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Development of Bioactive Microspheres for the Functionalization of Textile Materials

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Aos meus pais

aos meus irmãos

e ao meu avô,

“Algo só é impossível até que alguém o duvide e acabe provando o contrário”.

Albert Einstein

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Resumo

O desenvolvimento de têxteis médicos funcionais, incluindo os cosméticos, terá um impacto positivo na qualidade de vida da população já que os têxteis, ao funcionarem em contacto directo e não-obstrutivo com a superfície do corpo humano, podem funcionar como veiculadores de agentes terapêuticos ou ter um carácter de protecção contra infecções, adoptando uma acção preventiva aliada às características de conforto.

Desta forma, a funcionalização de materiais têxteis com microesferas bioactivas, permitirá obter produtos até agora impensáveis já que os agentes ou fármacos retidos no núcleo ou na membrana destas microesferas, podem exercer uma acção antimicrobiana e terapêutica.

Vários métodos, dependendo do agente activo e tipo de fibra, foram desenvolvidos ou encontram-se em desenvolvimento para conferir actividade antimicrobiana ao têxtil. Contudo, nem sempre as propriedades bioactivas pretendidas são conseguidas com elevada taxa de sucesso. Desta forma, uma nova abordagem passa pela investigação das condições mais adequadas de funcionalização da superfície de pensos de base têxtil, com microesferas bioactivas incorporadas, capazes de libertar agentes antimicrobianos e/ou fármacos, visando a prevenção e tratamento de infecções microbianas. Esta nova abordagem pretende estudar a formação de camadas superficiais antimicrobianas num processo sonoquímico para gerar e aplicar as microesferas no têxtil.

Este trabalho tem como objectivos o estudo da formação de microesferas através do processo sonoquimico com possíveis propriedades antimicrobianas para aplicação no material têxtil. Através do processo sonoquímico conseguiu-se a produção de microesferas estáveis com tamanho (2 µm) e morfologia (esferas) desejadas, em apenas 10 min. O segundo objectivo consistiu em avaliar as propriedades antimicrobianas no material têxtil através de métodos que fornecem informação qualitativa como o SEM e o método descrito na norma JIS L 1902:2002, tendo os resultados demonstrado que as amostras funcionalizadas possuem boa actividade antimicrobiana. A aplicação de novas técnicas como o LBL conferem às microesferas maior estabilidade. Este novo método torna as microesferas mais estáveis, contudo é mais moroso, uma vez que não se consegue fazer num único passo. O presente trabalho permitiu, ainda, o desenvolvimento de uma nova geração de materiais com propriedades antimicrobianas, à base de produtos totalmente naturais e mais amigos do ambiente, mais precisamente o óleo essencial *Lime oil*. O material têxtil funcionalizado com este agente antimicrobiano mostrou possuir excelentes propriedades antimicrobianas. Ficou provado que estes agentes são bastante promissores como agentes antimicrobianos para a funcionalização de fibras têxteis.

Palavras-Chave

Têxteis Antimicrobianos, Agentes Antimicrobianos, Concentração Mínima Inibitória (MIC), Infecções Microbianas, Biocidas, Quitosano, Microesferas, Ultra-sons, *Layer-by-Layer* (LBL).

Abstract

The development of functional medical textiles, including cosmetics, will have a positive impact on quality of life as textiles, when in direct contact and non-obstructive to the surface of the human body, can act as backers of therapeutic agents. Have adequate protection against infection by taking preventive action combined with the comfort features.

Thus, the functionalization of textile materials with bioactive microspheres will produce products previously unthinkable as agents or drugs in the nucleus or retained in the membrane of these microspheres may exert an antimicrobial and therapeutical action.

Several methods, depending on the active agent and type of fiber have been developed or are being developed to confer antimicrobial activity to textiles. However, not all the desired bioactive properties are achieved with high success rate. Thus, a new approach involves the investigation of more suitable conditions for surface functionalization of textile-based dressings with incorporated bioactive microspheres capable of releasing antimicrobial agents and/or drugs, preventing and treating microbial infections. This new approach aims to study the formation of surface layers antimicrobial sonochemical a process to generate and apply the microspheres in the textile industry.

This work aims to study the formation of microspheres by a sonochemical process with possible application in textile materials in order to provide antimicrobial properties. Through the sonochemical process it was possible to produce and bind to textile fibers stable microspheres with a size of 2 μm and a perfect spherical morphology, in a process that only takes 10 min. In addition it was evaluated the antimicrobial properties of the functionalized textile material using qualitative methods such as SEM and JIS L 1902:2002. These new antimicrobial agents have shown good antimicrobial activity, tested by these two methods mentioned. Moreover, and in order to obtain more stable microspheres, it was evaluated the application of new techniques such as LBL promotes. Results showed the success of this new approach by the greater stability of the LbL coated microspheres.
Finally, this investigation has contributed to the development of a new generation of antimicrobial materials developed tottaly with natural agents that are more environmentally friendly, in particular by the use of essential oils, more precisely Lime oil, which has shown excellent antimicrobial properties when incorporated in the microspheres that were linked onto the textiles.

Keywords

Antimicrobial Textiles, Antimicrobial Agents, Minimal Inhibitory Concentration (MIC), Microbial Infections, Biocides, Chitosan, Microspheres, Ultrasound, Layer-by-Layer (LBL).

List of Abbreviations

MW – Molecular Weight

MIC – Minimal Inhibitory Concentration

QACs – Quaternary Ammonium Compounds

PHMB – Polyhexamethylene Biguanides

E. coli – Escherichia coli

CS – Chitosan

Alg/CS – Alginate/Chitosan

Triclosan – 2,4,4'-trichloro-2'-hydroxydiphenyl ether

AATCC – American Association of Textile Chemists and Colorists

JIS L – Japanese Industrial Standard (Standard adjacent fabrics for staining of color fastness test)

SN – Standard Normal

ISO – International Organization for Standardization

UV – Ultra Violet

LBL – Layer-by-Layer

TA – Terephthalic Acid

HTA – Acid (2-hydroxyterephthalic)

OH – Hydroxyl

EM – Emission Activity

EX – Emission Wavelength

SEM – Scanning Electron Microscopy

US – Ultra-sons

PP – Polypropylene

PET – Polyester

PA – Polyamide

CO – Cotton

Wo – Wool

CA – Cellulose Acetate

AC – Acrylic

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Chapter 1 – Introduction

Textiles are substrates available to microbial growth and proliferation under appropriate conditions of moisture, nutrients and temperature. In the hospital environment, it can be a dangerous source of bacteria and fungi that infect patients and health professionals. The use of antimicrobial textiles, especially in direct contact with the body, can significantly reduce the risk of infections. However, several requirements should be met; bioactive textiles must have a broad spectrum of biocidal properties, be safe to use and highly effective in combating microorganisms resistant to antibiotics, including those used in the treatment of infections. It also should not allow the development of microorganisms resistant to the active compound used or to be the cause of skin irritation. The consumer demand for functional clothing and the growing market of medical textiles have stimulated research in this field.

Textile materials can be carriers of microorganisms such as fungi, bacteria, and viruses, and these microorganisms, if pathogenic or infectious, may cause health problems to users of the textile materials (Ren *et al.*, 2009).

The production of protective, decorative and technical textiles has become a consequence of its importance and as a consequence of this, the number of different antimicrobial agents suitable for textile application on the market increased significally. In recent years, antimicrobial finishing for textile fibers has attracted much attention (Tomsic *et al.*, 2009) but the first concept of ''antibacterial finishing for textiles'' emerged in 1941 and focused only on the protection of the quality and integrity of textile substrates. Recently, it becomes more important to protect wearers against the spread of bacteria and diseases. Textile industry has made significant advances in developing antibacterial fibers and agents (Ye *et al.*, 2006).

Antimicrobial textiles can be obtained by chemically or physically incorporating antimicrobial agents onto fibers or fabrics and can be grouped into two categories, temporary or durable (Ren *et al.*, 2009). An ideal textile antibacterial finishing should not only kill undesirable microorganisms and stop the spread of diseases, but also fulfill three other basic requirements, safety, compatibility and durability. Safety: because the product should not be excessively toxic to human and the environment and should not cause skin allergy and irritation. Compatibility: because the product must not present negative influences to textile properties or appearances and must be compatible with common textile processing. Durability: the product should be able to endure laundering, drying, and leaching (Ye *et al.*, 2006).

To select an antimicrobial agent for use on textile, one must take into account several criteria such as the mechanism of antimicrobial activity, its effectiveness for bacteria and fungi, toxicity, method of application, washing and cost (Tomsic *et al.*, 2009).

Today, textile materials are widely used in various environments, and antimicrobial treatment is rapidly becoming a prerequisite for textile goods used in several areas. However, there is an increasing public concern for possible side effects of antibacterial finishing on environmental and biological systems. Researchers are now focusing on safe, durable, and environmentally friendly natural substitutes (Ye *et al.*, 2006).

Chitosan is the second most abundant natural polymer in the world, is nontoxic, biodegradable, and biocompatible, and has long been used as a biopolymer or a crude material in pharmaceutical and medical field, papermaking, and food processing. It shows

good antibacterial activity against various bacteria and fungi. Experiments have proved that chitosan can stop the growth of a number of gram-positive and gram-negative bacteria by inhibiting the normal metabolism of microorganisms through the ionic interaction at cell surfaces and eventually killing the cell (Ye *et al.*, 2006). The mechanism for inhibition remains controversial because it is believed they should be affected by the chitosan molecular weight (MW) and two hypotheses are as follows. In the first mechanism the polycationic chitosan consumes the electronegative charges on cell surfaces and the cell permeability is changed, thus this interaction results in the leakage of intracellular electrolytes and proteinaceous constituents, for high MW. In second mechanism, chitosan enters fungal cells and then essential nutrients are adsorbed, which inhibit or slow down the synthesis of mRNA and protein, for low MW (Guo *et al.*, 2007).

Chitosan is one of the safest and most effective antibacterial agents. A number of textile materials including cotton, silk, nylon, polyester (PET), and nonwoven polypropylene (PP) fabrics have been modified with chitosan or its derivatives, and excellent antibacterial activity as well as good mechanical properties have been reported (Ye *et al.*, 2007). The chitosan can completely inhibit the growth of fungi at the concentration of 3% (Guo *et al.*, 2007). It has become of great interest as a new functional biomaterial with high potential in various fields and as an under-utilized resource (Pillai *et al.*, 2009).

In the development of durable antimicrobial or at least biostatic finishes for the hygienic sector an importance lies in the functionalization of fiber surfaces (Knittel and Schollmeyer, 2006).

In recent years, materials with nanometer domain size and a variety of chemical and physical preparative methods have been developed (Suslick *et al.*, 1996).

However, there are always new ways and strategies to improve the desired antimicrobial activity on textiles. With this investigation, a new strategy will be reported and may open new avenues for the design of novel antimicrobial textiles and *in situ* skin delivery systems.

Chapter 2 – Review

This chapter presents the literature review of different antimicrobial agents used in textiles, their requirements in terms of bioactivity, the application methods, the qualitative and quantitative standards to evaluate the effectiveness of the finishing, and some of the most recent developments in antibiotic treatment of textiles. The last sections of this chapter describe new approaches for the production and functionalization of antimicrobial agents and their application in textile.

2.1 Microorganisms in Textiles

Microorganisms such as bacteria and fungi cause microbial contamination on textiles, affecting not only its user out also as the textile material, causing undesirable effects with regard to its functionality, hygiene and aesthetics. Although microbial contamination is usually caused by those microorganisms, in specific conditions of humidity, it can occur development of algae, which are a source of nutrients for bacteria and fungi. Fungi cause many problems such as discoloration, staining and degradation of the fiber itself. The bacteria affect the properties of the fiber, causing foul odors and an unpleasant touch, though do not lead to complete deterioration (Schindler and Hauser, 2005; Teufel and Redl, 2006; Alonso *et al.*, 2009).

Most of the synthetic fibers are resistant to the attack by microorganisms due to its high hydrophobicity (Gao and Cranston, 2008; Purwar and Joshi, 2004) but not totally immune (Sun *et al.*, 2001). Natural fibers retain water and oxygen, because of their porous structure and hydrophilicity, acting as a source food for microorganisms. Therefore, the synthetic fibers are more resistant to attack by microorganisms than natural fibers (Gao and Cranston, 2008; Purwar and Joshi, 2004).

2.2 The Importance of Finishing on Antimicrobial Material Textile

Textile materials are used in many areas, although they are often prone to the growth of microorganisms. Therefore, there is a need to develop new standards aiming to meet the needs of users and protection of the textiles against microorganisms (Giri *et al.*, 2009).

Antimicrobial treatment arises naturally as a means of reducing microbial multiplication and may even lead to decrease or eliminate the risk of infections caused by pathogenic microorganisms. Inhibition of microbial metabolism, moreover, reduces the formation of unpleasant odors and protects the user textile (Michielsen, 2004; Schindler and Hauser, 2005).

Currently, there is already a wide range of commercially available compounds, and fibers with antimicrobial activity, (Ramachandran *et al.*, 2004). In most cases, the functionalization or antimicrobial treatment is done on a stage of completion and should act quickly in order to be effective (Tomsic *et al.*, 2009).

2.2.1 Requirements for an Antimicrobial Finish

An ideal textile with antimicrobial treatment must meet certain requirements. It should be effective against a broad spectrum of bacterial and fungal species, however, having low toxicity, to avoid toxic reactions, allergy or irritation to the user. It must meet standards in compatibility tests, including cytotoxicity, irritability and sensitivity. The finishing should be resistant to washing, dry cleaning and hot pressing, since textiles are often subjected to these processes. Moreover, the antimicrobial finishing should not adversely affect the quality or appearance of the textile and should preferably be compatible with other chemical processes such as dyeing, to be effective in terms of cost and not produce toxic substances to the manufacturer, user and environment (Gao and Cranston, 2008; Ramachandran *et al.*, 2004; Morin and Tomaselli, 2007; Wollin *et al.*, 2003; Ren *et al.*, 2009; Tomsic *et al.*, 2009; Kangwansupamonkon *et al.*, 2009).

The binding capacity of the compound in the textile material may or may not be a factor, depending on the value assigned to it. If a disposable textile is needed, it is sufficient that it possesses an antibacterial activity temporarily, so that the antimicrobial agent does not need to have a capacity for a permanent fixation. Conversely, if the textile is for continued use, it is essential that the antimicrobial compound has a high binding capacity and durability on the textile material. Thus, when the textile is washed, the antimicrobial effect is not removed (Ren *et al.*, 2009; Tomsic *et al.*, 2009).

It should also be considered that these antimicrobial finishes must not kill the resident, non-pathogenic flora of the user's skin. The normal skin flora includes bacteria that are important for skin health, lowering the pH of the skin surface and producing antibiotics that create an unfavorable environment to the growth of pathogenic bacteria. Thus, antimicrobials present in textiles should only reduce the density of resident flora of the skin, not to eliminate it completely. It is also important that there is no evidence of changes in the ecology of the resident flora of the skin due to the use of antimicrobial textiles (Gao and Cranston, 2008; Ramachandran *et al.*, 2004; Morin and Tomaselli, 2007).

In summary, the application of an antimicrobial finishing on the textile materials should not adversely affect their quality (color, etc.). It should be compatible with all methods and chemicals used in textile production, be resistant to atmospheric conditions (humidity, sunlight) and body fluids, and is expected to survive washings, dry cleaning and ironing (Ramachandran *et al.*, 2004; Schindler *et al.*, 2005). Furthermore, it should not kill non-pathogenic bacterial flora present on the surface of the skin and prevent allergic reactions or irritation. Thus, a material after functionalization has to be tested for compatibility,

cytotoxicity, irritability, among others, to offer maximum safety of the product use (Schindler *et al.*, 2005).

Finally, the method used for the impregnation of the bioactive compound in the textile material should be economically viable and show a reduced or no environmental impact. Furthermore, it must still meet the requirements demanded by regulators, be harmless for the producer and consumer (Sun *et al.*, 2001).

2.2.2 Methods of Introduction of Antimicrobial Compounds in Materials

Various finishing techniques are applied during the processing of textile materials, to give them antimicrobial properties. These are intended not only for user protection against bacteria, fungi and other microorganisms, but also to increase their resistance to microbial attack on the material, for example, biodeterioration caused by fungi, without affecting the other characteristics of the fiber (Giri *et al*, 2009).

The textile materials have different chemical composition and reactivity and during its life cycle, depending on the type of application, are subject to multiple factors of wear, such as repeated washing and drying, leaving the durability of the antimicrobial finishing compromised. Thus, the selection of a specific method for the incorporation of an antimicrobial compound is carried out according to the nature of the agent, its chemical and physical properties as well as fiber type and its intended purpose (Ramachandran *et al.*, 2004; Gao and Cranston, 2008).

Basically, the methods of incorporation of an antimicrobial agent in textiles can be divided into two main groups. The first group consists of methods in which the agent is introduced into the polymer matrix before or simultaneously with the extrusion processes of melt spinning, and through dry spinning and wet spinning. The second group contains methods of application of antimicrobial compounds during the finishing stage of materials and fibers on the impregnation process, exhaustion, or spray coating (Ramachandran *et al.*, 2004; Gao and Cranston, 2008).

The method of incorporation by introducing the agent in the polymer matrix confers antimicrobial properties to materials but also low durability. That is due to the fact that the antimicrobial compound is physically incorporated into the fiber structure and slowly released during use. This method is preferably used in materials of synthetic origin, due to the low reactivity of these fibers (Ramachandran *et al.*, 2004; Gao and Cranston, 2008).

The processes of exhaustion and impregnation are used in materials based on natural and synthetic fibers, for example for antimicrobial finishes. Methods of impregnation, foam and spray are used for specific situations with compounds based on quaternary silicone (Gao and Cranston, 2008).

More recently, techniques that provide increased durability of antimicrobial finishes were developed and refined (Mao, 2002; Ramachandran *et al*., 2004; Gao and Cranston 2008). These technologies can be summarized in three approaches: release, surface treatment and chemical bonding of the agent to fiber (Mao, 2002). Table 2.1 lists the substrates and mechanisms of insertion of functionalization with the methods that are currently used for increasing the durability of antimicrobial activity in fibers.

Table 2.1 – Mechanisms of insertion of the antimicrobial functionality, depending on the methods and textile substrates (adapted from Mao, 2002).

Functionality	*Process*	*Substrate*
Controlled release	Active substances insolubilization, inside and at the surface of the fiber; Addition of compounds to polymer matrix; Microencapsulation of active substance.	Synthetic fibers
Adsorption to the fiber surface	Impregnation resins; Coating; Ion Exchange;	Regenerated cellulose fibers and synthetic
Chemical bond to the fiber	Chemical modification of the fiber by introducing reactive groups; Chemical modification of the fiber by copolymerization or plasma;	Cellulosic fibers

The main objective of the technologies that operate by controlled release, in which the active substances are released into the surface of the material, causing the destruction of microorganisms, is to prevent deterioration of the textile material itself (Michielsen, 2004).

The insolubilization processes allow the spread of antimicrobial compound into the fiber, showing, however, limitations with regard to the consumption of antimicrobial agent and thus the durability of antimicrobial activity (Grabowsca *et al*., 2004; Schindler and Hauser, 2005).

The incorporation of microcapsules with the active antimicrobial agent is a methodology that allows for a prolonged duration of antimicrobial activity during the life cycle of the textile item, even after multiple washes (Grabowsca *et al.*, 2004, Schindler and Hauser, 2005).

The impregnation processes is applied to all fibers. This is usually a quick and low-cost method, and allows the use of a wide range of antimicrobial compounds. The performance of textile materials obtained by these processes depends on the permanence of the antimicrobial compound, which is related to its solubility (Michielsen, 2004).

Ion exchange processes used are ionic bonds created between the functional groups of the antimicrobial compound ionically charged and reactive sites of fiber (Mao, 2002).

The antimicrobial compound is chemically bounded to textile fibers when exist a total immobilization of these. The process of inhibition occurs when the microorganism contact exist with the surface of the treated textile material (Mao, 2002).

The sol-gel technique is a method commonly used for antimicrobial finishes on textile materials and is also well explored in other applications. It is a simple technique which consists in incorporating functional agents in sol-gel nanoparticles with subsequent inclusion in the material to be functionalized. As for its antimicrobial properties, several antimicrobial compounds have been encapsulated in sol-gel particles, which are then attached to the textile material to give it the desired functionality. It is assumed that this new process will allow the formation of textile finishing with unlimited features (Gao and Cranston, 2008).

2.3 Antimicrobial Compounds

Several classes of antimicrobial agents have been laboratory tested for application in textile industry. These are agents with potent bactericidal activity, as indicated by the value of MIC (Ma *et al.*, 2003; Wollina *et al.*, 2003; Ramachandran *et al.*, 2004; Thorn *et al.*, 2006; Morin and Tomaselli, 2007; Ovington, 2007; Gao and Cranston, 2008). The majority of the compounds mentioned in the literature, produces no lasting effect on the fabric and are limited in their amount of microbial species capable of inhibiting (Sun *et al.*, 2001). Moreover, a broad range of antimicrobial agents are now marketed and applied to different fibers with variable efficacy and toxicity (Table 2.2).

Table 2.2 – Some biocides in use and under development for the treatment of various fibers (adapted from Gao and Cranston, 2008).

Biocide	*Fiber*	*Application method*	*MIC*	*Comments*
Silver	Polyester Nylon Wool Regenerated cellulose	F/I I F F	0.05- 0.1 mg/L against *E. coli*	Slow release, durable but silver may be depleted
QACs	Cotton Polyester Nylon Wool	F F F F	10-100 mg/L against Gram-positive and Gram-negative	Covalent bond, very durable, possible bacterial resistance
PHMB	Cotton Polyester Nylon	F F F	Low toxicity (MIC=0.5-10 ppm) against Gram-positive and Gram-negative	Need for large quantities, potential bacterial resistance
Triclosan	Polyester Nylon Polypropylene Cellulose acetate Acrylic fiber	F/I F/I I I I	Less than 10 ppm against Gram-positive e Gram-negative	Need for large quantities, bacterial resistance, degradation dioxin toxic, banned in some European countries
Chitosan	Cotton Polyester Wool	F F F	0.05-0.1% (w/v) against many species of bacteria, although its activity could be affected by its molecular weight and degree of deacetylation	Adverse effects on handling, low durability
N-halamine	Cotton Polyester Nylon Wool	F F F F	ND	Need regeneration, odor of chlorine residue
Peroxyacids	Cotton Polyester	F F	ND	Need regeneration, poor durability
Dyes	Polyester	F	10-100 mg/L against Gram-positive and Gram-negative	Undesirable color effects and /or is not possible for all colors

"F" stands for biocides used as finishing agent and "I" represents biocides incorporated into the fiber during extrusion

Heavy metals, quaternary ammonium compounds, polyhexamethylene biguanides (PHMB), chitosan, triclosan, titanium dioxide, compounds of plant origin and some dyes are compounds with antimicrobial properties used in textiles and also exploited in the food

industry, cosmetics and in cleaning products (Gao and Cranston, 2008; Giri *et al*, 2009). However, its attachment to the surface of textiles or incorporation into the fibers substantially reduces its activity and limits their availability, requiring high concentrations in the process of functionalization. The biocide may gradually lose activity during use and washing of textiles. Thus, large quantities of biocides are applied to textiles to effectively control bacterial growth and maintain its durability (Ma *et al*., 2003; Wollina *et al*., 2003; Ramachandran *et al*., 2004; Thorn *et al*., 2006; Morin and Tomaselli, 2007; Ovington, 2007; Gao and Cranston, 2008; Kangwansupamonkon *et al*., 2009).

2.3.1 Titanium Dioxide

It is a photocatalytic compound, which expresses photocatalytic and oxidizing powerful, activity and stability, simultaneously, compared with compounds such as zinc oxide, cadmium sulfide and zinc sulfide, which also feature a high antimicrobial potential. It can cause a bacterial reduction of around 99.99% against *E.coli* (Shieh *et al*., 2006; Kangwansupamonkon *et al*., 2009) and continues to be used in research related to the degradation of organic mixtures and microorganisms like viruses, bacteria and cancer cells, as well as application in other areas (Kangwansupamonkon *et al*., 2009).

2.3.2 Quaternary Ammonium Compounds

The quaternary ammonium compounds have a positive charge at the nitrogen atom, which is responsible for the damage to the cell membrane, protein denaturation and disruption of the cell structure of microorganisms. It presents MIC values of 10-100 mg/L against Gram-negative and Gram-positive bacteria. The quaternary ammonium compounds with 12-18 carbons in the molecular chain are used as bacteriostatic and disinfectant agents in textile fibers (Gao and Cranston 2008).

The quaternary ammonium groups remain unchanged during the process of microbial cell inactivation, retaining their antimicrobial properties even after their connection to the fiber (Gao and Cranston, 2008.)

2.3.3 Dyes

Some dyes used in the textile industry are specifically designed for antimicrobial activity. As an example, dispersive azo dyes shown excellent results in dyeing and antimicrobial effect (Han and Yang, 2005; Gao and Cranston, 2008). The dyes can also attack the microorganisms selectively (Purwar and Joshi, 2004).

2.3.4 N-Halamine and Derivatives

N-Halamine is a polymer that has lately received particular attention because of the possibility of obtaining a lasting antimicrobial finish. Its lasting antibacterial activity is a consequence of its strong capacity for regeneration. It was demonstrated that this agent have capacity to eliminate a wide range of microorganisms without disrupting ecosystems, and the incorporation of compounds derived from N-Halamine textile materials has had excellent results. The oxidative properties of Halamine links (CI-N), gives a strong bactericidal action (Gao and Cranston, 2008).

2.3.5 Metals and Metal Salts

Metals such as copper, zinc and cobalt, have attracted attention as antimicrobial agents for application in textiles, silver being the most widely used. These metals, in their free state or combined with other compounds, are toxic to the metabolism of microorganisms, even at low concentrations. It presents values of MIC from 0.05 to 0.1 mg/L against *E. coli* (Michielsen, 2004; Gao and Cranston, 2008).

Textiles, in which the silver ions are used as final coating on the surface of the fiber, have high antimicrobial activity. But this adversely affects the color of the final articles (Michielsen, 2004). Under normal conditions, while using the material, and in the presence of moisture, the silver diffuses to the surface of the fiber forming non-toxic silver ions. However, with prolonged use on wounds, the silver ion may give rise to pigmentation more or less permanent in the body when the skin absorbs large amounts of it (Gao and Cranston, 2008).

To obtain a lasting antimicrobial effect, the silver ions in the form of zeolite, or silver salts, are incorporated into synthetic polymers in the form of nanoparticles, where the metal is released gradually to the fiber surface at a controlled rate. However, stability to washing is not satisfactory (Michielsen, 2004; Gao and Cranston, 2008).

It is also of importance that antimicrobial treatments based on metal ions present serious constraints due to environmental and technical issues that they can cause (Gao and Cranston, 2008).

2.3.6 Chitosan

Chitosan is a polymer that has aroused increasing interest in several areas such as chemistry, biochemistry, medicine, pharmacy, biotechnology and technology textiles. That is due to its properties of biodegradability, biocompatibility, non-toxicity, regenerative and antimicrobial activity. These properties depend on two basic parameters, degree of deacetylation and the

molecular weight of the polymer (Gao and Cranston, 2008). The antimicrobial activity of chitosan is influenced by other properties such as pH, ionic strength and addition of non-aqueous solvents (Joshi *et al.*, 2009; Aranaz *et al.*, 2009). Chitosan shows MIC values from 0.05 to 0.1% (w/v) as a function of molecular weight against the most common species of bacteria (Gao and Cranston, 2008).

Besides the pure chitosan, which is a derivative of chitin deacetylated, poly (1,4)-2-amino-2-deoxy-ß-D-glucose, many derivatives were synthesized and used as antimicrobials for use in textiles, particularly those based on chitosan oligosaccharides. Many of these derivatives contain more than one quaternary ammonium group to enhance antimicrobial activity (Gao and Cranston, 2008).

The first textile fibers in which the antimicrobial properties of chitosan were tested were cellulose fibers, more specifically cotton fibers. There was a slight antimicrobial effect on a wide range of organisms, which, however, was not durable after washing. The method of incorporation did not allow a direct covalent bond between the chitosan and cellulose molecules, and during washes, there is loss of the polymer and its consequent antimicrobial effect. Thus, studies have been conducted on the application of chitosan on cellulosic materials (cotton), with the use of cross-linking agents such as dimethyloldihydroxyethyleneurea, citric acid, 1,2,3,4-butanetetracarboxylic acid or glutaric dialdehyde to increase durability or permanence of the finish (Gao and Cranston, 2008). In addition to antimicrobial properties, studies have shown that chitosan also improves the absorption of dye by the fiber (Giri *et al.*, 2009).

2.3.7 Phenolic Compounds

The phenolic compound most used in the preparation of antimicrobial materials is triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether). It presents MIC values of 10 ppm against most common bacteria species. The triclosan is a relatively small molecule and can act as a disperse dye, which is applied before or during the dyeing process for textile fibers. To ensure an effective and continuous antimicrobial activity during use of textiles, the bioactive agent migrates to the surface of the tissue at a slow and regular rate (Gao and Cranston, 2008).

The triclosan, when exposed to sunlight, is a reactive and form toxic compound (dioxins), due to the disruption of bonds existing in the triclosan molecule. For this reason, their use was banned in some European countries, joined with the facts of their activity against fungi is not very significant and bacterial resistance to this compound pose a tremendous concern on part of researchers (Gao and Cranston, 2008).

2.3.8 Compounds Polyhexamethylene Biguanides (PHMB)

Compounds like Polyhexamethylene biguanides have a potent and broad spectrum against Gram-positive and Gram-negative bacteria and fungi revealed by its low MIC 0.5 to 10 ppm. They also have low toxicity and an acceptable environmental impact resulting from its application. Therefore, they are commercially used. However, the textiles they lose their antimicrobial activity after washing, and their properties, such as color, are changed (Gao and Cranston, 2008).

2.3.9 Peroxyacids

Regenerable antimicrobial agents such as peroxyacetic acid, which is a strong disinfectant, and often used in hospitals, are converted into carboxylic acids during the deactivation of microorganisms and can be regenerated by the reaction with an oxidant such as hydrogen peroxide.

The antimicrobial activity decreases after several washes, despite of the peroxyacid being stable for long periods, particularly during storage of tissues (Gao and Cranston, 2008).

2.4 Mechanisms Action of Antimicrobial Compounds vs. Microorganisms

Bacteria and fungi has an outer cell wall, composed mainly of polysaccharides. The cell wall maintains the integrity of cellular components, protecting it from the outside. Immediately below the cell wall is the cell membrane, semi-permeable, closing the organelles, enzymes and nucleic acids. Enzymes are responsible for chemical reactions occurring inside the cell, and nucleic acids contain the genetic information of the organism. The survival or growth of microorganisms is dependent on cell integrity and appropriate state and combined action of all components (Ramachandran *et al*., 2004; Gao and Cranston, 2008).

Bacteria can be separated into two classes, due to the presence of one or two cell membranes, the Gram-positive and Gram-negative, respectively (Ramachandran *et al*., 2004). The Gram-negative bacteria are usually less sensitive to bactericidal due to the presence of the outer membrane, since it is responsible for the intrinsic resistance of these microorganisms to antimicrobial compounds. Cytoplasmatic membrane is considered the main target of antimicrobial compounds, due their functions such as barrier of the selective permeability, bioenergetics functions, etc. (Maillard, 2002).

The negative effect on the vitality of microorganisms is generally referred to as antimicrobial. The activity that affects bacteria is named antibacterial, and the activity that

is known to affect the fungus antimycotic or antifungal. The degree of activity is differentiated by the suffix "cidal", indicating the destruction of microorganisms, and the suffix "static" representing the growth inhibition without significant levels of destruction. Treatments that inhibit the growth and development of microorganisms are called biostatic, bacteriostatic and fungistatic, and products that completely destroy the microorganisms are called biocides, bactericides and fungicides (Ramachandran *et al.*, 2004; Gao and Cranston 2008; Schindler and Hauser, 2005).

Antimicrobial textiles can be generally classified into two categories, namely, passive and active, based on their activity against microorganisms. Passive materials do not contain any active substance; however, the structure of its surface has a negative effect in terms of survival of microorganisms, for example, "Lotus effect" or anti-adherent effect. Active materials, are those that contain active antimicrobial substance, acting on or within the cell (Gao and Cranston, 2008; Ramachandran *et al.*, 2004; Morin and Tomaselli, 2007).

One of the criteria for selection of an antimicrobial agent is based on its mechanism of action. Most antimicrobial compounds act intracellular but some others may act outside the cell allowing for their detention cell surface. Thus, the first come into the cell, damaging it and the second act by breaking or destruction of the cell wall cross-linking, or by increasing its permeability, resulting in the release of their organelles, respectively (Michielsen, 2004).

The connection that the first contact antimicrobial agent involves with the cell surface triggers a series of amendments. This may be enough to induce the antimicrobial effect, or only allow the invasion of the agent to the intracellular space to reach this place to harm (Maillard, 2002). The mechanism of action of an antimicrobial agent is defined according to the structure which provides for bacterial activity. There can be described three types of interactions: (1) interaction with cellular components of the outer layer, (2) interaction with the cytoplasmatic membrane, and (3) interaction with the cytoplasmatic constituents. It is also possible, that an antimicrobial compound acts on more than one structure (Maillard, 2002). Those three kinds of interactions are described in detail below.

(1) There are many antimicrobial compounds that can interact with the outer cell layer, which does not mean that the cell viability is affected, i.e., after removing the biocide agent and supportive environment, microorganisms can grow again. Many of those compounds act specifically by increasing the permeability of this barrier, preventing normal cell metabolism, for example, the case of quaternary ammonium compounds (Jones *et al.*, 1991; Maillard, 2002). The antibacterial activity exhibited by these antimicrobial agents is not very strong, but their use can improve the action of others, since they promote the clearing of the outer

structures facilitating the access of other agents to specific sites leading to cell inactivation (Maillard, 2002).

(2) At the level of the cytoplasmatic membrane, antimicrobial interactions may cause damage to the bacterial cell, such as rupture of the cytoplasmatic membrane, dissipation of the proton and dealings with enzyme systems (Maillard, 2002). Disruption of the cytoplasmatic membrane causes only disturbances in permeability, thus reflecting a bacteriostatic effect rather than bactericidal one (Maillard, 2002).

(3) Certain selective bactericides have capacity to react with cytoplasmatic components. Interactions with cytoplasmatic components it highlights interactions with nucleic acids, and with the ribosomes (Maillard, 2002).

2.5 Evaluation of Antimicrobial Activity in Textiles

The methods for evaluating antimicrobial activity in textiles should be able to evaluate the effectiveness of antimicrobial treatment as well as ensuring that the performance of the textile item is not changed after treatment (Ramachandran *et al*., 2004).

The compounds differ physically and chemically, and act according to different mechanisms for microorganisms. It may be necessary to perform several tests of antimicrobial activity to properly assess an application of such compounds. It's not all advisable to assess two distinct antimicrobial products by an only assessment test (Ramachandran *et al*., 2004).

To determine the effectiveness of antimicrobial textiles a series of tests were developed. These can be classified into two categories: diffusion in agar and in suspension (Gao and Cranston, 2008).

2.5.1 Agar Diffusion Test

The tests based on agar diffusion estimate the antimicrobial activity by inhibiting bacterial growth, i.e. the area surrounding the sample treated with the antimicrobial compound forms a zone of inhibition, which is observed with the naked eye. Those tests are only qualitative but easy to perform, and because of that, widely used as preliminary tests and where a large number of samples is to be examined (Ramachandran *et al*., 2004; Gao and Cranston, 2008). In these methods, the textile samples to be tested are placed on nutrient agar plates on which bacterial cells are inoculated. The plates are incubated at specific temperature (37-38 °C) during a certain period of time, checking out, after this, whether or not the inhibition zone appearance. The appearance of an inhibition zone or halo depends on factors such as minimum inhibitory concentration (MIC), the concentration of an antimicrobial agent in

tissue, and the power of migration of the antimicrobial agent. The absence of halo does not necessarily mean that the sample does not have antimicrobial activity, since bacterial growth may not occur under the textile substrate. The efficiency of antimicrobial treatment does not show a proportional relation with the zone of inhibition and is therefore an indirect measure of the activity of the sample (Ramachandran *et al.*, 2004; Askew, 2009). The development of the zone of inhibition is also influenced by the rate of diffusion of the agent, and their size show the power of the antimicrobial activity or rate of release of active agent. A strong attachment of the agent to the substrate (formation of covalent bonds), prevents its spread to the inoculated medium, not allowing for an interaction between the agent and the organism, preventing the formation of inhibition zone (Gao and Cranston, 2008).

2.5.2 Suspension Test

These tests assess the antimicrobial effectiveness by reducing microbial population, using the technique of counting of microorganisms and are therefore quantitative. Compared with the agar tests these are more time consuming. The antimicrobial activity is evaluated by comparing the size of the initial population with the population after incubation (Ramachandran *et al.*, 2004). Table 2.3 lists the methods used to evaluate the efficiency of antimicrobial (antibacterial and antifungal) textile materials.

Table 2.3 – Methods for evaluation the antimicrobial activity of textile materials (Gao and Cranston, 2008; adapted Askew, 2009 and Teufel and Redl, 2006).

Tests	*Agar diffusion*	*Suspended*
Methods antibacterial	AATCC 147–1993 and 1998; AATCC 90–1982; AATCC 174–1993; JIS L 1902–2002; ISO 20645–2004; SN 195920–1992; ISO 20743-2007	AATCC 100–1993 and 1999; XP G 39–010–2000; JIS L 1902-1998 and 2002; SN 195924–1983; ISO 20743-2007
Methods antifungal	AATCC 30–1993 and 2004; SN 195921–1992	-

There is great difficulty in developing a single test that can satisfy all requirements because of the wide diversity of materials with properties of its own and it is therefore necessary to use several tests to thoroughly study the activity of a given material (Ramachandran *et al.*, 2004).

Before being commercialized, textiles have to be compulsorily subjected to other tests, including biocompatibility, which involves the cytotoxicity assays, sensitivity and irritability, since the antimicrobial tests only measure antimicrobial performance of the material (Gao and Cranston, 2008).

2.6 Ongoing Research and New Strategies: Natural Antimicrobial Agents for Textile Application

Antimicrobial agents based on natural products such as chitosan, natural dyes, neem extract and other products based on antibiotics for finishing of textile materials have been studied, since they not only help to effectively reduce the harmful effects associated with microbial growth in textiles, such as minimizing the potential for bacterial resistance, but also respect the legal requirements imposed by regulatory agencies (Joshi *et al.*, 2009.)

2.6.1 Chitosan

Chitosan is a deacetylated derivative of chitin, is natural, non toxic and is a biodegradable polymer. Chitin is one of the most abundant polysaccharides found in nature, and is found in structures of a large number of invertebrates (crustaceans, exoskeleton, insect and cuticle) and cell walls of fungi, among others. Chitosan appears naturally only in some fungi (Mucoraceae) (Aranaz *et al.*, 2009).

Chitosan has two advantages over chitin: is dissolved in diluted acetic acid, while chitin is dissolved in toxic solvents such as lithium chloride and dimethylacetamide, and has free amino groups, with several reactive sites available for many chemical reactions (Joshi *et al.*, 2009).

Chitosan is receiving great interest with regard to medical and pharmaceutical applications because it has properties that make it suitable for biomedical use, such as biocompatibility, biodegradability and no toxicity (Aranaz *et al.*, 2009). A compound biocompatible with living tissue, it does not cause allergic reactions and rejection, since it degrades slowly in harmless products (sugars, aminoacids) which are fully absorbed by human body. Due to its high biocompatibility, chitosan has been used in drug delivery systems, implants and injectable systems like orthopedic and periodontal compounds, wound healing and regenerative tissue engineering (Agnihotri *et al.*, 2004; Aranaz *et al.*, 2009).

The mechanism of biocidal action is still not completely understood but it is believed that the antimicrobial and antifungal properties of chitosan have origin in its polycationic nature which allows connections with the negatively charged residues of macromolecules on the

surface of cells of bacteria, inhibiting subsequently, the growth of these. The antibacterial activity of chitosan is also sensitive to pH, with higher activity to lower values (pKa 6.5) (Joshi *et al*. 2009; Aranaz *et al*., 2009). In basic conditions of pH, the chitosan containing free amino groups and thus is insoluble in water. In acid pH, the amino groups can undergo protonation, making it thus water soluble. Its solubility depends on the distribution of free amino acids and N-acetyl (Agnihotri *et al*., 2004).

Chitosan can be considered an agent for multifunctional textile finishing because its antimicrobial activity may be combined with other functions such as better fixation of dyes in the dyeing process, static and deodorant activity (Gao and Cranston, 2008; Joshi *et al*., 2009). However, its application in textile products is effective against microorganisms only at high concentrations, which causes the formation of a film on the surface of the fabric, causing the air permeability decrease. Another disadvantage is that the material has a low flexibility after application (Joshi *et al*., 2009).

2.6.2 Sericin

The sericin is a natural macromolecular protein produced by the silkworm *Bombyx mori* and constitutes 25-30% of the silk fiber. It is a biomolecule of great value, since it has antibacterial, UV resistant, resists oxidation and has hydrating properties. It has several applications, such as moisturizing agent in shampoos and creams and is also an important biomaterial for various applications, including textiles. However, the antibacterial properties in textiles with the application of sericin have not been reported (Zhang, 2002; Sarovart *et al*., 2003; Genç *et al*., 2008; Joshi *et al*., 2009).

2.6.3 Neem Extract

Neem (*Azadirachta indica*) is an evergreen tree of India, belonging to the plant family Meliaceae. This is recognized as one of the most promising sources of compounds for the control of proliferation of insects, with antimicrobial and medicinal properties. The active ingredients in neem are found in all parts of the tree. The neem extract has been widely used in herbal pesticide formulations, which due to their pest repellent properties has the potential to inhibit the growth of Gram-positive and Gram-negative. Currently, little has been reported of its use in textiles as an antimicrobial agent (Vaideki *et al*., 2007; Joshi *et al*., 2009).

2.6.4 Aloe Vera

Aloe Vera (*Aloe barbadensis* Miller), belonging to the family Liliaceae, is known as "Lily of the Desert". In modern times scientific research has shown that Aloe leaf contains a large number

and variety of nutrients and active compounds. It has been used in traditional medicinal practices for the purpose of healing wounds and burns, for medical and aesthetic as well as for general health. Aloe Vera also has antibacterial and antifungal properties and may be exploited in medical applications for the textile industry (Joshi *et al.*, 2009).

2.6.5 Tea Tree Oil

The Tea Tree (*Melaleuca alternifolia*) is the most famous natural Brazilian product in the world used for thousands of years by Aborigines, to help relieve bruises, bites, burns and other skin diseases. The Tea Tree oil has more than 100 different compounds and is recognized worldwide as a natural medicinal product, known for having the best natural antiseptic with antifungal properties in the world. It is active against a broad range of bacteria. Its therapeutic use in microbial and fungal infections is not yet regulated for medical use, and its activity in textile substrates is still being explored (Joshi *et al.*, 2009).

2.6.6 Eucalyptus Oil

Eucalyptus oil is natural and has extraordinary cleaning properties. There is evidence that it can effectively fight infections caused by bacteria, fungi and viruses. Its applications in textile substrates are also still to be explored (Joshi *et al.*, 2009).

2.6.7 Azuki Bean

Extract water from Azuki beans (*Vigna angularis*) green, black and red show antimicrobial effects against several bacteria species. In contrast, the extract of white bean Azuki shows no inhibition in relation to any organism ever studied. Extracts from Azuki color bean contain large amounts of polyphenols, which may be responsible for antibacterial activity. The antimicrobial activity of Azuki beans can be exploited in textile materials (Joshi *et al.*, 2009).

2.6.8 Prickly Chaff Flower

The prickly chaff flower (*Achysanthus rough*) is one of the herbs found in India. It presents antimicrobial activity against both Gram-negative and Gram-positive bacteria, but its activity level is lower than that of neem oil (Joshi *et al.*, 2009).

2.6.9 Tulsi Leaves

The leaves of Tulsi are used since the ancient age as antimicrobial, insecticidal, antiprotozoal, diaphoretic and expectorant and as aromatic carminative. Thilagavathi et al observed that the leaves of Tulsi have antimicrobial activity suitable for textile application (Joshi *et al.*, 2009).

2.6.10 Clove Oil

Clove oil (eugenol) is the main product of *Syzygium aromaticum*. The bioactivity of clove oil was explored as an agent for finishing of cotton fabrics to make them antibacterial (Joshi *et al.*, 2009).

2.6.11 Pulp Extracts and Onion Skin

Onions (Allium cepa), belonging to the family Liliaceae have antimicrobial properties. Have been founded antimicrobial properties of cotton fabrics treated with onion in studies by other authors (Joshi *et al.*, 2009).

2.6.12 Other Herbal Products

The Turmeric or cumin, a yellow fluorescent pigment extracted from the rhizomes of several species, has been used as a dye for dyeing wool, silk and cotton. Because of its bacterial activity, turmeric also confers antimicrobial properties. The antimicrobial activity of plant extracts such as peppermint, primrose and perilla oil, has been explored for applications in the textile industry (Joshi *et al.*, 2009).

2.7 Antimicrobial microsphere-based coatings for Textile: A New Approach

2.7.1 Background

Currently, the quality requirements in production of antimicrobial textiles are not only emphasizing the intrinsic functionality and long product life, but also a production process which is environment friendly and effective against a wide range of microorganisms (Joshi *et al.*, 2009).

Many of the antimicrobials used as finishing of textile materials are a toxic chemical, which means that there is a growing public concern about the possible effects of antimicrobial finishes in environmental and biological systems. Thus, the antimicrobial finishing of textiles should be able to destroy undesirable microorganisms, as well as safe and environmentally benign (Ye *et al.*, 2005).

In terms of research there is much work to exploit the antimicrobial activity of natural products in textile substrates, but there are still several problems that need to be discussed. The mechanism of bactericidal action of different natural antimicrobial agents is still unknown. Natural products have a complex chemical structure and not all its components have antimicrobial activity. Thus, the selective isolation of bioactive ingredients is an important way to reduce the dosage of antimicrobial agent. The dissolution of natural agents

for textile application is also a challenge because most products are not soluble in water. The attachment of bioactive substances in different types of textile substrates for increased durability of antimicrobial activity is also an important avenue to research. Despite the fact that some research has already been done to produce and develop encapsulated natural agents, design of bioactive textiles with slow release for a long activity will be a good area for innovation in the world of biotextiles (Joshi *et al*., 2009).

However, during the attachment of antimicrobial agents on textiles functional groups, which may be responsible for antimicrobial activity, can be blocked, causing the loss of bioactivity (Joshi *et al*., 2009).

To resolve this, more research is needed in the area of textiles, in particular, in the functionalization with bioactive natural products, to make them a viable alternative to antimicrobial textile based chemicals. Issues like ecotoxicity, cytotoxicity, coupled with the effect of bacterial multidrug resistance makes the use of natural antimicrobials, instead of chemicals, very desirable (Joshi *et al*., 2009).

Recently, several methods, depending on the active agent and type of fiber, have been developed or are being developed to confer antimicrobial activity to textiles. The antimicrobial agents can be applied to the textile substrate by techniques of exhaustion, impregnating, coating, spraying, microencapsulation and foams. However, desired bioactive properties related to the effectiveness of these application methods not all are achieved with high success rate. Thus, new developments and processes are expected around the world (Gao and Cranston, 2008).

A new approach is developed in this thesis and involves the investigation of more suitable conditions for surface functionalization of textile materials, with bioactive microspheres embedded, capable of providing a controlled release of antimicrobial agents, preventing and treating microbial infections. It is also of great importance to research the most suitable textile materials and fixing methods.

The area of functional fibers and technical textiles has encouraged industry to use microencapsulation processes as a mean of transmitting properties and finishes for textiles, which would not be profitable using other techniques. Microcapsules have been used in several areas such as adhesives, cosmetics, pesticides, pharmaceuticals, medicine, food, etc. But it is only recently that they have been introduced in textiles industry (Monllor *et al*., 2007). The controlled release of bioactive substances encapsulated in polymeric microspheres and microcapsules deposited on textiles opens new perspectives for applications of

textiles. The antibacterial effect of microspheres containing material with biocide depends on the capacity to release a set amount of biocides and during its use. The amount depends on the parameters of microsphere, the concentration of the biocide, as well as the quantity and dispersion of the microspheres after introduced into the textile substrate. The key factor for evaluating the effect obtained is the kinetics of release of the biocide, which characterizes the efficiency and durability of the antibacterial properties (Grabowska *et al.*, 2008).

2.7.2 Techniques for Microencapsulation

The most widely used methods for preparing encapsulated chitosan systems are: Emulsion cross-linking, coacervation/precipitation, spray-drying, emulsion-droplet coalescence method, ionic gelation, reverse micellar method, sieving method. The choice of the method depends on factors such as the requirement of particle size, thermal and chemical stability of active substances, reproducibility of kinetic profiles, the product stability and residual toxicity associated with the end product (Agnihotri *et al.*, 2004). Those methods are described in more detail in the fallowing sections.

Emulsion cross-linking

This method utilizes the aldehyde groups of the cross-linking agent with reactive functional amine group of CS to cross-link. In this method, is prepared water-in-oil (w/o) emulsion by emulsifying the CS aqueous solution in the oil phase. Using a suitable surfactant, aqueous droplets are stabilized. The stable emulsion is cross-linked by using an appropriate cross-linking agent to harden the droplets. Microspheres are filtered and washed repeatedly with n-hexane followed by alcohol and then dried. By this method, size of the particles can be controlled by controlling the size of aqueous droplets. However, the particle size of final product depends upon the extent of cross-linking agent used while hardening, and in addition by the speed of stirring during the formation of emulsion. The emulsion cross-linking method has few drawbacks as use of harsh cross-linking agents, which might possibly induce chemical reactions with the active agent (Agnihotri *et al.*, 2004). Figure 2.1 shows the schematic representation of this method.

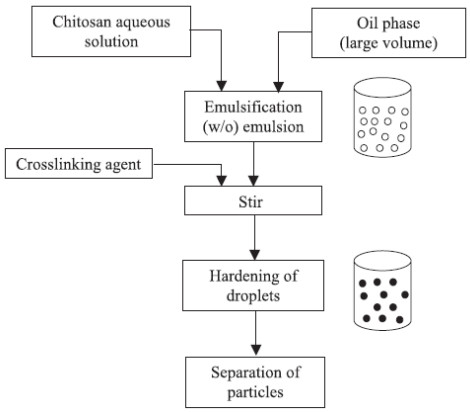


Figure 2.1 – Schematic representation of preparation of chitosan particle systems by emulsion cross-linking method (Agnihotri *et al.*, 2004).

Coacervation/precipitation

This method utilizes the physicochemical property of CS. Chitosan is insoluble in alkaline pH medium, but precipitates/coacervates when it comes in contact with alkaline solution. Particles are produced by blowing CS solution into an alkali solution using a compressed air nozzle to form coacervate droplets. By filtration/centrifugation followed by successive washing with hot and cold water is performed separation and purification of particles. Particle size can be controlled by varying the pressure of compressed air or spray diameter, and then to control the release of the drug using a cross-linking agent to harden the particles (Agnihotri *et al.*, 2004). Figure 2.2 presents the schematic representation of this method.

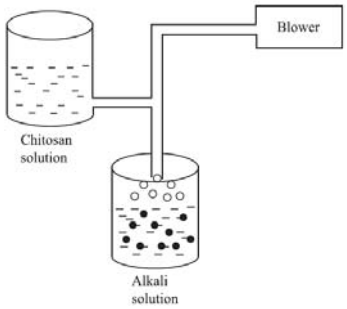


Figure 2.2 – Schematic representation of preparation of chitosan particle systems by coacervation/precipitation method (Agnihotri *et al.*, 2004).

Spray-drying

This method is based on the drying of the atomized droplets by a stream of hot air. It is a well-known technique to produce powders, granules or agglomerates from the mixture of drug and excipient solutions as well as suspensions. First the CS is dissolved in an aqueous

solution of acetic acid, then the drug is being dissolved or dispersed in the solution, and an appropriate cross-linking agent is added and atomized in a stream of hot air. Atomization leads to the formation of small droplets, from which solvent evaporates instantaneously leading to the formation of free flowing particles. To get the desired size of particles, various process parameters have to be controlled, such as; the size of nozzle, spray flow rate, atomization pressure, inlet air temperature and extent of cross-linking (Agnihotri *et al.*, 2004). Figure 2.3 presents the schematic representation of this method.

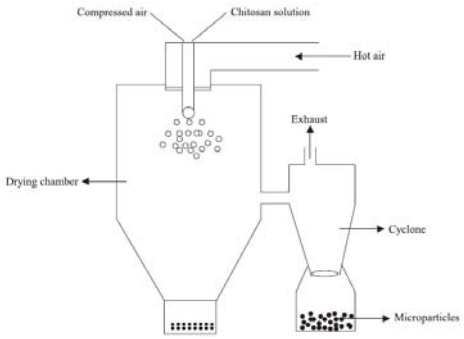


Figure 2.3 – Schematic representation of preparation of chitosan particle systems by spray drying method (Agnihotri *et al.*, 2004).

Emulsion-droplet coalescence method

This method uses the principles of both emulsion cross-linking and precipitation. Precipitation is induced, allowing the coalescence of droplets with CS drops of NaOH. Stable emulsion containing aqueous solution of CS together with the drug is produced in paraffin oil and then another stable emulsion CS aqueous solution of NaOH is produced similarly. When both emulsions are mixed by high speed stirring, the drops of each emulsion that collide at random and coalesce, which are precipitating CS drops to give small size particles. Size of the nanoparticles do not reflect the drop size, this depends upon the type of CS, i.e. as the % deacetylation degree of CS decreased, particle size increased, but drug content decreased. It is within the emulsion droplets that the nanoparticles are obtained (Agnihotri *et al.*, 2004). Figure 2.4 shows the schematic representation of this method.

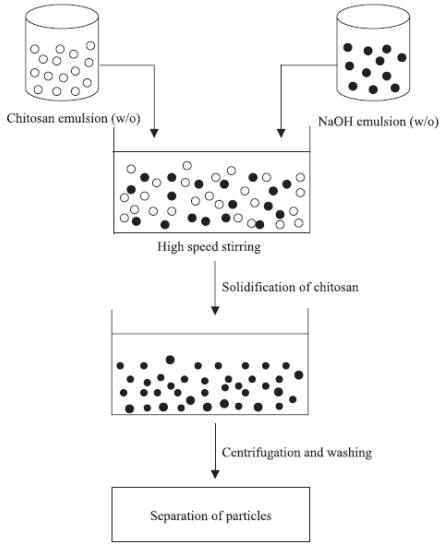


Figure 2.4 – Schematic representation of preparation of chitosan particle systems by emulsion coalescence method (Agnihotri *et al.*, 2004).

Ionic gelation

In this simple process, complexation between opposite charged macromolecules is used to prepare microspheres of CS. To avoid the possible toxicity of reagents and other undesirable effects, reversible physical cross-linking was applied by electrostatic interaction, rather than chemical cross-linking. In this method, CS is dissolved in acid aqueous solution to obtain the cation of CS, and then this solution is added dropwise under stirring to the polyanionic solution. CS undergoes ionic gelation and precipitates to form spherical particles due to the complexation between oppositely charged species. The microparticles formed have poor mechanical resistance which makes their use in drug delivery limited (Agnihotri *et al.*, 2004). Figure 2.5 presents the schematic representation of this method.

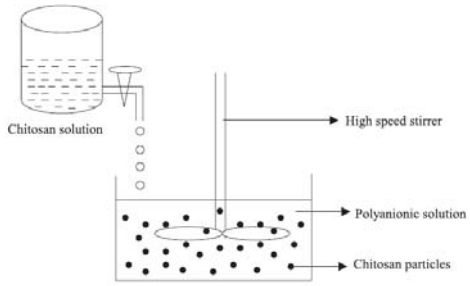


Figure 2.5 – Schematic representation of preparation of chitosan particle systems by ionic gelation method (Agnihotri *et al.*, 2004).

Reverse micellar method

Reverse micelles are thermodynamically stable liquid mixtures of water, oil and surfactant and one of the most important aspects of reverse micelle hosted systems is their dynamic behavior. Macroscopically, they are homogeneous and isotropic. By a rapid dynamic equilibrium, the size, polydispersity and thermodynamic stability of these droplets are maintained in the system. In this method, the surfactant is dissolved in an organic solvent and than the solutions of CS and drug is added with constant vortexing to avoid any turbidity. To keep the entire mixture in an optically transparent microemulsion phase, the aqueous phase is regulated. To obtain nanoparticles of larger size additional amount of water was to be added. To determine the maximum amount of drug that can be dissolved in reverse micelles an amount of drug is added with a gradual increase until the microemulsion is transformed into a clear transparent solution. The maximum amount of dissolved drug varies from drug to drug. After dissolving, the organic solvent is evaporated to obtain a transparent dry mass (Agnihotri *et al.*, 2004). Then the mixture is purified (subjected to centrifugation, sedimentation, dialysis and lyophilization) as described by Agnihotri et al. In figure 2.6, a schematic representation of this method is shown.

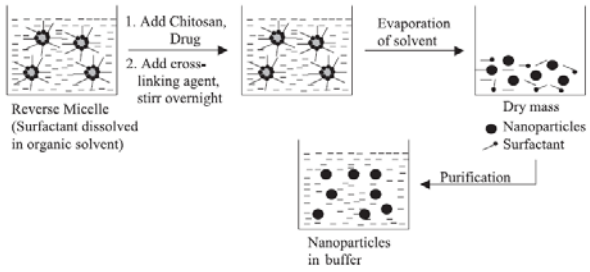


Figure 2.6 – Schematic representation of preparation of chitosan particle systems by reverse micellar method (Agnihotri *et al.*, 2004).

Sieving method

In this method, microparticles were produced by cross-linking of CS to obtain a non-sticky glassy hydrogel in order to get through a sieve. This method can be expanded easily and is devoid of tedious procedures (Agnihotri *et al.*, 2004). Figure 2.7 is the schematic representation of this method.

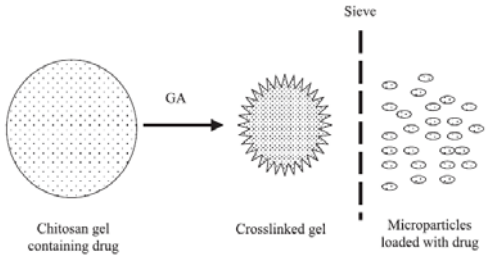


Figure 2.7 – Schematic representation of preparation of chitosan particle systems by sieving method (Agnihotri *et al.*, 2004).

In table 2.4, the different methods, conventionally used, for preparation of different types of systems based on chitosan are summarized.

Table 2.4 – Different preparation methods of systems based on chitosan (adapted Agnihotri *et al.*, 2004).

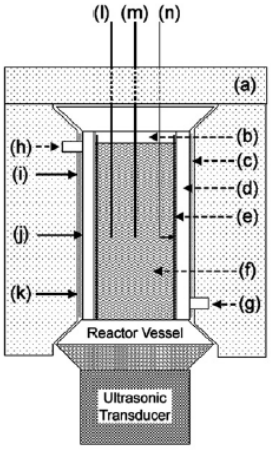
Type of system	*Method of preparation*
Tablets	Matrix; Coating
Capsules	Capsule Shell
Microspheres/Microparticles	Emulsion Cross-linking; Coacervation/Precipitation; Spray-drying; Ionic Gelation; Sieving method
Nanoparticles	Emulsion-droplet Coalescence; Coacervation/Precipitation; Ionic Gelation; Reverse Micellar method
Beads	Coacervation/Precipitation
Films	Solution Casting
Gel	Cross-linking

2.8 Ultra-Sonication

Ultrasound has recently been applied to obtain stable nano-suspensions. This can be applied to the other methods to increase efficiency in the formation of hydrated lipid vesicles of the smallest size. The ability to produce stable nanoparticles of desired size with great precision (narrow distribution and small scale) is the main factor of production of nano-suspensions, which emerged as a promising strategy for the efficient delivery of hydrophobic drugs, due to its characteristics versatile as the size of very small particles. To reduce the size of the nano-scale materials, extreme conditions generated within the collapsing cavitational bubbles have been used. In sonochemistry, an acoustic cavitation process can generate a localized hot zone with extremely high gradient of temperature and pressure. These sudden changes help to destruct the sonochemical precursor and allow for the formation of nanoparticles.

Nanoparticles can be produced by techniques such as sonochemical processing, cavitation processing and high-energy ball milling (Silva *et al.*, 2008).

Figure 2.8 shows the schematic representation of ultrasonic method.



Reactor Set Up:

(a) Expanded polystyrene insulation
(b) Air space
(c) Metallised card
(d) Water jacket
(e) Stainless steel liner
(f) Bulk solution
(g) Cooling water in
(h) Cooling water out

Thermocouple placement:

(i) Foam/metallised card top
(j) Outer jacket wall/metallised card
(k) Foam/metallised card
(l) SS probe off-centre
(m) SS probe centre
(n) Inner wall/stainless steel liner

Figure 2.8 – Schematic representation of reactor ultrasound (Little et al., 2007).

2.9 Chitosan Microspheres: a New Approach

An important application of chitosan in the industry is the development of systems or structures to release drugs or bioactive substances such as nanoparticles, hydrogels, microspheres, films and tablets (Agnihotri *et al.*, 2004; Joshi *et al.*, 2009).

Chitosan is a potential candidate for the production of bioactive microspheres due to characteristics such as low toxicity and good antimicrobial properties. Not only has a broad inhibitory spectrum against Gram-positive and Gram-negative but also sterilizes some yeast and molds (fungi). These properties, together with the wide applicability, do that, a promising candidate for use as an antimicrobial agent in dressings (Grinberg *et al.*, 2007;

Agnihotri *et al.*, 2004; Han *et al.*, 2008; Kong *et al.*, 2008; Chen e Schluesener, 2008; Morones *et al.*, 2005; Ye *et al.*, 2005).

Due to the positive charge of the carbon-2 of glucosamine monomer at a pH below six, the interaction between positively charged chitosan molecules and negatively charged cell membranes of microorganisms, leads to disruption of intracellular contents (Grinberg *et al.*, 2007).

Chitosan microspheres can be formed through various methods such as emulsification ionic gelation, spray drying, among others (Agnihotri *et al.*, 2004). However, microspheres formed by such methods, have a size difficult to manage and with high polydispersity. Thus, these methods involve limitations on the reproducibility. It has to be taken into account that microspheres of different sizes have different effects.

Moreover, the sonochemical method has potential advantages, (Gedanken, 2008). Despite the use of ultrasound technology in the formation of microspheres is not new, it is nevertheless necessary, to obtain a detailed description of specific methodology and optimization of experimental conditions, in order to improve the method (Silva *et al.*, 2008; Gouveia, 2009 (patent pending)).

Thus, this new approach aims to focus on the formation of surface layers of antimicrobial in a sonochemical process which will generate and apply the microspheres in the textile.

2.9.1 Layer-by-Layer (LbL) Functionalization

Recently a technology was developed that offers the opportunity to fabricate multifunctional microenvironments based on nano-engineering, called layer by layer deposition (LbL). This technology is based on the sequential deposition of oppositely charged polyelectrolytes on a surface, which allows the formation of multilayer from a wide range of compounds with nanometer precision (Shchukin *et al.*, 2004).

Microcapsules can be prepared by LbL, forming a multilayers structure containing different polyelectrolyte, making them multi-functional systems being implemented as a high potential for pharmaceutical use. In that way they can adapt to many different requirements in a single structure, in order to provide a supply of controlled substances at the right time in the right place and in proper concentration (Shao *et al.*, 2009).

Figure 2.9 shows a schematic representation of microspheres prepared by the method of Layer-by-Layer.

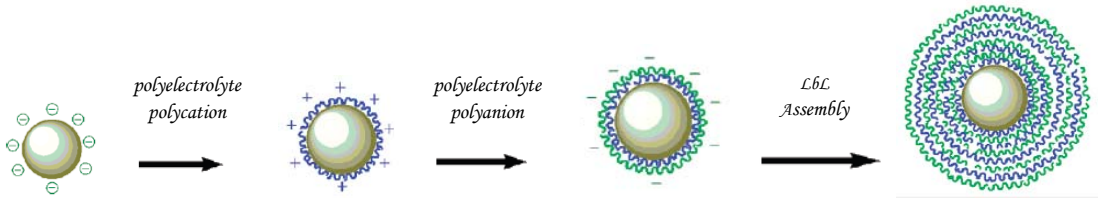


Figure 2.9 – Schematic representation of microspheres prepared by the LbL method (*Layer-By-Layer*) (adapted de Liu *et al.*, 2010).

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Graça Faustino and Isabel Gouveia, Polymers for Advanced Technologies, 2010 (submitted)

Chapter 3 – Synthesis and Characterization of a microsphere-based coating for textiles

In this chapter, it is used a low-molecular-chitosan to prepare chitosan-based micro/nanoparticle bioactive delivery systems, attached to a textile material. Characterization and optimization of ultrasound method was also performed in order to optimize the conditions for the formation of the microspheres. Characterization in terms of size, morphology and stability was also evaluated. The microspheres were approximately 2 μm in size and were further coated by the layer-by-layer (LBL) self-assembly deposition of polyelectrolytes including polyanion alginate (Alg) and polycation chitosan (CS) that were alternately deposited on chitosan microsphere templates. Scanning electron, inverted and optical microscopy were used to characterize the different produced bioactive microspheres.

The article was submitted to the journal Polymers for Advanced Technologies (Wiley) and is under review.

3.1 Introduction

Biodeterioration of textiles is a very wide ranging problem. By diversity in the conditions of usage, storage, washing, exposure to environmental conditions, etc., the mechanism of deterioration is of course different for each type of fiber, and protection is complicated (Kotowa, 2004).

Chitosan has a positive charge and is mucoadhesive compared many other natural polymers and can be produced in various forms such as powder, paste, film and fiber, is therefore it is used extensively in drug delivery applications. This, being a cationic polysaccharide in neutral or basic pH conditions (Agnihotri *et al*., 2004). It thus can react with many negatively charged molecules or surfaces such as biological membranes and it has considerable antimicrobial ability (Shao *et al*., 2009).

In recent years, alginate, a biodegradable polysaccharide, has attracted intense attention as an important class of biomaterials because of its unique properties including biocompatibility. It has been extensively investigated for many biomedical applications such as tissue engineering, drug delivery vehicles, and cell transplantation matrices. Since alginate contains carboxylic acid groups on polyguluronate units, alginate microspheres exhibit negative surface charge, allowing them to be used as negatively charged templates for polyelectrolyte layer-by-layer self-assembly (Liu *et al*., 2010).

The sonochemical method developed by Suslick and co-workers can be used to produced micrometer-sized gas-or liquid-filled proteinaceous microspheres from various kinds of proteins. This one-step procedure yields microspheres with a long shelf life and high stability (Grinberg *et al*., 2007). Ultrasound offers the prospect of an escalation of reaction rates, improved yields, or a better quality product due to improved homogenation of the constituent chemicals (Little *et al*., 2007).

On the other hand, the ability to readily tailor the properties (e.g., size, composition, porosity, stability, surface functionality, colloidal stability) capsules prepared by the layer-by-layer (LBL) technique has attracted particular interest. This has found application in diverse areas, ranging from medicine and biotechnology with micro- and nanometer-sized. These capsules allow the introduction of multiple functionalities, thus providing opportunities to engineer a new class of materials with unprecedented structure and function and can be assembled from suite materials, including synthetic and natural polyelectrolytes, nanoparticles and biomacromolecules. Various materials can be sequestered into the capsule interior for drug delivery, sensing or catalysis applications, and capsule surface can be

modified to alter the functionality and/or improve the colloidal stability of the capsules (Johnston *et al.*, 2006; Shao *et al.*, 2009). The polyelectrolyte multilayer coatings may serve multiple purposes such as stabilizing alginate hydrogels against dissolution in biological environments and providing barrier membranes for alginate hydrogels to slow release of encapsulated drug or biomolecules (Liu *et al.*, 2010).

On the other hand, a wide range of extracts and natural products with antimicrobial properties has been reported during the last years, due to the increased multidrug resistance of many human pathogenic microorganisms as well as the appearance of undesirable side-effects of certain antibiotics. The investigation of the chemical compounds within traditional plants has become desirable.

Essential oils and/or their components are becoming increasingly popular as natural antimicrobial agents to be used for a wide variety of purposes such as cancer treatment, food preservation, aromatherapy and fragrance industries. They are natural complex mixtures of volatile, lipophilic substances obtained from different parts of plants by different methods and are usually characterized by a strong odor and are composed by secondary metabolites of aromatic plants with oxygenated structures such as alcohols, ketones, aldehydes and esters. Besides the eco-friendly and biodegradable nature, these have been shown to possess antibacterial, antifungal, antiviral and antioxidant properties, but the specific advantage of essential oils appears to be in synergistic effects of their compounds as evidenced in greater activity when applied as natural essential oil compared with summary of the effects of the individual substances (Pedro *et al.*, 2009). The majority of the oils showed antibacterial activity, however cinnamon, clove and lime oils were found to be inhibiting both gram-positive and gram-negative bacteria (Prabuseenivasan *et al.*, 2006).

Lime oil is extracted from *Citrus aurantium* of the Rutaceae family) and has properties such as stomachic, carminative, flavouring agent, antiseptic, antiviral, bactericidal, disinfectant and haemostatic, and has also shown immunomodulatory effect in humans (Prabuseenivasan *et al.*, 2006).

The association between chitosan and essential oils may be suitable for further enhancing the antimicrobial properties of chitosan. However, there are few studies with the association of chitosan with essential oils. More studies have to be performed for developing new formulations taking benefic of the potential of essential oil entrapment (Pedro *et al.*, 2009).

Therefore, this study aims to investigate the development of chitosan-based microspheres using the sonochemical and LbL techniques in order to produce more stable and resistant

microspheres to be used in the functionalization of textile materials, to authors knowledge, this is the first report on the chitosan-microsphere coating for textiles as a potential for the design of new bioactive textiles.

3.2 Experimental

3.2.1 Materials

Terephthalic Acid (98%), Sodium Hydroxide (> 98%), Phosphate Buffer pH 7.4, Chitosan (low molecular weight), Alginic Acid sodium salt, Acetic Acid glacial (95%), Dodecane (≥ 90%), Sodium Chloride (≥ 99.5%) and Lime oil was purchased from Sigma-Aldrich. Coomassie blue (Bradford), Methylene blue and Astrazon red were supplied by Bayer.

Different textile materials (yarns) with the following composition: 100% Cellulose Acetate (CA), 100% Cotton (CO), 100% Polyamide (PA), 100% Polyester (PET), 100% Acrylic (AC) and 100% Wool (WO) were selected due to their common use in medical and hospital textiles as well as for their different chemical composition.

3.2.2 Optimization of the operating conditions of the Ultrasonic Device

In order to characterize the ultrasonic bath, the dosimetric method was followed as described by Little *et al.*, (2007). A solution of terephthalic acid (TA) 1.5 M was prepared at pH 7.4 in a phosphate buffer solution ($NaH_2PO_4.H_2O$) 0.1 M

TA solution was placed in test-tubes at various points in the ultrasonic bath, with a distance of 1cm between them, to evaluate the amount of radicals in each point and each position of the test-tube. Figure 3.1 shows the positions where the best results were placed (1, 2, 3, 4 and 5 points in positions A, O and B).

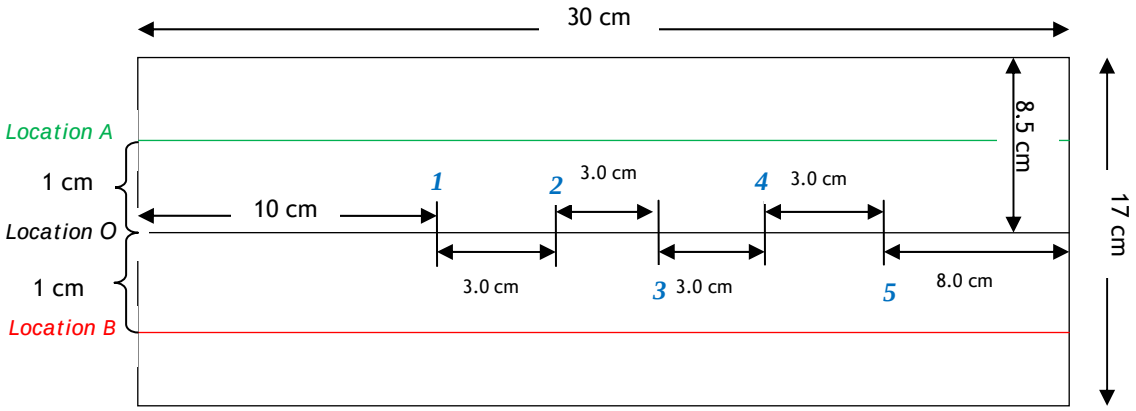


Figure 3.1 – Best positions of tubes in different parts of the ultrasonic bath.

Each of the positions was studied to optimize the volume of the solution to be added in test-tubes. For to frequency used (32 kHz) it was determined the height of the points under study and the volume the wavelength of the equation $\lambda = c/f$. Therefore volumes studied ranged from 5, 5.5, 6 to 6.5 mL. It was assumed a wave velocity (c) 1498 m/s, as described by Little *et al.*, (2007).

Figure 3.2 shows the schematic representation of a test-tube and the different positions of nodal (λ) and anti-nodal ($(3/4)\lambda$) points.

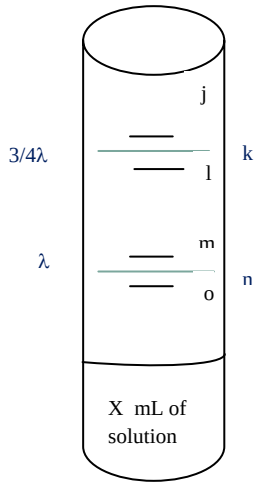


Figure 3.2 – Schematic representation of a test-tube study.

Hydroxyl radicals were analyzed in a fluorescence spectrophotometer (Spectrofluorometer RF-1501, Shimadzu) with emission activity (EM) 420 nm and an excitation wavelength (EX) of 314 nm. The nodal points and anti-nodal wave is obtained by analyzing the amount of -OH radicals according to the study of Little and colleagues (2007).

3.2.3 Preparation of the Chitosan Microspheres

After characterization and optimization of the ultrasonic bath (Ultrasons-H, Selecta), the study of the formation and characterization of protein microspheres, based on certain studies carried out by several authors using the sonochemical method (Avivi and Gedanken, 2002; Suslick and Grinstaff, 1990; Grinstaff and Suslick, 1992; Suslick *et al.*, 1994), was performed.

Chitosan microspheres were synthesized sonochemically from aqueous solution of chitosan. Concentration ranged from 0.20 to 0.80 mg/mL. The n-dodecane was used as the co-solvent to make the emulsion which is necessary for microsphere formation. In place of the dodecane

it was also tested an essential oil (Lime oil) in the same quantity and in the same conditions. Temperature ranged from 20, 25, 30, 35 and 40 ºC, and sonication time from 5, 10, 15, 20, 25 and 30 min.

After the synthesis, the mixture solution was stored at 4ºC during 12 hours to able the separation of all phases (dodecane/oil, microspheres and chitosan solution).

3.2.4 LBL Self-Assembly of Alg/CS on Chitosan Microspheres

The polyelectrolyte solutions used for alternating deposition of micro layers of Alg/CS on the CS microspheres were prepared at concentrations of 1 mg/mL, based in the study of Shao et al, (2009). Alginate was dissolved in an aqueous solution of 0.5M sodium chloride as described elsewhere (Xie et al, 2009) and Chitosan was dissolved in aqueous solution of 0.1 M acetic acid. The pH values were adjusted to 3 using HCl 0.1 M and NaOH 1 M solutions.

For the deposition of each layer, 900 µL of polyelectrolyte solution was added to a test-tube containing 100 µL of microsphere suspension and stirred for 10 min on a magnetic stirrer. After woods they were washed with 400 µL of desioniated water, and stirred on a magnetic stirrer for 5 min. This process was repeated using the oppositely charged polyelectrolyte until expected multilayer pattern was obtained, where chitosan was used as polycation and alginate as polyanion. The layer sequence was $(Alg/CS)_n$. As described by Shao et al, (2009) and illustrated in figure 3.3.

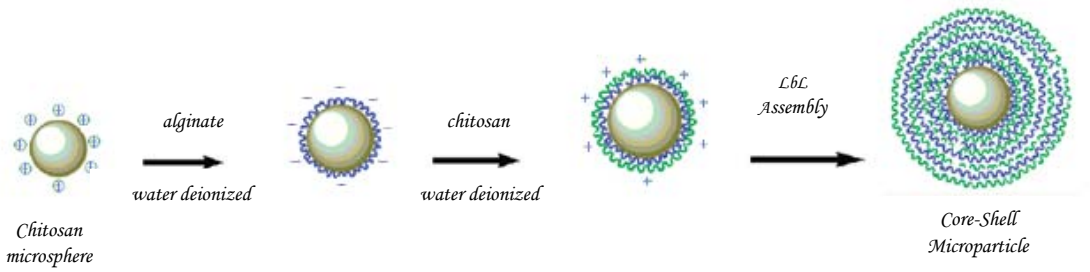


Figure 3.3 – Chitosan-template polyelectrolyte multilayer microcapsules loaded with Alginate (Adapted from Liu *et al.*, 2010).

In order to evaluate the alternatively layer deposition, the positive charges due to the presence of free amino groups present in CS were stained with a solution of Coomassie blue (Bradford). Microspheres in which the ultimate layer was of Alg were stained with a solution of a cationic dye Astrazon red, as is visible in table 3.6. All dye solutions were prepared in the same concentration of the 0.0005% (w/v).

3.2.5 Functionalization of the textile fibers

Optimum conditions, namely: 3.9 mL of CS solution (0.8 mg/L) and 2.6 mL of n-dodecane or oil, in the case of Lime oil core, were used to produce and bind simultaneously the microspheres onto textile materials (50mg), which were immersed in the solution, before sonication, irrespectively the fiber composition and sonicated for 10 min at 37 ºC.

For the case of the LbL CS/Alg coated CS microspheres textile material (50 mg), irrespectively the fiber composition, was immersed into a test-tube with microsphere suspension in a proportion to obtain a solution volume of 6.5 mL (5.85 mL polyelectrolyte and 0.65 mL microspheres) and was sonicated with 32 kHz for 2, 5 and 10 min at 37 ºC.

3.2.6 Characterization of microspheres

The size and the morphology and the different layers of the microspheres were observed under a HITACHI S2700 scanning electron microscopy (SEM), an inverted microscopy OLYMPUS CKX 41 and an optical microscopy (Zeiss, West Germany). SEM samples were prepared by the application of a drop of microspheres suspension onto the glass and then dried at room temperature (20±2 ºC); which were further coated with a thin layer of sputtered gold in a gold EMITECH-K550 evaporator. The samples for the inverted microscopy were prepared by the application of a drop of microspheres suspension in a plate and observed under a microscope.

The microsphere coating onto the textile fibers was observed on an optical microscopy (Zeiss, West Germany).

3.2.7 Measurement of the Stability of Chitosan Microspheres and LBL Alg/CS Chitosan Microspheres

The stability of the chitosan microspheres and LBL Alginate/CS coated chitosan microspheres, was assessed by following the changes in the microspheres which were monitored under different temperatures (-5, 5, 20, 35, 37 and 40ºC), taking into account the conditions of storage (-5, 5 and 20 ºC) and the possible contact with human body (35, 37 and 40ºC), pH of (4.5, 7 and 9.5) was also evaluated due to the different pH of the skin. Stability in all conditions was monitorized for 30 days.

3.3 Results and Discussion

3.3.1 Optimization of the operating conditions of the Ultrasonic Device

Is of interest to study the ways in which the operating conditions of the sonochemical reactor can be optimized for better performance (Little et al., 2007).

To optimize the device, the characterization of ultrasonic bath was based on a dosimetric study, as described by Little et al (2007). The dosimetric method determined the maximum amount of hydroxyl radicals-OH, to obtain the nodal points (low cavitation) and the anti-nodal points (higher cavitation) of the wave. The detection of hydroxyl radicals is due to the conversion of TA in HTA acid (2-hydroxyterephthalic) (Little et al., 2007).

The values of absorbance of the radical-OH obtained in three different positions of the bracket (A, 0 and B) at different points in the ultrasonic bath (1, 2, 3, 4 and 5) were read in a fluorescence spectrophotometer at 420 nm, and are shown in table 3.1.

Table 3.1 - Values of absorbance of the radical-OH obtained in three different positions of the bracket (A, 0 and B) at different points in the ultrasonic bath.

Positions of the tubes in the ultrasonic bath		Absorbance radical-OH
1	A	581.173
	0	587.509
	B	557.626
2	A	440.017
	0	129.439
	B	66.807
3	A	275.975
	0	958.200
	B	1016.897
4	A	863.180
	0	893.550
	B	344.779
5	A	787.293
	0	1016.897
	B	71.035

After analysis of the results of the table 3.1 it was found that most of the radical-OH formation occurs in position 3 and 5, at positions B and 0 respectively, reaching a value of absorbance in the range of 1016.897.

In accordance to this, the synthesis of the microspheres was performed always in the same position, position 5, due to the major microsphere formation caused by the ionization of radical-OH, in that case, of the chitosan, in order to promote the desired crosslinking to give a more stable shell, as described by Avivi and Gedanken 2002.

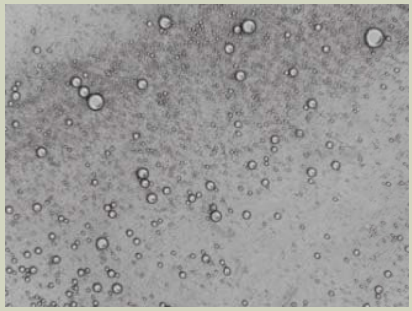
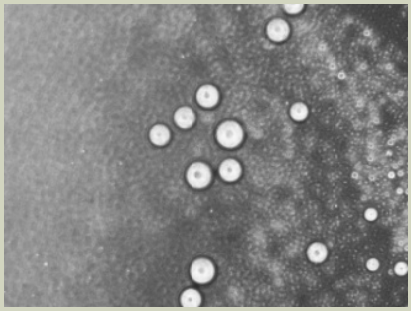
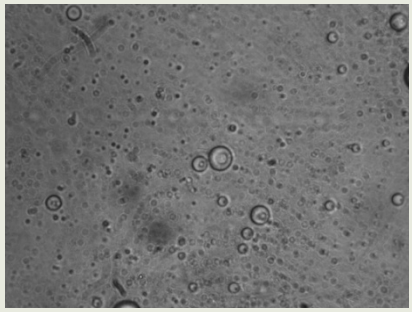
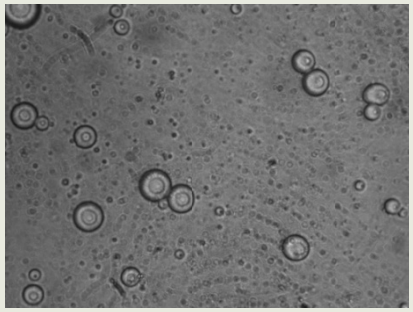
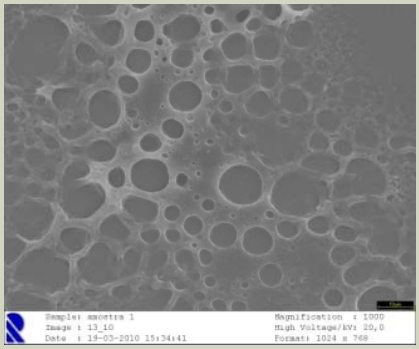
3.3.2 Production and characterization of the Chitosan Microspheres

Chitosan microspheres were prepared by sonication (Ultrasons-H, Selecta) in optimal conditions at frequency of the 32 kHz, for 10 min. The optimum conditions were found to be, 3.9 mL of chitosan 0.8 mg/mL dissolved in aqueous solution of 1 M acetic acid placed in a test-tube with 2.6 mL dodecane, to give a total volume of 6.5 mL, which was also found to be the best amount in the equipment used (data not shown). The results showed that in position 5 at temperature of 37 ºC sonicated for 10 min, occurred the higher formation of CS microspheres, i.e., ± 1 mL. It was also found that at temperature of 40 ºC formation of microspheres doesn´t occur. With regards with the sonication time, results showed that the best time was 10 min, where microspheres resulted stable for many hours.

In the above described conditions, the CS microspheres have spherical shape and a size about 2 µm, as it can be seen in the inverted microscopy images and SEM images presented in table 3.2. Thus they have the shape and size that are in accordance with the previous results obtained by Shao et al 2009 and Agnihotri et al 2004. , with the advantage of being obtained in a much faster and simple method than the reported by Agnihotri et al (2004) who studied the preparation of chitosan nanoparticles by emulsion cross-linking or by Shao et al (2009) whereas ultrasonication required 30 min to generate monodisperse CS microspheres with 1 µm size and spherical shape.

Therefore, in comparison with other methods the results obtained are promising since they show microspheres with size ± 2 µm through a simple and fast process (only 10 minutes sonication).

Table 3.2 – Images of CS microspheres (magnification: 40 and 100).

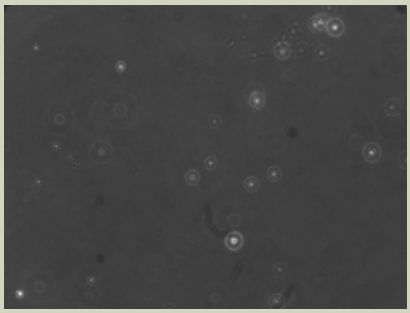
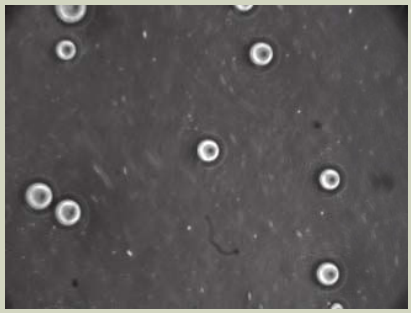
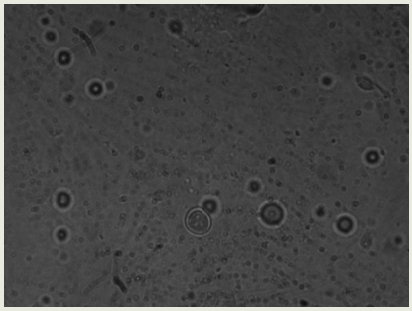
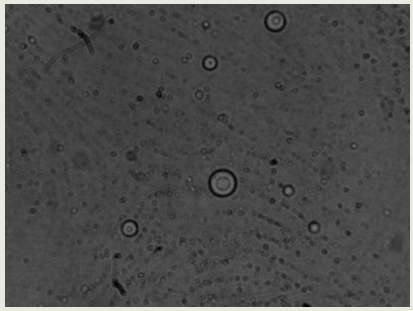
Chitosan microspheres	*Amplification 40X*	*Amplification 100X*
Inverted microscopy		
Optical microscopy		
SEM		

3.3.3 LBL deposition of Alg/CS nanolayers on Chitosan Microspheres

The morphology of microspheres with deposition of Alg/CS layers was characterized by inverted and optical microscopy and SEM. optical and inverted microscope images show that it appears that the microspheres of chitosan with LBL (Alg/CS) are, as well, spherical and the

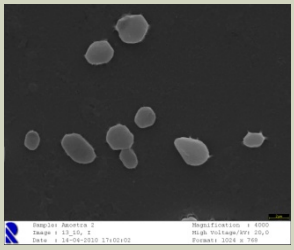
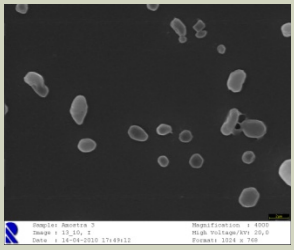
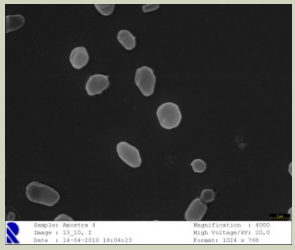
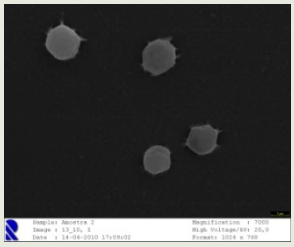
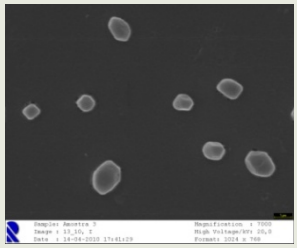
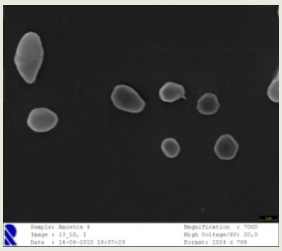
layers are deposited on the shell of the CS microspheres as shown in the table 3.3, especially in the lower magnification (40X).

Table 3.3 – Images of LBL $(Alg/CS)_4$ layers on CS microspheres magnification (40 and 100).

Chitosan microspheres with LBL	*Ampliação 40X*	*Ampliação 100X*
Inverted microscopy		
Optical microscopy		

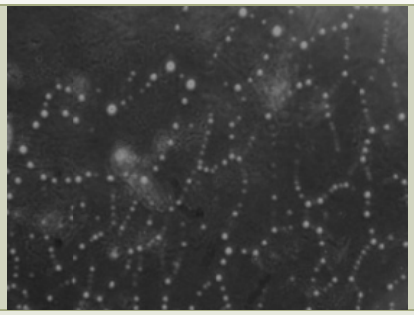
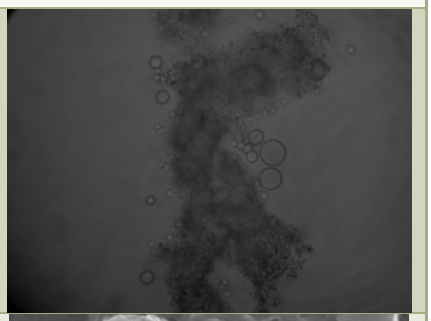
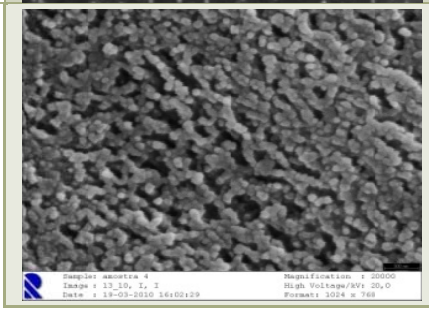
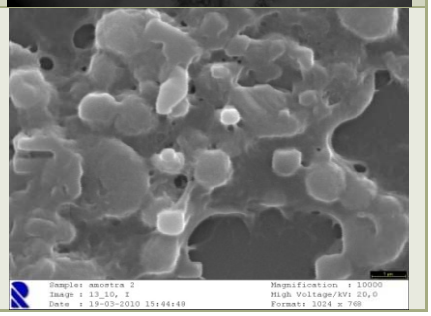
Observing the SEM images (table 3.4) it appears that the microspheres with LBL have spherical shape and monodispersitivity of about 1 µm in size, although it was also visible some microspheres with non-spherical shape most probably because the microsphere cores were totally removed when completely dried before testing in SEM analysis, resulting in microcapsules with shrunken surfaces as reported by with Xie et al 2009.

Table 3.4 – SEM images of LBL Alg/CS coated CS microspheres.

	LBL *2 camadas*	LBL *4 camadas*	LBL *6 camadas*
Ampliação 4000			
Ampliação 7000			

Chitosan microspheres with LBL $(Alg/CS)_6$ presented some problems such cross-linking, as visible in table 3.5 due to the agglomeration promoted by the solutions of CS and Alg for the LbL deposition. However, cross-linking of chitosan microspheres was expected, in this case, because it's in agreement with the results obtained by Mei et al (2009). CS microspheres took the shape of irregular long strips interconnecting with each other, which is typical for macromolecular structure because of the straight chain. The agglomeration is in agreement with results of Genç et al (2008) because the small particles tendency to form agglomerate.

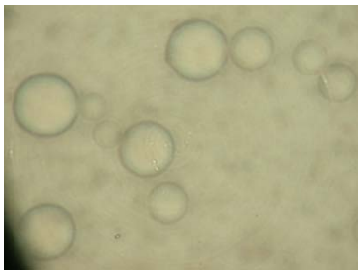
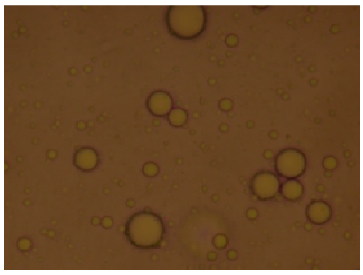
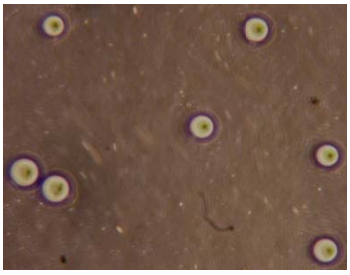
Table 3.5 – Images of the cross-linking and agglomeration effect of the microspheres (inverted microscopy and SEM).

	Cross-linking	*Glomeration*
Inverted microscopy		
SEM		

As described before and in order to evaluate the alternatively layer deposition, the positive charges were stained with a solution of Coomassie blue (Bradford). Microspheres in which the ultimate layer was of Alg were stained with a solution of a cationic dye Astrazon red, as is visible in table 3.6. All dye solutions were prepared in the same concentration of the 0.0005% (w/v). Figures show the thin blue line around the shell of the microsphere, in the case of CS microsphere and in the case of CS/Alg LbL coated CS microsphere where the ultimate layer was of chitosan (positive charged surface); whereas for the ultimate Alg layer (negative charged surface), a red line appears at the surface of the shell.

The results show the success of the LbL assembly of the polyelectrolytes on the CS microsphere shell as expected, increasing the thickness of the membrane and the stability of the microspheres, as shown in the stability assays discussed below.

Table 3.6 – Images of CS microspheres and LBL of Alg/CS on CS microspheres with dyes to positive and negative charge stained.

Chitosan microspheres	Negative Charge (LBL)	Positive Charge (LBL)
		

3.3.4 Stability of the microspheres

CS microspheres

In all conditions studied for the production of CS microspheres, they have low stability. For the different concentrations, temperatures and sonication time, microspheres are stable for only several hours. In contrast, the microspheres produced under the optimum conditions are very stable, in solution, at all pH and all temperature studied for at least 1 month. The pH 7 and temperature 40 ºC presents lower stability (only some hours). This is agreeing with previous studies performed by Avivi and Gedanken 2002. The microspheres have to stay in solution in order to last several weeks, otherwise, as for example in dry air, they break out very easily (in few hours).

Microspheres with multilayers Alg/CS

The microspheres with multilayers Alg/CS have high stability at all pH and temperatures for at least 1 month, in solution. It was agreed that obtained in previous studies by Shao et al 2009. For temperature at 40 ºC was formed interconnection, as is visible in table 3.5.

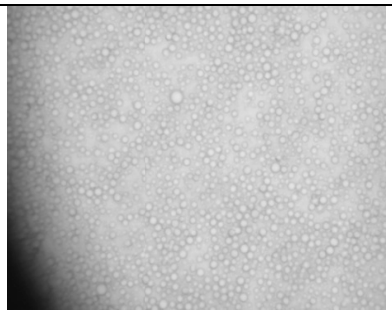
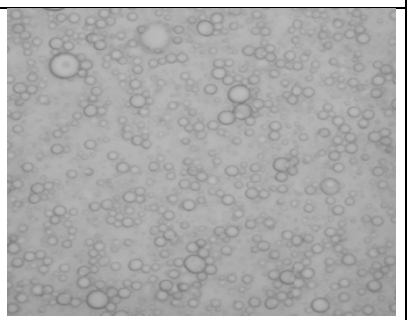
In addition, the microspheres with LBL Alg/CS layers do not break out for one week, when exposed to dry air, which is in agreement with the study of Shao et al (2009), being much more stable than the microspheres of chitosan that only last in moisture conditions.

3.3.5 Chitosan Microspheres with Lime Oil

Chitosan microspheres with essential oil were sonicated using the same optimal conditions studied for CS microspheres. The chitosan solution (3.9 mL) was placed in a test-tube and overlayered with lime oil (2.6 mL), and sonicated for 10 minutes at 37 ºC.

The images acquired of CS microspheres with essential oil in the optical microscope are shown in table 3.7. The microspheres present a spherical shape and monodispersivity, being in agreement with the previous results where dodecane was used instead of Lime oil, because they present the same size (2 µm). Pedro et al (2009) studied the entrapping of citronella oil in chitosan microspheres produced by the emulsionating technique, with different particles sizes, ranging from 11 ± 3 µm to 225 ± 24 µm. In addition, it was also reported that the smallest microparticles showed the biggest release rate, most probably because they have a larger specific surface area, causing the oil release rate to be faster. Therefore, for certain applications in which a smaller size might be required, this sonication process reveals to be very effective.

Table 3.7 – Images of chitosan microspheres with essential oil (Lime Oil) in optical microscope.

Amplification 40x	Amplification 40x
	

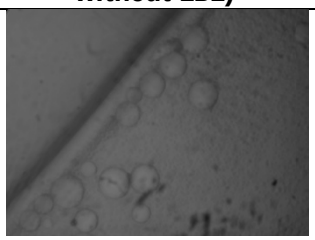
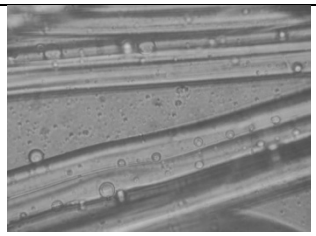
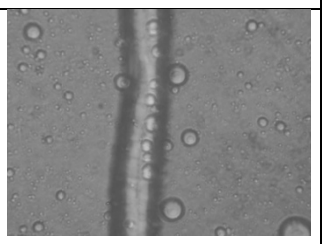
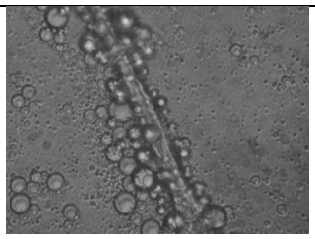
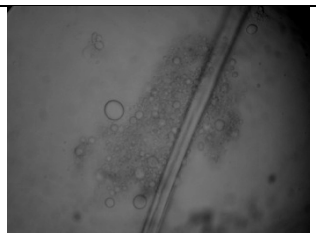
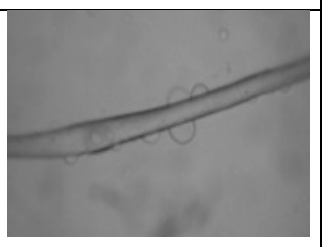
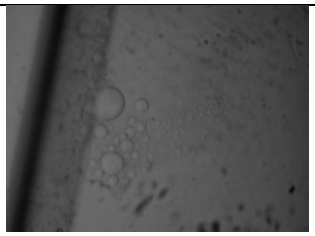
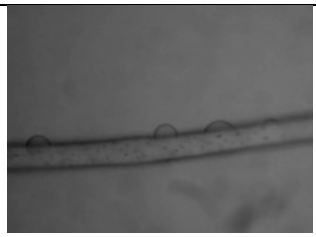
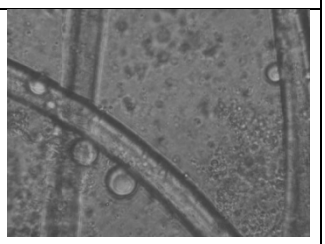
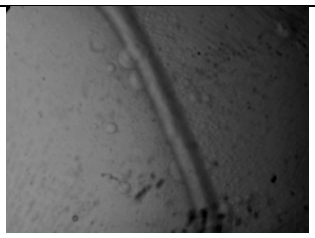
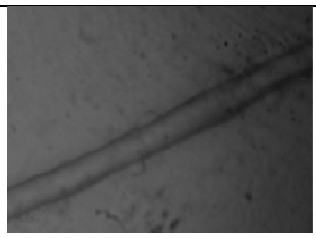
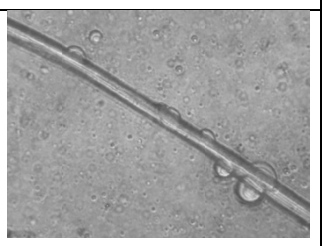
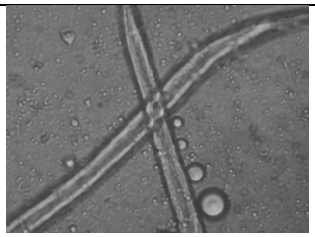
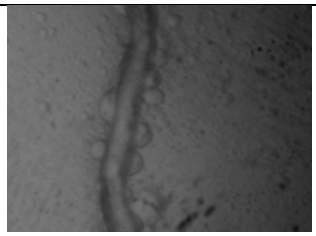
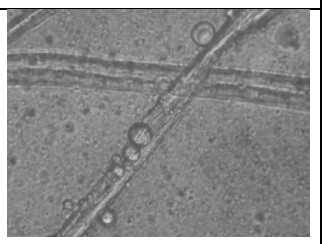
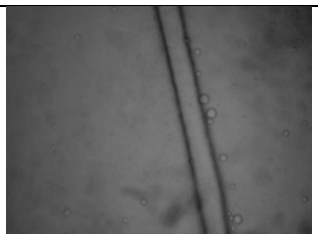
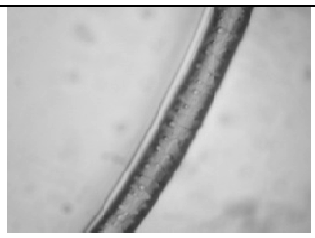
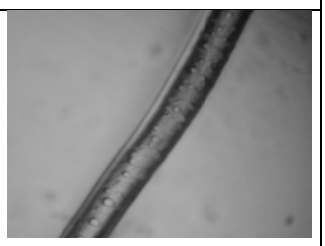
3.3.6 Microsphere-based Coating for Textile Materials

The adhesion of the microspheres onto the fibres was evaluated by the optical microscope. Table 3.8 shows the microspheres adhered to all the fibres tested. However, adhesion is more visible in the case of cotton fibres, most probably due to the surface structure of the fibre and the negative charged exhibit by these cellulosic fibres, especially in the case of CS microspheres which has an opposite charge. Images also show a slight decrease in the amount of microspheres adhered to the fibres in the case of LbL coated microspheres. However this is

because the amount of microspheres used is lower when compared with the CS microsphere simultaneous formation and fixation of the fibres.

All these results are in agreement with the expected and the LbL deposition has shown to be fundamental to provide microsphere´s stability. The coating appears to be independent of fibre composition although in the case of synthetic and less reactive fibres like polyester the microspheres´ amount that is linkage onto the fibres is lower.

Table 3.8 – Images of microspheres coating in textile materials.

Fibres	Microspheres with positive charged (CS without LBL)	Microspheres with negative charged (LBL)	Microspheres with positive charged (LBL)
cellulose acetate			
cotton			
polyamide			
polyester			
acrylic			
wool			

3.4 Conclusions

This work describes a novel method to give a microsphere-based coating for different textile fibers using non-toxic and biodegradable agents. To the best of author's knowledge, this is the first report on the simultaneous formation and coating of textiles trough a single-step sonochemical method, giving homogeneous coating of stable microspheres.

The major advantages of this method in comparison with other techniques that are commonly used to incorporate microspheres/microcapsules onto textile materials, are the non-toxicity both to the potential users and to the environment, and the possibility of being carried out in a simple step process with short reaction time and without using cross-linking agents such glutaraldehyde or epoxy resins that are normally required to produce microspheres or to bind them onto the textile materials. The other advantage of this sonochemical process is that the microspheres are stable at different pH solutions and temperatures in a range that is usually required for medical textiles that are in close contact with the skin.

There is a great potential for the use of this new method since it will be able to encapsulate drugs or other bioactive agents into microspheres and bind them to textiles that can be used in medical applications, in particular, in the design of textile-based wound-dressings carefully developed according to different stages of the healing process. Consequently, this novel process can be a very promising strategy that may open new avenues for the design of in situ textile-based bioactive delivery systems for skin-contact interaction.

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Chapter 4 – Activity of Antimicrobial Textiles

This chapter encloses the study of JIS L 1902:2002 method for each both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella pneumoniae*) bacteria were used. in addition, SEM was also used to assess antibacterial activity, by analyzing the morphological changes in *Staphylococcus aureus* and *Klebsiella pneumonia* and their adhesion to textile fibers.

4.1 Introduction

In the last few years the number of biofunctional textiles with an antimicrobial activity has increased considerably (Gao and Cranston, 2008; Papaspyrides *et al*., 2009; Singh *et al*., 2005). The largest application of antimicrobial textiles is perhaps the biomedical area (Kramer *et al*., 2006; Zilberman and Elsner, 2008). Textiles provide a good contact area and can absorb moisture both required for microbial growth. This growth can lead to malodours, dermal infections, allergic responses and fabric deterioration (Singh *et al*., 2005; Gao and Cranston; Ammayappan and Moses, 2009). Thus, the incorporation of antimicrobial agents on textile products, to be overcome these problems, is of the utmost importance (Askew, 2009).

Several different types of antimicrobial agents have increased drastically in recent years to confer antimicrobial properties in the textile industry (Kim *et al*., 2009).

Moreover, several standard methods are being used to assess antimicrobial activity on textile products. The most common standards include qualitative (AATCC 147:1998, ISO 2045:2004 and JIS L 1902:2002 – Halo method) and quantitative methods (AATCC 100:1999 and JIS L 1902:2002 – Absorption method) (Askew, 2009).

The qualitative methods consist of placing the textile samples in contact with nutrient agar plates containing bacterial which are then incubated under moist conditions at 37ºC for 24 – 48 hours (Askew, 2009; Gao and Cranston, 2008). These methods are simples to perform, quick and useful when a large number of samples have to be screened (Gao and Cranston, 2008). In addition to the qualitative tests, quantitative can be provide values of antimicrobial activity based on the reduction of planktonic bacterial growth (Askew, 2009; Gao and Cranston, 2008). This method requires more time to be realized and the procedure is very complex. The complexity also increases when the number of samples increases.

To evaluate the effectiveness of the antimicrobial activity of textile materials as well as bacteria adhesion on textiles, Gomes et al (2010) showed that Scanning Electron Microscopy, can be a useful and simple tool.

Accordingly, the observation and characterization of heterogeneous organic and inorganic materials and surfaces, SEM is a powerful instrument. The area/volume to be examined is irradiated with a finely focused electron beam, which may be static or swept in a raster across the surface of the specimen. SEM offers a relatively simple method of studying the surface morphology of samples at high magnification under optimal conditions and with three dimensional appearance of the specimen image. Therefore, one of the potentials of SEM, which remains largely unexplored, is the evaluation of the effectiveness of the antimicrobial

activity of textiles, in particular, through the analysis of bacteria adhesion on textiles and of the morphological consequences of exposure of bacteria to antimicrobial agents (Goldstein *et al.*, 1992). Some studies that use SEM to characterize different microorganisms and different antimicrobial agents were undertaken by several authors to assess antimicrobial and morphological changes caused in the microorganism by antibiotics (Didenko *et al.*, 2005; Rajeshwari *et al.*, 2009; Chan *et al.*, 1998; Monson *et al.*, 2010; Rasul *et al.*, 2010; Greenwood and O'grady, 1972; Cvelbar *et al.*, 2009). However to authors knowledge, only one study was reported recently concerning the SEM surface analysis of antimicrobial textiles. Hence, this investigation aims to evaluate the potential of SEM as a tool for the assessment of antimicrobial activity of textiles. Therefore, here we report the potential of SEM in the assessment of antibacterial activity, by analyzing the morphological changes in *Staphylococcus aureus* and *Klebsiella pneumoniae* by the functionalized textiles which were coated with chitosan-based microspheres.

4.2 Experimental

4.2.1 Materials

Acetic Acid glacial (CH_3COOH), Dodecane (≥ 90%), Sodium Chloride (≥ 99.5%), Glutaraldehyde (25 %) and Ethanol (96 % (v/v)) Chitosan with low molecular weigh, Alginic Acid and Lime oil were purchase from Sigma Aldrich.

The textile materials used were 100% Cotton and Gauze and were selected due to their common use in medical and hospital textiles.

4.2.2 Microorganisms

The microorganisms used in all assays were *Staphylococcus aureus* (ATCC 25923) and *Klebsiella pneumoniae* (ATCC 13883) as described in the JIS L 1902-2002.

4.2.3 Antimicrobial Treatment

Chitosan microspheres, LBL self-assembly Alg/CS chitosan microspheres, and chitosan microspheres produced with Lime oil were used to functionalized the textile materials as described previously in chapter 3.

Briefly microspheres were synthesized sonochemically from aqueous solution of chitosan. The n-dodecane was used as the co-solvent. In place of the dodecane it was also tested an essential oil (Lime oil) in the same quantity and in the same conditions. Chitosan microspheres were prepared by sonication in optimal conditions at frequency of the 32 kHz, for 10 min, as described by Faustino and Gouveia, (2010).

The polyelectrolyte solutions used for alternating deposition of micro layers of Alg/CS on the CS microspheres were prepared at concentrations of 1 mg/mL. For the deposition of each layer, 900 µL of polyelectrolyte solution was added to a test-tube containing 100 µL of microsphere suspension and stirred for 10 min on a magnetic stirrer. Afterwards they were washed with 400 µL of desioniated water, and stirred on a magnetic stirrer for 5 min. This process was repeated using the oppositely charged polyelectrolyte until expected multilayer pattern was obtained, where chitosan was used as polycation and alginate as polyanion, as described by Faustino and Gouveia, 2010 (pending).

4.2.4 Assessment of antibacterial activity by the JIS L 1902-2002 Halo-method

The culture medium Brain Heart Infusion (BIH) used for the cultivation of the bacterial strains deployed in this work, were prepared according to the instructions of the manufacturer. Culture media were dissolved directly after being weighed in deionized distilled water, and then sterilized by autoclaving for 15 min at 121 ºC. Agar was used to solidify the media before autoclaving. The strains of Staphylococcus aureus and Klebsiella pneumoniae used were obtained from the American Type Culture Collection, ATCC 25923 and ATCC 13883 respectively. The cell density of bacterial suspensions was determined by measuring the optical density (OD) of appropriately diluted samples using a spectrophotometer at a wavelength of 640 nm [OD640] The number of viable cells in a bacterial suspension was estimated and diluted in physiological saline (0.9% wt/vol, NaCl). 100 µL aliquots of the appropriate dilutions were plated onto the surface of agar plates with a Mueller-Hinton medium, and after approximately 10 min the functionalized cotton samples were placed on top of agar plates and incubated for 24h at 37 ºC.

4.2.5 Assessment of antibacterial activity with Scanning Electron Microscopy

After completing the previous procedure, samples were removed from agar plates and fixed with 3% glutaraldehyde at 4ºC overnight. Dehydration of the samples was then conducted by a series of 10, 25, 50, 70, 100 % ethanol solutions. Using a Critical Point Dryer the samples were dried further (CPD, Emitech). These samples were mounted on aluminum stubs and then coated with gold using a Sputter Coater (Emitech). Finally the samples were examined using a Hitachi (S 2700) Electron scanning microscopy. Samples of culture medium Mueller – Hinton (agar plates) were also prepared to observe in the SEM, by the same method described previously.

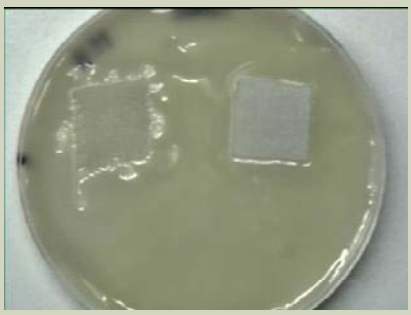
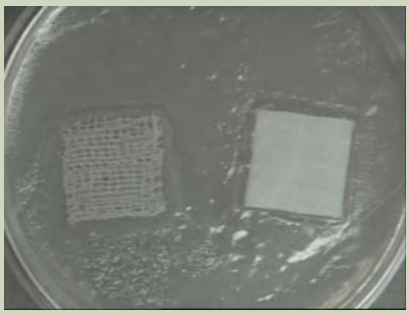
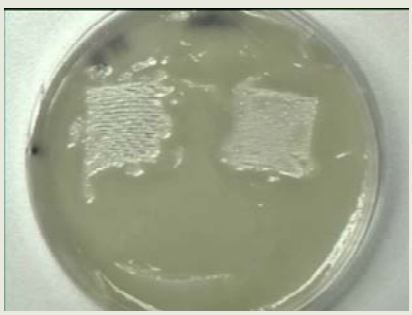
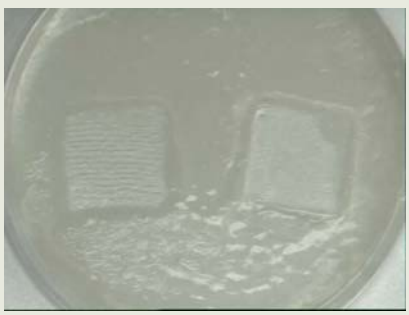
4.3 Results and Discussion

4.3.1 Antibacterial activity

In this work, the antibacterial efficacy of the chitosan microspheres and Alg/CS multilayer's was evaluated by assessing the reduction in bacterial attachment and growth on the functionalized gauze and cotton substrates, by SEM analysis, and by the evaluation of bacterial activity according with qualitative method JIS L 1902-2002 (Halo method). Halo size provides some indication of the potency of the antimicrobial activity of textile samples.

The Control samples (cotton and gauze without microspheres) did not present any halo formation indicating that they don´t exhibit any antibacterial activity against the tested as it can be observed in table 4.1. These results are in agreement with the expected.

Table 4.1 – Images of control samples analyzed by standard method JIS L 1902-2002 – Halo method, against *K. pneumoniae* and *S. aureus*.

	Control (Klebsiella)	*Control (Aureus)*
With samples		
Without samples		

Quite different were the results presented by the samples treated with chitosan microspheres. In this case, a halo around the contours of the test specimen was clearly identified showing the antibacterial effect of this functionalized material against *K. pneumonia* and *S. aureus (*table 4.2).

Samples treated with Alg/CS multilayers in chitosan microspheres also showed a good antibacterial activity due to the clear formation of a halo around them, against *K. pneumonia and S. aureus*. But samples without drying showed a higher halo, as it can be observed in table 4.3.

The results show that the samples treated with LBL CS/Alg microspheres have higher efficiency than the samples treated only with the CS microspheres against *Staphylococcus aureus* and *Klebsiella pneumoniae, most probably due to the fact that* i the latter have low stability when subjected to drying.

The results also showed that the antibacterial effect of Alg/CS occurred without migration of the active agents in the samples of the cotton with drying. Only microorganisms in direct contact with the active sites of Alg/CS are inhibited.

Table 4.2 – Images of samples treated with chitosan microspheres analized by the standard method JIS L 1902-2002 – Halo method, against *K. pneumoniae* and *S. aureus*.

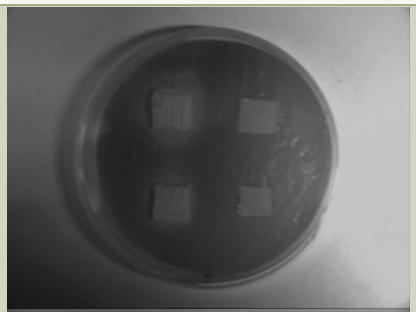
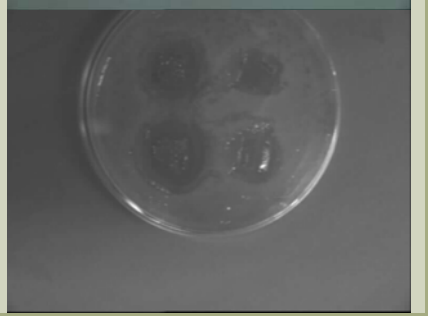
	With samples	*Without samples*
K.pneumoniae		
S. aureus		

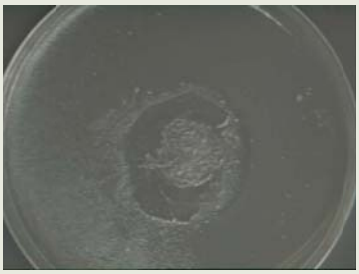
Table 4.3 – Images of samples treated with LBL Alg/CS chitosan microspheres analized by standard method JIS L 1902-2002 – Halo method. All assays were performed using Gram-positive bacteria *S.aureus*.

	With samples	*Without samples*
		

The antimicrobial activity of essential oils and plant extracts iswell documented in several studies (Hammer *et al.*, 1999; Joshi 2009). They are also promising candidates as antimicrobial agents for textiles. In the previous chapter, lime oil (*Citrus aurantifolia*) was used as the co-solvent for the formation of chitosan microspheres. Here further insvestigation was undertaken to evaluate the antimicrobial properties of the functionalized textiles with these microspheres, against *Klebsiella pneumoniae* and *Staphylococcus aureus*.

Samples treated with chitosan microspheres produced with the essential oil, lime oil, showed a higher halo around the test specimens, in comparison with the microspehres produced with n-dodecane as the co-solvent. This is in accordance with the expected due to the release of the oil that is entrapted in the microspheres and can be released trought time, increasing the antibacterial effect in the surrounding surfaces of the textile. Resultof these samples tested, against *K. pneumoniae* and *S. aureus* can be observed in table 4.4.

Table 4.4 – Images of samples treated with chitosan microspheres produced with essential oil, lime oil analyzed by standard method JIS L 1902-2002 – Halo method, against *K. pneumoniae* and *S.aureus*.

	With the sample	*Without the sample*
K.pneumoniae		
S.aureus		

4.3.2 Scanning Electron Microscopy

The antibacterial efficacy of the chitosan microspheres and Alg/CS multilayer's was evaluated by assessing the reduction in bacterial attachment on the functionalization cotton substrates, by SEM analysis. Results are in agreement with the expected and with the antibacterial activity of the samples evaluated by the Halo method, as it can be observed in the images of table 4.5.

In tables 4.5 and 4.6 it can be observed that in cotton samples that were previously functionalized with chitosan microspheres, the amount of the attached bacteria is lower when compared with that of the control. This is agreeing with the expected showing that the microsphere coated surfaces are effective at restricting or preventing the formation of *Staphylococcus aureus* and *Klebsiella pneumoniae* biofilms. However, this inhibition is not higly effective, most probably, due to low stability of the dehydrated CS microspheres.

Table 4.5 - SEM images of control samples (cotton and gauze) tested by standard method JIS L 1902-2002 – Halo method, against *K. pneumoniae* and *S. aureus*.

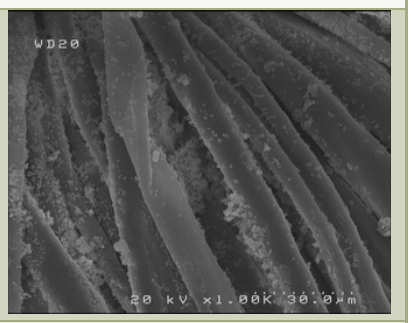
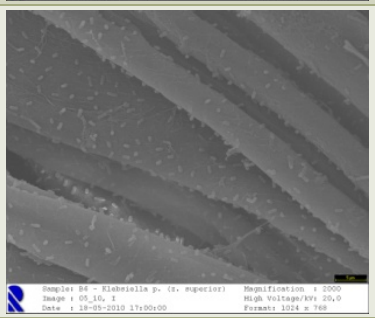
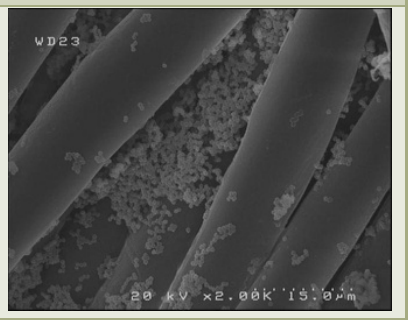
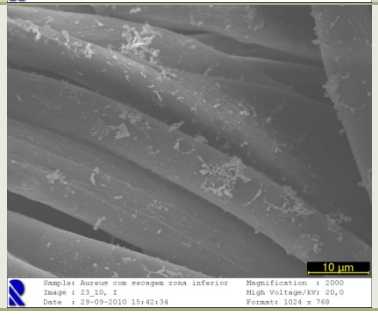
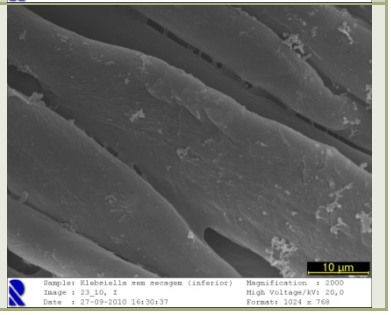
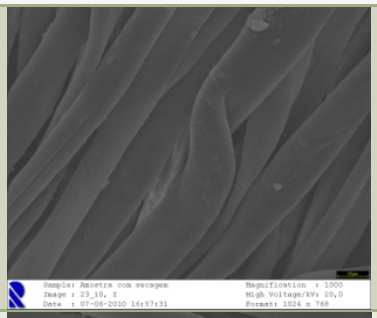
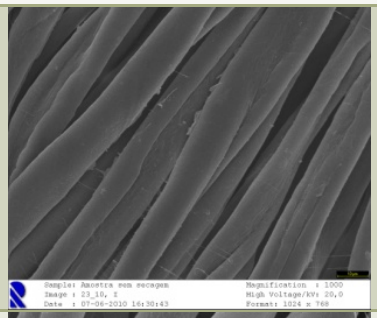
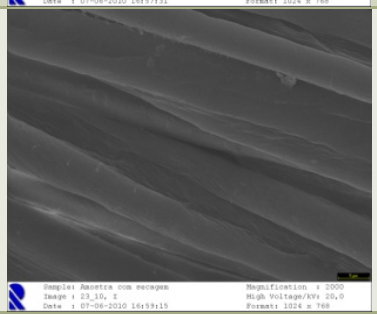
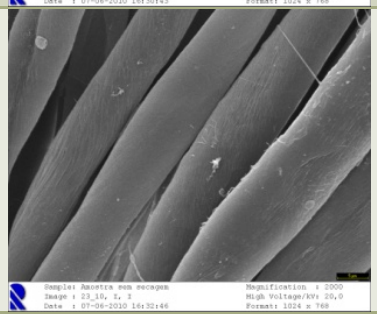
	Control K. pneumoniae	*Control S.aureus*
1000x		
2000x		

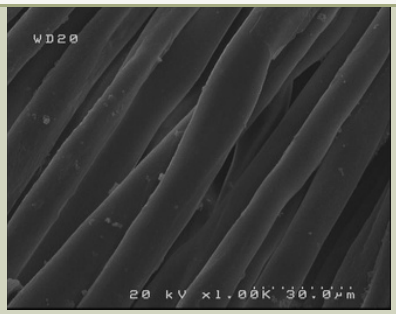
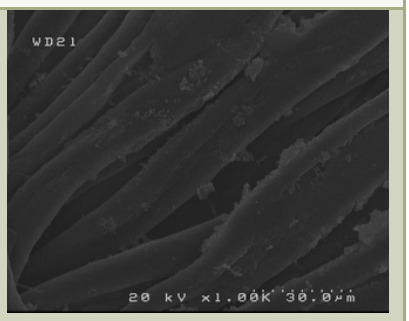
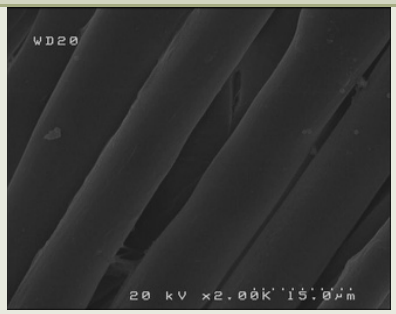
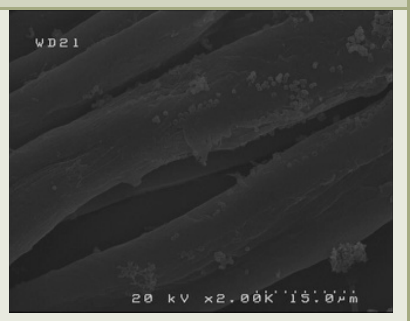
Table 4.6 – SEM images of CS-coated samples (cotton) tested by standard method JIS L 1902-2002 – Halo method, against *K. pneumoniae* and *S. aureus*.

	S. aureus	*K. pneumoniae*
1000x		
2000x		

The Alg/CS functionalized gauze and cotton, provided a much lower amount of bacteria attached to the fibers when compared with that of the control and of the CS-micrsophere coated samples. Coated surfaces with more stable microspheres are highly effective at restricting or preventing the formation *Staphylococcus aureus* and *Klebsiella pneumoniae* biofilms, as visible in table 4.7.

Table 4.7 - SEM images of Alg/CS multilayered CS-coated samples tested by standard method JIS L 1902-2002 – Halo method, against *K. pneumoniae* and *S. aureus*.

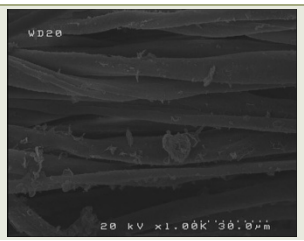
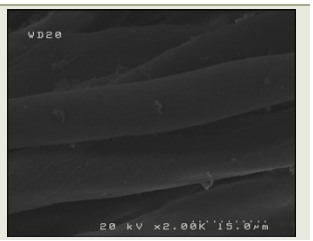
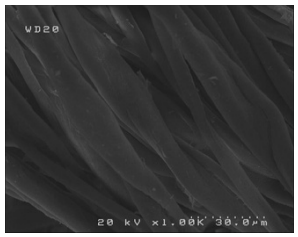
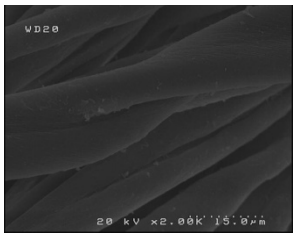
K. pneumoniae	*cotton*	*gauze*
1000x		
2000x		

S. aureus	*cotton*	*gauze*
1000x		
2000x		

Lime oil CS microspheres

SEM analysis (table 4.8) showed that the chitosan microspheres with essential oil also decrease the amount of attached bacteria decreased and is very similar to the behavior of the multilayered CS microspheres. This is agreeing with the expected because essential lime oil as an effective antimicrobial effect against a wide range of microorganisms as decribed by Prabuseenivasan et al 2006.

Table 4.8 - Images of samples (gauze) treated with CS microspheres with essential oil such antimicrobial agent analyzed by standard method JIS L 1902-2002 – Halo method, against *K. pneumoniae* and *S. aureus*.

	1000x	*2000x*
K. pneumoniae		
S. aureus		

4.4 Conclusion

The antibacterial qualitative tests determine antibacterial activity of textiles in a relatively quickly and easy way. In this study, to assess antibacterial activity of textiles. When a halo is not so evident the samples may have antibacterial activity by direct contact when there is also no bacteria growth under the samples.

The results reported in this investigation showed the added value of the SEM in studying the effect of bioactive textiles on Staphylococcus aureus and Klebsiella pneumoniae. Information about the mode of action is not obtained, as in the case of the qualitative and quantitative standards to assess antibacterial activity of textiles, but direct observation of morphological changes following antibacterial action can be observed. These changes cannot be evaluated by those standards and are not adequately seen in the light microscope, while the high magnifications and three-dimensional effect obtained with the SEM make it ideal for this purpose.

SEM analysis is able to show the effectiveness of the several functionalized samples in preventing bacterial adhesion besides bacteria growth.

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Chapter 5 – Concluding Remarks and Work Perspectives

In this last chapter, the most important conclusions withdrawn from the work reported in the present dissertation are addressed. In addition and considering the conclusions of the work developed, some suggestions for further research in this field are given.

5.1 Concluding Remarks

Antimicrobial products have been developed that provides an antimicrobial finish for textile products consistent with claims and the needs of target consumers but many textiles are susceptible to spoilage by microbial growth when used in conditions that allow their growth despite advances in modern materials technology. A wide range of tests exist that can be used to model such growth and determine the efficacy of preventative treatments.

Chitosan, which is very useful non-toxic biopolymer, can be used as an effective biopolymer to form microspheres through several production techniques, in particular, by ultrasound irradiation. Moreover, these microspheres have a high potential to be used as antimicrobial finishing agents or to entrap bioactive formulations for skin contact delivery.

The new method here developed aiming the production of chitosan microspheres is very simple, easy and economic in comparison with other conventional processes which require for more equipment and supplies and are sometimes of complex implementation. Moreover this technique does not require the use of toxic reagents making it more ecologically and environmentally friendly.

Stability, size and morphology of microspheres were observed by SEM and optical microscopy. The antibacterial activity was evaluated according to JIS L 1902:2002 and SEM.

In conclusion, the results of this study demonstrate that chitosan microspheres have antibacterial activity.

5.2 Work Perspectives

This work led to a growing interest in its continuation, with the aim of assessing and studying in greater detail, the bioactivity involved in the functionalization of textile materials. Thus, this issue remains open for future developments. In fact, these new antimicrobial agents enable the study and scientific grounds, in particular the following aspects:

- Evaluation of antimicrobial activity by quantitative methods.
- Drug microencapsulation and bioactivity evaluation.
- Delivery rate.
- Cytotoxicity analysis.