol, and each was distributed into 3 beakers. Approximately 60 capsules from each of the three samples, represented on table 4.1, were distributed into the 3 different pH solutions. After 4 hours they were removed, and the absorbance of these solutions was acquired in the UV-Vis spectrophotometer, at the range of 350-750 nm.

Alginate capsules time-dependent release in a pH 5 buffer:

Approximately 60 capsules of alginate with blue colouring (sample 1, represented on table 4.1), were suspended in an acetic acid buffer solution with the pH of 5. The solution was then collected every 30 minutes for 2 hours and also, after 9h 30 mins and their absorbance were acquired in the UV-Vis spectrophotometer in a range of 350-750 nm (the solutions were put back in the beaker with the capsules right after the readings).

Effect of pH on the multi-layered capsules time-dependent release:

Three buffer solutions were made with potassium dihydrogen phosphate (KH_2PO_4) and NaOH (1M) and respectively adjusted to the pH of 6, 7.5 and 9. Approximately 50 capsules from each of the three samples (represented on table 4.1), were separately suspended in the solution with the pH of 6. The solutions were collected every 15 minutes for 2 hours and also, after 17 hours and their absorbance was acquired in the UV-Vis spectrophotometer in a range of 350-800 nm. This procedure was repeated with the other two solutions, with the pH of 7.5 and 9 (the solutions were put back in the beaker with the capsules right after the readings).

pH time-dependent study on the release of alginate capsules with and without *Aloe vera* extract and Eudragit-coated alginate capsules

The three buffer solutions with pHs of 6, 7.5 and 9 prepared above was used again in this assay. Approximately 0.255g of capsules were weighed, for a more correct measurement, from each sample (the alginate capsules with and without *Aloe vera* extract, and alginate capsules uncoated and coated with Eudragit). These were then separately suspended in the buffer solution with pH 6. The solutions were collected every 15 minutes for 2 hours and their absorbance was acquired in the UV-Vis spectrophotometer in a range of 350-800 nm. This procedure was repeated with the other two solutions, with pHs 7.5 and 9 (the solutions were put back in the beaker with the capsules right after the readings).

pH time-dependent study on the effect of shellac on the capsules' release

Three phosphate buffer solutions were made, with $Na_2HPO_4 \cdot 7H_2O$, $NaH_2PO_4 \cdot H_2O$ and using NaOH (1_M) to adjust the pH till 6, 7.5 and 9. Approximately 0.3284g of capsules were weighed (around 50 capsules), for a more correct measurement, from each sample (the shellac-alginate and alginate capsules with sunflower oil). These were then separately suspended in the buffer solution with pH 6.

The solutions were collected every 15 minutes for 2 hours and their absorbance was acquired in the UV-Vis spectrophotometer in a range of 350-800 nm. This procedure was repeated with the other two solutions, with pHs 7.5 and 9 (the solutions were put back in the beaker with the capsules right after the readings).

pH time-dependent study on the release of sunflower oil

The alginate capsules with sunflower oil made with compressed air were dried in the drying and heating chamber at 40°C until completely dry, to simulate their state after impregnation into the textile structure. Three new phosphate buffer solutions were prepared only using the salts $Na_2HPO_4 \cdot 7H_2O$ and $NaH_2PO_4 \cdot H_2O$, in distilled water, to obtain buffers with pHs of 6, 7.5 and 8.7. Three beakers were set with each buffer solution, to be filtered at different times, and approximately 0.03 g of the capsules were submersed in each one. From all pH solutions, each beaker was filtered with a half an hour time interval between each other, so the capsules were filtered at 30, 60 and 90 minutes. The capsules were then dried at the same drying and heating chamber at 40°C until they were completely dry and ready to be studied in the FTIR Spectrometer.

4.3. FTIR analysis

Fourier Transform Infrared - Attenuated Total Reflectance (ATR-FTIR) analysis was also used to detect the encapsulated additives and obtain their chemical profile. The spectra were acquired using a Spectrum 100 FTIR Spectrometer (PerkinElmer®) equipped with an Attenuated Total Reflectance (ATR) device. The software was adjusted to analyse each spectrum between 4000 and 650 cm^{-1} at a resolution of 8 cm^{-1}. The first step, to diminish the detection of water in the spectra was to dry all the capsules in the drying and heating chamber at 40°C until completely dry. The analysis were performed in the capsules made with the optimized method, using compressed air, being thus analysed the alginate capsules, alginate capsules with *Aloe vera* extract, alginate capsules with sunflower oil and alginate capsules without sunflower oil.

Additionally, the capsules that resulted from the previously described time-dependent study at different pHs were also analysed, in order to try to detect the release of sunflower oil encapsulated in them. For comparison reasons, the ATR-FTIR spectra of sunflower oil and *Aloe vera* extract were also obtained, as well as the spectra of the sunflower lecithin and alginate SMT, used for the preparation of the capsules.

4.4. Impregnation in the textile structure

4.4.1. Impregnation of the chitosan-alginate 8-layered alginate capsules

The application of the capsules was made into the textile structures on a laboratory scale, using the textile structure in the size 6 x 6 cm.

Impregnation with pressure:

The textile (sample 1) was immersed for around one minute in a suspension of chitosan-alginate 8-layered alginate capsules with dye in distilled water (at a concentration of 5 g/L) using a textile to liquor ratio of approximately 1:20 and then, passed between the rollers of the *Foulard* with a pressure of 0.3 bar. The samples were then dried at 100°C for 7 minutes at the dryer/vaporizer (figure 4.3).

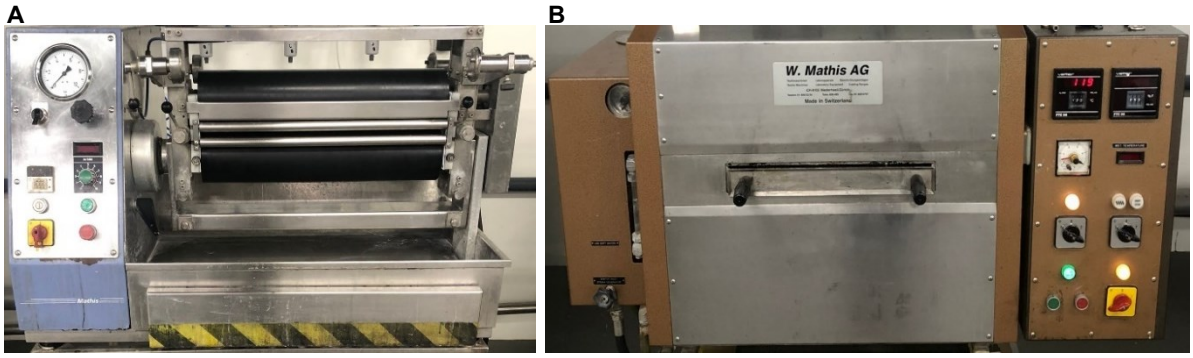


Figure 4.3- Lab padder, horizontal and vertical (*Foulard*), used for impregnations (A) and lab dryer and vaporizer machine, which was used as a dryer (B).

Impregnation with solutions with different crosslinkers:

For this impregnation, as well as the following ones, two types of capsules were used: the alginate capsules with 8 layers without dye, prepared with a regular bevel 25G syringe, and the alginate capsules with 8 layers with dye, produced using the same protocol but with the addition of red Remazol RGB 2% (w/v) to the alginate aqueous solution.

To access the effect of various crosslinkers, different aqueous impregnation dispersions were prepared: sample 2 - glutaraldehyde (20 g/L) and alginate capsules with dye and 8 layers (10 g/L); sample 3 - glutaraldehyde (20 g/L) and alginate capsules without dye and 8 layers (10 g/L); sample 4 - glyoxal (10 g/L) and alginate capsules with dye and 8 layers (10 g/L); sample 5 - glyoxal (10 g/L) and alginate capsules without dye and 8 layers (10 g/L); sample 6 - citric acid (9 g/L), sodium hypophosphite (SHP) (0.3 g/L) and alginate capsules with dye and 8 layers (10 g/L); sample 7 - citric acid (9 g/L), SHP (0.3 g/L) and alginate capsules without dye and 8 layers (10 g/L), as represented in table 4.2.

Table 4.2- Description of the components in the impregnation solutions of each sample.

Sample no.	Type of capsules	Solution parameters	
		Crosslinker	Catalyst
2	Alginate capsules with 8 layers with dye	Glutaraldehyde	–
3	Alginate capsules with 8 layers without dye	Glutaraldehyde	–
4	Alginate capsules with 8 layers with dye	Glyoxal	–
5	Alginate capsules with 8 layers without dye	Glyoxal	–
6	Alginate capsules with 8 layers with dye	Citric acid	SHP
7	Alginate capsules with 8 layers without dye	Citric acid	SHP

The solutions were then poured with caution into each textile sample and always on the right side of the textile, which is the smoothest one, simulating a dispenser of solutions or creams as used in the

cosmetic industry (from which FORUMAG, the company that provided the additives belongs to). Afterwards, the samples were dried at 100°C for 7 minutes at the dryer/vaporizer equipment.

Impregnation in solutions with different surfactants:

The addition of surfactants to the solutions was also studied, utilizing initially Tween 80 and Imerol JET. Two aqueous solutions were prepared: sample 8 - glutaraldehyde (20 g/L), Tween 80 (1% w/v) and the alginate capsules with dye and 8 layers (10 g/L); sample 9 - glutaraldehyde (20 g/L), Tween 80 (1% w/v) and the alginate capsules without dye and 8 layers (10 g/L); sample 10 - glutaraldehyde (20 g/L), Imerol JET (3 g/L) and the alginate capsules with dye and 8 layers (10 g/L); sample 11 - glutaraldehyde (20 g/L), Imerol JET (3 g/L) and the alginate capsules with dye and 8 layers (10 g/L), as represented in table 4.3.

Table 4.3- Description of the components in the impregnation solutions of each sample.

Sample no.	Type of capsules	Solution parameters	
		Crosslinker	Surfactant
8	Alginate capsules with 8 layers with dye	Glutaraldehyde	Tween 80
9	Alginate capsules with 8 layers without dye	Glutaraldehyde	Tween 80
10	Alginate capsules with 8 layers with dye	Glutaraldehyde	Imerol
11	Alginate capsules with 8 layers without dye	Glutaraldehyde	Imerol

The solutions were equally poured over each textile sample and into the same side of the textile. This was followed by the drying process at 100°C for 7 minutes at the dryer/vaporizer.

Impregnation in solutions with adjusted pH:

Four other samples were studied with the addition of Na_2CO_3 to the impregnation solutions until the pH of 9: sample 12 - glyoxal (10 g/L), Na_2CO_3 (1M) till reaching pH 9 and the alginate capsules with dye with 8 layers (10 g/L); sample 13 - glyoxal (10 g/L), Na_2CO_3 (1M) till reaching pH 9 and the alginate capsules without dye with 8 layers (10 g/L); sample 14 - glutaraldehyde (20 g/L), Na_2CO_3 (1M) till reaching pH 9 and the alginate capsules with dye with 8 layers (10 g/L); sample 15 – glutaraldehyde (20 g/L), Na_2CO_3 (1M) till reaching pH 9 and the alginate capsules without dye with 8 layers (10 g/L). The solutions were poured into each textile sample, in the same way as described previously and were then dried at 100°C for 7 minutes at the dryer/vaporizer.

Impregnation in solutions with dried capsules:

Both types of 8-layered alginate capsules, with and without dye, were left to dry at room temperature for approximately 60 hours, until complete dryness. Then, two aqueous solutions were prepared with glutaraldehyde (20 g/L) and the capsules (1.65 g/L) with and without dye, corresponding to samples 16 and 17, respectively. After poring the solutions into the textile samples, these were dried at 100°C for 5 minutes, followed by 5 more minutes at 120°C at the dryer/vaporizer.

4.4.2. Impregnation of the capsules made with the compressed air method

Impregnation with and without pressure:

Two samples were prepared and impregnated with dispersions that contained the capsules made with the compressed air method. Sample 18 was prepared by pouring a glutaraldehyde (20 g/L) aqueous solution with the alginate-dye capsules made with compressed air (approximately 21 g/L), and was not exposed to pressure. Another aqueous dispersion was prepared using glutaraldehyde (20 g/L), Tween 80 (3.46 g/L), Span 80 (6.54 g/L) and alginate-dye capsules made with compressed air (21 g/L). This solution was then poured over the textile (sample 19) which went through the *Foulard* machine with a pressure of 0.3 bar. Both samples were then dried at 100°C for 3 minutes, followed by 3 minutes at 120°C at the dryer/vaporizer.

Impregnation with an attempt to homogenize the distribution of capsules in the textile:

In order to achieve a more even distribution of the capsules over the samples, three samples were impregnated with thicker solutions. To prepare sample 20, a solution was made using an 89% w/w alginate (1% w/v) aqueous solution, Tween 80 (0.346% w/w), Span 80 (0.654% w/w) and alginate-dye capsules made with compressed air (10% w/w). Then this sample was dried at 100°C for 3 minutes and 3 more minutes at 120°C at the dryer/vaporizer. The other two samples were prepared with Tubicoat HEC in different concentrations. For sample 21 a solution was made with glutaraldehyde (20 g/L) aqueous solution, 21 g/L of the alginate capsules with *Aloe vera* extract made with compressed air and 92% v/v of a Tubicoat HEC aqueous solution (2.5% w/v), that was previously stirred with a magnetic stirrer for around 1h 30 mins, until completely uniform. Another solution, for sample 22, was prepared using glutaraldehyde (20 g/L) aqueous solution, 21 g/L of the alginate capsules made with compressed air and 92% v/v of a Tubicoat HEC aqueous solution (1.25% w/v), that was also left to stir for 1h30 mins beforehand. After the solutions were poured into the corresponding textile sample, these two samples went through the *Foulard* machine with a pressure of 0.3 bar. Afterwards, the samples were dried at 100°C for 5 minutes, followed by 5 minutes at 120°C at the dryer/vaporizer.

Impregnation with solutions containing different crosslinkers:

The impregnation of two types of capsules made with the compressed air method was tested using different crosslinkers in order to optimize the process. Two aqueous solutions were prepared with glutaraldehyde (20 g/L) and 21 g/L of the alginate capsules with dye (sample 23) and without dye (sample 24). Two other solutions were prepared using a glyoxal (10 g/L) aqueous solution with alginate capsules (21 g/L) with and without dye, corresponding respectively to samples 25 and 26. For samples 27 and 28 two other aqueous solutions were made with citric acid (9 g/L), SHP (0.3 g/L) and the two types of alginate capsules (21 g/L) with dye (sample 27) and without dye (sample 28). The last sample, sample 29, was the blank, using only an aqueous solution with 21 g/L of alginate capsules for the impregnation, as represented in table 4.4.

Table 4.4- Description of the components in the impregnation solutions of each sample.

Sample no.	Type of capsules	Solution parameters	
		Crosslinker	Catalyst
23	Alginate-dye capsules	Glutaraldehyde	–
24	Alginate capsules	Glutaraldehyde	–
25	Alginate-dye capsules	Glyoxal	–
26	Alginate capsules	Glyoxal	–
27	Alginate-dye capsules	Citric acid	SHP
28	Alginate capsules	Citric acid	SHP
29	Alginate capsules	–	–

In this case, all the solutions were stirred in a magnetic stirrer settled to minimal agitation for 10 minutes to achieve more uniform solutions for impregnation. The solutions were then poured carefully into each textile sample. Afterwards they were dried completely in the dryer/vaporizer at 80°C and then, to finish, at 120°C for 3 minutes.

4.4.3. Delicate wash of the impregnated textile structures

The last impregnated samples with alginate capsules, samples 23, 25, 27 and 29, were submitted to a very subtle wash using a magnetic stirrer and a beaker containing water. Each sample was initially cut in half and, for comparison, only one of the parts of each sample was gently placed in the water with the side that contained the capsules facing upwards. The proportion used was 70 mL of water per gram of textile and the magnetic stirrer was settled to 60 rpm for 10 minutes. Afterwards, the samples were placed in the dryer/vaporizer at 80°C until completely dry.

5. Results and Discussion

5.1. Morphology of the capsules

Alginate-dye capsules dry:

It was possible to obtain capsules, although they appeared extremely stiff, which would hamper the impregnation in the textile structures (figure 5.1). Therefore, it was settled that the best option would be not to put the capsules through the drying process.

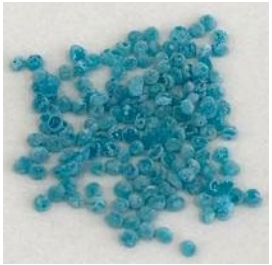


Figure 5.1- Alginate capsules with food colouring made by ionic gelation, after the drying process.

Chitosan-Alginate Multilayer Alginate Capsules with dye:

The capsules are more malleable and possibly more suitable for the impregnation in terms of structure and consistency (figure 5.2). A smaller size needle, from 21 G to 25 G, also made a significant difference in the capsule's size, although they might still be too big for incorporation in textile structures, with sizes between 2 and 2.5 mm.

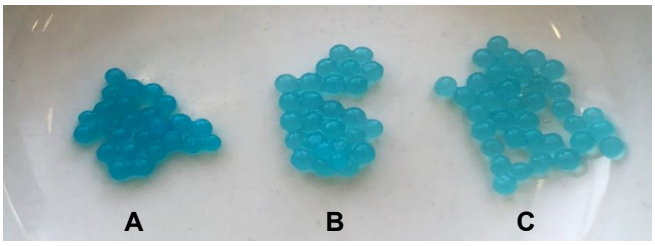


Figure 5.2- Three types of capsules made by ionic gelation: A) Alginate capsules (sample 1); B) Alginate capsules with 4 alternating chitosan-alginate layers (sample 2); C) Alginate capsules with 8 alternating chitosan-alginate layers (sample 3).

Comparing the three types of capsules, the size and shape are very similar between each other. It's also noticeable that the alginate capsules without layers have a darker colour. Through the microscopic images (figure 5.3) it is possible to observe the colouring distribution and how the content happens to be more spread out on the outer part of the capsules.

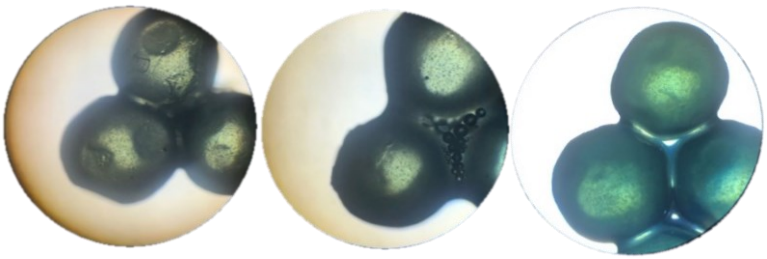


Figure 5.3- Images of the three types of alginate capsules, without layers (sample 1), with 4 alternating chitosan-alginate layers (sample 2) and with 8 alternating chitosan-alginate layers (sample 3), respectively, from left to right, captured using optic microscopy (40x magnification).

Alginate Capsules with and without *Aloe vera* extract:

The capsules prepared with the spraying method are much smaller compared to the capsules made with a syringe (figure 5.4). They also show a more irregular form and size differences between each other. On the other side, they appear to be much easier to impregnate in the textiles.

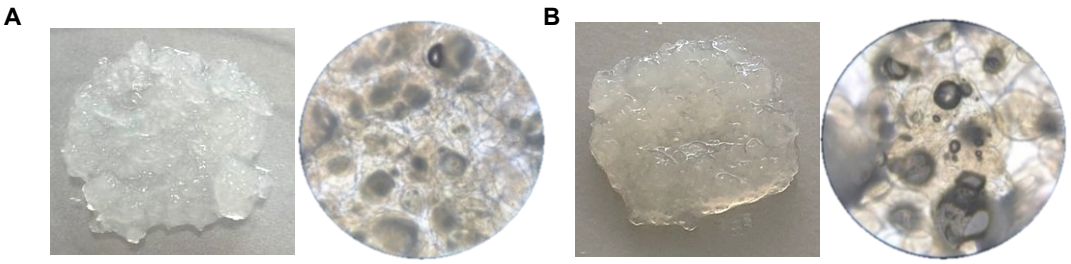


Figure 5.4- Picture of the capsules made with the spraying method and the corresponding microscopic image (100x magnification), using optic microscopy: A) Alginate capsules; B) Alginate capsules with *Aloe vera*.

The spraying method, as it was used, made the production of the capsules a lot easier and less time consuming, however, one possible disadvantage would be the uneven or possibly even unsuccessful encapsulation of the additive. Nevertheless, the use of a controlled spray system could allow the obtention of uniform capsules, and even control the range of sizes.

Eudragit-coated alginate capsules:

The Eudragit-coated alginate capsules, as shown in figure 5.5, appear to present a very similar morphology to the previous capsules, the alginate capsules with and without the *Aloe vera* extract. Therefore, although the size range seems a lot more suitable for impregnation, the variety in shape and size of the capsules, visible in the microscopic images, indicates that this spraying method might not be the most reliable for the encapsulation of additives.

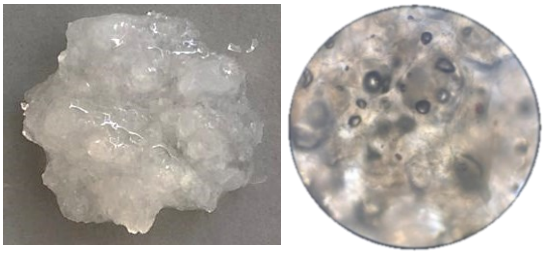


Figure 5.5- Picture of the Eudragit-coated alginate capsules made with the spraying method and the corresponding microscopic image, using optic microscopy (100x magnification).

While other methods to decrease evenly the size of the capsules were being investigated, other components and procedures were tested in order to successfully encapsulate the sunflower oil.

Shellac-Alginate and alginate capsules with sunflower oil:

It can be observed that shellac changed the colour of the capsules to a light brown colour, when comparing them with the alginate capsules (figure 5.6), which was a result of the shellac solution used in the procedure.

Figure 5.6- Shellac-alginate (on the left) and alginate (on the right) capsules with sunflower oil, made by ionic gelation, using the syringe method.

The sunflower oil seems to have been effectively encapsulated in both types of capsules, judging by the way the capsules were formed and the homogeneity between them. The sunflower lecithin fluid worked well as a natural emulsifier and the capsules, in terms of appearance, are consistent.

With the next step of the work outline being the impregnation of the capsules in the textile structure, the capsules with the best results, for the intended goal of being used in a wound environment, were made. Therefore, based on the release studies, the chosen capsules for this step were the chitosan-alginate 8-layered alginate capsules with and without dye, for comparison.

Chitosan-alginate 8-layered alginate capsules with and without dye:

The capsules have similar appearance to the others previously prepared with the same method, as presented in figure 5.7.

Figure 5.7- Chitosan-alginate 8-layered alginate capsules with and without dye, from the left to the right, made by ionic gelation using the syringe method.

As mentioned, a smaller size is needed to perform impregnation in the textile structure. Although layering has shown advantages (results presented in section 5.3.1), since the main focus for this next stage was to be able to analyse an efficient way to impregnate the capsules, the wall of the capsules made with compressed air was of alginate only, to facilitate the process at this early stage.

Alginate-dye capsules made with compressed air:

The method using compressed air worked very well, since the compressed air disperses the droplet that forms at the tip of the needle into several small uniform beads that solidify when in contact with the calcium solution. The capsules came out looking homogeneous in terms of morphology, the size

decreased significantly and the shape, although in the microscope the dye did not allow a very clear image (figure 5.8), appears to be less spherical than expected but similar between them.

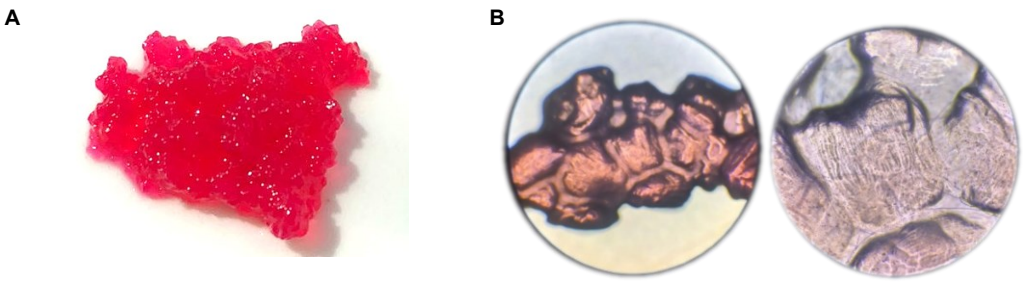


Figure 5.8- Alginate capsules with dye made by using compressed air (A) and the corresponding microscopic images (B), using optic microscopy (magnification of 40x (on the left) and 100x (on the right)).

Since the method was a success and since the more irregular shape might be from the incorporation of the dye, the encapsulation of the additives was performed next.

Alginate capsules with and without *Aloe vera* extract made with compressed air and a peristaltic pump:

The same method used, with compressed air, allowed the formation of particles with a similar size when compared with the other types of capsules by this technique (figures 5.8 and 5.9), even though it is not possible to control or quantify exactly the pressure rate being exerted by the compressed air hose. Regarding the size, although it slightly varied between particles, the produced alginate capsules presented approximately 1022 µm of diameter and the alginate capsules with *Aloe vera* extract around 983 µm. This is a much more adequate size for the intended activity, the impregnation into the textile structure, being much closer to the typical size range used to describe microcapsules, which is approximately between 1 and 1000 µm of diameter (Cheng *et al.*, 2009; Lengyel *et al.*, 2019).

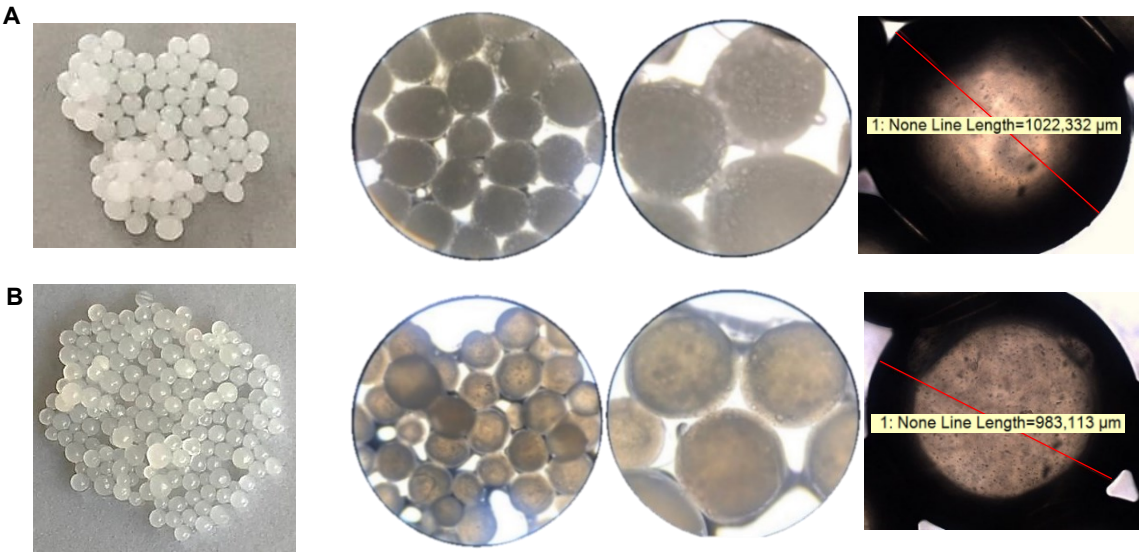


Figure 5.9- Alginate capsules (A) and alginate capsules with *Aloe vera* extract (B), made by using compressed air and a peristaltic pump, and the corresponding microscopic images (40x, 100x and 1000x magnification, from the left to the right) with the measurements of the capsules.

The resulted particles also presented a much rounder and even shape and appear to have properly encapsulated the additive, since the alginate capsules with *Aloe vera* have a clearer separation between the core and the wall material. The observed separation is created because the gelation starts with the contact of the droplet into the gelling bath, containing calcium, beginning from the external surface, therefore, the cations diffuse towards the interior of the particle. The chemical bonding between the alginate and the calcium is, therefore, the highest at the surface and decreases throughout the core, forming core-shell structured microcapsules as detected in figure 5.9 (Ching *et al.*, 2017).

Alginate capsules with and without sunflower oil made with compressed air and a peristaltic pump:

The capsules with sunflower oil, as it can be observed in figure 5.10, present a whitish colour and the capsules without the oil have a brown tone, a colour that is related with the presence of lecithin.

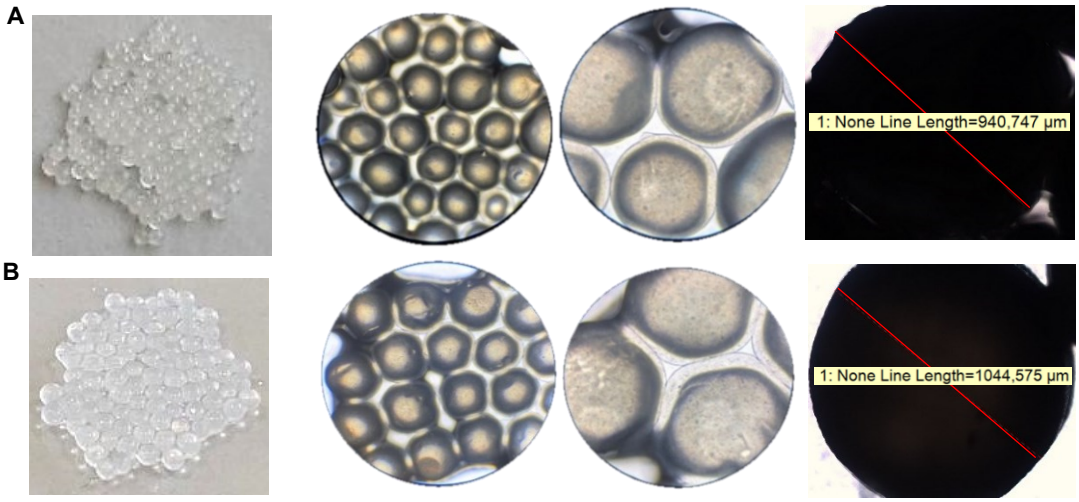


Figure 5.10- Alginate capsules with sunflower oil (A) and without sunflower oil (B), made by using compressed air and a peristaltic pump, and the corresponding microscopic images (40x, 100x and 1000x magnification, from the left to the right) with the measurements of the capsules.

Additionally, although in the microscopic image with a higher magnification it is not especially visible, in the other images, in similarity to the capsules with and without *Aloe vera*, it appears to be a separation between the wall material and the core of the capsules. For verification, future studies involving scanning electron microscope (SEM) could be performed, acquiring high resolution images concerning the microstructure of the shell membrane and interior. Concerning the size range, the diameter of the alginate capsules with and without sunflower oil are approximately 940 µm and 1044 µm, respectively, which are similar to the ones obtained with the *Aloe vera* extract.

The addition of ethanol in the gelling bath was also noticeable during the capsule formation process, resulting in the sinking of the capsules as soon as they entered the solution, as well as in their movement to a more centred position in the beaker. The addition of this component was carried out with the aim of reducing the instability of capsule production, since unstable emulsions are associated with a greater probability of undergoing phase separation during the dripping process. Organic solvents, such as ethanol, function as emulsion stabilizers to facilitate the contact between the alginate and the calcium, so that the capsules are consistent, which appears to have worked based on their homogeneity. This effect of ethanol on the capsule's morphology was also verified in the research made by J. Li *et al.* (2015), regarding alginate films, demonstrating that the use of this solvent in the calcium chloride solution resulted in visual improvements regarding the thickness, the uniformity of the surface and even the mechanical properties of the films (J. Li *et al.*, 2015).

5.2. Analysis of the UV-Vis spectra of the additives and the alginate

The absorbance of the sunflower oil and *Aloe vera* extract was analysed in the UV-Vis spectrophotometer. The analysis of the oils and extracts' spectra allowed to understand in which wavelength each additive had greater absorbance, as demonstrated in figure 5.11 and table 5.1, and if

it was possible to detect their presence in the capsules with alginate. For comparison, the absorbance of an aqueous alginate solution was also analysed.

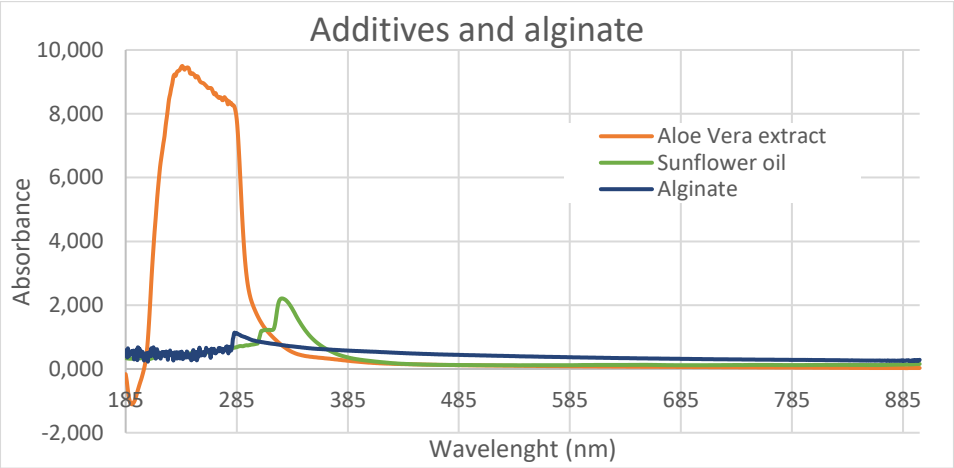


Figure 5.11- UV-Vis absorption spectra of an alginate aqueous solution and additives: sunflower oil and *Aloe vera* extract, with accounted dilution factor.

Table 5.1- Summary table with the peak values from the obtained UV-Vis absorption spectra of the additives with accounted dilution factor.

	Absorbance	**Wavelength (nm)**
Sunflower oil	1.228	312
	2.210	326
***Aloe vera* extract**	9.453	241
Alginate	1.14	283

In the case of the sunflower oil, two absorption bands are detected, approximately around 312 nm and 326 nm, which, according to other publications, are both related to the phenolic compounds, although the second band could additionally be related with tocopherols, organic compounds involving many methylated phenols (Didham *et al.*, 2020; J. B. Oliveira *et al.*, 2019). The *Aloe vera* extract shows a large band around 241 nm, which is most likely associated with a phenolic anthraquinone, such as aloin, a compound that has a yellow tone to it, also showing a much higher absorbance compared to the others (Ravi *et al.*, 2011).

Regarding the alginate spectrum, it showed a band at the wavelength of 283 nm, although not very prominent since this aqueous solution had to be extremely diluted in order to allow the reading in the UV-Vis spectrophotometer. With this, it is clear that the absorption band is very close to the bands of the additives, which could interfere with their detection in UV-Vis absorption spectra, used in posterior capsule analysis. The noise prior to the band is probably related to a reading error associated with the utilization of a disposable cuvette, that does not allow analysis at lower wavelengths.

5.3. Study on the capsule release kinetics and biodegradation

The pH-dependent release was evaluated by submerging the capsules in each buffer solution. The tests were initially made with buffer solutions of pHs 5, 7 and 9, since the healthy skin has a pH between 4 and 6, the pH of non-infected wounds is around 6-7.5, while an infected wound presents a more alkaline pH, generally ranging between 7 and 9 (Farahani & Shafiee, 2021). This allowed to make some conclusions around the goal of the project, which is to have the release of the desired active agents during different stages of the wound development: e.g., if it is an antibacterial agent, its release should occur preferably when the infection starts; if it is a hydrating or wound healing promoting agent, they should be released at any stage, but in a controlled manner along the time, in order to promote the lasting of the effects.

5.3.1. Influence of layers on the capsule's release

a) Effect of pH on the multi-layered capsule release:

The release of the dye from the alginate capsules, after these were submerged for 4h in different pH buffer solutions is clear, just by the observation of the colouring of the capsules, as demonstrated in figure 5.12.

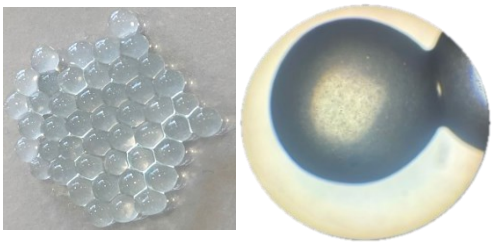


Figure 5.12- Picture and microscopic image, using optic microscopy (40x magnification), of alginate capsules after being in solution (pH 5) and releasing their content.

The release of the blue dye was analysed for the different capsules, in each pH, after the 4 hours described. The absorbance spectra are shown in figure 5.13, and the maximum absorbance for each capsule and pH, at approximately 630 nm, is shown in table 5.2.

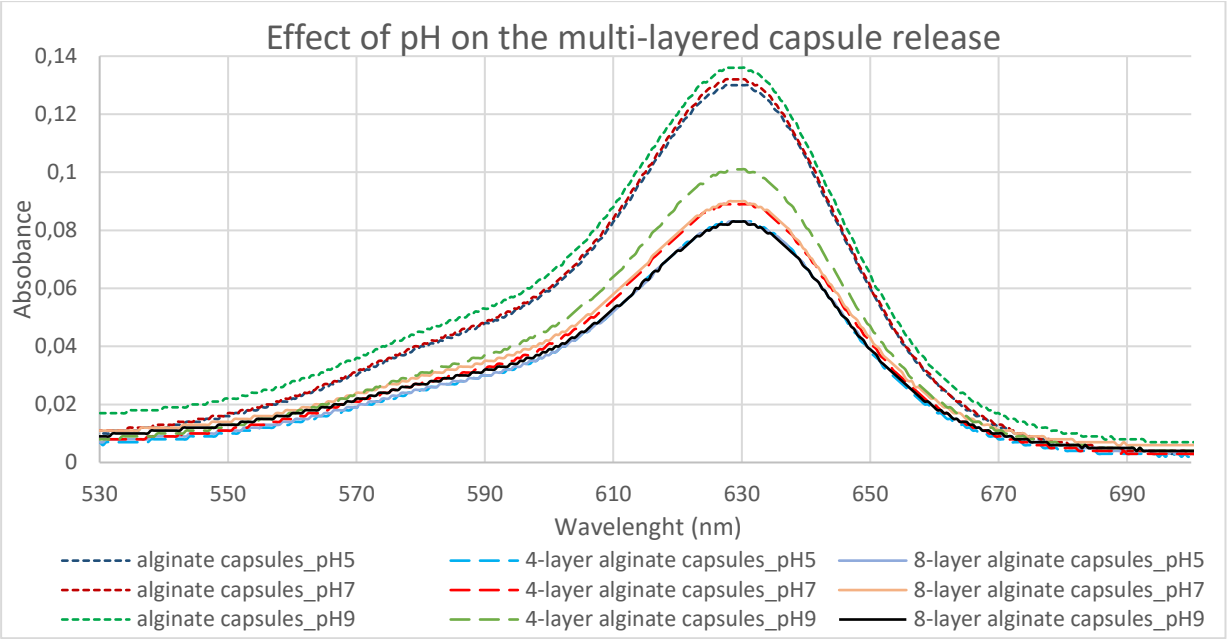


Figure 5.13- UV-Vis absorption spectra of the alginate capsules without, with four and with eight layers after 4 hours in buffers with different pHs (5,7 and 9).

Table 5.2- Summary table with the peak values from the obtained UV-Vis absorption spectra of the multi-layered alginate capsules in different pHs

Capsules	pH5	pH7	pH9
Alginate capsules	0,13	0,132	0,136
4-layer alginate capsules	0,083	0,089	0,101
8-layer alginate capsules	0,083	0,09	0,083

The results show that the release is faster in capsules made only of alginate, as expected, with a greater control regarding the release by the capsules with chitosan-alginate layers, which would be advantageous for the intended application. It was also verified that the release is similar between capsules with 4 and 8 layers, varying only slightly at pH 9. Also, as a general trend, the release was higher at pH 9, and smaller at pH 5. These results stimulated interest in an analysis over time, with smaller intervals in between samples, in order to study the release kinetics.

b) Alginate capsules time-dependent release in a pH 5 buffer:

From the collected data, this study allowed the analysis of the release rate of the dye from the capsules, and the results are shown in figure 5.14 and table 5.3.

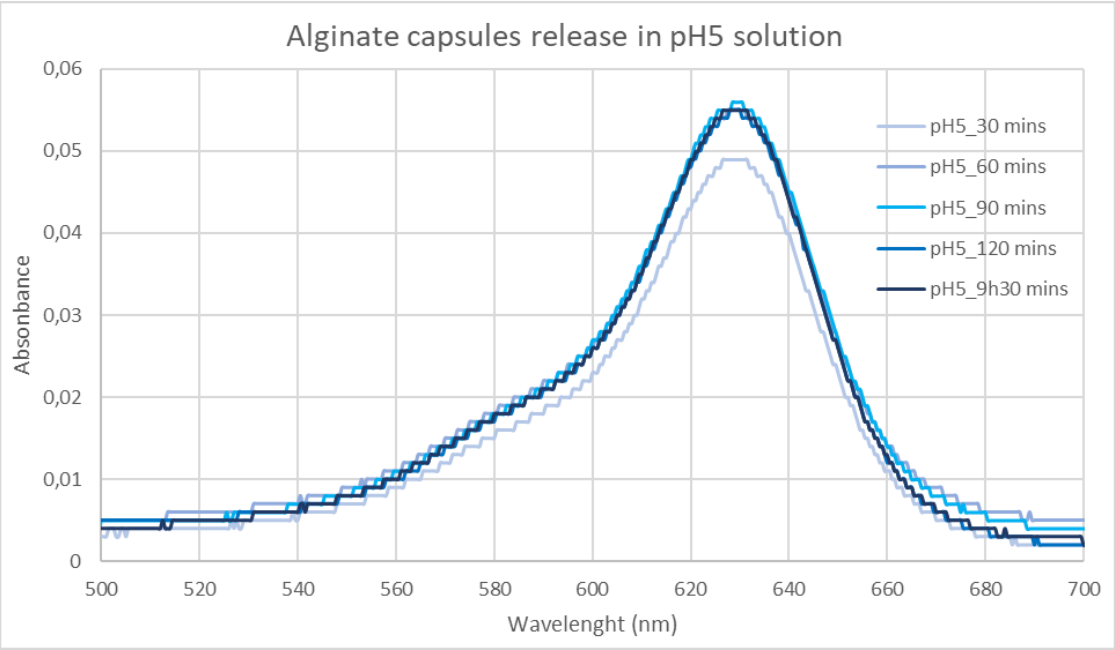


Figure 5.14- UV-Vis absorption spectra of the alginate capsules in a pH 5 acetate buffer at different times.

Table 5.3- Summary table with the peak values from the obtained UV-Vis absorption spectra of the alginate capsules along the time

Time	30mins	60mins	90mins	120mins	9h30mins
Absorbance	0,049	0,055	0,056	0,055	0,055

It is possible to observe that the complete release, with a peak at approximately 630 nm, occurs mostly within the first hour, which is not the desired behaviour for wound treatment, especially for high-absorption dressings that are frequently used to cover the wound for more than one day. Thus, studies on the multi-layered capsules were performed afterwards, as an attempt to observe improvements in this behaviour.

After this test, a change in the acetate solutions, used for the pH-related release tests, was made. It was acknowledged that a phosphate solution would be more suitable for the replication of the bioenvironment of the wound and that would also be more stable at the range of high pH needed. The forward tests were performed for the pHs of 6, 7.5 and 9 since these would be more fitting for this solution in order to make it steadier.

c) Effect of pH on the multi-layered capsules time-dependent release:

A great amount of data was collected in this study, as it is possible to observe in figure 5.15 and table 5.4.

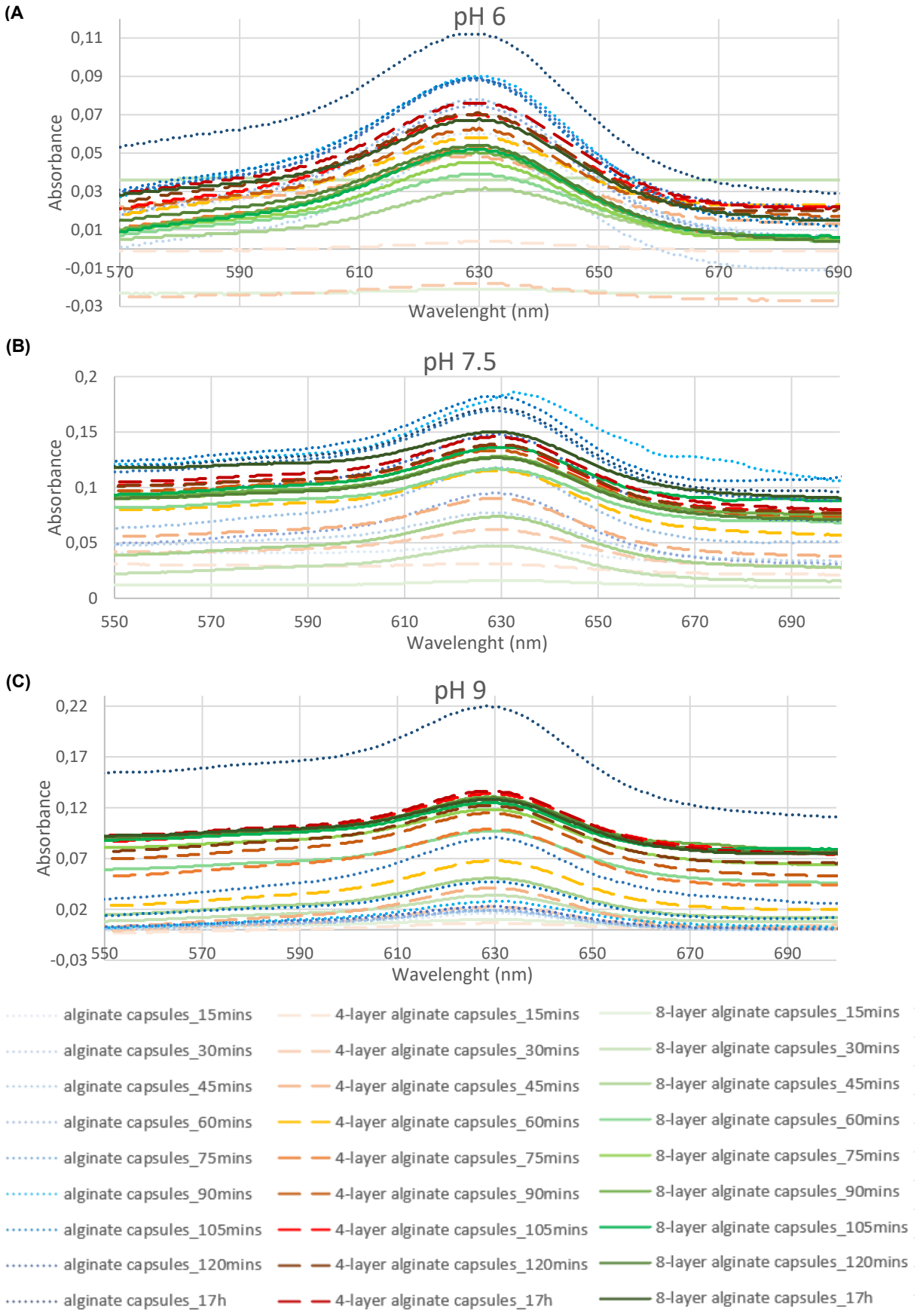


Figure 5.15- UV-Vis absorption spectra of the alginate capsules, alginate capsules with 4 layers and alginate capsules with 8 layers along the time at different pHs: (A) pH 6; (B) pH 7.5 and (C) pH 9.

Table 5.4- Summary table with the peak values from the obtained UV-Vis absorption spectra of the multi-layered alginate capsules at pHs of 6, 7.5 and 9, along the time.

		15 mins	30 mins	45 mins	60 mins	75 mins	90 mins	105 mins	120 mins	17h
pH 6.0	Alginate capsules	0,06	0,052	0,075	0,078	0,089	0,09	0,089	0,088	0,112
	4-layer alginate capsules	0,004	-0,018	0,048	0,058	0,053	0,063	0,07	0,071	0,076
	8-layer alginate capsules	-0,021	0,036	0,031	0,039	0,045	0,05	0,052	0,054	0,068
pH 7.5	Alginate capsules	0,047	0,077	0,094	0,118	0,148	0,183	0,182	0,169	0,172
	4-layer alginate capsules	0,031	0,062	0,09	0,115	0,137	0,133	0,137	0,139	0,145
	8-layer alginate capsules	0,016	0,047	0,074	0,116	0,127	0,128	0,136	0,127	0,15
pH 9.0	Alginate capsules	0,016	0,018	0,019	0,019	0,023	0,028	0,047	0,091	0,219
	4-layer alginate capsules	0,007	0,023	0,041	0,068	0,099	0,115	0,134	0,122	0,136
	8-layer alginate capsules	0,01	0,034	0,051	0,097	0,118	0,131	0,125	0,13	0,128

With these results, it is apparent that there is some inaccuracy with the initial values of absorbance in the pH 6 solution, presenting a few negative values, although still very close to zero, possibly due to it being further from the optimal pH range of the phosphate solution used.

Despite that, while performing this study it was visible that the degradation rates of the capsules were different depending on the pH they were in. All the capsules submersed in the pH 6 solution were intact after 17 hours, while all the capsules in the solution at pH 7.5 were completely degraded after that time. At pH 9, the capsules with layers started to degrade after approximately 75 minutes and were completely degraded after the 17 hours. The alginate capsules, at this pH, without layers, took a while to degrade, starting around the 120 minutes mark but were also completely degraded at the end of the test. This degradation of the capsules, accompanied by the increase of the polymer in the solution over time, could be an explanation for the increase in the baseline values, and is associated with a greater release of the dye. Before and after the 630 nm peak they gradually move away from zero, after nearly 75 mins in the case of capsules with layers, and after 17 hours in the case of the alginate ones, being consistent with the degradation rate described above.

Another important observation regarding the pH-dependent release is that the capsules with layers have a greater and faster release at pH 7.5, followed by pH 9 and lastly in the solution with pH 6. The capsules composed by only alginate have a superior and quicker release at pH 7.5 and lastly at pH 9, although, in the next studies this order of release is not accurate, possibly being related to a measurement error. Posterior results reveal a faster release around pH 7.5, followed by pH 9 and finally pH 6, as expected. On the other hand, besides the results regarding pH 9, the alginate capsules, in general, revealed a faster release of their content, when comparing to the layered capsules, showing disadvantages. With this, it is possible to assume that the layered capsules, additionally to having an easier release at pH 7.5-9.0, possess a more controlled release that could be useful for a more ongoing effect. The referred pH-dependent release properties could be useful for manufacturing wound

dressings, since the pH of a wound ranges between pH 6 and 7.5, becoming even more alkaline when infected, possibly increasing until pH 9. The pH differences, when compared to a healthy skin pH, usually between 4 and 6, allows a greater release of the desired content when necessary (Farahani & Shafiee, 2021).

However, the capsules are releasing all their content in the first 2 hours, still being too fast for the intended activity, although with the oils the release rate could be quite different. Furthermore, these tests are being made with the capsules completely submerged in the solutions, which does not precisely simulate their use in a textile structure over a (chronic) wound, where the textile will touch an exudating wound, but which does not have so much solution.

After the preliminary tests with only alginate capsules with food dye, the work evolved to the tests with capsules with the additives encapsulated.

5.3.2. pH time-dependent study on the release of alginate capsules with and without *Aloe vera* extract and Eudragit-coated alginate capsules

The data obtained from the absorbance measurements on the spectrophotometer allowed the study of the effect of *Aloe vera* extract and Eudragit in the spectra, exhibited in figure 5.16 and table 5.5.

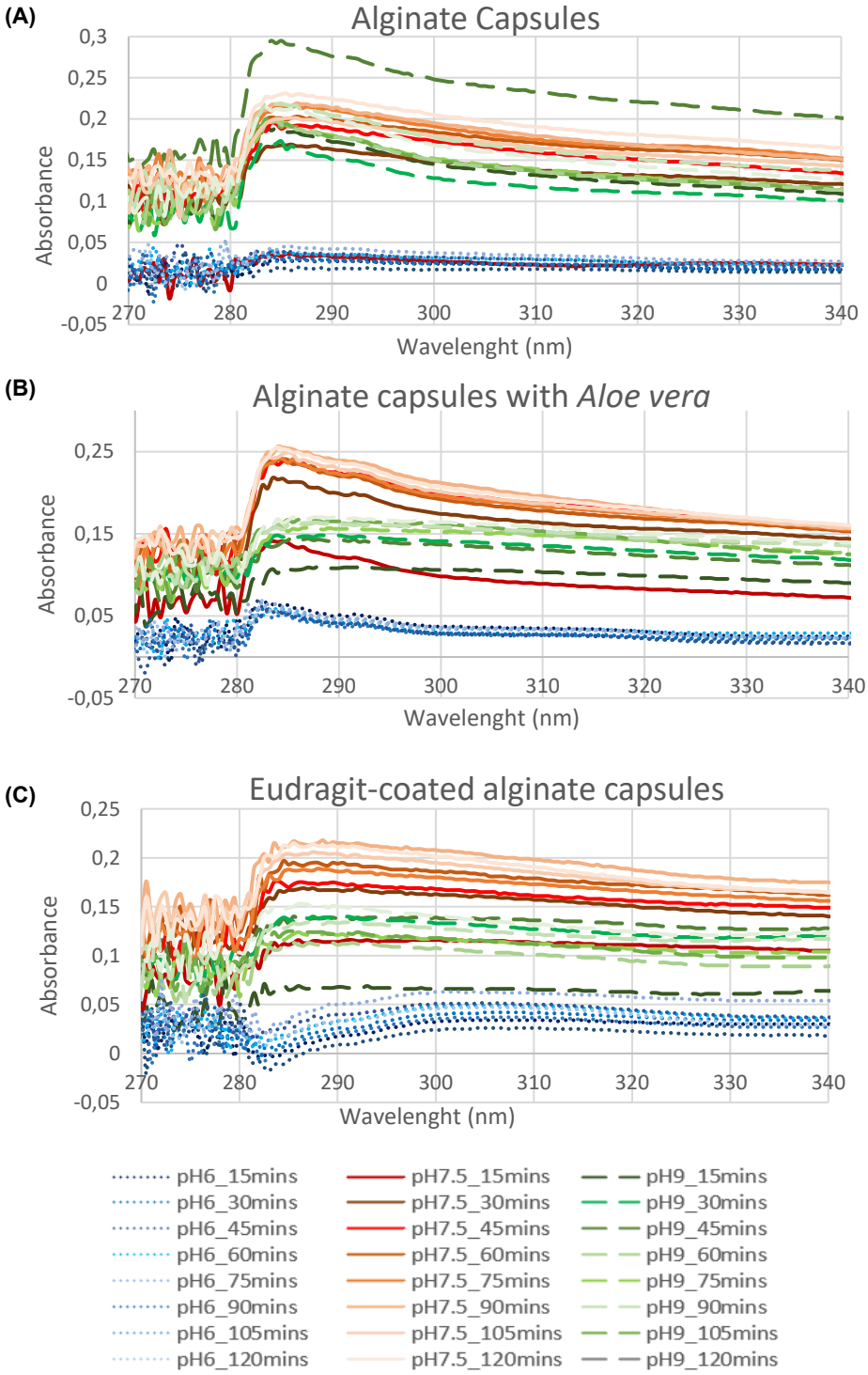


Figure 5.16- UV-Vis absorption spectra of the alginate capsules (A), alginate capsules with *Aloe vera* (B) and Eudragit-coated alginate capsules (C), along the time at different pHs (6, 7.5 and 9).

Table 5.5- Summary table with the peak values from the obtained UV-Vis absorption spectra of alginate capsules with and without *Aloe vera* and Eudragit-coated alginate capsules, along the time at different pHs.

		15 mins	30 mins	45 mins	60 mins	75 mins	90 mins	105 mins	120 mins
Alginate capsules	**pH 6**	0,03	0,029	0,032	0,037	0,038	0,039	0,041	0,045
	pH 7.5	0,036	0,169	0,195	0,203	0,216	0,219	0,201	0,231
	pH 9	0,192	0,295	0,173	0,198	0,194	0,197	0,222	0,212
Alginate capsules with *Aloe vera*	**pH 6**	0,068	0,07	0,055	0,058	0,06	0,069	0,058	0,067
	pH 7.5	0,141	0,218	0,239	0,241	0,242	0,256	0,249	0,255
	pH 9	0,11	0,146	0,148	0,157	0,167	0,164	0,164	0,17
Eudragit-coated alginate capsules	**pH 6**	0,05	0,035	0,023	0,018	0,024	0,034	0,034	0,043
	pH 7.5	0,116	0,169	0,176	0,197	0,188	0,217	0,204	0,215
	pH 9	0,071	0,138	0,14	0,124	0,125	0,113	0,134	0,153

With these results, concerning the alginate capsules, it is noticeable that the absorbance is higher at pH 7.5, followed by pH 9 and lastly pH 6, although there was a more prominent peak, correspondent to the 30 minutes in pH 9, most likely from a measurement error since it is very misplaced. As mentioned before, this is the most expected release rate for alginate capsules, which was not obtained in the previous study, but are in agreement with the results from the remaining analyses in this work. It was also detected a lot of noise prior to 280 nm, which could probably be improved with the use of quartz cuvettes instead of disposable ones, which supposedly only measure absorbance correctly above 340 nm. The capsules were also visibly completely degraded from early on, probably influencing the baseline values as described in the studies previously presented.

Most importantly, it can be observed that the results are very similar between the three samples. All the spectra have the peak at approximately the same wavelength, approximately between 280-290 nm. Additionally, in regard to the alginate capsules with *Aloe vera*, the extract was not detected or represented by its own peak in the spectra, that should have been around 241 nm according to previous measurements. This indicates that the method of evaluation in question might not be the most appropriate for this type of analysis, or that the *Aloe vera* extract might not be the best out of the additives to evaluate by UV-Vis, having an absorbance peak so close to the alginate peak, as observed previously in figure 5.11. Either way, more studies need to be performed with different capsules and other additives to see if it is possible to have a more accurate analysis of the oils and extracts' release. Therefore, it was concluded that the peaks represented in figure 5.16 and table 5.5 are most likely to be related with the alginate, instead of the *Aloe vera* extract.

In another test, Eudragit RLPO was used with the aim to reduce the degradation rate of the capsules, since the final objective is a release for a long period of time, and the capsules with alginate alone have already shown to be almost completely degraded after only 120 minutes. The addition of an Eudragit coating to the capsules was based on some published research studies that stated that Eudragit RLPO, although pH independent, could be used as a time-dependent release control. Among these studies, one provided by Hasan *et al.* (2013) revealed that a higher percentage of Eudragit RLPO on the prepared microspheres, although being less efficient than Eudragit RSPO, retarded the release of metformin HCl, an oral antidiabetic drug (Hasan *et al.*, 2013). Another research by Puttegowda *et al.* (2016) revealed that the release of Pravastatin Sodium, which is a group of lipid's regulating drug, was

much slower in Eudragit RLPO and Eudragit RSPO coated capsules compared to uncoated ones, achieving the desired purpose which was a controlled release in the small intestine (Puttegowda *et al.*, 2016).

Nevertheless, the results obtained relatively to the Eudragit-coated alginate capsules did not show any significant delay on the degradation rate, since the absorption spectrum observed in figure 5.16 is quite similar to the one for the alginate capsules, not contributing to the intended aim of the project.

In order to find a more suitable capsule formulation for the additives' release, other protocols were posteriorly tested. One of the goals now was to discover a way to encapsulate sunflower oil, and with this research an interesting concept was found. The use of shellac to adapt the release in capsules has been tested and, depending on the concentrations, it can increase the pH at which the alginate capsules release the encapsulated additive, as pretended. With this, shellac-alginate and alginate capsules with sunflower oil were prepared, to test the effect of shellac in the release of the additive.

After the acquisition of other phosphate salts, a new phosphate buffer solution, made with sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) and sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$), was used. This buffer provides a more adequate and reliable way to measure pH-dependent release of the capsules' content, being adjusted to pH 6, 7.5 and 9 for the following tests.

5.3.3. pH time-dependent study on the effect of shellac on the capsules' release

Regarding shellac applications in drug-delivery systems, there are some interesting studies involving shellac capsules, such as the one described by Morales *et al.* (2017), which aim at intestinal release, used pH values of 1, 4 and 7 for the pH-dependency test of capsules developed from alginate-shellac. The environment of the human digestive tract varies greatly in terms of pH, considering the stomach, small intestine, and colon, the last one being the main target of release in most studies, having a more alkaline pH than the others. This goal was achieved, and the capsules presented an unaltered swelling percentage at acidic conditions and a higher swelling percentage at pH 7, which consequently resulted in a higher release of the oil content. With this, shellac is used by many studies as a way to increase the pH at which occurs the release of the active agent in the capsules, frequently used to achieve release at intestinal digestion (Morales *et al.*, 2017).

Although many of these studies don't investigate the release of the capsules at alkaline conditions since the main explored application has been linked to gastro-resistance, the effect of shellac in increasing the release of the capsules' content when at higher pHs, suggests that this system could potentially work for wound healing applications. With the analysis of the obtained absorption spectra, exhibited in figure 5.17 and table 5.6, the effect of shellac on the release of the capsules was evaluated, based on the pH time-dependent study performed at pH 6, 7.5 and 9 during the course of 2 hours.

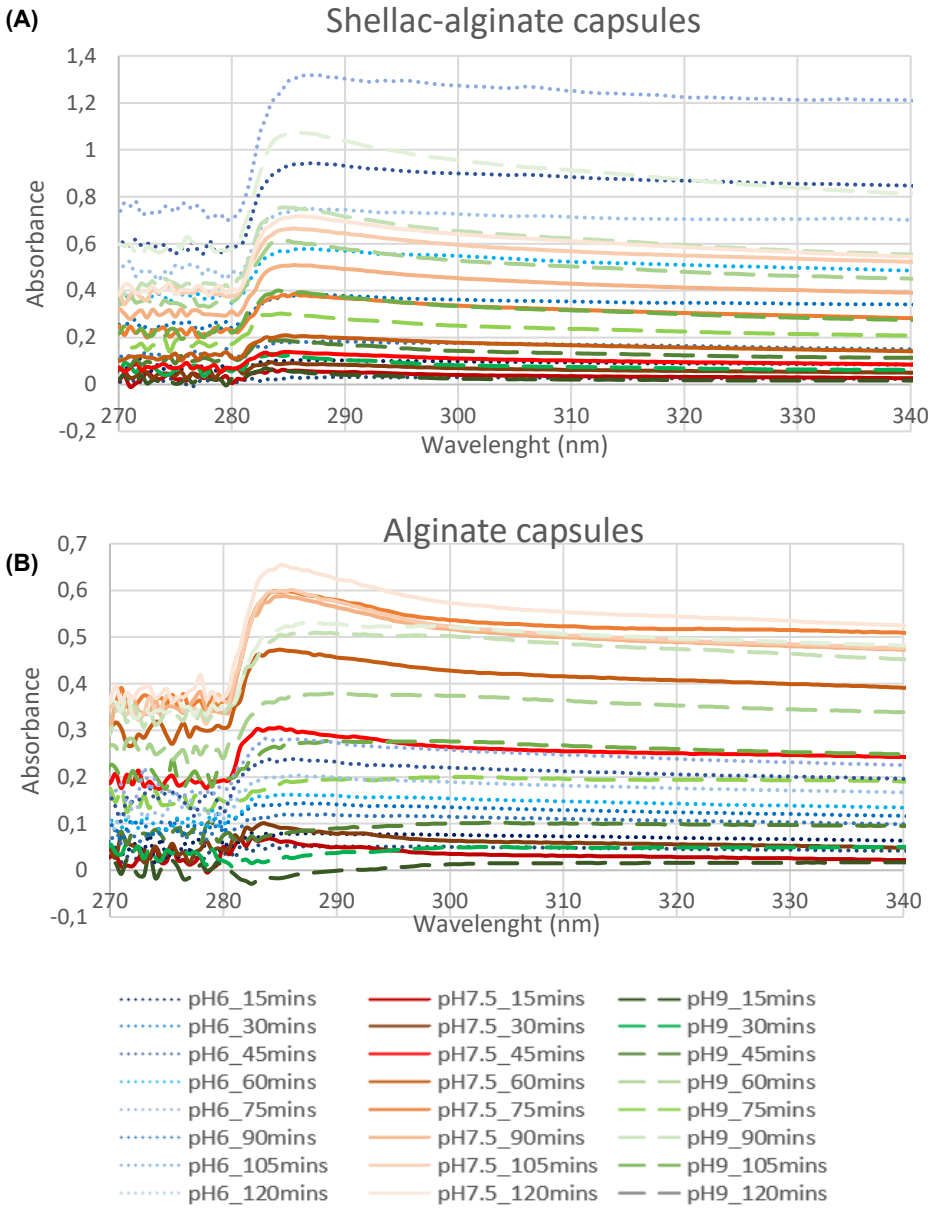


Figure 5.17- UV-Vis absorption spectra of the shellac-alginate capsules (A) and alginate capsules (B), along the time at different pHs (6, 7.5 and 9).

Table 5.6- Summary table with the peak values from the obtained UV-Vis absorption spectra of shellac-alginate and alginate capsules, along the time at different pHs.

		15 mins	30 mins	45 mins	60 mins	75 mins	90 mins	105 mins	120 mins
Shellac-alginate capsules	**pH 6**	0,033	0,105	0,184	0,383	0,578	0,75	0,943	1,317
	pH 7.5	0,065	0,095	0,139	0,209	0,386	0,509	0,665	0,717
	pH 9	0,07	0,131	0,188	0,302	0,4	0,612	0,755	1,073
Alginate capsules	**pH 6**	0,055	0,08	0,12	0,145	0,164	0,204	0,239	0,283
	pH 7.5	0,069	0,101	0,306	0,471	0,598	0,588	0,601	0,654
	pH 9	0,001	0,037	0,09	0,195	0,277	0,379	0,509	0,53

The alginate capsules demonstrated the behaviour already detected in previous studies, being dependent on the pH of the solution in which they are found, dissolving more easily, in descending

order, at pH 7.5, 9 and 6. On the other hand, all the capsules with the shellac layer resisted total degradation after two hours, but a greater degradation of the submerged capsules was visible at pH 6, then at pH 9 and finally at pH 7.5, as also represented in the results obtained.

The objective of this study was not achieved with the method used, considering that both the degradation and the release were lower at pH 7.5. This could be due to the interactions between the shellac used and the alginate, being able to alter disintegration, mechanical and swelling properties. It could also be related to the need for a higher shellac concentration and even the use of other components to improve the properties and connection of the shellac layer, such as plasticizers or other water-soluble polymers.

With the results obtained, it is also possible to verify that the absorption peak of sunflower oil is not visible, permitting the analysis of only the alginate, since this compound, most likely, overlaps the spectrum of the oil, which is also not ideal for the analysis of the intended release. The correlation between the degradation of alginate and the release of the active agent was used throughout the UV-Vis analysis, allowing to obtain some conclusions. However, a way to detect the additives is still needed to assure their encapsulation, and eventually quantify the amount of encapsulated and released additive.

5.4. Fourier-transform infrared spectroscopy analysis (ATR-FTIR)

All the capsules made with the optimized process, with compressed air, were analysed through ATR-FTIR in order to explore whether the encapsulation was successful and the detection of the additives in the spectra was possible, which could be very valuable for future tests regarding the release of the additive. The first analysis was concerning the presence of *Aloe vera* extract, which, for this matter, a graphic demonstrating the spectra of the alginate capsules, alginate capsules with *Aloe vera* extract and the spectrum of the *Aloe vera* extract was studied (figure 5.18).

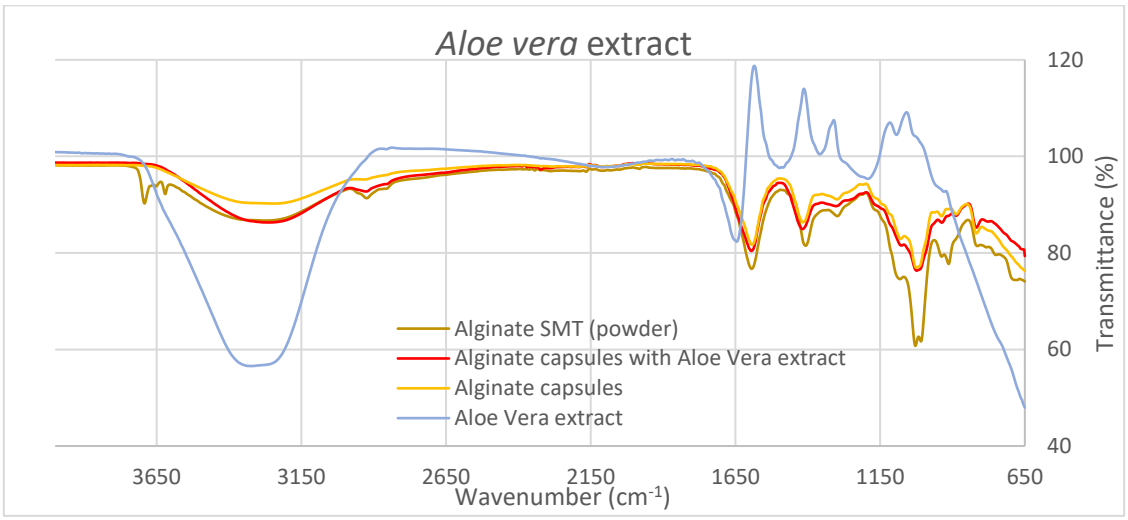


Figure 5.18- ATR-FTIR spectra of alginate capsules with and without *Aloe vera* extract, *Aloe vera* extract, and Alginate SMT in powder form.

As it can be observed, the *Aloe vera* extract is associated with two main bands, a large one between the wavenumbers of 3350 cm^{-1} and 3245 cm^{-1}, which is associated with the stretching vibration of the hydroxyl group (O-H), and another one at 1645 cm^{-1} which is related to the absorption band of carboxyl groups, specifically to the asymmetrical COO^- stretching vibration (Nejatzadeh-Barandozi & Enferadi, 2012; Pereira *et al.*, 2011).

By observing the alginate capsules with *Aloe vera* and comparing them with the alginate capsules, the bands are primarily coincidental. The band associated with the O-H groups is still predominant, around 3263 cm^{-1}, other bands are present, approximately at the wavenumbers of 1595 cm^{-1} and 1418 cm^{-1}, attributed to the asymmetrical and symmetrical COO^- stretching vibrations, respectively. The small band around 1302 cm^{-1} is due to the C-O group and the one at 1025 cm^{-1} is associated with the C-O-C group. These results of the alginate capsules' spectrum are very similar to the ones in the literature (Lawrie *et al.*, 2007).

The showed graphic, as mentioned, demonstrates fewer differences between the bands of the alginate capsules and the alginate capsules with *Aloe vera*, which indicates that it was not possible to detect this additive, substantially at least, using ATR-FTIR. Although the obtained results did not allow to significantly detect *Aloe vera* or show any alterations that were indicative of chemical structure changes in the alginate, some studies correlate the addition of *Aloe vera* to the alginate with the decrease on the absorption band of some groups. This is described mainly in relation to the larger band, linked to the hydroxyl group, explained by the presence of polysaccharides and higher strength hydrogen bonds. This is stated along with the acknowledgement of the slight shift regarding the wavenumber of the bands, when comparing the alginate with and without *Aloe vera*, associating these facts to the establishment of interactions between these two components (Pereira *et al.*, 2011; Bialik-Was, Raftopoulos and Pielichowski, 2022).

The second FTIR analysis was made regarding the presence of the sunflower oil in the capsules. For this matter, a graphic with the spectra of the alginate capsules with and without sunflower oil was obtained (figure 5.19), along with the spectra regarding the sunflower lecithin, alginate powder and sunflower oil used in the protocols.

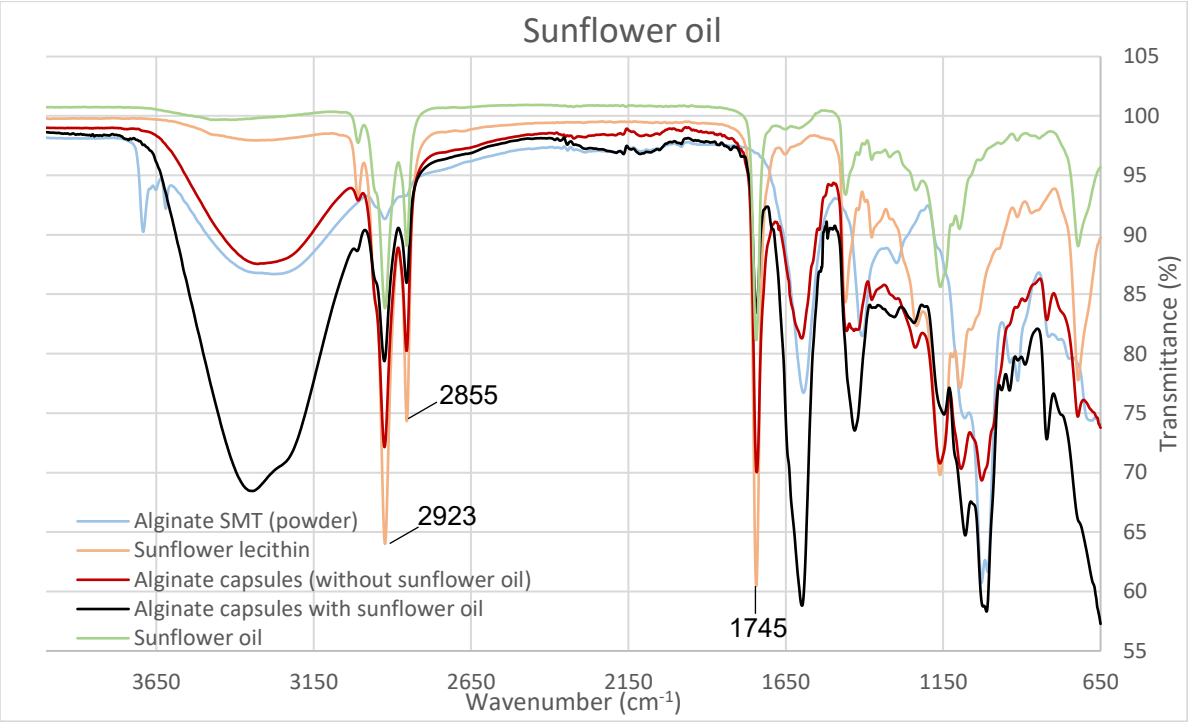


Figure 5.19- ATR-FTIR spectra of the alginate capsules with and without sunflower oil, sunflower lecithin, alginate powder and sunflower oil.

At the obtained graphic some important bands can be observed in the sunflower oil spectrum. A small band is shown around 3473 cm^{-1}, associated with the overtone of -C-O ester, an intermediate band at the wavenumber of 3009 cm^{-1}, related to the stretching of -C-H (cis) , two other bands at 2923 cm^{-1} and 2854 cm^{-1}, representative of the asymmetric and symmetric stretching of -C-H (CH_2), respectively, a strong one at 1744 cm^{-1}, characteristic of the stretching of -C-O ester, another band at 1460 cm^{-1}, associated with the bending of -C-H (CH_2, CH_3) and one at 1161 cm^{-1}, along with a few more near that wavenumber, that are related to the stretching vibrations of -C-O groups of esters. A very similar sunflower oil spectrum was also exhibited by Guille and Cabo, corroborating the acquired results (Guille & Cabo, 2002; Hamed & Allam, 2006).

It is also evident that, although the bands are more prominent in the lecithin spectrum, the bands of lecithin and sunflower oil coincide, probably since both derive from sunflower, so it may not be possible to observe differences between these components using this type of analysis.

With this, it is apparent that the spectrum for alginate capsules (without sunflower oil) corresponds, as expected, to the junction of the spectra corresponding to alginate and lecithin. The spectrum presents the bands related to alginate, already described above when the alginate capsules were referenced, with the addition of certain bands characteristic of lecithin, such as a small one with a wavenumber of 3009 cm^{-1}, associated with the stretching of the -C-H, the stronger bands at 1745 $cm^{-1}$and 1161 cm^{-1} assigned to the stretching of -C-O groups of esters, as described earlier. Additionally, although the bands represented at around 2923 cm^{-1} and 2855 cm^{-1}, concerning the stretching of -C-H, are common bands between the lecithin and the alginate, they present a much higher intensity in the lecithin spectrum.

Furthermore, it is possible to observe, by the spectrum of the alginate capsules with sunflower oil, that the spectrum is similar to the correspondent to the alginate capsules without sunflower oil, for the reasons described previously, but presents lower absorption bands at the lecithin characteristic wavenumbers and higher at the ones associated with the alginate. This could be explained due to the different quantities of alginate and lecithin since the capsules with sunflower oil have less lecithin.

After analysing all the spectra, the main conclusion is that it was not possible to detect the sunflower oil separately in the capsules, however, it can be confirmed that the encapsulation of the additive was successful, since the emulsion prepared with lecithin and sunflower oil was detected, although combined, in the capsules. This means that, by correlation, the study of the capsules' oil release could be done by analysing the emulsion, if prepared correctly, as a whole. For future studies the use of a different type of lecithin, such as soy lecithin, could be utilized in order to possibly analyse the additive separately.

The final study performed was a pH and time-dependent evaluation on the release of sunflower oil from the alginate capsules (figure 5.20), as an attempt to seeing results based on the previous statements. The pHs of the solutions were, once again, used to simulate the different wound environments during various stages of infection. Healthy skin/ normal wound healing environment is associated with the pH of 6 and the pHs of 7.5 and 8.7 representing a wound and infected wound at different degrees.

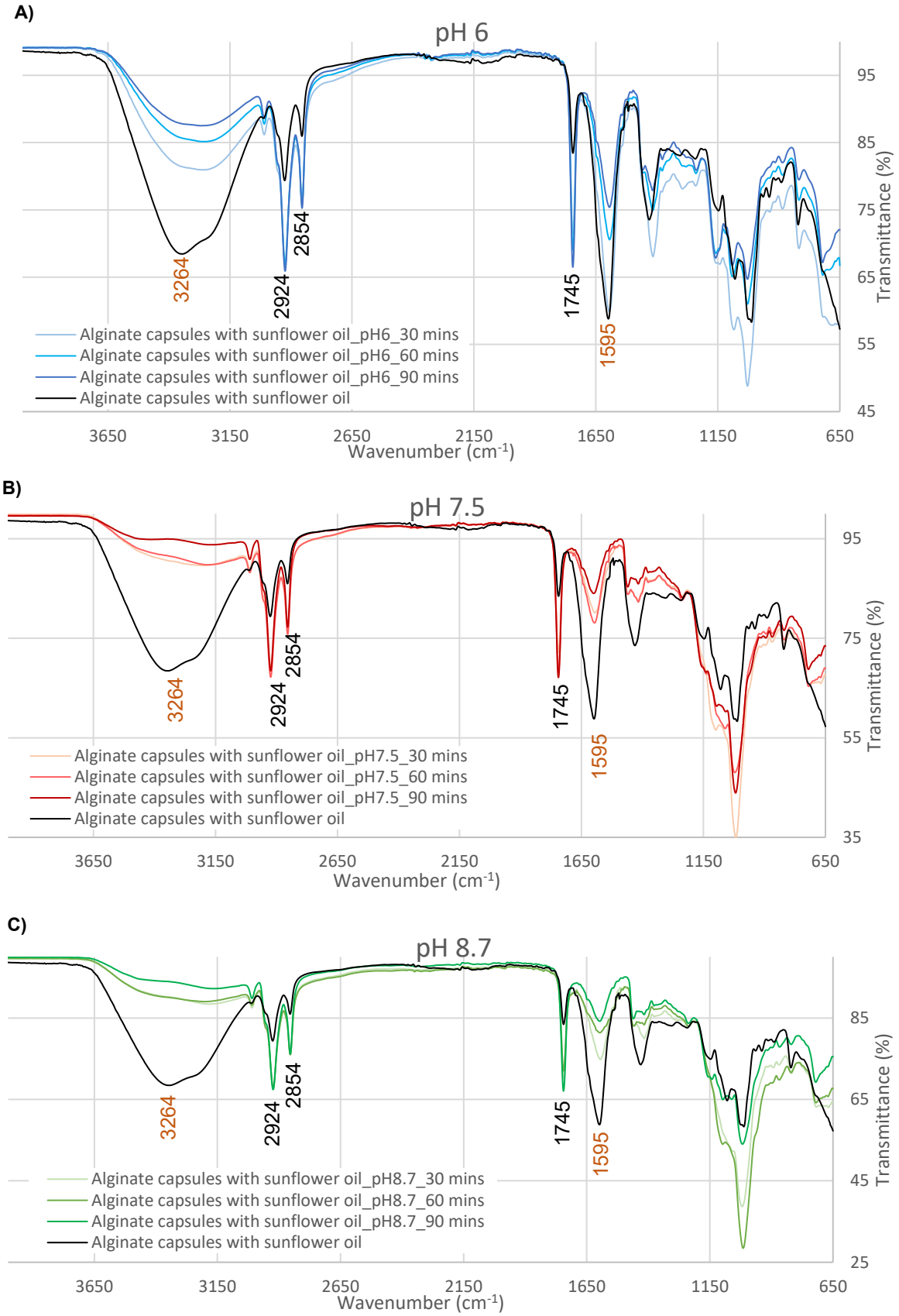


Figure 5.20- ATR-FTIR spectra of the alginate capsules with sunflower oil along the time at different pHs: (A) pH 6; (B) pH 7.5 and (C) pH 8.7.

The analysis of the graphs was performed by observing the bands representative and exclusive of alginate, such as 3264 cm^{-1} and 1595 cm^{-1} and the bands associated with lecithin and sunflower oil, represented by the wavenumbers of 2924 cm^{-1}, 2854 cm^{-1} and 1745 cm^{-1}, in order to compare the behaviour of the capsule wall material with the release of the additive.

Considering the fact that weaker bands, represented in the graph, are indicative of smaller quantities of the component associated with that band, it can be observed that the amount of alginate in the capsules gradually decreases with time. This is verified at all pHs, in the great majority of the bands, following the expected pattern, since it is associated with the degradation of the alginate. As already proven at the previously performed tests of alginate capsules using the UV-Vis spectrophotometer, it is also evident the difference in the degradation rates at each pH, showing a higher rate of alginate degradation at pH 7.5, followed by pH of 8.7 and finally pH 6. Consequently, by analysing the main and most distinguished band of the sunflower oil, at the wavenumber of 1745 cm^{-1}, the increase on the concentration of it along the time is clear in all the graphs. This would be anticipated based on the drop in alginate concentration, but also indicates that the release of the additive is not occurring as expected. The rest of the bands associated mainly with the additive, at the wavenumbers 2924 cm^{-1}, 2854 cm^{-1}, do not alter significantly throughout the time, not providing many indicative results. This is probably related to the fact that these peaks, although with a much lower intensity, are also observed in the alginate spectrum, influencing the values at all the pHs.

The release of the sunflower oil was not evident through this study since the concentration did not decrease significantly in any solution and would require more studies on this matter. These results could be related to the degradation of the wall of the capsules not being fully completed after the 90 minutes, not allowing the oil to be released or even with the unsuccessful or uneven encapsulation of the active agent. Therefore, other evaluations could be made with more samples throughout a longer period and with more adequate equipment, possibly allowing a more informative and accurate analysis.

5.5. Textile analysis after capsule's impregnation

5.5.1. Impregnation of the chitosan-alginate 8-layered alginate capsules

The first attempt to perform the conventional method for impregnation, dipping the textile into the dispersion with the capsules and using the *Foulard* machine, did not work for the capsules used (obtained with the syringe method). Even with the lowest pressure setting, that still allows the two rollers to move, the capsules collapsed. Immersing the textile in the impregnation dispersion was also proved to not be the most efficient method for the dispersion in question, as most of the capsules did not get bonded in any way into the textile, remaining in the dispersion when removing the textile.

Therefore, the next attempts included pouring the impregnation dispersions into the softer side of the textile and the samples did not go through the *Foulard* machine. Using this method, different dispersions were studied in order to optimize the impregnation of the capsules. Dispersions with and without crosslinkers were tested, using glutaraldehyde, glyoxal and citric acid with SHP (as a catalyst), as demonstrated in figure 5.21. The analysis, by visual inspection of the samples obtained, indicated that the use of crosslinkers improves slightly the fixation of the capsules to the textile.

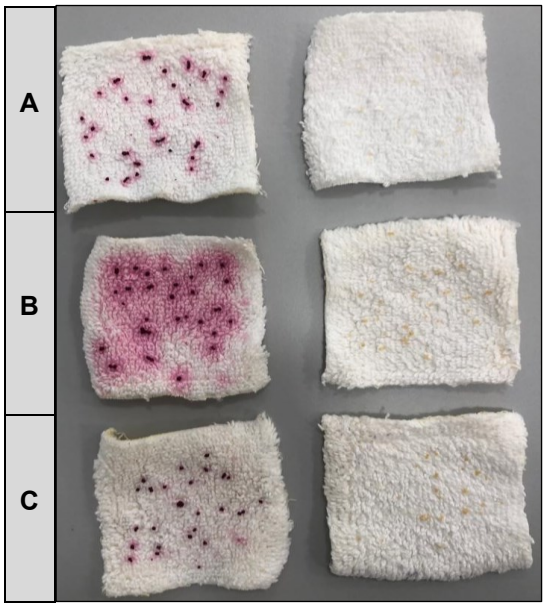


Figure 5.21- Impregnation of the chitosan-alginate 8-layered alginate capsules. Impregnation aqueous dispersion composition: A) Glutaraldehyde and capsules with dye, on the left (sample 2), and without dye, on the right (sample 3); B) Glyoxal and capsules with dye, on the left (sample 4), and without dye, on the right (sample 5); C) Citric acid and SHP and capsules with dye, on the left (sample 6), and without dye, on the right (sample 7).

Despite everything, the impregnated capsules are not very attached, possibly because they are too big and heavy. Although there is no apparent difference between the crosslinkers, regarding the fixation of the capsules, fabric washing tests are likely to be necessary to detect if these differences exist or not. Additionally, by observing the results, the only visual parameter that distinguishes the samples is the quantity of dye that is left around the capsules after the drying procedure. It can be seen that the sample with glyoxal contains a bigger stain of dye around the capsules, giving the idea that glyoxal might destroy slightly the capsules. With this, the glutaraldehyde and citric acid capsules present a more sustained release, appearing to show a higher degree of crosslinking in comparison with glyoxal crosslinked capsules.

The addition of surfactants was also tested, in this case Tween 80 and Imerol JET were used (figure 5.22), as an attempt to homogenize the dispersion and improve the spreading of the capsules through the textile. However, no significant differences were observed between impregnations with and without surfactants, except the fact that sample with Tween 80 contains a larger stain of dye around the capsules. This could mean that Tween 80 worked better as a surfactant, reducing surface tension of liquids and resulting in better wetting of the capsules by the liquid solution, possibly being a greater

option for the future studies with the capsules containing the additives. On the other hand, it can also mean that it destroyed slightly the capsules, similarly to what happened with glyoxal.

Figure 5.22- Impregnation of the chitosan-alginate 8-layered alginate capsules with dye. Represented on the left is sample 8, which impregnation dispersion composed by glutaraldehyde and Tween 80. On the right is sample 10, impregnated with a glutaraldehyde and imerol dispersion.

The posterior analysis on the adjustment of the pH to 9 with Na_2CO_3 was made (figure 5.23) to compare all the solutions with the same pH, since it has been proven to influence the release of the capsules content and, in addition, to simulate the pH of an infected wound.

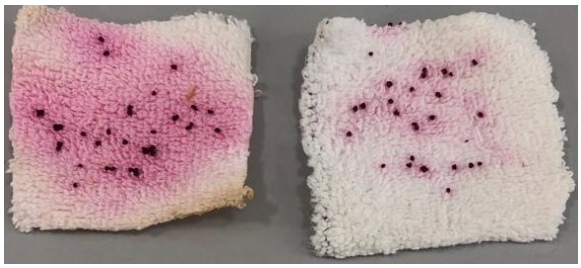


Figure 5.23- Impregnation of the chitosan-alginate 8-layered alginate capsules with dye. Represented on the left is sample 12, which impregnation dispersion was composed by glyoxal, with the addition of Na_2CO_3 to adjust the pH till 9. On the right is sample 14, impregnated with a glutaraldehyde with the addition of Na_2CO_3 to adjust the pH till 9.

The pH adjustment happens to, as predicted, only contribute to the release of the capsules' content into the dispersion at a higher rate, showing an even greater dispersion of the dye in the sample with glyoxal. Since both solutions had the same pH, it only emphasises the fact that glutaraldehyde presents a higher crosslinking degree, as described before.

Regarding fixation, all the previously explored samples showed that it is not the desired attachment for the intended goal and that the capsules would need decrease greatly in size in order for the impregnation on the textile to function. The last parameter that was tested with these capsules was the addition of another step before impregnation, the drying of the capsules. The capsules decreased greatly in size but difficulted the incorporation in the structure of the textile since they hardened quite significantly, confirming the fact that the size of the capsules itself needs to decrease. Therefore, the next impregnation tests were made with the capsules obtained with the compressed air method.

5.5.2. Impregnation of the capsules made with the compressed air method

Although the resulting capsules presented a very significantly smaller size, the first impregnation using the *Foulard* machine still caused the capsules to collapse. On the other hand, the sample that was not exposed to pressure, sample 18, during the impregnation procedure, showed a much more suitable fixation of the capsules. Even though the size of the capsules has improved majorly, the distribution of the capsules on the textile structure could still be improved.

The first attempt to obtain better results regarding this matter involved thickening the impregnation dispersion. The use of alginate and Tubicoat HEC (a natural based thickening agent) in different concentrations was tested, as exhibited in figure 5.24.

Figure 5.24- Impregnation of the alginate capsules with *Aloe vera* extract, corresponding to sample 21 (on the right), and of the alginate capsules made with the same method, representing sample 22 (on the left). Both impregnation solutions containing Tubicoat HEC at the concentration of 2.5% w/v and 1.25%w/v, for sample 21 and 22 respectively.

The alginate in the solution caused the capsules to stick together, since they are also made of alginate, which is not the intended result. The Tubicoat HEC in the solutions facilitated the even distribution of the capsules on the textile, although it still did not function with the pressure of the *Foulard* machine, as the textiles sample stick to the rolls and did not pass between them. Therefore, the method of pouring the dispersion over the textile and dry this directly without passing in the *Foulard* was used. However, even with the lowest amount of Tubicoat HEC used, the textile samples became hard and not very malleable after drying, so this thickener was discarded.

The best option according to the last results was to test the solutions with different crosslinkers in order to optimize the impregnation process, which in this case were glutaraldehyde, glyoxal and citric acid (figure 5.25), and stir them with the capsules for a certain amount of time to assure the evenness of the dispersion.

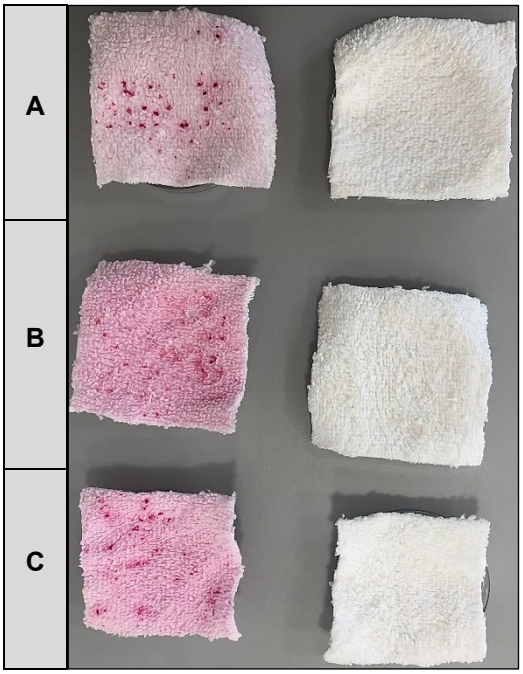


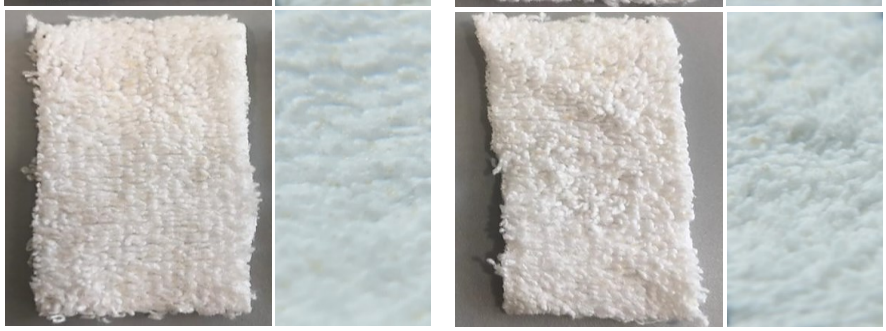
Figure 5.25- Impregnation of the alginate capsules, with and without dye, made with the compressed air method, using there different crosslinkers. Composition of the impregnation aqueous dispersion: A) Glutaraldehyde and capsules with dye, on the left (sample 23), and without dye, on the right (sample 24); B) Glyoxal and capsules with dye, on the left (sample 25), and without dye, on the right (sample 26); C) Citric acid and SHP and capsules with dye, on the left (sample 27), and without dye, on the right (sample 28).

The capsules with dye were not very homogeneous between themselves, so the impregnation of the alginate capsules without dye worked much better. When visualizing at the naked eye, all samples seemed to be quite impregnated, with the capsules intact and fixed to the textile, even if the distribution was still not with the desired uniformity. However, it was not possible to observe any significant difference between them regarding the fixation of the capsules, including the capsules of the control sample (sample 29) - impregnation without any crosslinker.

5.6. Textile analysis after a gentle wash

The gentle wash was performed to evaluate the different crosslinkers (glutaraldehyde, glyoxal and citric acid) used and therefore optimize the impregnation dispersion. The water from the wash was absorbed through all the samples and the great majority of the capsules stayed fixated and still well impregnated on the textiles, including the control sample (sample 29), without crosslinkers (table 5.7).

Table 5.7- Images of the resulting textile samples before and after the gentle wash

Sample no.	Before the gentle wash	After the gentle wash
24		
26		
28		
29		

The results confirmed that the impregnation was successful, since the capsules remained attached, at least during the course of a subtle wash. As these textiles are intended to be used as wound dressings, they will not be reusable, so there is not aim in washing resistance. This gentle wash was just an attempt to simulate some movement in a wound bed and see if there is a different between capsules bonding to the textile between crosslinkers. The resultant samples were not exceptionally clear in regard to assessing impregnation dispersions, mainly because the distribution of the capsules was not even throughout the textile structure, making the comparison between samples challenging. In an effort to explore this topic further, a more extensive analysis was made by observing the textiles through the stereoscope (figure 5.26), also acquiring conclusions about the shape, size and distribution of the impregnated capsules from the different samples.

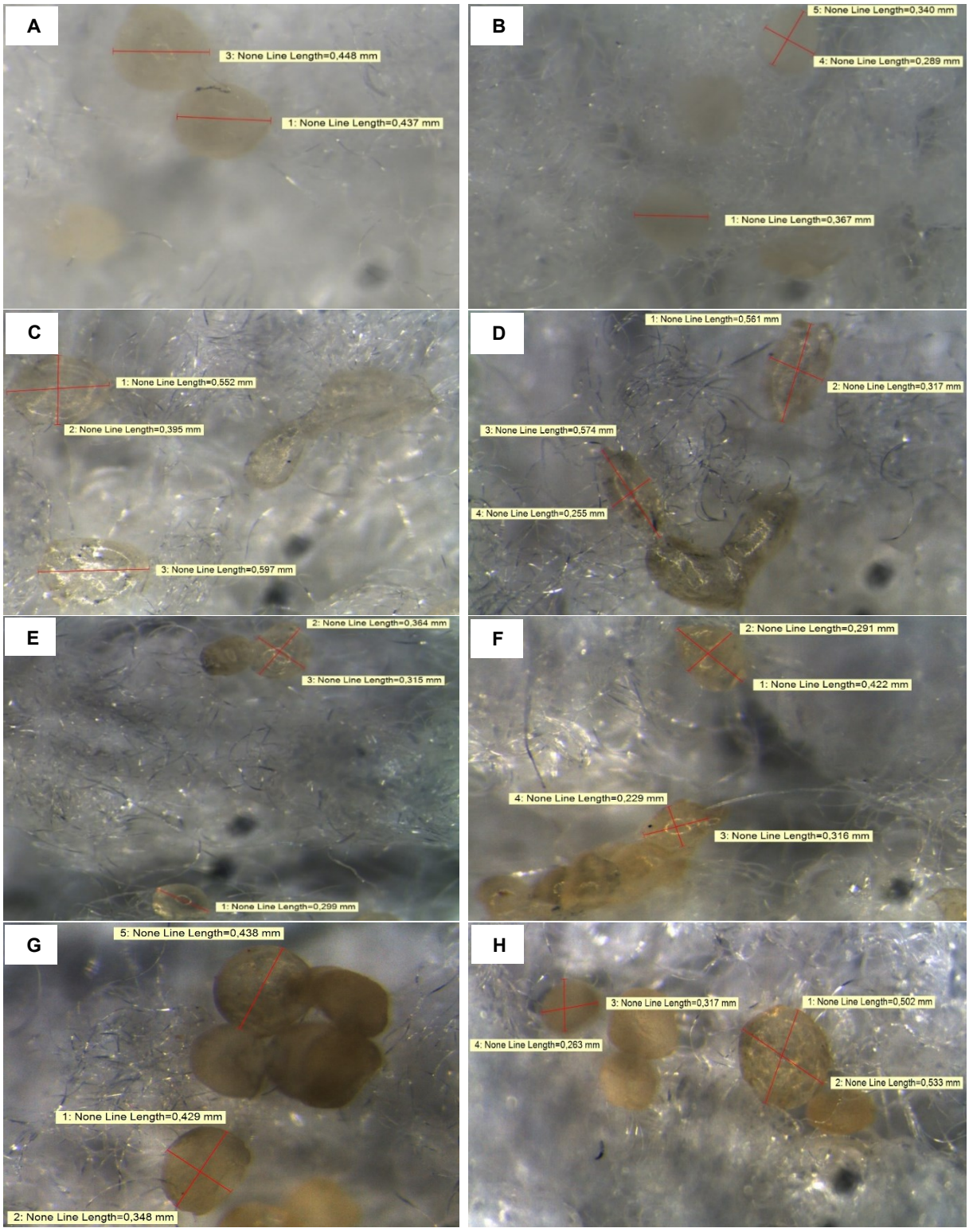


Figure 5.26- Microscopic image of the alginate capsules impregnated in each of the textile samples, before and after the gentle wash, with the corresponding measurements, using a stereo microscope (32x magnification): A) Sample 24 before the gentle wash (glutaraldehyde solution); B) Sample 24 after the gentle wash (glutaraldehyde solution); C) Sample 26 before the gentle wash (glyoxal solution); D) Sample 26 after the gentle wash (glyoxal solution); E) Sample 28 before the gentle wash (citric acid solution); F) Sample 28 after the gentle wash (citric acid solution); G) Sample 29 before the gentle wash (without crosslinkers); H) Sample 29 after the gentle wash (without crosslinkers).

The obtained images did not clarify the differences between the samples with distinct impregnation dispersions, since the distribution of the capsules was extremely uneven and their fixation was not homogeneous throughout the whole textile structures, making the microscopic images not reliable for conclusions on this matter. In order for this comparison to be possible, future tests would have to be done using an optimized method of capsule impregnation or a more adequate consistency for the capsules to spread homogeneously, as for example, applying the dispersions as a low viscosity hydrogel, containing a surfactant (or mixture of) that would assure the even dispersion of the capsules throughout the gel.

Furthermore, the before and after the gentle wash microscopic images, taken from different parts of the sample, allowed the analysis of other important parameters such as the size and shape of the capsules after the impregnation process. Samples 24, which contained glutaraldehyde, sample 28, with citric acid and sample 29, the control sample, all appear to present capsules with a much rounder shape when comparing with the other sample. Even though the size of the capsules can slightly vary, not being the most consistent, all these samples show capsules with similar measurement proportions such as 429 µm by 348 µm and 289 µm by 340 µm.

On the other hand, the capsules captured from sample 26, impregnated with glyoxal, appear to be often longer and more irregular, showing measurements such as 561 µm by 317 µm and 552 µm by 395 µm. With this, it is apparent that glyoxal might destroy slightly the capsules, as described previously, which would explain the non-ability to achieve a more spherical shape. Regarding this matter, a more in-depth analysis with a larger number of replicated samples would have to be made to produce a clear conclusion.

6. Conclusions

The wound healing process is extremely complex, and it is crucial to provide a suitable wound dressing that can create a wound-healing microenvironment by possessing all the important properties such as exudate extraction, moisture control, reduction of bacterial infections, long-lasting application and easy removal, while maintaining the material volume. It this work, progresses have been made towards developing the interface layer of an optimized high-absorption multi-layered dressing, containing natural encapsulated functional additives, namely sunflower oil and *Aloe vera* extract, with a controlled release.

The capsules were successfully produced by the external ionic gelation method and the incorporation of compressed air benefited the process, resulting in more homogeneous, spherical and smaller capsules, being able to achieve capsules with diameters bellow 1000 µm, which are more suitable for impregnation in the textile than larger capsules.

Between all the wall materials tested in order to obtain a pH-dependent control release of the active agents, the alginate-chitosan capsules formed through the layer-by-layer method have proven to be superior. They demonstrated a greater and faster release around pH 7.5, followed by pH 9, when compared to a more acidic pH, which is the desired range, since the pH of a wound is around 6-7.5, increasing to more alkaline pHs, between pH 7-9 when infected. The layering with chitosan has shown advantages, especially with a higher number of layers, in relation to the capsules containing only

alginate because the release is slower in general, providing a more long-lasting effect. Additionally, the other materials tested for the wall of the capsules, eudragit and shellac, did not show potential of application for this purpose. The eudragit coating on alginate capsules did not contribute for a delayed degradation of the capsules and the shellac-alginate capsules, although more resistant to degradation, did not provide the pH-dependent release at the suitable pH range for wound healing.

Additionally, the detection of the additives in the capsules, essential to assure their encapsulation, was not achieved through UV-Vis spectrophotometry, since the alginate spectrum most likely overlaps the additives' spectra. For this same reason, the ATR-FTIR analysis was also not able to detect *Aloe vera* and, concerning the sunflower oil, the detection was only possible in conjunction with the sunflower lecithin.

Moreover, concerning the impregnation process, the most effective way was discovered to be through the pouring of the dispersions directly over the textile. All the capsules made with compressed air were fixated to the textile structure, the majority even after a very gentle wash. Regarding crosslinkers, glutaraldehyde and citric acid appeared to have been more suitable than glyoxal, which might destroy slightly the capsules, although further studies would have to be made.

7. Future work

The present work, as part of a very early stage in the development of the functionalized textile that will be incorporated into the final dressing, still exhibits several aspects that can be explored further and improved. One of the aspects that deserves a more extensive analysis is the concentration of some solutions involved in the capsules' development, such as the concentration of the calcium solution of the gelling bath and the alginate concentration used as a wall material. These, along with parameters such as the working distance and a controlled pressure of the compressed air, which was not possible to precisely define in this work, can all influence the capsules' morphology and properties. Other encapsulation techniques such as internal gelation, instead of external, could also be analyzed as an option to experiment if the capsules morphology and consistency is enhanced.

Concerning the process of impregnation, for a more homogenized distribution of the capsules in the textile structure, a more appropriate consistency is required, for example, by applying the dispersion as a low viscosity hydrogel with surfactants. This would allow a more controlled and reliable process, facilitating the upgrade of the dispenser method, also used in the cosmetic industry, to a possibly bigger scale in the future.

Furthermore, a crucial limitation that requires a solution to enable future release studies is the detection of the additives, which was not successful by using UV-Vis and ATR-FTIR. For the sunflower oil, the detection of future capsules could be made with a different type of lecithin, such as soy lecithin, so that, potentially, the bands do not overlap in the FTIR analysis. Primarily, further characterization methods would have to be used for the detection of sunflower oil and *Aloe vera* in the capsules. For example, gas and liquid chromatography could be used to separate and detect the components in the release solutions of the capsules, allowing an easy identification and quantification, facilitating the performance of the release studies (Moreda *et al.*, 2001; Nazeam *et al.*, 2017). Regarding the

morphology, a SEM analysis could also be performed in order to accurately evaluate the thickness of the wall and the type of capsule structure in question (Pedroso-Santana & Fleitas-Salazar, 2020).

As it is possible to conclude from the previous statements, there are numerous opportunities for improvement and development regarding the processes of encapsulation and incorporation of the additives in the textile structure. The researches for interactive antibacterial wound dressings, that provide suitable environments for efficient wound healing, are increasing, especially since the wound healing process and biochemical pathways involved in it are understood to a deeper extent nowadays. With this, continuous attempts need to be made to improve the limitations of the commercially available dressings.

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