Aroma encapsulation for eco-friendly textile application

Asma Sharkawy

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Aroma Encapsulation for Eco-friendly Textile Application

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemistry

By

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Livraria Lello, Porto
April 29, 2016
To my parents and siblings
Abstract

The textile industry sector has shown a growing interest in the functionalization of conventional fabrics to produce innovative products that enhance health, safety and ergonomics. This research is concerned with developing a functional fabric with durable antibacterial and fragrant properties by employing green chemistry materials and processes. This was achieved by microencapsulation of aroma compounds in biodegradable polymers by the complex coacervation method. Afterwards, the produced microcapsules were covalently attached to cotton fabrics by means of thermofixation grafting process using a polycarboxylic acid. The effects of different processing parameters, including the type and amount of the emulsifier, the type and amount of the hardening agent, and the wall to core ratio, on the morphology, size, dispersion, encapsulation efficiencies (EE%) of the produced microcapsules were examined. The release profiles of the active agents were investigated. The impact of different grafting conditions on the microcapsules adhesion was inspected. Scanning electron microscopy (SEM) and Fourier Transform Infrared (FTIR) spectroscopy confirmed the adhesion of the produced microcapsules on the cotton fabrics. The antibacterial assays of both the produced microcapsules and the functionalized fabrics demonstrated that they exhibited a sustained antibacterial activity.
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<tr>
<td>ASTM</td>
<td>American Society for Testing &amp; Materials</td>
</tr>
<tr>
<td>ATR</td>
<td>attenuated total reflectance</td>
</tr>
<tr>
<td>β-CD</td>
<td>β-cyclodextrin</td>
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<tr>
<td>C</td>
<td>chitosan</td>
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<tr>
<td>CFU</td>
<td>colony forming unit</td>
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<tr>
<td>CR</td>
<td>cumulative release</td>
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<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
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<tr>
<td>EE</td>
<td>encapsulation efficiency</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration (United States of America)</td>
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<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>GA</td>
<td>gum Arabic</td>
</tr>
<tr>
<td>GC-FID</td>
<td>gas chromatography-flame ionization detector</td>
</tr>
<tr>
<td>GRAS</td>
<td>generally regarded as safe</td>
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<tr>
<td>HLB</td>
<td>hydrophilic-lipophilic balance</td>
</tr>
<tr>
<td>µm</td>
<td>micrometer</td>
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<tr>
<td>MPa</td>
<td>megapascal</td>
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<tr>
<td>O/W</td>
<td>oil-in-water</td>
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<tr>
<td>PGPR</td>
<td>polyglycerol polyricinoleate</td>
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<tr>
<td>rpm</td>
<td>rotation per minute</td>
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<td>SEM</td>
<td>scanning electron microscopy</td>
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<td>TA</td>
<td>tannic acid</td>
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<tr>
<td>TPP</td>
<td>tripolyphosphate</td>
</tr>
<tr>
<td>W/O</td>
<td>water-in-oil</td>
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<tr>
<td>W/O/W</td>
<td>water-in-oil-in-water</td>
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<td>WPI</td>
<td>whey protein isolate</td>
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Chapter 1: Introduction

1.1. Relevance and motivation

The increase in the market competitiveness along with the diversity of consumers’ demands has created a challenging environment in the textile industry sector. This subsequently led to the production of innovative textile products with new properties that enhance ergonomics, health and safety. Functional textiles are one of those novel products in the textile area. They are obtained by either incorporating active ingredients to conventional fabrics, such as cotton, wool, silk or polyester, or by manufacturing new materials (e.g., nanofibers and nanocomposites). Innovative technologies in textiles have succeeded in offering a wide variety of fabrics with unprecedented functions. The most common applications of functional textiles include phase change materials, insect repellents, antimicrobials, fragrances, dyes and colorants, skin softeners and moisturizers, some medicines, and flame retardants.

Enhancing the durability and prolonging the lifetime of functional textiles have been always one of the most challenging missions for the manufacturers of these types of textiles. This is due to the fact that these textiles are non-disposable and need to be washed after use. Microencapsulation techniques have been known to provide textiles with long-lasting properties and added value. This process involves the coating of the active ingredient with one or more polymeric materials to form microcapsules whose size range between 1µm and 1000µm. These microcapsules when later fixed onto the fabrics, they create a product with new properties and added value. In fact, each microcapsule acts as a minute reservoir for the active ingredient which would be liberated under specific conditions, such as pH, mechanical factors, temperature or diffusion. Therefore, the process of microencapsulation allows the controlled release of the active substances and their protection against the surrounding environmental conditions, such as heat, oxygen, and light. This process thus, remarkably increases the durability and long lastingness of the effect of the functional ingredient incorporated onto these textiles.

Nowadays, researchers and manufacturers are increasingly interested in green chemistry protocols, taking into account the growing public concern and awareness of the importance of the utilization and application of safe and eco-friendly materials and processes. However, the majority of the commercially available microcapsules that are intended for textile applications
are made of melamine-formaldehyde, urea-formaldehyde or phenol-formaldehyde resins.\textsuperscript{12,13} Regardless of the fact that these polymers are used because of their good thermal stabilities and their ability to be modified according to the desired release profiles, they represent a serious threat for the environment and human health. This is due to their being non-recyclable thermosetting polymers, and also due to the carcinogenicity and toxicity of formaldehyde.\textsuperscript{14,15} Thus, the replacement of such resins with safe and environmentally benign materials has become extremely important.\textsuperscript{16} Biocompatible and biodegradable polymers, such as alginate, gum Arabic, gelatin, chitosan, and cyclodextrins, have recently become promising alternatives for the previously mentioned toxic polymers; as they are known to be eco-friendly, abundant, and safe to human health.\textsuperscript{17}

Fabrics of natural origins, such as cotton are known to be more susceptible to colonization by invasive microbes than synthetic ones.\textsuperscript{16} This is due to their high hydrophilic and porous composition that tends to retain humidity, nutrients, and oxygen, which is indeed considered as an ideal environment for the growth of high number of microorganisms.\textsuperscript{16,18} Consequently, these microorganisms result in unpleasant odors, transmission of diseases and allergic responses in some individuals. Additionally, they cause the deterioration of fabrics in terms of color degradation, loss of elasticity and tensile strength, and interference with the dyeing and printing processes.\textsuperscript{16,19} Hence, it is crucial to combat these undesired effects through imparting antimicrobial additives to textiles.

1.2. Statement of purpose

This study aims to confer antimicrobial and fragrant properties to cotton fabrics by means of microencapsulation, depending on green and eco-friendly materials, and thus contribute to the ongoing advancements in the fields of both microencapsulation and functional textiles.

The main investigations that were conducted in this thesis are as follows:

The preparation of the microcapsules and formulation optimization was achieved by means of the complex coacervation microencapsulation method; using chitosan and gum Arabic as shell materials. Vanillin and limonene were incorporated independently as core (active) materials. The influence of using different process parameters (e.g., type and amount of emulsifier and type of hardening agent) was studied to optimize the final formulation of the microcapsules. This was
followed by the characterization of the prepared microcapsules through the analysis of the morphology, particle size distribution and encapsulation efficiency of the prepared microcapsules. The release of active agents was also investigated through release profiles of both limonene and vanillin microcapsules employing GC-FID. The grafting of the microcapsules to cotton fabrics was done by the pad-dry-cure method using citric acid as a cross-linker. SEM was used to examine the treated fabrics before and after washing and FTIR spectroscopy was used to confirm the covalent attachment of the produced microcapsules to the cotton fabrics. Finally, antimicrobial assays were conducted to examine the antibacterial activities of the produced vanillin and limonene microcapsules. These were evaluated using the Agar Diffusion Method, whereas the antibacterial activity of the treated fabrics was assessed by the Standard Test Method under Dynamic Contact Conditions (American Society for Testing & Materials, ASTM Standard E 2149-01).

To the best of our knowledge, no reports previously investigated the microencapsulation of vanillin powder by the complex coacervation microencapsulation technique. Furthermore, limonene encapsulation using chitosan and gum Arabic as a complex coacervation pair was not previously reported in the literature.

1.3. References


Chapter 2: State of the Art

2.1. Microencapsulation: Definition and Purpose

Microencapsulation involves the entrapment of solid, liquid or gaseous materials in small particles that release their contents at a controlled rate over a prolonged period of time and under certain conditions. These particles are called microcapsules. The word “encapsulate” is derived from the Latin words; “en” which means in and “capsula” which means a small box. Thus, the overall meaning of to encapsulate is to place something in a box. Microcapsules have a diameter of 1-1000 µm. They are made up of a core (inner part) which contains the active material, and a wall material (outer part) that surrounds the core and creates a physical barrier around it. The core is also called the nucleus, payload phase, fill or internal phase; whereas the wall material is sometimes called the shell, encapsulant, carrier or external phase. The core material can be in the form of solid, liquid, or gas. The morphological structure of microcapsules depends on different parameters, e.g., the type of the shell material, its rigidity, and the preparation method used. The shape of the microcapsule may range from being spherical, irregular, with one core or multicore, with single coating or coatings that consist of more than one layer. Microencapsulation protects the active agent from different adverse conditions in the surrounding environment, such as humidity, air, heat, light and exposure to changes in pH. Additionally, it guards against the rapid evaporation of highly volatile active agents and helps in controlling the rate of their release.

2.2 Microencapsulation Techniques

There are different techniques used for the preparation of microcapsules. They can be classified into three main categories being the chemical methods, the physico-chemical methods, and the physico-mechanical methods.

2.2.1. Microencapsulation by Chemical methods

- *In situ* polymerization

In this method the wall material of the microcapsule is chemically produced due to the polymerization of monomers added to an emulsion that is formed of a dispersed core material
and a continuous phase. Subsequently, polymerization starts to occur at the interface between the continuous phase and the immiscible core material, on the continuous phase side. In the beginning of the process, a prepolymer of low molecular weight is formed then it grows with time and deposits on the interface between the continuous phase and core material creating a solid shell. In this method no reagents are added to the core material and the polymerization occurs exclusively in the continuous phase.

- **Interfacial polymerization**

In interfacial polymerization, the shell of the capsule is formed on the surface of a core droplet as a result of the polymerization of reactive monomers. Two sets of monomers that are able to react with each other are used. This method basically involves the formation of an emulsion of two immiscible phases, where one of the monomers is solubilized in the core material and a co-monomer is added to the continuous phase. The reaction conditions are then set to promote the polymerization and the wall formation at the interface.

The most commonly used monomers are the multifunctional isocyanates and acid chlorides. The reaction of isocyanate with an amine results in the formation of a polyurea shell, whereas the reaction of acid chloride with an amine results in the formation of a polyamide or a polynylon microcapsule shell. Polyurethane shell materials are obtained by the reaction of isocyanate with a hydroxyl containing monomer. Both liquid and solid core materials can be encapsulated by this method.

2.2.2. Microencapsulation by Physico-mechanical methods

- **Spray drying**

Spray drying involves the atomization of a solution or suspension of the active agent and the coating material into a heated drying gas which causes the water to evaporate rapidly, leaving dried microcapsules. The microcapsules are then obtained by continuous discharge from a collecting chamber in the spray dryer. The initial temperature of the air is usually 150-220°C which allows evaporation to occur quickly, then it decreases to be within the typical range of 50-80°C. The spray drying technique is suitable for the encapsulation of labile and thermally sensitive substances due to the short exposure time to heat, which typically does not exceed few seconds. This method produces small microcapsules in the form of very fine powder (10-50
µm) or large particles that are 2-3 mm in diameter.\textsuperscript{11} Spray drying is considered the most popular microencapsulation technique used in food industry.\textsuperscript{13} It has the advantage of being economic, rapid and efficient. However, this method has the disadvantage of requiring a coating agent that should be soluble in water to a certain extent. The use of shell materials with low water solubility (e.g., sodium caseinate, whey protein, carboxymethyl cellulose, and guar gum) makes the process expensive due to the need of increasing the amount of water used, and thus requiring more time for its evaporation.\textsuperscript{11}

- Spray chilling

Spray chilling involves the loading of the core material into a warm bath of the wall material followed by spraying through a heated nozzle allowing the shell to solidify and form the microcapsules. Spray chilling is carried out by equipment similar to the one used in spray drying but the air used is not heated.\textsuperscript{5}

- Solvent evaporation/extraction

Microencapsulation by solvent evaporation is carried out through four main steps: (1) dissolution of the active core in an organic solvent that contains the polymeric shell material; (2) emulsification of the organic (dispersed) phase in an aqueous (continuous) phase; (3) extraction of the solvent from the dispersed phase by the continuous phase, and evaporation of the solvent from the organic phase by heating; which causes the coating material to shrink around and encapsulate the core agent; (4) recovery and harvesting of the formed microcapsules.\textsuperscript{8,14,15} The solvent evaporation technique is most commonly applied in the pharmaceutical industry because it provides a controlled release profile of drugs.\textsuperscript{15}

- Air suspension coating

The air suspension technique involves suspending solid core particles in a chamber supported with an upward flowing air stream, and spraying of the coating material onto the suspended particles.\textsuperscript{8,12} The air stream carries the particles in a repetitive circulating pattern to the coating zone in the chamber, where a polymeric coating material is being sprayed to the moving particles. The number of the cycles applied depends on the desired thickness of the wall material.\textsuperscript{9,12} The coating material used can be in the form of aqueous or solvent solution, emulsion, suspension or hot melt. The supporting air stream aids in the solidification of the
coating and drying of the produced microcapsules.\textsuperscript{12} This technique is used to encapsulate solid cores, such as granules, crystals and powders. This technique however cannot be used to coat liquid droplets unless they are first absorbed on a porous solid.\textsuperscript{9}

- Pan coating

The pan coating technique is one of the oldest methods used in the pharmaceutical industry to produce coated particles and tablets. The solid cores are initially tumbled in a rotating pan-like container in which the coating material is slowly applied in the form of solution or atomized spray. The solvent is then removed through passing warm air over the produced coated particles, or by means of drying oven.\textsuperscript{12} The method is also used in microencapsulation to produce microparticles of size greater than 600 µm, usually for controlled-release purposes.\textsuperscript{16,17}

- Centrifugal extrusion

The centrifugal extrusion technique is used to encapsulate liquid cores. It involves the usage of a rotating extrusion head with concentric nozzles. The core material is pumped through a central tube and the wall material in a liquid form is pumped through a surrounding circular space.\textsuperscript{9,12} Consequently, the coating material comes out at the end of the nozzle forming a membrane that surrounds the core material which flows out of the nozzle simultaneously causing the extrusion of a liquid jet (Figure 2.1). This jet later breaks into droplets of the core material coated with the wall material. Hardening of the droplets takes place later by allowing their passage into a heat exchanger.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{diagram.png}
\caption{Schematic diagram of a centrifugal extrusion device with two-fluid nozzle. Adapted from Ref. 9.}
\end{figure}
The centrifugal extrusion method is known to form microcapsules of 400-2000 µm in size.\(^9\) Centrifugal extrusion can be applied for large scale production; since 22.5 kg of microcapsules can be formed per hour using special nozzles, additionally, multiple-nozzle systems can be used, e.g., heads of 16 nozzles are already available.\(^{12}\)

### 2.2.3. Microencapsulation by Physico-chemical methods

- **Supercritical fluids assisted microencapsulation**

  This technique is considered environmentally friendly where supercritical fluids are used as green solvents. Supercritical fluids are highly compressed gases, such as CO\(_2\), N\(_2\)O, and alkanes (C\(_2\) to C\(_4\)), and are known to possess the properties of both gases and liquids in terms of viscosity and diffusivity. Supercritical fluids are known as fluids kept at conditions above their critical point. They have almost zero surface tension.\(^{18}\) Supercritical fluid technology is suitable for shell materials that dissolve in supercritical fluids, and polymers that do not dissolve in them. In practice, the process involves maintaining the core material and the shell material at high pressure, followed by their release at atmospheric pressure through a nozzle. The quick drop in pressure causes the desolvation of the shell and its deposition around the core agent.\(^{19}\)

  Microencapsulation using supercritical fluids provides the advantage of their high solvating power, and hence the solubilization of the coating and core materials is fast and is done in the absence of water.\(^{20}\)

- **Coacervation**

  Coacervation is based on the separation of an initial homogenous polymer solution into two phases: a polymer-rich phase (coacervate) and the other is almost polymer free and called the equilibrium solution. The origin of the word coacervation is derived from the Latin word “acervus” which means heap.\(^8\) Microencapsulation by coacervation provides controlled release of the active agent and high encapsulation efficiency.\(^{20}\) Microencapsulation by coacervation is divided into simple and complex coacervation. The former is carried out using a single polymer as the shell material, while the latter occurs by the interaction of two oppositely charged polymers.\(^{21}\)
i) Simple Coacervation

Simple coacervation is induced by changing the pH or temperature, or the addition of inorganic salts (e.g., sodium sulfate) in order to precipitate the initial solubilized wall material.\textsuperscript{20,22} Phase separation and formation of coacervate of the polymeric wall material is followed by its adsorption around the core material.\textsuperscript{23}

ii) Complex Coacervation

The complex coacervation technique is described in depth here since it is the microencapsulation method used in this study. It is one of the important microencapsulation techniques due to its high loading capacity, mild reaction conditions and controlled release possibilities.\textsuperscript{24,25} It is also considered a green method that does not require the use of organic solvents.\textsuperscript{26} Complex coacervation phenomenon was first reported in 1911 and was then studied more comprehensively in the 40s in the light of the polymer system gelatin/gum Arabic. Nevertheless, its use and application in the food industry only started in the 50s.\textsuperscript{27} More detailed and extensive studies on the method have emerged during the last two decades. Figure 2.2 shows the number of articles related to microencapsulation by coacervation in the period from 1995 to 2016. Complex coacervation is defined as a fluid-fluid phase separation process that occurs as a result of electrostatic attraction between two oppositely charged polymers. It may also involve hydrogen bonding and hydrophobic interactions between the oppositely charged polymers.\textsuperscript{7}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{complex_coacervation狀況.png}
\caption{Number of scientific documents on microencapsulation/complex coacervation published since 1995. (Obtained from Scopus database, February 2016; Search field: microencapsulation complex coacervation; “in article title, abstract, and keywords”).}
\end{figure}
Complex coacervation proceeds by mixing two polymer solutions with opposite charges, which leads to their phase separation from the initial solution and subsequently their deposition around an active core (Figure 2.3). The phase separation takes place by changing the pH or temperature, or the addition of an electrolyte solution which results in the formation of coacervates that tend to precipitate at the oil/water interface as a result of repulsion with the solvent. Consequently, two phases are obtained; one is rich in the coacervate and is called the polymer-rich phase and the other contains the solvent and is called solvent-rich phase.7

Figure 2.3 Schematic representation of complex coacervation method showing the formation of coacervates between two oppositely charged polymers and their deposition at the core interface creating the shell. Adapted from Ref. 29.

The classical and most extensively studied complex coacervation model is the gum Arabic (negatively charged polyelectrolyte) and gelatin (positively charged polyelectrolyte). This system has received considerable attention in industrial applications. It has been used in the manufacture of carbonless paper, fragrance strips and samplers, and flavor incorporation.30 However, gelatin is not always preferred due to its high viscosity and the new regulations that restrict the use of some animal-derived proteins in some countries.24 Hence, other complex coacervation pairs that exhibit new properties have emerged recently. These new systems are based on proteins other than gelatin, and polysaccharides. The proteins are either of animal origin, such as silk fibroin, albumin, and whey proteins, or of plant origin such as, soy and pea proteins.7 The polysaccharides include gum Arabic, chitosan, alginate, sodium carboxymethyl cellulose, and carrageenan.7,26,31 Complex coacervation is used mainly for the encapsulation of essential oils, some drugs, vitamins, nutraceuticals (e.g., omega-3) and different various food ingredients.7
The complex coacervation method typically involves the five following steps: \(^7,^{27}\)

1. Dissolution and hydration of polymers to obtain aqueous solutions of the two polymers, (either a protein and a polysaccharide or two oppositely charged polysaccharides) at a temperature and pH above their gelling point.

2. Emulsification step where creating an emulsion of the hydrophobic core material in the aqueous phase is accomplished. The emulsion is typically stabilized by a surfactant and/or one of the polymers.

3. Coacervation and shell formation due to changing the reaction conditions, such as lowering the pH, the two polymers would interact by electrostatic attraction and form a complex that would eventually deposit on the oily droplets of the core. The pH is one of the important parameters in this step as it directly affects the degree of ionization of the functional groups of the polymers.

4. Hardening by means of adding a cross-linker to harden the shell.

5. Separation of the microcapsules which are isolated from the reaction mixture by either decantation or centrifugation. The microcapsules are usually dried to get a sample in a powder form.

In spite of the advantages that the complex coacervation technique offers, there are two main limitations for this method. Firstly, the method is mainly suitable for the encapsulation of hydrophobic core materials and is not ideal for encapsulating hydrophilic cores. However, the introduction of some changes to the conventional coacervation method enables the incorporation of hydrophilic cores, such as including a double emulsion step which comprises the formation of a primary w/o emulsion in the beginning of the process before coacervation, followed by a double w/o/w emulsion.\(^{32-34}\) Although the double emulsion method is reported to be effective in encapsulating hydrophilic substances, it adds to the cost of the process.\(^7\) The second disadvantage of this method is that the majority of the articles in the literature cited the use of toxic aldehydes (e.g., formaldehyde, glyoxal and glutaraldehyde) as cross-linkers for the wall polymers, which is not legislated in some countries.\(^{35}\) This problem could be solved by using non-toxic hardening agents, such as transglutaminase enzyme\(^{36}\), genipin\(^{37}\) or tripolyphosphate.\(^{38}\)

In this study, two hardening agents were used; the polyanion tripolyphosphate and tannic acid, which are both reported to be non-toxic and environmentally friendly.\(^{38,39}\)
2.3 Microcapsules wall material: Biodegradable polymers

There is a wide variety of natural and synthetic shell materials that are used in microencapsulation. The choice of the appropriate coating material depends on the final product objective and requirements; as it will determine the final properties of the resulting microcapsules. The product requirements may include a specific controlled release profile, reduction of volatility, adding value and functionalization or stabilization of an active agent. Moreover, the resulting encapsulation efficiency should be taken into consideration. The ideal polymer should be non-toxic, insoluble and non-reactive with the material intended to be encapsulated and should readily deposit on its surface to form a cohesive film of a suitable thickness around it. The selection criteria may also be influenced by economic factors. The use of natural biodegradable polymers has been recently of high interest; mainly because they are naturally abundant, eco-friendly and biocompatible. Biodegradable polymers, both natural and the synthetic, are known to be eco-friendly due to their capability to cleave into biocompatible products through chemical or enzymatic hydrolysis. Biodegradable polymers are also able to release the active agent in a controlled pattern. Examples of synthetic biodegradable polymers that are used in drug microencapsulation include polyesters, polyorthoesters, polyphosphazenes and polyanhydrides, while natural biodegradable polymers are either based on proteins (such as gelatin, albumin, and collagen) or polysaccharides (such as, chitosan, hyaluronic acid, gum Arabic, alginate, maltodextrin, starch, ethyl cellulose and carrageenan). Owing to the fact that one wall material may not possess all the desired requirements for a certain application, a combination of different encapsulating materials is sometimes used.

2.3.1. Chitosan

Chitosan has recently acquired great attention in different areas due to its interesting properties, such as being non-toxic, biodegradable, non-allergic, mucoadhesive, excellent film forming ability and being a potent antimicrobial agent. It has been widely used in several industrial applications, such as food engineering and packaging, wastewater treatment, cosmetics, textile coating, pharmaceuticals, biomedicine and tissue engineering. Chitosan is a linear polysaccharide made of β- (1→4) linked monosaccharide units of β-(1,4)-2- amino-2-deoxy-D-glucose (Figure 2.4). It is a biodegradable polymer derived from chitin, which is the second most
abundant polysaccharide in nature.\textsuperscript{11,40} Chitin occurs mainly in the skeletons of crustaceans, insects and in the cell wall of some fungi. The acetamide (N-acetyl) group in chitin is chemically converted by alkaline treatment into an amino group to obtain chitosan. The degree of deacetylation “DDA” is a term that shows the amount of 2-amino-2-deoxy-D-glucose (D-glucosamine) units in a molecule of chitosan.\textsuperscript{43} The commercially available chitosan is known to have a DDA of 66\% to 98\%. At alkaline and neutral pH, chitosan has free amino groups and therefore, it is insoluble in water. At acidic conditions, the amino groups become protonated, and hence chitosan becomes soluble.\textsuperscript{44}

![Chemical structure of (a) chitin and (b) chitosan. Adapted from Ref. 44.](image)

The DDA of chitosan affects its solubility; since it reflects the amount and distribution of the free amino groups in relation to the remaining N-acetyl groups.\textsuperscript{42,43} The DDA also influences the complexation ability of chitosan since a higher DDA results in a higher charge density and greater complex formation capability.\textsuperscript{43} Additionally, the biodegradability of chitosan is affected by its DDA.\textsuperscript{11} The biodegradability of chitosan is of high importance for the release of the encapsulated material. It has been reported that the variations in the DDA of chitosan affects the
release profile of the drug isoniazid, and that the higher the DDA of the chitosan used, the faster
the reported rate of release.\textsuperscript{45}

The antibacterial activity of chitosan pertains to the positive charge on its protonated amino
groups.\textsuperscript{41,46} The DDA and the pH define the extent of the charge density of chitosan, and hence,
its antibacterial activity. The majority of amino groups on C-2 of chitosan acquire cationic
charge at low pH (lower than its pKa ~ 6). This protonation enhances the interaction between
chitosan and the anionic charges of lipopolysaccharides on the surface of the Gram negative
bacteria, and with the anionic peptidoglycans in Gram positive bacteria.\textsuperscript{47} Consequently, these
interactions lead to changes and disruption in the permeability of the bacterial cell and leakage of
the vital intracellular substances; which affects the functions and bioactive processes of the
microorganism and finally its death.\textsuperscript{48,49}

Cross-linking of chitosan involves the introduction of intermolecular bridges between the
polysaccharide macromolecules by using specific reagents known as cross-linkers.\textsuperscript{50} Cross-linking
reactions are known to decrease the mobility of the polymer segment and results in the
interconnection between the polymer chains through new linkages.\textsuperscript{11,50} It has been reported that
the cross-linked chitosan microcapsules are more efficient for the controlled release applications
as compared with those that are not cross-linked.\textsuperscript{50} The cross-linking reaction is affected by the
size and kind of the cross-linking agent used. Generally, the smaller the size of the cross-linker,
the faster the reaction; as its diffusion between the function groups of the polymer becomes
easier.\textsuperscript{51} According to the interaction of the cross-linker with chitosan, the cross-linking reaction
can be classified into chemical or physical. The chemical cross-linking results in networks made
by permanent covalent bonding between the chitosan chains. The amino and the hydroxyl
groups of chitosan are the cross-linking sites that form ester linkages, amide linkages, and
sometimes Schiff bases. The chemical cross-linking reaction depends mainly on the
concentration of the cross-linkers, as well as the exposure time. Examples of well-known
chemical cross-linkers are genipin, glutaraldehyde, vanillin which forms a Schiff base, and
epichlorohydrin.\textsuperscript{50} The physical cross-linking depends on electrostatic interactions between the
chitosan and the counterions. The physical cross-linkers are polyanionic compounds and they
include phosphoric acid salts (e.g., tripolyphosphate), citric acid, and sulfate.\textsuperscript{11,50,51}
2.3.2. Gum Arabic

Gum Arabic (GA) is the dried exudate of *Acacia senegal* and *Acacia seyal* trees, Family Leguminosae.\textsuperscript{52} Sudan, Nigeria, Chad, Ethiopia and Senegal are the main producers. It is harvested as dried sap (Figure 2.5). The use of GA dates back to the time of ancient Egyptians; as they used it as a binder in paints, inks, cosmetics and in embalming mummies.\textsuperscript{53} It is now widely used in food, pharmaceutical and cosmetic industries for its notable stabilizing, binding, thickening and emulsifying properties. It is also used in the textile industry to thicken the printing pastes which are used in the coloration of cellulose fabrics.\textsuperscript{53} GA is also reported to have antimicrobial effects against some fungal pathogens, such as *Candida albicans* and *Cryptococcus neoformans*.\textsuperscript{52}

GA is a branched heteropolysaccharide polymer that exists as a mixture of calcium, potassium and magnesium salts.\textsuperscript{53} The main fraction of GA (88-90\%) is composed of two chains: the main chain consists of 1,3-linked β-D-galactopyranosyl units, while the side chains consist of two to five units of 1,3-linked β-D-galactopyranosyl units joined by 1,6-linkages. Both chains contain α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl and 4-O-methyl-β-D-glucuronopyranosyl units.\textsuperscript{52-54} The secondary fractions (10\%) is composed of both complex arabinogalactan-protein (AGP) and glycoprotein (GP).\textsuperscript{55}

![Gum Arabic exudate on Acacia tree. Adapted from Ref. 52.](image)

**Figure 2.5** Gum Arabic exudate on Acacia tree. Adapted from Ref. 52.

GA is widely used in microencapsulation due to its low viscosity, excellent emulsification properties, high solubility in water, film-forming capability and high ability to retain and protect
volatile compounds.\textsuperscript{56} It is believed that the emulsifying ability of gum Arabic is related to the AGP complex in its structure; since it is an amphiphilic protein component that can deposit on the surface of the oil droplets, while the carbohydrate fraction remains directed to the aqueous phase preventing the coalescence of the oil phase droplets.\textsuperscript{55}

2.4. Microcapsules core material

2.4.1. Vanillin

The core material is the substance present in the inner part of the microcapsule over which the shell material is applied. Vanillin (Figure 2.6) is one of the most widely used flavoring agents and it is generally regarded as safe (GRAS). It is a plant metabolite obtained from the beans or pods of the tropical orchid \textit{Vanilla planifolia}. It is native to Mexico and Central America, and is now cultivated in Madagascar, Indonesia, Uganda and Guinea. Madagascar is the largest producer followed by Indonesia.\textsuperscript{57,58} Due to the high cost of the process of growing and harvesting the vanilla orchid, most of the vanillin used in pharmaceuticals, perfumery, food products, and cosmetics is chemically synthesized. In fact, the naturally used vanillin makes up only less than 1\% of the total vanillin produced worldwide.\textsuperscript{59}

\begin{figure}[h]
\centering
\includegraphics[width=0.3\textwidth]{vanillin_structure.png}
\caption{Structure of vanillin (4-hydroxy-3-methoxybenzaldehyde).}
\end{figure}

The volatile nature of vanillin and its limited solubility in water make its use in different applications problematic.\textsuperscript{60,61} Moreover, the presence of a phenolic and an aldehydic group in its structure makes it highly susceptible to oxidation and thermally unstable.\textsuperscript{62} Therefore, its protection by encapsulation is worth investigating to increase its stability and functionality.
Table 2.1 represents examples of the different methods that have been reported on vanillin microencapsulation.

Vanillin has been reported to possess several bioactive effects, such as antioxidant, antimicrobial, anticarcinogenic and antimutagenic properties.\textsuperscript{57} Vanillin is incorporated in a lot of food preparations not only for being a sweet flavoring agent, but also due to its antimicrobial property making it into a “phytopreservative” or a “green preservative”,\textsuperscript{63} which satisfies the needs of consumers who look for natural additives rather than chemical ones. It has been reported to have a potent antimicrobial activity against gram negative and gram positive bacteria,\textsuperscript{64,65} yeasts and molds. Vanillin antibacterial activity has been recognized against \textit{Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter aerogenes, Escherichia coli, Listeria monocytogenes and Yersinia enterocolitica} which are known to cause skin diseases and gastrointestinal tract problems.\textsuperscript{62,66} The antibacterial activity of vanillin depends on its concentration, exposure time and the target microorganism.\textsuperscript{46} It has also been reported that the antifungal activity of vanillin is attributed to its aldehyde moiety and the position of the side groups on its benzene ring.\textsuperscript{67}

Recent studies also demonstrated the ability of vanillin to protect against chronic depression and induce relaxation through olfaction and oral administration.\textsuperscript{68,69} It is claimed that the antidepressant effect of vanillin is connected to its adrenergic agonistic activity.\textsuperscript{69} Furthermore, it was found that the exposure to a familiar vanillin scent decreases neonates’ crying that accompany pain attacks and alleviates the concomitant symptoms, such as energy depletion and increased risk of hypoxemia.\textsuperscript{70}

To the best of our knowledge, the microencapsulation of vanillin using complex coacervation method has not been previously reported.
Table 2.1 Examples of different methods of vanillin microencapsulation and their area of application.

<table>
<thead>
<tr>
<th>Method</th>
<th>Wall material</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray Drying</td>
<td>Chitosan</td>
<td>Textiles</td>
<td>60</td>
</tr>
<tr>
<td>Phase Inversion by Polysulfone</td>
<td>immersion precipitation</td>
<td>Textiles</td>
<td>64,71</td>
</tr>
<tr>
<td>Spray-freeze drying β-CD</td>
<td>Food preparations</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>β-CD + WPI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spray Drying</td>
<td>Sodium alginate</td>
<td>Food preparations</td>
<td>73</td>
</tr>
<tr>
<td>Methyl β-CD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxypropylmethyl cellulose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spray Drying</td>
<td>Soy protein isolate-maltodextrin</td>
<td>Food preparations</td>
<td>13</td>
</tr>
<tr>
<td>Emulsion stabilization</td>
<td>Carnauba wax</td>
<td>Food preparations</td>
<td>74</td>
</tr>
<tr>
<td>Maltodextrin β-CD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gum Arabic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freeze-drying β-CD</td>
<td>Food preparations</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Multilayer emulsion</td>
<td>Soy protein isolate</td>
<td>Food preparations</td>
<td>76</td>
</tr>
<tr>
<td>and spray drying</td>
<td>Modified starch</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chitosan</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.4.2. Limonene

D-limonene (Figure 2.7) is one of the most abundant terpenes that occur naturally. It represents the main constituent in the essential oils of citrus fruits, such as orange, lemon, lime and grapefruit. Its optical isomer is L-limonene; it has a turpentine odor and is mainly found in the volatile oils of pine and oak trees. D-limonene is listed as a GRAS compound in the Code of Federal Regulations, and is used widely in the food and cosmetic industries as an important flavor and fragrance. It is clinically used to dissolve gallstones and treat heartburns. It has been also reported to have an anti-tumor and chemo-preventive activity against mammary, colon and lung cancers. D-limonene has also been reported to lower the risk of skin carcinoma.

![Structure of d-limonene (4-isopropenyl-1-methylcyclohexene).](image)

D-limonene has a potent antimicrobial activity against several bacteria such as *Salmonella* spp., *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Antifungal properties of d-limonene have been also reported. The chemical structure of the essential oil denotes its antibacterial behavior. Limonene has more potent antibacterial activity than p-cymene; because the alkenyl group of the former is oxidized upon exposure to air and forms ions that disturbs cell wall, which is not the case of the alkyl group of the latter. Limonene is capable of penetrating the lipid barrier in the bacterial cell wall. This results in the denaturation of the protein components of the bacterial cell membrane, causing the leakage of the cytoplasmic constituents which in turn disrupts the energy status and eventually leads to cell death.

Table 2.2 shows different methods that have been reported on limonene microencapsulation and the wall materials used in each method. Much has been published about the encapsulation of limonene with various methods including complex coacervation; yet most of the literature
available on the microencapsulation by complex coacervation refers to the use of pairs of wall materials other than the chitosan and gum Arabic together. Leclercq et al.\textsuperscript{87} compared limonene protection against oxidation to limonene oxide by both complex coacervation and spray drying methods. They reported that microencapsulation by complex coacervation has fully protected limonene against oxidation to limonene oxide during 25 days of storage, whereas high amount of limonene oxide was produced over the same duration when spray drying technique was used.\textsuperscript{87}

**Table 2.2** Examples of different methods used for limonene microencapsulation and their area of application.

<table>
<thead>
<tr>
<th>Method</th>
<th>Wall material</th>
<th>Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex coacervation</td>
<td>Chitosan/gelatin</td>
<td>Textiles</td>
<td>\textsuperscript{88}</td>
</tr>
<tr>
<td>Complex coacervation</td>
<td>Gelatin/carboxymethyl cellulose</td>
<td>Footwear</td>
<td>\textsuperscript{89}</td>
</tr>
<tr>
<td>\textit{In situ} polymerization</td>
<td>Melamine-formaldehyde resin</td>
<td>Footwear</td>
<td>\textsuperscript{89}</td>
</tr>
<tr>
<td>Complex coacervation</td>
<td>Gelatin/gum Arabic</td>
<td>Flavors</td>
<td>\textsuperscript{87}</td>
</tr>
<tr>
<td>Spray drying</td>
<td>Gelatin/gum Arabic</td>
<td>Flavors</td>
<td>\textsuperscript{87}</td>
</tr>
<tr>
<td>Interfacial polymerization</td>
<td>Polyyurethane-urea</td>
<td>Textiles</td>
<td>\textsuperscript{90}</td>
</tr>
<tr>
<td>Simple coacervation</td>
<td>Chitosan</td>
<td>Non-woven Fabrics</td>
<td>\textsuperscript{91}</td>
</tr>
<tr>
<td>Spray drying</td>
<td>Gum Arabic/maltodextrin</td>
<td>Flavors</td>
<td>\textsuperscript{92}</td>
</tr>
<tr>
<td>Ultra-high pressure homogenization and freeze drying</td>
<td>Gelatin/sucrose/gum Arabic</td>
<td>Flavors</td>
<td>\textsuperscript{93}</td>
</tr>
<tr>
<td>Molecular inclusion</td>
<td>β-CD</td>
<td>Flavors</td>
<td>\textsuperscript{94}</td>
</tr>
<tr>
<td>Simple coacervation</td>
<td>Gum Arabic</td>
<td>Textiles</td>
<td>\textsuperscript{95}</td>
</tr>
</tbody>
</table>

**2.5. Release Mechanisms**

Microencapsulation allows effective shielding and protection of the active agent from degradation by the surrounding environment. At the same time, it offers controlled release of the active agent over long periods. The rate of the controlled release depends on the nature of the coating material, its thickness and porosity.\textsuperscript{17,96} Moreover, it depends on the physiochemical
properties of the active agent, such as its solubility, partition coefficient and diffusibility across the polymeric matrix.\textsuperscript{96}

The term controlled release includes different types of release profiles such as sustained release, triggered release, pulsed release and targeted release.\textsuperscript{97} There are several release mechanisms by which the core material is being gradually liberated out of the microcapsules, and in general, the release mechanism depends on the intended application of the microcapsules.\textsuperscript{19} The following are examples of the main release mechanisms associated with microencapsulation:

1. **Diffusion through the wall material**

   Diffusion involves the penetration of the shell material by the dissolution fluid surrounding the microcapsules, which subsequently dissolves the core and results in the leaking out of the core material through the pores or interstitial channels of the wall material.\textsuperscript{17} Diffusion is the most commonly involved release mechanism in pharmaceutical preparations as it allows for the sustained release of the microencapsulated drug.\textsuperscript{17,19}

2. **Dissolution of the wall material**

   In this mechanism, the release of the active agent occurs when the coating material dissolves in the dissolution fluid. The thickness of the microcapsule wall and its degree of solubility in the dissolution fluid determines the release rate.\textsuperscript{17} This release mechanism is typically utilized in detergent preparations where the dissolution of the wall material of the microcapsules in the detergent powder liberates the encapsulated protease enzyme which has the ability to remove the stains from clothes. It is also utilized widely in the food industry, where microencapsulated ingredients such as nutrients, taste enhancers, or flavoring agents (e.g., in chewing gums) are liberated through the melting of the wall material.\textsuperscript{19}

3. **Mechanical rupture of the wall**

   The mechanical rupture of the microcapsule shell immediately releases the core contents. The rupture can be either achieved by applying pressure (such as in carbonless copy paper) or by scratching (as in case of scratch and sniff perfumes).\textsuperscript{19}

4. **Degradation and Erosion of the wall**

   The erosion of certain coating materials causes the gradual release of the core material.\textsuperscript{17,98} Erosion can be classified into surface and bulk erosion, according to the chemical structure of the
polymer. When the rate of the polymer erosion is higher than the rate by which water permeates into the bulk of the polymer, the erosion is said to be surface erosion and the release follows zero order kinetics. However, bulk erosion occurs when the rate of water permeation is higher than the polymeric system erosion causing complex release kinetics.\textsuperscript{99,100} The majority of the biodegradable polymers exhibit bulk erosion. Erosion is a physical phenomenon that depends on diffusion and dissolution, whereas degradation is a chemical phenomenon. The degradation of the majority of the biodegradable polymers occurs by the bulk hydrolysis of the polymer into smaller fragments. Nevertheless, some biodegradable polymers, such as polyanhydrides and polyorthoesters, degrade only at the surface, exhibiting a release rate for the core material proportional to the surface area of the coating material.\textsuperscript{100}

2.6. Applications of microencapsulation

2.6.1. Food Industry

Microencapsulation technology has recently been employed in the food industry.\textsuperscript{20} It allows for the incorporation of important ingredients, such as flavoring agents, polyphenols, vitamins, volatile additives, antioxidants and enzymes in different food products, and thus protecting these ingredients from degradation. Microencapsulation is also used in food packaging technologies where it permits the incorporation of antimicrobial agents in the packaging material to protect food against different foodborne pathogens.\textsuperscript{5} It is also used in the food industry to retard the rate of evaporation of volatile cores to the surrounding environment or to control their release rate over time or until reaching a specific stimulus.\textsuperscript{11} In addition, microencapsulation is applied in the food industry to mask the flavor of the core material; to separate different components that are reactive with one another within the product; or to modify the physical characteristics of the core material so that it becomes easier and more convenient to handle.\textsuperscript{1,11} Microencapsulation of probiotic bacteria has been recently used to enhance their bioavailability and targeted delivery in the gastrointestinal tract.\textsuperscript{101}

2.6.2. Pharmaceuticals

Microencapsulation has various applications in the pharmaceutical industry. It provides a lot of advantages over the conventional drug systems, such as sustained release, protection of the drug, prolongation of shelf-life and targeted drug delivery.\textsuperscript{4} It is also used to mask the unpleasant taste
of some components in the pediatric and geriatric drug formulations. Bioencapsulation is one of the important pharmaceutical and medical applications of microencapsulation. It involves the entrapment of biologically active materials, such as DNA and cells. Encapsulation of DNA vaccines in polymeric microcapsules, such as poly lactic-co-glycolic acid (PLGA), offers slow release rate, and thus prolonged immune response. Another example includes the encapsulation of certain human tissues by natural polymers prior to their transplantation to manage some hormone deficient diseases (e.g., diabetes).

2.6.3. Cosmetics

Microencapsulation in cosmetics covers a wide range of applications, including hair and skin care and maintenance products, oral cavity and mucous membrane products (e.g., toothpaste), perfumes, hair products, cleansing products, correcting body odor products, makeup and decorative cosmetics. Innovation, cost, and meeting the consumer demands drive the microencapsulation research and development in the cosmetic industry and perfumery.

There are various examples of core ingredients that are being microencapsulated in cosmetics, such as skin moisturizers, vitamins, essential oils, antimicrobials, and colorants.

2.6.4. Agriculture

Microencapsulation is widely applied in the area of agriculture; either for crop protection or promoting plant growth. Microencapsulation of nitrogen-fixing bacteria in biodegradable polymers was investigated in one of the recent studies using spray drying methods which help in the growth of plants without the use of excessive chemical fertilizers. Furthermore, microencapsulation of pesticides and insecticides allows for plant protection while reducing human, animal and soil toxicity. Additionally, it helps in elongating the duration of activity of the pesticide and controls its rate of evaporation.

2.6.5 Textiles

The application of microencapsulation in textile industry started in the early 90s; with very few commercial products. However, by the beginning of the 21st century, the number of applications has shown a visible growth, specifically in Japan, North America, and the countries of Western Europe. Microencapsulation permits the incorporation of different active agents onto textiles, and thus provides an added value to them. The core material is responsible for the new properties
of the fabric, and hence defines its function. Examples of microencapsulation applications in textiles include durable fragrances, skin softeners, vitamins, insect repellents, phase-change materials, color changing fabrics, cosmetotextiles, and medical textiles.

Different attempts to incorporate aroma onto textiles have been carried out for many years. Microencapsulation allows for higher durability and release of fragrances over a longer period. Fragrant textiles are frequently used in aromatherapy; a field which relates fragrances with psychology and how they can trigger specific feelings, such as happiness, relaxation, excitement, or well-being. Numerous essential oils such as, lavender, citrus, vanilla and rose have been applied to fabrics designed for aromatherapy. Each essential oil is reported to initiate a particular emotion and acts as a healing element.

‘Cosmetotextiles’ is a term given to fabrics designed to be in direct contact with the skin, and contain personal care active agents, such as skin softeners, slimming agents, moisturizers, anti-cellulite, UV-protecting and anti-ageing ingredients. Those active ingredients are integrated in the fabrics by microencapsulation techniques, and are released to the skin by mechanical means, such as friction and abrasion.

Imparting antimicrobial properties to textiles has been one of the important applications of microencapsulation in textile industry. Textiles that are made of natural fabrics, such as cotton and wool are known to furnish excellent media for microbial growth due to their ability to hold humidity and highly porous structure that increases their surface area. A myriad of antimicrobial substances have been incorporated onto textiles. Natural antimicrobials have recently gained increasing interest due to their being safe, eco-friendly and originating from renewable sources.

Another important textile application of microencapsulation is in the area of defense where chemical decontaminants are microencapsulated within certain polymeric shells. Theses shells are selectively permeable to toxic substances and partially permeable for the decontaminating core materials, and hence allowing the diffusion of the toxic chemicals into the core of the microcapsules where they become irreversibly detoxified by means of chemical reactions.

The process of adhesion of the microcapsules onto the fabric is an important factor to consider in microencapsulation application in textiles; because it directly affects the stability and the durability of the finished product. There are different methods by which microcapsules can be
applied to the fabrics, such as spraying, foaming, grafting through chemical links, impregnation, coating, printing, padding and bath exhaustion. Padding is usually followed by drying and curing of the treated fabrics, and hence, the conventional process is known as the pad-dry-cure method. The most widely used binding method in industry is padding. It involves soaking of the fabric in a bath that contains the microcapsules, resin and water and then passing the fabric through a padder (foulard). This subsequently entails applying a stream of hot air as a thermal treatment to cure the binding resin and adhere the microcapsules to the fabric.

2.7. References


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94. Fang, Z.; Comino, P. R.; Bhandari, B. Effect of encapsulation of d- limonene on the moisture adsorption property of β-cyclodextrin. *LWT - Food Science and Technology* **2013**, 51, 164-169.


Chapter 3: Materials and Methods

3.1. Materials

The reagents and chemicals used in the preparation and characterization of the microcapsules are listed in table 3.1.

Table 3.1 List of used chemical compounds, their functions and suppliers.

<table>
<thead>
<tr>
<th>Chemical Compound</th>
<th>Function</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gum Arabic</td>
<td>Wall material</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Chitosan (Degree of deacetylation 88-95%)</td>
<td>Wall material</td>
<td>BioLog Biotechnologie Und Logistik GmbH (Germany)</td>
</tr>
<tr>
<td>Vanillin</td>
<td>Core material</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>(R)-(+-)-Limonene</td>
<td>Core material</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Tween® 20</td>
<td>Emulsifier</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Tergitol, type 15-S-9</td>
<td>Emulsifier</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Span 85</td>
<td>Emulsifier</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>PGPR 4150</td>
<td>Emulsifier</td>
<td>Palsgaard® (Denmark)</td>
</tr>
<tr>
<td>Corn oil</td>
<td>Carrier and solvent for vanillin</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Hydrochloric acid 0.2 N</td>
<td>pH adjustment</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Acetic acid 0.1 N</td>
<td>Solvent for chitosan</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Sodium TPP</td>
<td>Hardening agent</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>Hardening agent</td>
<td>Merck</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>Washing reagent</td>
<td>Carlo Erba Reagents</td>
</tr>
<tr>
<td>Citric acid</td>
<td>Grafting of microcapsules onto fabrics</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Sodium phosphate monobasic monohydrate</td>
<td>Catalyst for grafting reaction</td>
<td>Sigma Aldrich</td>
</tr>
</tbody>
</table>
Table 3.2 lists the cotton fabrics that were used for the application of the produced microcapsules. Fabrics dimensions were chosen according to the required impregnation method (whether simple coating or thermofixation method). All the fabrics were purchased from SDC Enterprises Limited, UK.

**Table 3.2** List of the cotton fabrics used.

<table>
<thead>
<tr>
<th>Fabric</th>
<th>Dimensions</th>
<th>Product Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton Lawn</td>
<td>30cm x 25cm</td>
<td>1305 SDC</td>
</tr>
<tr>
<td>Cotton Lawn Rubbing Fabric</td>
<td>20cm x 10cm</td>
<td>1351 SDC</td>
</tr>
<tr>
<td>Cotton Limbric Gimped</td>
<td>10cm x 4cm</td>
<td>1531 SDC</td>
</tr>
</tbody>
</table>

3.2. Experimental Methods

3.2.1. Production of Microcapsules

The process of both vanillin and limonene microcapsule formation involved three main steps: emulsification, complex coacervation induction and finally, the hardening of the formed microcapsules. Owing to the fact that vanillin is in a solid form and hydrophobic, corn oil was used to dissolve it and to permit its emulsification. Several preliminary formulations were produced until a final optimized formulation was obtained. These trials will be discussed in chapter 4, whereas the final microencapsulation formulation is discussed in this section. The method for the production of microcapsules was adapted from procedures described in previous reports\textsuperscript{1,2} but with some modifications.

3.2.1.1. Production of Vanillin Microcapsules

1. Emulsification

1.0% (w/v) of chitosan solution was prepared by dissolving 0.5 g of chitosan in 50 mL of 0.1N acetic acid and was left overnight to be completely dissolved. 0.12 g of vanillin was dissolved in 4.5 g of corn oil which was heated at 40°C and was added to the chitosan solution. 50 mL gum Arabic solution (2.0%, w/v) (which was prepared in deionized water), and a specified amount of the emulsifier was added to the mixture. Then, emulsification was generated by continuous mechanical agitation; through keeping the system under stirring at 8000 rpm with a homogenizer
(an ultraturrax IKA DI 25 Basic, yellow line) (Figure 3.1; Table 3.3), for 1 minute at 40°C to form an O/W emulsion.

2. Induction of complex coacervation

Complex coacervation was initiated by decreasing the stirring speed to 400 rpm, and the pH value of the mixture was adjusted from 6 to 3.5 with 0.2 N HCl. The mixture was left under continuous stirring for 30 minutes, and then it was cooled to 5°C by means of an ice bath.

3. Hardening of microcapsules

Consolidation of the produced microcapsules was done by the dropwise addition of the hardening agent (either solution sodium TPP or tannic acid) to the suspension of the microcapsules with continuous stirring (400 rpm) for 3 hours at 5°C. The microcapsules mixture was then left for decantation to allow phase separation.

Figure 3.1 IKA® DI 25 Basic Ultraturrax.
### Table 3.3 Specifications of the homogenizer used in the formation of microcapsules

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>IKA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>DI 25 Basic</td>
</tr>
<tr>
<td>Dispersion tool working range</td>
<td>10-1500 ml</td>
</tr>
<tr>
<td>Speed range</td>
<td>8000-24000 rpm</td>
</tr>
</tbody>
</table>

#### 3.2.1.2. Production of Limonene Microcapsules

The final method used to produce limonene microcapsules was similar to that used for the preparation of vanillin microcapsules by complex coacervation (in Section 3.2.1.1), except for the volume and nature of the core material. Being a liquid, limonene was used directly as the oil phase in the O/W emulsion without the need to dissolve it in a suitable oil, as was previously done with vanillin.

1. **Emulsification**

   1.0% (w/v) of chitosan solution was prepared by dissolving 0.5 g of chitosan in 50 mL of 0.1N acetic acid and was left overnight. One gram of limonene was added to the prepared chitosan solution. Then 50 mL gum Arabic solution (2.0%, w/v) (which was prepared in deionized water), and a specified amount of the emulsifier was added to the mixture. The system was maintained under stirring at 8000 rpm with the homogenizer for 1 minute at 40°C to form O/W emulsion.

2. **Induction of complex coacervation**

   To start complex coacervation, the stirring speed was set to 400 rpm, and the pH value of the mixture was adjusted from 6 to 3.5 with 0.2 N HCl. After stirring for 30 min, the mixture solution was cooled gradually to 5°C by means of an ice bath.

3. **Hardening of microcapsules**

   The method used for the hardening of vanillin microcapsules was also applied for limonene microcapsules (Section 3.2.1.1) and the microcapsules mixture was then left for decantation to allow phase separation.
3.2.2. Characterization Methods

3.2.2.1. Optical Microscopy

The morphology of the obtained microcapsules was examined by means of a Leica DM 2000 optical microscope equipped with Leica Application Suite Interactive Measurement imaging software (Figure 3.2). Different magnifications (100x, 200x, 400x, and 1000x) were used to observe the produced microcapsules.

![Optical microscope, Leica DM 2000.](image)

**Figure 3.2** Optical microscope, Leica DM 2000.

3.2.2.2. Particle Size Evaluation

Mean particle size and size distribution of the produced microcapsules were determined by a Beckman Coulter Laser Diffraction Particle Size Analyzer LS 230 (Figure 3.3). The Coulter LS 200 Series uses reverse Fourier lens optics incorporated in a binocular lens system, which enables the optimization of light scattering across a wide size range in a single scan with high data reproducibility. The size distribution measurements were done in both volume and number for all samples.
3.2.2.3. Scanning Electron Microscope (SEM)

A high-resolution (Schottky) Environmental Scanning Electron Microscope with X-Ray Microanalysis and Electron Backscattered Diffraction Analysis: Quanta 400 FEG ESEM/EDAX Genesis X4M operating at 15.00 kV was used to examine the morphological features of the produced microcapsules (Figure 3.5). Microcapsules were freeze-dried and then coated with a thin film of gold and palladium, by sputtering, using the SPI Module Sputter Coater equipment (Figure 3.6) prior to SEM analysis. Textile samples were directly examined without being previously coated.

For some SEM examinations, a Phenom ProX desktop scanning electron microscope (Figure 3.7), equipped with Elemental Identification (EID) software with Energy Dispersive Spectrometer (EDS) was used.

Freeze-drying was done by placing the microcapsules for 24 hours in a Scanvac CoolSafe 55-4 Basic 4lt freeze dryer, operating at about -50°C and 0.2 mbar, after being separated by decantation.
Figure 3.5 Scanning electron microscope FEI Quanta 400 FEG ESEM / EDAX Genesis X4M.®

Figure 3.6 SPI-Module™ Sputter Coater of the Materials Center of the University of Porto.
3.2.2.4. Gas chromatography (GC-FID)

Gas chromatography (GC-FID) was used to quantify the encapsulated and the released vanillin and limonene. A Varian CP-3800 gas chromatographer equipped with split/splitless injector, two CP-Wax 52CB bonded fused silica polar columns (50 m x 0.25 mm with 0.2 μm film thickness) and a Varian FID detector operated by the Saturn 2000 WS software was used for the analysis (Figure 3.8). The injectors were set at 240°C, with a split ratio of 1:50 for FID. The FID detector was maintained at 250°C. The volume of each injected sample was 0.1μL. The carrier gas was helium He N60 with a constant flow rate of 1 mL/min.

For the analysis of vanillin, the oven temperature was kept isothermal at 50°C for 5 minutes, and then increased gradually from 50°C up to 120°C at a rate of 10°C/min, followed by a second gradual increase to 200°C with a rate of 2°C/min. The total running time for a sample was 73 minutes.

During limonene quantification, the oven temperature was maintained isothermal at 175°C for 7 minutes, and then increased to 220°C with a rate of 10°C /min with a hold of 5 minutes. The total running time for each sample was 16.5 minutes.
Figure 3.8 Varian CP-3800 GC/FID equipment.

3.2.2.5. pH Measurement

Determination of pH was done by a Crison pH meter, model Basic 20 (Figure 3.9), which was calibrated to pH 4, pH 7 and pH 9 prior to all experiments.

Figure 3.9 Crison Basic 20 pH meter.
3.2.2.6. Solid Content Determination

Solid content of the microcapsules was determined according to the European Standard EN 827 for the determination of solid content in a water based adhesive. It involves measuring the difference between the initial mass and the final mass of microcapsules samples after water evaporation. The test was done by placing about one gram of the microcapsule suspension on a watch glass and allowing it to be dried in an oven at 100°C for 30 minutes, then placing the sample in a desiccator for 15 minutes and weighing the residual mass. The drying step was repeated until the difference between the initial mass and the residual mass (mass \textit{final}) did not exceed 2 mg.\textsuperscript{6} The solid content was calculated according to equation 3.1:

\[
\text{% Solid Content} = \frac{\text{mass } \text{final}}{\text{mass } \text{initial}} \times 100 \tag{3.1}
\]

3.2.3. Release Measurements

The release studies of vanillin and limonene were performed by using the produced microcapsules suspensions after being washed with hexane. The measurements involved quantification of the free active material (vanillin or limonene) after being released from the core of the microcapsules to the microcapsules surrounding phase. The method was adapted from a previously reported study,\textsuperscript{7} but with few alterations.

Throughout the work, known volumes of washed microcapsules were placed in sealed bottles and placed in an incubator (Figure 3.10), which was set to specific temperatures and shaking speeds over a certain duration of time. Samples of the incubated microcapsules suspensions were collected at predetermined time intervals. Subsequently, the microcapsules surrounding phase was separated from the loaded microcapsules in the suspension by a syringe equipped with a 0.2 µm pore size polypropylene filter (VWR International - Material de Laboratório, Lda). The concentration of the free vanillin and limonene in the filtered microcapsules surrounding phase was eventually measured by GC-FID chromatography. Vanillin and limonene release profiles are presented and discussed in detail in chapter 5.
3.2.4. Fixation of Microcapsules onto Fabrics

3.2.4.1. Simple coating method

Cotton fabrics (10 cm x 4 cm) were submerged in the microcapsules suspension for five minutes and then were left to dry at room temperature. Samples were cut from different locations on each fabric. SEM was used to confirm the presence and evaluate the morphology of the microcapsules on the fabrics. Elemental analysis of the chemical constituents of the microcapsules that coated the fabric was also done by SEM.

3.2.4.2. Grafting followed by pad-dry-cure method

The application of microcapsules onto the fabrics was also carried out by a chemical grafting method followed by an impregnation process using a ‘pad-dry-cure’ technique. The method was adapted from a previous report. The chemical adhesion process involved the use of citric acid as the linker, and sodium hypophosphite as a catalyst. The padding step was done by a Roaches
foulard, model EHP Padder (Figure 3.11). The padding pressure varied between 0.1 MPa and 0.3 MPa, followed by a drying process in a Roaches thermofixation oven (Figure 3.12), in which the treated samples were adjusted on a pin frame and exposed to a recirculating heat current that allowed optimized drying and curing of the microcapsules at different selected temperatures for specific time.

**Figure 3.11** Roaches EHP Padder, the laboratory foulard used in the fixation of the formed microcapsules onto the fabrics.

**Figure 3.12** Roaches laboratory thermofixation oven, model Mini Thermo.
3.3. References


3. IKA Yellow Line


Chapter 4: Production and Characterization of Vanillin and Limonene Microcapsules by Complex Coacervation

4.1. Introduction

Complex coacervation is one of the most suitable methods used to encapsulate fragrances and flavors; because it reduces or prevents the loss of the volatile compounds since it does not require high temperature. In spite of the fact that the exact mechanism has not yet been fully elucidated, complex coacervation is regarded as a phase separation process that depends on complex formation between oppositely charged polymers via electrostatic attractions, formation of hydrogen bonds or hydrophobic interactions. The majority of the studies reported in literature have investigated the combination of a protein and a polysaccharide in complex coacervation, namely gelatin (an example of a positively charged polymer) and gum Arabic (an example of a negatively charged polymer). Gelatin/gum Arabic is considered the most common pair of complex coacervation and has been widely applied in carbonless paper production and in the encapsulation of flavors and fragrances. However, due to some religious and ethnic constraints, and some health concerns related to emerging diseases, such prion diseases, the use of gelatin is sometimes not preferred and is replaced with a different positively charged polymer, which can be a protein or a polysaccharide.

A cross-linking agent is added in the last step of the coacervation process to harden the formed shells of the microcapsules and stabilize their structure. Formaldehyde and glutaraldehyde are widely used as hardening agents for the microcapsules, and are reported to be toxic and are banned in some countries. Thus, they are being replaced with eco-friendly hardening agents. Moreover, it has been reported that when glutaraldehyde was used to harden the microcapsules of gelatin and gum Arabic, the crosslinked microcapsules had a tendency to aggregate and showed a poor state of dispersion. Therefore, the use of safe and eco-friendly microcapsules hardening agents has recently substituted these conventional cross-linking agents. These hardening agents include sodium tripolyphosphate, tannic acid, glycerol, transglutaminase and genipin.

In the work presented here, sodium tripolyphosphate and tannic acid (Figure 4.1) were examined individually as hardening agents instead of the conventional aldehyde compounds. Sodium
tripolyphosphate (TPP) is a multivalent anion; carrying five negative charges and hence, is able to interact with the positively charged $-\text{NH}_3^+$ groups of chitosan through electrostatic attraction.\textsuperscript{8,10} Sodium TPP is a non-toxic FDA approved compound and is ‘generally regarded as safe’ (GRAS).\textsuperscript{11}

Tannic acid is a natural plant polyphenol, which has the ability to bind to polymers, e.g., gelatin, carrageenan and chitosan through hydrogen bonding and hydrophobic interactions.\textsuperscript{7,12-14}

![Figure 4.1](image1.png)  
(a) Structure of (a) sodium TPP and (b) tannic acid. Adapted from Ref. 10 and 14.

According to their internal structure,\textsuperscript{15} microcapsules can be classified into two types either reservoir or monolithic (Figure 4.2). Reservoir microcapsules can be either mononuclear or polynuclear (multinuclear), whereas the monolithic microcapsules are formed of a matrix of the internal phase and the wall material.\textsuperscript{16}

![Figure 4.2](image2.png)  
Figure 4.2 Types of microcapsules. Adapted from Ref.16.
Mononuclear microcapsules are produced when an oil droplet becomes encapsulated with the polymer(s) whereas the polynuclear microcapsules are formed as a result of aggregating mononuclear microcapsules.\textsuperscript{5,15} This aggregation can be hindered by modifying the hardening process, using particular wall materials or making changes in the coacervation process parameters.\textsuperscript{15} It has been reported in literature that the change in the agitation speed of dispersion during the preparation of the microcapsules by complex coacervation could lead to the change in their morphology. According to Jégat et al.,\textsuperscript{17} multinuclear microcapsules were formed when low stirring speed was applied (less than 1000 rpm) while the proportion of mononuclear microcapsules increased when the speed exceeded this value. Nevertheless, Dong et al.\textsuperscript{15} obtained multinuclear microcapsules by complex coacervation when a stirring speed of 10,000 rpm was applied for 3 minutes.

Current investigations on microencapsulation by coacervation typically focuses on mononuclear microcapsules and overlooks the study of the polynuclear microcapsules, although the polynuclear microcapsules have been recently reported to exhibit better sustained release than the mononuclear ones.\textsuperscript{5,15}

Factorial designs are often applied in the experiments related to the microencapsulation research. These designs involve changing one factor, which will act in an additive manner when keeping the other variables constant within the same study.\textsuperscript{18}

This chapter investigates the encapsulation of vanillin and limonene by complex coacervation method using chitosan/gum Arabic as encapsulants. Two green hardening agents; tannic acid and sodium tripolyphosphate were investigated and their effect on the microcapsule size, morphology and encapsulation efficiency was examined. Moreover, the influence of different emulsifiers (Tween 20, Tergitol, Span 85 and polyglycerol polyricinoleate (PGPR)) on the morphology, dispersion, encapsulation efficiency and the size of the formed microcapsules were studied.

\section*{4.2. Materials and Methods}

\subsection*{4.2.1. Materials}

The chemical compounds and reagents used in this chapter were previously mentioned along with their suppliers in Chapter 3, Section 3.1.
4.2.2. Methods

The general method of the preparation of the microcapsules is comprised of four main steps and was adapted from some methods previously described in the literature\textsuperscript{8,19,20} but with some modifications that principally involved changing the type and amount of the core material, along with the type and amount of the emulsifier and the masses of the chitosan and gum Arabic used. The preparation process of the microcapsules is schematically illustrated in Figure 4.3.

The first step involved the dissolution of certain amount of chitosan and gum Arabic. 1% (w/v) chitosan solution was prepared by dissolving known amount of chitosan in 0.1N acetic acid and left under magnetic stirring overnight (15 hours) to ensure complete dissolution. 2% (w/v) gum Arabic solution was obtained by dissolving certain amount of gum Arabic in deionized water with continuous magnetic stirring at 45ºC for 2 hours.

In the second step, the polymer solutions were mixed together and a known amount of the core material with a known volume of the emulsifier were added to them. Then, they were all mixed at a speed of 8000 rpm at 40ºC for 1 minute with a homogenizer to form an O/W emulsion. Taking into consideration that vanillin is in the form of solid crystals; so it was previously dissolved in a certain amount of corn oil at 40ºC in a covered beaker for 10 minutes before being added to the mixture.

\begin{figure*}[h]
\centering
\includegraphics[width=\textwidth]{diagram.png}
\caption{Schematic representation of the method of microcapsules preparation.}
\end{figure*}
The third step entailed the induction of complex coacervation by decreasing the pH value to 3.5 with 0.2N HCl and setting the stirring speed of the formed emulsion to 400 rpm. Complex coacervation is strongly affected by the pH, and it is reported in the literature that the interaction between chitosan and gum Arabic is maximized within a pH range of 3.5 to 5. The pH in this study was reduced to 3.5; because chitosan has a maximum amount of positive charge in the pH range of 2.8 to 4, and gum Arabic has negative charges only if the pH is above 2.2. After 30 minutes of continuous magnetic stirring, the temperature was gradually decreased from 40ºC to 5ºC with the help of an ice bath.

The last step involved the hardening of the formed microcapsules by drop wisely adding the cross-linker solution (tannic acid or sodium TPP) at 5ºC and stirring at 400 rpm for 3 hours. Sodium TPP solution was prepared by dissolving a specific amount in deionized water followed by the adjustment of pH from 8 to 3.5 by 0.2N HCl before adding it to the final microcapsules mixture. The ratio of sodium TPP to chitosan in all formulations was 1:2 (wt%).

Here we discuss 15 formulations that were prepared by using either vanillin or limonene as the core material and examining different emulsifiers (Tween® 20, Tergitol 15-S-9, Span 85, Tween 80 and PGPR) and two hardening agents separately (tannic acid and sodium TPP). The chemical system of each formulation is summarized in Table 4.1.

4.2.3. Characterization of Microcapsules

The morphology and the dispersion of the microcapsules were investigated by optical microscope and the mean particle size was determined by laser diffraction particle size analysis. The encapsulation efficiency of microcapsules was determined by the quantification of the non-encapsulated vanillin or limonene with GC-FID analysis and calculating the masses of the encapsulated core agents through a method reported previously in Chapter 3 (Section 3.2.2.4.).

4.3. Results and discussion

4.3.1. Encapsulation efficiency

The encapsulation efficiency (EE %) was determined using the following equation:

\[
EE\% = \frac{\text{mass (total)} - \text{mass (non-encapsulated)}}{\text{mass (total)}} \times 100
\]  

(4.1)
The values of EE % of the formulations are shown in Table 4.1.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Active agent</th>
<th>Amount (g)</th>
<th>C</th>
<th>GA</th>
<th>Emulsifier</th>
<th>Amount (g)</th>
<th>Hardening agent</th>
<th>Amount (g)</th>
<th>EE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>V</td>
<td>0.3*</td>
<td>2</td>
<td>8</td>
<td>Tween 20</td>
<td>2.58</td>
<td>TPP</td>
<td>1</td>
<td>64.2</td>
</tr>
<tr>
<td>2</td>
<td>V</td>
<td>0.3*</td>
<td>2</td>
<td>8</td>
<td>Tergitol</td>
<td>2.58</td>
<td>TPP</td>
<td>1</td>
<td>62.0</td>
</tr>
<tr>
<td>3</td>
<td>V</td>
<td>0.3*</td>
<td>-</td>
<td>-</td>
<td>Tween 20</td>
<td>2.58</td>
<td>TPP</td>
<td>1</td>
<td>30.2</td>
</tr>
<tr>
<td>4</td>
<td>V</td>
<td>0.02**</td>
<td>0.5</td>
<td>1</td>
<td>PGPR</td>
<td>0.35</td>
<td>TA</td>
<td>0.2</td>
<td>93.4</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>0.12***</td>
<td>0.5</td>
<td>1</td>
<td>PGPR</td>
<td>0.6</td>
<td>TA</td>
<td>0.2</td>
<td>95.2</td>
</tr>
<tr>
<td>6</td>
<td>L</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>PGPR</td>
<td>0.35</td>
<td>TA</td>
<td>0.2</td>
<td>90.4</td>
</tr>
<tr>
<td>7</td>
<td>L</td>
<td>4.5</td>
<td>0.5</td>
<td>1</td>
<td>PGPR</td>
<td>0.6</td>
<td>TA</td>
<td>0.2</td>
<td>94.1</td>
</tr>
<tr>
<td>8</td>
<td>L</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>PGPR</td>
<td>0.35</td>
<td>TA</td>
<td>0.4</td>
<td>97.3</td>
</tr>
<tr>
<td>9</td>
<td>L</td>
<td>4.5</td>
<td>0.5</td>
<td>1</td>
<td>Span 85</td>
<td>0.6</td>
<td>TA</td>
<td>0.2</td>
<td>98.7</td>
</tr>
<tr>
<td>10</td>
<td>L</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>Span 85</td>
<td>0.35</td>
<td>TA</td>
<td>0.2</td>
<td>98.6</td>
</tr>
<tr>
<td>11</td>
<td>L</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>Span 85</td>
<td>0.35</td>
<td>TPP</td>
<td>0.2</td>
<td>--</td>
</tr>
<tr>
<td>12</td>
<td>L</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>PGPR</td>
<td>0.35</td>
<td>TPP</td>
<td>0.25</td>
<td>85.3</td>
</tr>
<tr>
<td>13</td>
<td>V</td>
<td>0.02***</td>
<td>0.5</td>
<td>1</td>
<td>Span 85</td>
<td>0.35</td>
<td>TA</td>
<td>0.2</td>
<td>100a</td>
</tr>
<tr>
<td>14</td>
<td>V</td>
<td>0.12***</td>
<td>0.5</td>
<td>1</td>
<td>Tween 20</td>
<td>0.6</td>
<td>TA</td>
<td>0.2</td>
<td>--</td>
</tr>
<tr>
<td>15</td>
<td>V</td>
<td>0.12***</td>
<td>0.5</td>
<td>1</td>
<td>Tween 80</td>
<td>0.6</td>
<td>TA</td>
<td>0.2</td>
<td>--</td>
</tr>
</tbody>
</table>

V=vanillin, L=limonene, C=chitosan, GA= gum Arabic, TA= tannic acid, TPP=tripolyphosphate

a GC/FID peak for the non-encapsulated vanillin could not be detected, and thus the concentration and mass of the non-encapsulated vanillin was considered zero.

*0.3 g vanillin was dissolved in 20 ml corn oil

**0.02 g vanillin was dissolved in 1 g corn oil

***0.12 g vanillin was dissolved in 4.5 g of corn oil; amount of corn oil is comparable to limonene.

Comparing the EE% of the first three formulations (where one factor in each was changed and the others were kept the same), it could be observed that the values showed no big difference when Tergitol was used as the emulsifier instead of Tween 20. However, when the wall material contained only chitosan (formulation 3) and no gum Arabic was included, there was a dramatic decrease in the EE% from 64.2% to 30.2%. This indicates that the presence of gum Arabic in the shell material along with chitosan not only adds to the compactness and strength of the microcapsules wall, but also increases the EE%. It could be also explained in terms that the availability of more polymers in the formulation mixture results in encapsulating higher amounts of core materials.1
The use of sodium TPP as hardening agent resulted in the appearance of dark patches, perhaps of polymer precipitation, that could be seen when the formulations were examined by the optical microscope without centrifugation (Figure 4.4).

It was also noticed that formulations which contained vanillin as the core material generally had higher EE% than those which contained limonene. This could be observed when comparing the formulations that contained the same amounts of limonene and corn oil that was used to dissolve certain amounts of vanillin, i.e., formulations 4, 5 and 12 with formulations 6, 7 and 9 respectively. (In these formulations, the preparation conditions and chemical systems were kept the same and only the core material was changed). This might be because vanillin was dissolved in corn oil that resulted in decreasing its diffusivity through the wall and more core material was successfully encapsulated. In contrast, in the case of limonene microcapsules limonene was used purely as the core material, and hence diffused more readily.

The hydrophilic-lipophilic balance (HLB) is a numerical value that reflects the composition of the emulsifier, regarding its content of the hydrophilic and hydrophobic moieties. Emulsifiers with low HLB values (4.7-6.7) are usually used to obtain w/o emulsions, whereas o/w emulsions are obtained by emulsifiers with higher HLB values (9.6-17.6). However, some articles in the literature reported the formation of o/w emulsion followed by complex coacervation by using emulsifiers with low HLB values (e.g., Span 83). It was noticed in this work that the formulations prepared with Span 85 (HLB=1.8) emulsifier exhibited relatively higher EE% than formulations prepared with PGPR (HLB=2-4) that had the same other preparation factors. This could be observed by comparing the EE% of vanillin formulations 5 and 12 (95.2% and 100%), and limonene formulation pairs 6 and 10 (90.4% and 98.6%), 7 and 9 (94.1% and 98.7%). These results are in agreement with those obtained by Rabisková et al who stated that the use of emulsifiers with low HLB values (1.8 and 6.7) in the preparation of o/w emulsions for complex coacervation results in higher values of EE%, indicating the preference of the encapsulation of hydrophobic materials for emulsifiers with low HLB value. The authors reported the inability of emulsifiers with high HLB values, such as Tween 81 and Tween 80 (HLB=10 and 15, respectively) to encapsulate oils by complex coacervation using gelatin and gum Arabic as wall materials.
The encapsulation efficiency obtained by using tannic acid as hardening agent in this study ranged between 90.4% and 100%. This is significantly higher than what was reported by Pakzad et al\textsuperscript{12}; who obtained an EE\% falling in the range of 53\% to 80\% by using tannic acid as a hardening agent for menthol microcapsules produced by complex coacervation using gum Arabic and gelatin as the wall materials. Comparing the EE\% of limonene formulations 6 and 12, in which only the type of the hardening agent was different and all the other formulation factors were kept the same, it was found that tannic acid resulted in a higher encapsulation efficiency (EE\% of formulation 6=90.4\%) that sodium tripolyphosphate (EE\% of formulation 12= 85.3\%). This suggests that tannic acid has higher ability to cross-link with the microcapsules walls than sodium tripolyphosphate. Additionally, tannic acid did not result in the precipitation around the microcapsules that could be seen in the optical microscopy images of formulation 12 (Figure 4.7.B).

Increasing the amount of tannic acid from 0.2 g to 0.4 g in formulations 6 and 8, and keeping the amounts of all other ingredients constant, resulted in the increase of EE\% from 90.4\% to 97.3\%. This is in line with what was reported by Devi et al\textsuperscript{13} who observed an increase in the EE\% from 42.6\% to 71.6\% when the amount of tannic acid increased from 0.2 to 0.8 mmol, and explained that the increase in the EE\% that occur by increasing the amount of the hardening agent is due to the presence of more cross-linkable groups able to form more covalent bonding with the polymers of the wall materials of the microcapsules, and thus making them have higher core material retention ability.

4.3.2. Optical microscopy

It was observed from the optical microscope images of the formulations that the type of the emulsifier used had an influence on the dispersion of the microcapsules and their morphology. Formulations obtained by the emulsifier Tween 20 (HLB=16.5)\textsuperscript{24} encountered formation of aggregates of the microcapsules which were mononuclear (Figure 4.4.A). The degree of aggregation increased when Tergitol (HLB= 13.3)\textsuperscript{24} was used instead of Tween 20. Tergitol also produced mononuclear microcapsules but with smaller particle size (Figure 4.4.B). Formulation 3 that was produced with Tween 20 and using chitosan solely as the wall material (without adding gum Arabic) also had bunches-of-grapes-like aggregation (Figure 4.4.C), and had bigger size of microcapsules than formulation 1.
Figure 4.4 Optical microscope images of formulations: A) 1 (Tween 20), B) 2 (Tergitol) and C) 3 (Tween 20 and no gum Arabic). Images on the left show the emulsions and on the right side are the formulations after adding the hardening agent sodium TPP without sample centrifugation. Magnification: A) 200 x, B) 200 x and C) 400 x.
The appearance of this aggregation in formulation 3 indicated that the presence of gum Arabic in formulation 1 contributed to a better dispersion of the particles in the emulsion than in 3. Moreover, the absence of gum Arabic resulted in unstable formulation and the appearance of oil drops on the surface after 2 months. Gum Arabic has been reported in the literature to be highly beneficial in the stabilization of oil-in-water emulsions and preventing the flocculation of droplets during the storage at room temperatures; because of its ability to be adsorbed at the droplet surface.\textsuperscript{25,26} It could be noticed in the optical microscope images of the formulation 1, 2 and 3, in which sodium TPP was used as hardening agent, the presence of a dark patches probably of polymer precipitation or gelation between sodium TPP and excess chitosan dissolved in the medium. The same dark precipitation was also observed when chitosan amount was reduced from 2g to 0.5g (formulation 11) and another emulsifier was used. However, this precipitation was removed by centrifugation in some preparations which allowed the measurement of EE\%. All the formulations that were prepared with PGPR are rounded with polynuclear morphology (Figure 4.5). PGPR is a hydrophobic emulsifier (HLB=2-4), and its use to form polynuclear complex coacervate microcapsules through (w/o) or (w/o/w) emulsions has been reported in the literature.\textsuperscript{27-29} Polynuclear microcapsules are known to grant more protection for the encapsulated core material.\textsuperscript{29} Formulations prepared with Span 85 emulsifier (HLB=1.8) have shown mononuclear morphology and good dispersion without any aggregation (Figure 4.6). Moreover, the use of tannic acid as the hardening agent in these preparations resulted in the highest EE\% values compared with other formulations. Nevertheless, when sodium TTP was used as the hardening agent instead of the tannic acid and Span 85 was kept as the surfactant (formulation 11), the dark patches of polymer precipitation appeared again (Figure 4.7.A.). It also appeared when sodium TTP was added to an emulsion obtained with PGR in formulation 12, (Figure 4.7.B). It was observed that sodium TTP resulted in the aggregation of the microcapsules prepared with Span 85, which previously had good dispersion. Therefore, the hardening agent also seems to have a big influence on the dispersion of the final microcapsules; not just the emulsifier. Tannic acid was examined with formulations prepared with Tween 20 and Tween 80 (HLB=15) and appeared to be incompatible with these emulsifiers since it resulted in the flocculation of the microcapsules as shown in Figure 4.8.
Figure 4.5 Optical microscope images of vanillin and limonene microcapsules of formulations prepared with PGPR: A) 4, B) 5, C) 6 and D) 7. Magnification: A) 200 x, B) 200 x, C) 200 x and D) 200 x.

Figure 4.6 Optical microscope images of limonene and vanillin microcapsules of formulations: A) 9 and B) 13, in which Span 85 was used as the emulsifier. Magnification: A) 400 x and B) 400 x.
Figure 4.7 Optical microscope images of limonene microcapsules of formulations: A) 11 and B) 12; prepared with Span 85 and PGPR, respectively, using sodium TPP as the hardening agent. Magnification: A) 100 x and B) 100 x.

Figure 4.8 Optical microscope images of vanillin microcapsules of formulations: A) 14 and B) 15; prepared with Tween20 and Tween 80, respectively, using tannic acid as the hardening agent, in same amounts. Magnification: A) 100 x and B) 100 x.

4.3.3. Particle size

It was observed that the type of the emulsifier greatly influenced the size of the microcapsules. Tergitol resulted in very small sized microcapsules, Tween 20 and Span 85 produced medium sized microcapsules and PGPR produced microcapsules with larger sizes than the formers. Table 4.2 lists the mean diameter of the formulations that showed good dispersion. The particle size distribution was also affected by the core-wall ratio. In the present study, it was noticed that keeping the amount of wall materials constant and increasing the amount of oil used (from 1 to
4.5 g) resulted in a significant increase in the mean diameter of the microcapsules which were prepared with the PGPR (Vanillin formulations 4 and 5 and limonene formulation 6 and 7). However, a very slight increase was noticed for the mononuclear microcapsules when Span 85 was used (Formulations 9 and 10). The increase in the size of the microcapsules with increasing the core-wall ratio has been reported in the literature in preparations of microcapsules by complex coacervation.\textsuperscript{19,20,29} Dong et al.,\textsuperscript{30} stated that concerning the multinuclear microcapsules, the increase in the ratio of core to wall material results in an increase in the amount of emulsion droplets available in the suspension during the preparation, which subsequently forms larger spherical coacervate polynuclear microcapsules.

It was also notable that increasing the amount of tannic acid from 0.2 g to 0.4 g (in formulation 6 and 8) resulted in smaller size of microcapsules. This result is consistent with the findings obtained in the literature.\textsuperscript{12,31} This may be due to the fact that the availability of more tannic acid results in an increased degree of cross-linking with the wall materials and consequently makes the wall more compact and with smaller sizes.

Table 4.2 Mean diameters of produced microcapsules.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mean diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.2</td>
</tr>
<tr>
<td>2</td>
<td>4.1</td>
</tr>
<tr>
<td>3</td>
<td>(-)*</td>
</tr>
<tr>
<td>4</td>
<td>15.7</td>
</tr>
<tr>
<td>5</td>
<td>38.2</td>
</tr>
<tr>
<td>6</td>
<td>18.4</td>
</tr>
<tr>
<td>7</td>
<td>39.0</td>
</tr>
<tr>
<td>8</td>
<td>10.9</td>
</tr>
<tr>
<td>9</td>
<td>12.9</td>
</tr>
<tr>
<td>10</td>
<td>11.1</td>
</tr>
<tr>
<td>11</td>
<td>(-)*</td>
</tr>
<tr>
<td>12</td>
<td>35.4</td>
</tr>
<tr>
<td>13</td>
<td>10.4</td>
</tr>
<tr>
<td>14</td>
<td>(-)*</td>
</tr>
<tr>
<td>15</td>
<td>(-)*</td>
</tr>
</tbody>
</table>

(-)* Particle size of these formulations was not measured since they did not have good dispersion or did not seem stable.
4.4. Conclusion

Here, the production of chitosan/gum Arabic microcapsules loaded with vanillin or limonene was successfully achieved by the complex coacervation method. The EE% value ranged from to 62 to 100%, and was shown to be about 30% when gum Arabic was not included. The process parameters involved the examination of various emulsifiers with significant difference in their HLB values. Span 85 emulsifier resulted in the highest values of vanillin and limonene encapsulation, followed by PGPR and then Tween 20 (Span 85 > PGPR > Tween 20). The type of the emulsifier also affected the morphological characteristics of the produced microcapsules; as Span 85 and Tween 20 resulted in the formation of mononuclear microcapsules whereas PGPR produced multinucleated particles. Two green hardening agents; sodium TPP and tannic acid were used separately to cross-link with the microcapsules shell materials. The type and amount of the hardening agent was found to affect the size and EE% as well as the dispersion of the particles in the final product. Formulations in which tannic acid was used as the hardening agent acquired higher EE% values than those which have been treated with sodium TPP.

4.5. References


Appendix 4.1: Particle size distribution charts of formulation 2.

Figure 4.a Particle size distribution of microcapsules of formulation 2 in volume and in number.
Appendix 4.2: Calibration Curves for GC-FID Analysis

**Figure 4.b** Calibration curve of standard limonene.

**Figure 4.c** Calibration curve of standard vanillin.
Chapter 5: Release of Active Agents

5.1. Introduction

The controlled release of the active agents from the microcapsules intended for textile applications is crucial to be investigated. The release pattern should provide a slow release from the fabric microcapsules to the skin without overdosing, and at the same time maintaining a durable effect.\footnote{1} In fact, the longer the time the active agent can be retained within the core of the microcapsules, the longer the shelf-life of the product/fabric would be.\footnote{2} The characteristics of the polymers of the microcapsule wall, such as its type, thickness, swellability, degree of hardening, mechanical strength, and interactions between the wall polymers and the active agent all affect the diffusion, and hence, the release rate of the core material.\footnote{3,4}

The controlled release based on diffusion processes can be described mathematically by release kinetics equations. The most frequently discussed release rate models in literature are the zero order, first order and square root order systems (Figure 5.1). The zero order kinetics involves the release of the core material at a constant rate over time until total depletion.\footnote{2,5} The zero release can be expressed by the following simple equation:\footnote{6}

\[
\frac{M_t}{M_0} = K_0 t \quad (5.1)
\]

where $M_t$ is the amount of the core material/drug released at time $t$, $M_0$ is the initial amount of the core material/drug in the solution and $K_0$ is the zero order release constant. The plot of the zero release kinetics shows the percentage of cumulative release of core material/drug versus time.

In the first order kinetics (Equation (5.2)),\footnote{7} the release of the core material is relatively faster in the beginning, and then decreases with time.\footnote{2} This kinetic model implies that the change in the concentration of the released core material/drug with time is dependent on the concentration.

\[
\frac{M_t}{M_0} = 1 - e^{-kt} \quad (5.2)
\]

The release profile in the square root order kinetics (Higuchi model) exhibits a behavior between the zero and first order models.\footnote{2} The Higuchi diffusion model (Equation (5.3)) relates the concentration of the core material or drug released to the square root of time as follows:
\[
\frac{M_t}{M_0} = k_H t^{1/2} \tag{5.3}
\]

where \( \frac{M_t}{M_0} \) represents the fraction of the cumulative amount of the encapsulated material released in time \( t \) and \( k_H \) is the Higuchi rate constant.\(^8\)

**Figure 5.1** Graphical representation of the cumulative release of core material as a function of time. Adapted from Ref. 2.

The Higuchi model was first developed to relate the release of a drug from an ointment, but later it has been used to study the pseudo-steady diffusion of solid drugs from granular and homogenous matrices.\(^9\) The Higuchi model assumes that no interactions occur between the drug and the matrix, and that the diffusion coefficient is constant.\(^9,10\)

The Korsmeyer-Peppas release model was originally developed to describe the release of a drug molecule from a polymeric system. According to Korsmeyer et al., the release rate can be represented mathematically by the following equation:\(^10\)

\[
\frac{M_t}{M_\infty} = K_k t^n \tag{5.4}
\]

where \( M_t \) and \( M_\infty \) are the cumulative amounts of the drug in the release solution at time \( t \) and infinity, respectively, \( K_k \) is the release rate constant, and \( n \) is the diffusional exponent that describes the obtained release mechanism.\(^11\)
The Korsmeyer-Peppas equation is also called the “power law”; as the equation suggests that the fractional release of core material/drug is exponentially related to time, and the value of diffusional exponent ($n$) indicates the diffusional release mechanism of the core agent/drug from the polymer as shown in Table 5.1.\textsuperscript{12,13}

**Table 5.1** The indicative values of the release exponent “n” of Korsmeyer-Peppas equation and the related release mechanism of the core material/drug from polymers of different geometries. Adapted from Ref. 12 and 13.

<table>
<thead>
<tr>
<th>Thin film</th>
<th>Exponent, $n$</th>
<th>Drug release mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cylinder</td>
<td>Sphere</td>
</tr>
<tr>
<td>0.5</td>
<td>0.45</td>
<td>0.43</td>
</tr>
<tr>
<td>$0.5 &lt; n &lt; 1.0$</td>
<td>0.45 $&lt; n &lt; 0.89$</td>
<td>0.43 $&lt; n &lt; 0.85$</td>
</tr>
<tr>
<td>1.0</td>
<td>0.89</td>
<td>0.85</td>
</tr>
</tbody>
</table>

In this chapter, limonene and vanillin microcapsules were prepared by complex coacervation method. Characterization of the produced microcapsules, regarding the size, morphology and encapsulation efficiency was evaluated. The cumulative release profiles and the release kinetics of the core materials were investigated.

### 5.2. Materials and methods

#### 5.2.1. Materials

Chitosan (Degree of deacetylation 88-95\%) and gum Arabic were used as wall materials, and were purchased from BioLog Biotechnologie Und Logistik (Germany), and Sigma Aldrich, respectively. The molecular weight range of the chitosan used in all the formulations was between 80,000 and 200,000 Da as claimed by the manufacturer. Vanillin and limonene were separately used as the active agents, and were purchased from Sigma Aldrich. Three different emulsifiers were used Tween 20, Span 85 and Polyglycerol polyricinoleate (PGPR 4150). Tween 20 and Span 85 were supplied by Sigma Aldrich, and PGPR was obtained as a gift from Palsgaard\textsuperscript{®}, Denmark. Pure corn oil was purchased from Sigma Aldrich. Sodium tripolyphosphate (TPP) (Sigma Aldrich) and tannic acid (Merck) were used individually as hardening agents in different preparations. n-Hexane was used a washing reagent for the microcapsules and was supplied by Carlo Erba Reagents.
5.2.2. Microcapsules preparation

Three release studies were conducted, each with two formulations produced by changing the amount of the wall material used, type of the emulsifier, and the hardening agent. The amounts and different types of materials used in each formulation are mentioned in each study. Vanillin and limonene formulations were produced independently by the complex coacervation method according to the general procedure detailed in Chapter 3 (Sections 3.2.1.1 and 3.2.1.2).

5.2.3. Characterization of Microcapsules

The morphology of the prepared microcapsules was examined by optical microscopy and the mean diameter of each formulation was determined by laser diffraction particle size analysis. The encapsulation efficiency of microcapsules was determined by quantifying the non-encapsulated core material with GC-FID analysis and calculating the masses of the encapsulated active agents as reported previously in Chapter 3 (Section 3.2.2.4).

The GC-FID injected samples were prepared by taking 2 ml from the whole formulation and then mixing them with a particular volume of n-hexane, followed by centrifugation at 3000 rpm for 5 minutes. This was performed to extract the non-encapsulated vanillin or limonene from the medium. The supernatant was then collected, filtered through 0.2 µm pore size polypropylene filter and a volume of 0.1 µL was injected in the GC.

The encapsulation efficiency (EE %) was calculated as the difference between the total mass of the core material initially used for each preparation, and the non-encapsulated mass using the following equation:

\[
EE\% = \frac{mass\ (total) - mass\ (non-encapsulated) \times 100}{mass\ (total)} \tag{5.5}
\]

where mass (total) is the mass of the loaded core material (g), and mass (non-encapsulated) is the mass of the non-encapsulated core material (g). The masses of the non-encapsulated active principles were calculated from the formula (mass= V. C); where V is the volume of the microcapsules suspension and C is the concentration of the non-encapsulated core materials, obtained from the corresponding areas of the chromatograms that were quantified by the standard calibration curve. All measurements were done in triplicates.
5.2.4. Release studies

In this chapter, three release studies are discussed. These studies examined the effects of varying some factors during the preparation of the microcapsules, and investigating the influence of these changes on the release behavior of vanillin and limonene.

**Release Study 1: Effect of changing the concentration of the wall materials**

Here we examined the effect of decreasing the concentration of chitosan and gum Arabic on the release rate of limonene from the microcapsules. Table 5.2 summarizes the preparation conditions for the two formulations A and B that were involved in this study.

**Table 5.2** The chemical composition of formulations A and B.

<table>
<thead>
<tr>
<th></th>
<th>Formulation A</th>
<th>Formulation B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsifier</td>
<td>Tween® 20</td>
<td>Tween® 20</td>
</tr>
<tr>
<td>Mass of emulsifier</td>
<td>2.58 g</td>
<td>2.58 g</td>
</tr>
<tr>
<td>Limonene (Core)</td>
<td>32 g</td>
<td>32 g</td>
</tr>
<tr>
<td>Chitosan</td>
<td>1 g</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Gum Arabic</td>
<td>2 g</td>
<td>1 g</td>
</tr>
<tr>
<td>Hardening agent</td>
<td>Sodium TPP (0.5 g)</td>
<td>Sodium TPP (0.25 g)</td>
</tr>
</tbody>
</table>

**Release Study 2: Effect of changing the type of emulsifier on the release of limonene**

In this study, two different emulsifiers Span 85 and PGPR were used in the preparation of two formulations; C and D. Both emulsifiers were used in same amounts as shown in Table 5.3.

**Table 5.3** The chemical systems of formulations C and D.

<table>
<thead>
<tr>
<th></th>
<th>Formulation C</th>
<th>Formulation D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsifier</td>
<td>Span 85</td>
<td>PGPR</td>
</tr>
<tr>
<td>Mass of emulsifier</td>
<td>0.35 g</td>
<td>0.35 g</td>
</tr>
<tr>
<td>Limonene (Core)</td>
<td>1 g</td>
<td>1 g</td>
</tr>
<tr>
<td>Chitosan</td>
<td>0.5 g</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Gum Arabic</td>
<td>1 g</td>
<td>1 g</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>0.2 g/ 2 ml deionized water</td>
<td>0.2 g/ 2 ml deionized water</td>
</tr>
</tbody>
</table>
Release Study 3: Effect of changing the type of the core material

In this study vanillin was used as the encapsulated core material instead of limonene. As indicated in chapter 3, (section 3.2.1), vanillin crystals were first dissolved in corn oil to prepare the emulsion before initiating the complex coacervation process. Table 5.4 shows the chemical system in formulations E. This formulation is comparable to formulation D in the second release study, where limonene was encapsulated using the same emulsifier.

Table 5.4 The chemical composition of formulation E.

<table>
<thead>
<tr>
<th></th>
<th>Formulation E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsifier</td>
<td>PGPR</td>
</tr>
<tr>
<td>Mass of emulsifier</td>
<td>0.6 g</td>
</tr>
<tr>
<td>Corn oil</td>
<td>4.5 g</td>
</tr>
<tr>
<td>Vanillin (Core)</td>
<td>0.12 g</td>
</tr>
<tr>
<td>Chitosan</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Gum Arabic</td>
<td>1 g</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>0.2 g/ 2 ml deionized water</td>
</tr>
</tbody>
</table>

5.2.4.1. Cumulative release profiles

The release studies of the active agents from the microcapsules were conducted directly after the preparation of each formulation. In each study, equal volumes of microcapsules suspensions were washed with deionized water and n-hexane (¾ of the volume of the microcapsules suspension) in order to remove all the non-encapsulated core material from the microcapsules surrounding phase, and then placed in a sealed glass container that contained a fresh 30 ml of hexane. Samples were incubated at a constant temperature (50ºC for the first release study and 37ºC during the second and third release studies) and mild continuous stirring at 100 rpm, for a specific duration according to each study (7-40 days). At predetermined time intervals, samples were taken out of the incubating chamber; certain volume of hexane (2 ml) in the supernatant was filtered through 0.2 μm pore size polypropylene filter and placed in a sealed vial for GC-FID analysis. The volume of each GC-FID injected sample was 0.1μL. Injections were carried out in triplicates and the mean value of the peak area corresponding to the active agent (either limonene or vanillin) in each acquired chromatogram was determined. The average areas were then
quantified to the corresponding concentration in view of the standard calibration curve of the core material being measured. Then the masses of the released active agents were calculated from the formula (mass = volume x concentration). The cumulative release from the microcapsules suspension for each time interval was calculated from the following equation:

\[
\text{Cumulative Release} \% \ (CR \%) = \frac{M_x}{M_0} \times 100
\]  

(5.6)

Where \(M_x\) is the mass of released limonene or vanillin at a certain time interval and \(M_0\) is the initial mass of limonene or vanillin present in the microcapsules.

### 5.2.4.2. Kinetic analysis of the release profiles

The obtained cumulative release data were kinetically examined to determine the release order of limonene and vanillin from the microcapsules using Excel, Microsoft Office 2010. Four release kinetics models were used to assess and fit the obtained release profiles of limonene and vanillin microcapsules; the zero-order model, the first order model, the Higuchi model and the Korsmeyer-Peppas model. Thereafter, upon comparison of the linear regressions of the four models for a given release profile, the kinetic model with higher regression coefficient (\(r^2\)) was considered as the most appropriate release kinetic model for that release profile. This method is frequently used in the literature to determine the kinetic model of the in-vitro drug release and active principles from different dosage systems, and nano- and microcapsules.

### 5.3. Results and discussion

#### 5.3.1 Optical Microscopy

The morphology of the microcapsules of the three release studies has been examined by means of optical microscopy. Figures 5.2, 5.3 and 5.4 show the micrographic images of the microcapsules of the first, second and third release studies, respectively. It was observed that the microcapsules in all the formulations are spherical. Limonene microcapsules in formulations A and B of release study 1 are mononuclear and have a regular and smooth appearance. Images (a) and (b) in Figure 5.2 show that the microcapsules of formulation A have a larger size than those of formulation B (images (c) and (d) in Figure 5.2). The appearance of the limonene microcapsules in formulation D (images (c) and (d) in Figure 5.3) would suggest that they are
polynuclear in nature. The same morphology was also observed for the vanillin microcapsules in formulation E (images (a) and (b) in Figure 5.4). However, the apparent polynuclear vanillin microcapsules look denser than the limonene ones which were prepared using the same emulsifier; this might be due to the different nature, including the rheological properties and densities between limonene and corn oil (than was used to dissolve vanillin) which comprise the core of the microcapsules and also the relatively higher amount of vanillin and corn oil used than the limonene in formulation D.

**5.3.2 Particle Size Evaluation**

Particle size distributions in both, volume and number of the microcapsules of the release studies 1, 2 and 3 are shown in Figures 5.5, 5.6, and 5.7, respectively. The mean diameter values of the six formulations in the three studies are given in Table 5.5.

For formulation A (Figure 5.5 (a)), a bimodal distribution was observed with a mean particle size around 8 μm. A bimodal distribution was also seen in the particle size distribution of formulation B (Figure 5.5 (c)), with a mean particle size of 3.8 μm. This shows that the particle size increased by doubling the concentration of the wall material in formulation A, and thus follows the same trend observed in previous reports in the literature$^{21,22}$ where it was concluded that the size of the microcapsules prepared by the complex coacervation method increases by increasing the wall material concentration.
Figure 5.2 (a) and (b) Optical microscopy images of limonene microcapsules of formulation A, (c) and (d) of formulation B after hardening and centrifugation. Magnification of images: (a) 400x; (b) 1000x; (c) 400x and (d) 1000x.
Figure 5.3 (a) and (b) Optical microscopy images of limonene microcapsules solution of formulation C (formed with Span 85), (c) and (d) of formulation D (forms with PGPR) after hardening and centrifugation. Magnification of images: (a) 400x; (b) 1000x; (c) 200x and (d) 400x.
The use of different emulsifiers in the second release study showed dissimilarities in particle size distributions, although the other chemical system and preparation conditions were the same within the study. It was observed that the emulsifier PGPR which was used in the preparation of formulations D has led to the formation of microcapsules not just with a different morphology, but also with a greater mean diameter than those obtained by the Span 85 emulsifier (formulation C).

Table 5.5 Mean diameters of produced microcapsules.

<table>
<thead>
<tr>
<th>Release Study</th>
<th>Formulation</th>
<th>Volume mean diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>3.8</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>18.4</td>
</tr>
<tr>
<td>3</td>
<td>E</td>
<td>38.2</td>
</tr>
</tbody>
</table>
5.3.3. Scanning Electron Microscope

The SEM micrographs of formulation B (Figure 5.9 (1) and (2)) show that the fabricated microcapsules have spherical shape and are covered with a thick layer. This might reveal that some of the wall materials could not deposit completely on the core material during the preparation process, but eventually deposited on the surface of the formed microcapsules later covering it with this layer. Similar images were observed by Yang et al., who reported that such “conglutination” in appearance is due to the use of high viscosity chitosan.\textsuperscript{23}

5.3.4. Encapsulation efficiency of microcapsules

In general, the microencapsulation of hydrophobic materials by the complex coacervation method results in high encapsulation efficiency.\textsuperscript{24} Table 5.6 compares the percentage of the core material (limonene or vanillin) entrapped in the microcapsules in the six formulations.

In the first release study, the difference in the EE\% is related to the difference in the concentration of the wall material, while in the second and third release studies, the encapsulation efficiency was influenced by the type of the emulsifier used.

Decreasing the concentration of the wall materials in formulation B to half the amount of formulation A resulted in the reduction of the EE\% from 98.4\% to 93.2\%. It is worth noting that previous studies reported that encapsulation efficiency increases with increasing the concentration of the polymers. A possible explanation of this is that higher concentrations of the dispersion phase result in higher viscosity and enhance faster precipitation of the polymer on the oil phase, and thus delays its diffusion from the microcapsules to the outer phase.\textsuperscript{25}

In the encapsulation efficiencies of the second release study; formulations C (98.6\%) and D (90.4\%), it could be observed that changing the type of emulsifier had an influence on how much core material could be entrapped. This could also be related to the difference in the morphology of the formed microcapsules (mononuclear versus multinuclear).
Figure 5.6 Particle size distribution of limonene microcapsules of the first release study: formulation A; distribution in volume (a) and in number (b); and formulation B; distribution in volume (c) and in number (d).
Figure 5.7 Particle size distribution of limonene microcapsules of the second release study: formulation C; distribution in volume (a) and in number (b); and formulation D; distribution in volume (c) and in number (d)
Figure 5.8 Particle size distribution of vanillin microcapsules of the third release study: formulation E; distribution in volume (a) and in number (b).
Figure 5.9 SEM micrographs of limonene microcapsules of formulation B after freeze-drying.

The effect of the emulsifier on the characterization properties of microcapsules is often mentioned in the literature. For example recently it was reported the effect of the HLB
(hydrophilic-lipophilic balance) of the surfactants Tween 20, Tween 80, Tergitol 15-S-9, Span 85, and their combinations on the encapsulation efficiency of thymol oil/PLA microcapsules prepared by simple coacervation.\textsuperscript{25} The HLB range of the emulsifiers used in that study was between 11 and 16.5 and the highest EE\% obtained was 65 \% when Tergitol 15-S-9 (HLB=13.3) was used. The influence of the HLB value on the encapsulation efficiency of microcapsules obtained by complex coacervation was investigated by Rabisková et al.,\textsuperscript{26} where they reported that emulsifiers of HLB values between 1.8 and 6.7 resulted in encapsulation efficiencies, while using an emulsifier with an HLB value of 9.6 caused a significant decrease in the amount of the encapsulated oil. Higher HLB values of 10 and 15 failed to incorporate the oil inside the microcapsules. This explains the high EE \% obtained with the lipophilic surfactants PGPR (HLB= 2-4)\textsuperscript{27} and Span 85 (HLB=1.8).\textsuperscript{26}

Table 5.6 Encapsulation efficiencies percentages of the formulations used in the three release studies.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Core material</th>
<th>Emulsifier</th>
<th>Total mass (g)</th>
<th>Non-encapsulated mass (g)</th>
<th>Encapsulated mass (g)</th>
<th>EE%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Limonene</td>
<td>Tween 20</td>
<td>32.5</td>
<td>0.5</td>
<td>32</td>
<td>98.4</td>
</tr>
<tr>
<td>B</td>
<td>Limonene</td>
<td>Tween 20</td>
<td>32.5</td>
<td>2.18</td>
<td>30.32</td>
<td>93.0</td>
</tr>
<tr>
<td>C</td>
<td>Limonene</td>
<td>Span 85</td>
<td>1</td>
<td>0.014</td>
<td>0.986</td>
<td>98.6</td>
</tr>
<tr>
<td>D</td>
<td>Limonene</td>
<td>PGPR</td>
<td>1</td>
<td>0.096</td>
<td>0.904</td>
<td>90.4</td>
</tr>
<tr>
<td>E</td>
<td>Vanillin</td>
<td>PGPR</td>
<td>0.12</td>
<td>0.0058</td>
<td>0.1142</td>
<td>95.2</td>
</tr>
</tbody>
</table>

5.3.5 Release Studies

5.3.5.1. Effect of changing concentration of the wall materials

The release profiles of microcapsules in formulations A and B were investigated throughout the course of 30 and 40 days, respectively. It was notable from the cumulative release graphs (Figure 5.10 and 5.11) of both formulations that the release rate was very slow and prolonged, and did not follow the expected biphasic release behavior which is usually obtained from microcapsules produced by the complex coacervation method. In formulation A, approximately 0.61\% of the total encapsulated limonene was released after incubating the microcapsules suspension for 30
days (at 50° C and 180 rpm). For formulation B, it was observed that a mass of only 0.443 g was released, which corresponds to 1.5% of the initial encapsulated mass of 30.32 g.

This difference between the amounts of limonene released in the two formulations is attributed to the difference in the thickness of the microcapsules wall. Lower concentration of wall materials results in decreasing the microcapsules membrane, making the diffusion of the core material easier and hence, gives higher release rate.28 Furthermore, the smaller particle size of the microcapsules of formulation B also contributed to the faster percentage release of the internal phase; the diffusional path length for the oil becomes smaller and the contact surface area between the microcapsules and the dissolution medium becomes larger.29

![Figure 5.10](image) Cumulative release profile of limonene in formulation A.
Figure 5.11 Cumulative release profile of limonene in formulation B.

The release curves obtained from this study which demonstrated a very slow release behavior, along with the SEM images (Figure 5.9) suggest that the release of limonene was hindered by the layer that appeared to surround the microcapsules. This layer might be due to the high viscosity of chitosan as discussed earlier. It might be also due to the gelation caused by the hardening agent sodium TPP or due to excess limonene oil that might have leaked with time.

5.3.5.2. Effect of the type of emulsifier on the release pattern of limonene

The release manner of limonene from microcapsules in formulations C and D varied with the type of the emulsifier used in each formulation. Both formulations exhibited biphasic release behavior; one that is characterized initially by a burst release effect that is followed by a plateau of gradual sustained release. Nevertheless, the initial burst release effect took a longer time in formulation C (Figure 5.12) than in D (Figure 5.13). The burst release effect is described as a high initial delivery of the encapsulated active agent before the release profile reaches a steady rate. The reason of this fast initial release phase might be due to the distribution of some of the core material on the surface of microcapsules. Once this adsorbed surface oil is released, the release pattern becomes stable and the remaining oil is then liberated mainly by penetration through the microcapsules wall. The burst effect may also occur because of the low
molecular weight of the active agent and as a result of the high osmotic pressure and difference in concentration gradient.\textsuperscript{30} Although the initial burst effect might contribute to the reduction of the lifetime of the product, it is desired in specific applications, such as encapsulated flavors, wound treatment, targeted delivery (where a targeted burst release is required) and pulsatile release.\textsuperscript{32}

It has been previously reported that the multinuclear microcapsules have better controlled release behavior than the mononuclear microcapsules, which makes them more favorable in the applications that require prolonged release.\textsuperscript{22} By analyzing the curves of limonene release in our study, it could be observed that the release was initially faster in formulation D than in C. However, the stable sustained release phase started earlier (after almost 24 hours) in formulation D (Figure 5.13); whereby 43\% of the incorporated limonene was released. The same phase started in formulation C (Figure 5.12) after 120 hours (5 days) where about 74\% of the encapsulated limonene was released. It was notable that after incubating both formulations for 7 days (168 hours), at 37 °C and 100 rpm, the overall cumulative release for the mononuclear microcapsules was about 75\%, whereas it was 52\% for the polynuclear microcapsules. In this context, it could be concluded that the release rate is lower in the polynuclear microcapsules than in the case of mononuclear microcapsules. These results are in agreement with those described by Jégat et al.; who used different stirring speed to produce mononuclear and polynuclear microcapsules, and reported a lower release rate for the polynuclear microcapsules than the mononuclear microcapsules.\textsuperscript{33}
Figure 5.12 Cumulative release profile of limonene in formulation C (with Span 85).

Figure 5.13 Cumulative release profile of limonene in formulation D (PGPR).
5.3.5.3 Effect of the type of the core material

This study was performed to investigate the release profile of vanillin from the chitosan/gum Arabic microcapsules (Formulation E) in which PGPR was used as the emulsifiers. It was found that there was a change in the morphology of vanillin microcapsules of formulation E after 10 days of incubating the sample at 37°C and stirring at 100 rpm to study the release. Figure 5.14 shows the alteration that occurred to the appearance of the internal phase of the multinuclear microcapsules by the end of the study. There seemed to be a collapse/disintegration in the encapsulated core droplets inside the main membrane of each microcapsule.

![Figure 5.14 Optical microscope images of vanillin microcapsules of formulation E: (a) before and (b) after 10 days after incubating at 37°C ± 1 and 100 rpm during the third release study.](image)

It could be observed from the cumulative release profile of vanillin in formulation E that it also demonstrated a biphasic release pattern but is much prolonged than the limonene released from the polynuclear microcapsules in formulation D (prepared with the same emulsifier and have similar morphologies). The release plot (Figure 5.15) shows a high initial release followed by a plateau. However, in this formulation the plateau was attained after about 48 hours; whereby approximately only 16% of the total encapsulated vanillin was released. This result is relatively lower than what was observed in the case of the release profile of limonene in formulation D. Furthermore, it was observed that after 7 days (168 hours), the total cumulative % of vanillin released was about 19.4% of the total amount of encapsulated vanillin, unlike limonene formulation D in which 52% of the total encapsulated limonene was released within the first 7
days of incubating at the same conditions (37ºC and 100 rpm). This slower release rate behavior is probably due to the difference in the chemical structure of vanillin and limonene, and also their ability to diffuse through the wall polymers, added to that the fact that vanillin, unlike limonene, which was dissolved formerly in corn oil and shares the microcapsules core with it, this have might participated in the slow transport of vanillin out of the microcapsules shell. The results obtained here are similar to the slow and sustained release profile of vanillin that was reported by Dalmolin et al.,\textsuperscript{34} who used poly-lactic acid nanoparticles to encapsulate vanillin and obtained a biphasic slow pattern with 20% cumulative vanillin release after 120 hours. In the same study, the authors mentioned the importance of the biphasic release in pharmaceutical applications; where the initial burst effect is needed in reaching suitable plasma concentration and triggering the therapeutic onset, whilst the plateau phase is important in maintaining a stable drug concentration for long duration. Prolonged sustained release of vanillin aroma from polysaccharide polymeric hydrogels was also described in the literature as desirable in the perspective of controlled release applications.\textsuperscript{35}

![Figure 5.15](image-url) Cumulative release profile of vanillin in formulation E (PGPR).
5.3.5.4 Kinetic analysis of the release profiles

The data obtained from the former cumulative release profiles were fitted to the zero-order, the first-order, the square root of time (Higuchi) and the Korsmeyer-Peppas model equations to determine the kinetics and the mechanism of limonene and vanillin release from the microcapsules. Table 5.7 lists the regression coefficients ($r^2$) and the diffusion exponent (n) of the determinations. The release kinetic of formulation A suggests a zero-order release; since it has shown a higher $r^2$ value than the other two models. However, it was found that the release data of formulations B and C could be best fitted into the first order release kinetics. The highest coefficient for the release data of formulations D and E (who have the same morphology) was observed for the Higuchi release, but it was also noticed that the values of regression coefficients of formulation D for all of the three models were relatively lower than the coefficients of formulations A, B, C and E. Probably the release data of formulation D would be better fitted in a different kinetic model.

**Table 5.7** Correlation Coefficient ($r^2$) of release kinetics and diffusion exponent (n) of active agents from the chitosan/gum Arabic microcapsules.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zero Order ($r^2$)</th>
<th>First Order ($r^2$)</th>
<th>Higuchi ($r^2$)</th>
<th>Korsmeyer-Peppas (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.8903</td>
<td>0.8899</td>
<td>0.7233</td>
<td>≈ 0.45</td>
</tr>
<tr>
<td>B</td>
<td>0.9866</td>
<td>0.9869</td>
<td>0.9443</td>
<td>≈ 0.59</td>
</tr>
<tr>
<td>C</td>
<td>0.9260</td>
<td>0.9547</td>
<td>0.9250</td>
<td>≈ 0.78</td>
</tr>
<tr>
<td>D</td>
<td>0.7828</td>
<td>0.8331</td>
<td>0.8697</td>
<td>≈ 0.45</td>
</tr>
<tr>
<td>E</td>
<td>0.8244</td>
<td>0.8401</td>
<td>0.9733</td>
<td>≈ 0.09 (&lt; 0.43)</td>
</tr>
</tbody>
</table>

Despite the differences in their release profiles, the (n) value “release exponent” of the Korsmeyer–Peppas equation plot for the formulations A, B, C and D fell between (0.43 and 0.85) which indicates that the mechanism of limonene release in these formulations most probably follows an anomalous transport pattern, one that is controlled by non-Fickian diffusion and involves a combination of the diffusion and erosion mechanisms.\(^{36}\) This is consistent with the fact that the diffusion of materials through polymers is not always controlled by the standard Fickian diffusion model.\(^{37}\) However, the release exponent value (n) for formulation E was found
to be about 0.09 (i.e., $\leq 0.43$); which suggests that the release of vanillin from the microcapsules was controlled by diffusion. This value is similar for the one reported in the literature for the vanillin release form poly-lactic acid nanoparticles;\textsuperscript{34} and thus, the adopted controlling release mechanism might be related to the type and nature of the core material in this case, i.e., vanillin.

5.4. Conclusion

In this chapter, chitosan/gum Arabic limonene and vanillin microcapsules were prepared by the complex coacervation method. The encapsulation efficiency ranged between 90.4% and 100%. Optical microscopy confirmed the spherical shape of the microcapsules. However, the morphology and the particle size of the microcapsules varied remarkably according to the chemical system of each formulation. The mean diameter of microcapsules based on volume distribution ranged from 3.8 µm to 38 µm. Tween 20 and Span 85 emulsifiers produced mononuclear microcapsules, while PGPR gave rise to multinuclear ones. Notably, the release profiles of the active agents from the microcapsules have shown different release characteristics and were dependent on the concentration of the encapsulating polymers, the nature of the incorporated core material and the morphology of the microcapsules. Additionally, they demonstrated a considerable controlled release patterns that would make their use appropriate for many applications.

5.5. References


Chapter 6: Impregnation of Microcapsules on Textiles and Evaluation of the Antimicrobial Activity

6.1. Introduction

Developing innovative textiles with added-value properties is a production engineering challenge that enhances market competitiveness and growth. Microencapsulation introduces new durable properties to fabrics through the incorporation of active compounds with various functions, such as antimicrobials, dyes, phase change materials, insect repellents, flame retardants, thermo- and photo-chromatic finishes and fragrances.

The process of fixing the microcapsules onto textile substrates is critical in ensuring durability, wash-ability and the effectiveness of the added value to the fabric. The commonly known industrial methods that are used to apply microcapsules to fabrics are the spraying, padding/curing, bath exhaustion and coating. The bath exhaustion method is commonly applied when a chemical reaction between the fabric and microcapsules is required. These adhesion methods involve the use of two main groups of binders; polymeric resins, which possess a film-forming ability, and polyfunctional crosslinking agents. The first group includes polymers, such as carboxymethyl cellulose, silicon, acrylate resin, polyether polyurethane and polyvinyl acetate, while the second group includes the chemical cross-linkers, which can be subdivided into formaldehyde based cross-linkers, e.g., formaldehyde and glutaraldehyde, and non-formaldehyde based cross-linkers, such as polycarboxylic acids, such as citric acid, succinic acid and 1,2,3,4-butanetetracarboxylic acid.

Although film-forming binders provide a three dimensional network that strongly adheres microcapsules to the fabric, they may hinder the release of the encapsulated active agent and reduce the aroma intensity of the fragrance microcapsules after adhesion on fabrics. Therefore, chemical grafting by means of one of the members of the second group is sometimes preferred. Grafting or crosslinking of microcapsules to cotton fabrics via polycarboxylic acids occurs covalently through an esterification reaction between the carboxylic groups of the cross-linker and hydroxyl groups of the cotton cellulose and/or the hydroxyl groups of the polymers of the shell of the microcapsules. Figure 6.1 and Figure 6.2
help illustrate this reaction which occurs in the presence of heat and a catalyst. \(^5,8-10\) The reaction can also be aided by microwave radiation \(^11\) and ultraviolet radiation. \(^12\)

**Figure 6.1** The cross-linking reaction between chitosan and cellulose of cotton using citric acid. Adapted from Ref 12.

**Figure 6.2** Representation of the proposed grafting reaction of the produced microcapsules on cotton fabrics. Adapted from Ref 10.

Recently, more attention has been directed towards the production of antibacterial functionalized textiles for medical and hygienic uses. \(^13,14\) Vanillin encapsulated in a polysulfone polymer and incorporated onto cotton fabrics was reported to provide the fabrics with durable aromatic properties and antibacterial activity against *Staphylococcus aureus*. \(^15\) Rodrigues and coworkers used interfacial polymerization technology to encapsulate limonene in polyurethane-urea microcapsules for the purpose of producing durable fragrant fabrics. \(^16\) Sundrarajan also reported the preparation of limonene/gum Arabic microcapsules by the coacervation method for antibacterial textile application using citric acid for the grafting reaction. \(^14,17\)

This chapter describes the strategy employed to achieve the immobilization of the produced limonene and vanillin microcapsules on cotton fabrics by using green materials and processes.
The work aimed at overcoming the effect of extreme thermal treatment on microcapsules during the drying and curing steps; as the temperature of the linking reaction seemed to greatly influence the amount and morphology of the microcapsules grafted onto the fabrics. The main challenge encountered was in reaching a formulation to prepare microcapsules with sufficient strength to endure the fixation process and thus, maximize their deposition on the fabrics. The antimicrobial activity of both the free microcapsules (before grafting) and the functionalized fabrics was investigated respectively by means of the agar diffusion test and the standard test method under dynamic contact conditions.

6.2. Materials and methods

6.2.1. Materials

Chitosan (Degree of deacetylation 88-95%) and gum Arabic were used as shell-forming materials, and were purchased from BioLog Biotechnologie Und Logistik (Germany), and Sigma Aldrich, respectively. Standard 100% cotton fabric was purchased from SDC Enterprises Limited, UK. Vanillin and limonene, which were separately used as core agents, were purchased from Sigma Aldrich. Citric acid, sodium hypophosphite and sodium phosphate monobasic monohydrate were purchased from Sigma Aldrich and were used in the chemical grafting reaction. Tween 20 and Span 85 were used as emulsifying agents and were supplied from Sigma Aldrich. Polyglycerol polyricinoleate (PGPR 4150), also used as emulsifier in some preparations, was a gift from Palsgaard®, Denmark. Pure corn oil was used in the preparation of microcapsules to dissolve vanillin, and was obtained from Sigma Aldrich. Sodium tripolyphosphate (TPP) (Sigma Aldrich) and tannic acid (Merck) were used individually as hardening agents in different preparations. N-hexane was used as the washing medium for the microcapsules and was supplied by Carlo Erba Reagents. Baypret USV®, a commercial polyurethane binder, supplied from Bayer, was also used to evaluate the textile impregnation with the produced microcapsules.
6.2.2. Microcapsules preparation

Vanillin and limonene microcapsules were produced independently by the complex coacervation technique according to the general procedure described in Chapter 3 (Sections 3.2.1.1 and 3.2.1.2) and Chapter 4 (Section 4.2.2). In order to reach an optimized microcapsules formulation that could be successfully adhered to cotton fabrics, several trials were conducted by altering the amount of the core material incorporated, type of the emulsifier, and the hardening agent used. The amounts and different types of materials used in each formulation are listed in Table 6.1.

**Table 6.1 Used chemicals and formulations in the preparation of microcapsules.**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Core material</th>
<th>Amount of core material</th>
<th>C (g)</th>
<th>GA (g)</th>
<th>Emulsifier (g)</th>
<th>Hardening agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vanillin</td>
<td>0.3g/20ml corn oil</td>
<td>2</td>
<td>8</td>
<td>Tween 20 2.58 g</td>
<td>Sodium TPP Ratio TPP : Chitosan (1:2)</td>
</tr>
<tr>
<td>2</td>
<td>Vanillin</td>
<td>0.3g/20ml corn oil</td>
<td>0.5</td>
<td>1</td>
<td>Tween 20 3.9 g</td>
<td></td>
</tr>
<tr>
<td>3*</td>
<td>Limonene</td>
<td>32.5 g</td>
<td>1</td>
<td>2</td>
<td>Tween 20 3.9 g</td>
<td></td>
</tr>
<tr>
<td>4**</td>
<td>Limonene</td>
<td>1 g</td>
<td>0.5</td>
<td>1</td>
<td>PGPR (0.35 g)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Limonene</td>
<td>4.5</td>
<td>0.5</td>
<td>1</td>
<td>PGPR (0.35 g)</td>
<td></td>
</tr>
<tr>
<td>6**</td>
<td>Limonene</td>
<td>1 g</td>
<td>0.5</td>
<td>1</td>
<td>Span 85 (0.35 g)</td>
<td>Tannic acid 0.2 g/ 2ml H₂O (10%)</td>
</tr>
<tr>
<td>7</td>
<td>Vanillin</td>
<td>0.02g/ 1 g corn oil</td>
<td>0.5</td>
<td>1</td>
<td>PGPR (0.35 g)</td>
<td></td>
</tr>
<tr>
<td>8***</td>
<td>Vanillin</td>
<td>0.12g/4.5 g corn oil</td>
<td>0.5</td>
<td>1</td>
<td>PGPR (0.35 g)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Vanillin</td>
<td>0.02g/ 1 g corn oil</td>
<td>0.5</td>
<td>1</td>
<td>Span 85 (0.35 g)</td>
<td></td>
</tr>
</tbody>
</table>

*Limonene formulation 3 is formulation A that was investigated in the first release study in chapter 5.

**Limonene formulations 4 and 6 are formulations D and C which were investigated in the second release study in chapter 5.

***Vanillin formulation 8 is formulation E which was investigated in the third release study in chapter 5.
6.2.3. Fabric treatment with microcapsules

6.2.3.1. Simple coating

This method was discussed in chapter 3, section (3.2.4.1). It involves immersing the cotton fabrics in a suspension containing the microcapsules without the addition of binders or grafting agents.

6.2.3.2. Fixation with binder

The method applied was described by Rodrigues et al.\textsuperscript{16} for the fixation of polyurethane-urea microcapsules on wool/polyester fabrics at a laboratory scale while simulating the industrial impregnation conditions. An impregnation bath was prepared as shown in Table 6.2. The cotton fabrics were immersed in the bath and left for three minutes to allow for the penetration of the microcapsules and other bath constituents. The treated fabrics were passed through a two rollers foulard at a pressure of 3 bars and a speed of 3m/minute to squeeze the excess liquid from the fabric samples. The samples were then dried and cured thermally at the temperatures and time durations specified in Table 6.2.

**Table 6.2** Impregnation conditions with polymeric binder.

<table>
<thead>
<tr>
<th>Textile type</th>
<th>Cotton lawn fabric</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bath Volume</td>
<td>250 ml</td>
</tr>
<tr>
<td>Bath Ingredients</td>
<td></td>
</tr>
<tr>
<td>Microcapsules (formulation 1 and 2)</td>
<td>50 g/L</td>
</tr>
<tr>
<td>Baypret\textsuperscript{®} USV (polyurethane binder)</td>
<td>50 g/L</td>
</tr>
<tr>
<td>Perisoftal\textsuperscript{®} Nano (softener)</td>
<td>10 g/L</td>
</tr>
<tr>
<td>Padding by Foulard</td>
<td>3 bar (0.3 MPa)</td>
</tr>
<tr>
<td>Drying “Thermofixation”</td>
<td>100°C-3 minutes</td>
</tr>
<tr>
<td>Curing</td>
<td>140°C -3 minutes</td>
</tr>
</tbody>
</table>
6.2.3.3. Fixation using citric acid

Citric acid was used as a polycarboxylic acid cross-linker to covalently join the wall materials of the microcapsules (chitosan and gum Arabic) to the cotton fabrics by ester bonds. The procedure applied here is based on methods previously reported in the literature,9,10,18 but with some modifications. Briefly, the test fabrics were firstly immersed in a bath containing the 10 % w/v microcapsules suspension, 3% w/v citric acid, 1.5 % w/v catalyst (sodium phosphate monobasic monohydrate ) and thereafter heated at 50ºC for 5 minutes. Fabrics were then washed thoroughly with deionized water and passed through a 2 roller foulard with 1 bar pressure at a speed of 3 m/min. Subsequently, fixation was achieved by placing the fabric samples in a thermofixation chamber with circulating air at a temperature of 90ºC for 2 minutes. After drying, the curing process was investigated at two different temperatures (120ºC and 150ºC for three and two minutes, respectively) to obtain the maximum number of microcapsules immobilized onto the fabrics. Table 6.3 summarizes the curing process conditions of different fabric samples. The fixation reaction was also investigated for samples 5 and 8 using a domestic microwave oven (MS-2029UW, LG), with an output of 800 Watts for one minute. The wet pick up percentage of the impregnated samples ranged between 95% and 100% and was determined after passing through the foulard by the following formula: 16

\[
\text{Wet pick }\% = \frac{\text{mass of impregnation bath taken by the fabric}}{\text{mass of the dry fabric}} \times 100 \quad (6.1)
\]

Table 6.3 Curing conditions of microcapsules fixation using citric acid as a cross-linker.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curing temp (ºC)</td>
<td>120</td>
<td>120</td>
<td>120</td>
<td>150</td>
<td>120</td>
<td>150</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Curing time (minutes)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
6.2.4. Characterization

6.2.4.1. Optical microscopy

The morphology of the microcapsules was analyzed by using a Leica DM 2000 optical microscope, working in transmission light mode, and equipped with a Leica Application Suite Interactive Measurement imaging software. Optical microscopy was also used to examine the impregnated fabrics prior to their examination with SEM.

6.2.4.2. Particle size analysis

The mean particle size and size distribution of the microcapsules suspensions were determined by means of a Beckman Coulter Laser Diffraction Particle Size Analyzer LS 230.

6.2.4.3. Gas chromatography (GC-FID)

GC-FID was used to determine the encapsulation efficiency of vanillin and limonene microcapsules. The method was previously reported in Chapter 3 (Section 3.2.2.4). The encapsulation efficiency (EE %) was determined based on the difference between the total mass of the core material initially used for each preparation, and the non-encapsulated mass, calculated according to the following formula:

\[
EE\% = \frac{\text{mass (total)} - \text{mass (non-encapsulated)}}{\text{mass (total)}} \times 100 \quad (6.1)
\]

where mass (total) is the mass of the loaded core material (g), and mass (non-encapsulated) is the mass of the non-encapsulated core material (g). The masses of the non-encapsulated active principles were calculated from the formula (mass = volume x concentration). The concentration of the non-encapsulated core materials were obtained from the corresponding areas under the curves of the respective chromatograms.

6.2.4.4. Effect of heat on the morphology of microcapsules

A simple test was done to examine the effect of temperature on the morphology of microcapsules prior to their chemical grafting onto fabrics. A sample of about one gram of each formulation was placed on a watch glass and introduced in an oven where they were exposed consecutively
to temperatures of 50ºC, 120ºC, and 160ºC for 5, 2 and 2 minutes, respectively. Dry samples were then dispersed in deionized water and examined with the optical microscope to investigate possible deterioration in microcapsules’ morphology.

6.2.4.5. Scanning electron microscope (SEM)

A high-resolution (Schottky) Environmental Scanning Electron Microscope with X-Ray Microanalysis and Electron Backscattered Diffraction Analysis: Quanta 400 FEG ESEM/EDAX Genesis X4M operating at 15.00 kV was used to examine the morphological features of the microcapsules grafted onto the cotton fabrics.

6.2.4.6. Solid Content Determination

The solid content of the microcapsules was determined according to the European Standard EN 827 method- “Determination of solid content in water based adhesives”. The steps of the test were previously discussed in detail in Chapter 3, Section 3.2.2.6.

6.2.4.7. Effect of washing on treated fabrics

In order to investigate the effect of washing on the grafted microcapsules and their antibacterial activity, after drying and curing, some fabric samples were washed with deionized water containing 2% commercial soap for 15 minutes at 40ºC, rinsed in deionized water, and then washed with 0.1N acetic acid and re-rinsed with deionized water. Acetic acid was used to remove any residual unreacted chitosan from the surface of the fabrics. The washing was done according to methods described in the literature;19,20 and was repeated three times to remove any excess of polymer (chitosan or gum Arabic) and any unattached microcapsules.

6.2.4.8. FTIR spectroscopy

FTIR spectroscopic assay was used to examine the grafting reaction of the microcapsules on the cotton fabric. FTIR spectra were collected for the freeze-dried microcapsules, tannic acid, citric acid, control (untreated) cotton fabric, and the impregnated cotton fabric. The microcapsules were separated from the original microcapsule suspension by decantation and then freeze-dried into a powder form prior to the analysis. The analysis was conducted using a Jasco FT/IR-6800 spectrometer, (Jasco Analytical Instruments, USA), equipped with a MIRacle™ Single
Reflection ATR (attenuated total reflectance ZnSe crystal plate) accessory (PIKE Technologies, USA) and a TGS (triglycine sulfate) detector. Cosine apodization function was used to suppress leakage side lobes on the sampled signal. FTIR spectra of 56 scans at a resolution of 4 cm\(^{-1}\) were collected and averaged to obtain the single-beam background and sample ATR spectra. The spectra were collected over the spectral range of 4000–500 cm\(^{-1}\) in the Absorbance mode. The fabrics were used as they are and certain parts were randomly sampled to ensure consistent analysis and reproducibility. Aliquot of powdered samples (citric acid, tannic acid and limonene microcapsules) were analyzed without dilution to obtain the corresponding analyte standard FTIR spectrum using the same ATR accessory.

6.2.5. Evaluation of antibacterial activity

6.2.5.1. Agar diffusion method

This assay was conducted with the limonene and vanillin microcapsules suspensions obtained from formulations 4 to 9, after washing the microcapsules suspensions with deionized water and hexane to remove any excess oil or unreacted substances. *Staphylococcus aureus* (ATCC 19213) and *Escherichia coli* (ATCC 10536) were used as the test microorganisms, as representatives for Gram positive and Gram negative bacteria, respectively. The bacterial inoculums were prepared by transferring 4 isolated colonies of each type, under aseptic conditions, to separate test tubes containing nutrient broth that were then incubated at 37ºC for 24 hours. The inoculums were then diluted by sterilized Ringer solution to a concentration of 0.5 McFarland turbidity, which is comparable to a bacterial concentration of 1.5–3.0 \(\times\) \(10^8\) CFU/ ml. The concentration of the bacteria dilutions were also ascertained through UV spectrophotometry by measuring the absorbance at a wavelength of 625 nm. The absorbance of *S. aureus* was 0.0938, while *E. coli* was 0.0940. The bacterial solutions were then inoculated on the surface of Mueller Hinton Agar plates, using sterilized cotton swabs, and the plates were allowed to dry. Then, a well of 6 mm diameter was made in the center of each inoculated plate; the plug was removed, and filled with 100 µl of microcapsules suspension.

The free active agents were also tested separately (not incorporated in microcapsules). The limonene oil was diluted in dimethyl sulfoxide (DMSO) (7:3 ratio), and the vanillin was dissolved in corn oil (0.03 g vanillin in 1g of oil). The plates were incubated at 37ºC for 24 h.
After this period, the diameter of the inhibition zone was measured and incubation maintained for more 4 days in order to evaluate the possible change in the inhibition zone. The clear zone of inhibition formed around each hole after the incubation (inhibition halo), indicates an antimicrobial activity and its diameter is an indication of the inhibitory effect. All of the tests were done in duplicates.

6.2.5.2. Standard test method under dynamic contact conditions

This test was performed to evaluate the antibacterial activity of the fabrics impregnated with vanillin and limonene microcapsules. It is based on the American Society for Testing and Materials standard (ASTM) Designation: E 2149-01 standard method, with a slight modification. This standard method is originally designed to investigate the ability of samples treated with non-leaching (substrate-bound) antimicrobial agents to resist the growth of microbes under dynamic contact conditions.21

In this work the bacterial inoculum was adjusted to 0.5 McFarland turbidity standard (which corresponds to a concentration of 1.5–3.0 x 10^8 CFU/mL) using sterilized Ringer solution. The concentration of the bacteria dilutions were measured spectrophotometrically by determining the absorbance at 625 nm. This solution was then diluted in a sterile buffer of 0.3 mM KH_2PO_4 (pH = 7.2 ± 0.1) to reach a concentration of 1.5-3.0 x10^5 CFU/ml, and used as the working bacterial dilution employed in the assays. For the determination of bacterial inhibition, a fabric sample impregnated with the microcapsules with dimensions of 2 cm x 2 cm was introduced to 50 ml of the working bacterial dilution placed in a sterile 250 ml flask. The flask was then capped and placed in an orbital stirring bath at 37°C. After one minute of stirring, 1 ml of the solution was aseptically collected to determine bacterial concentration by the standard plate counting technique; which involves using serial dilutions and incorporation in Petri dishes with nutrient agar.

The obtained value was considered as the bacteria concentration at the initial contact time, t_0. After taking the sample, the flask was returned to the bath immediately and stirred for a further 15 minutes. Then, a new sample of the solution was aseptically collected for bacteria counting. The results of colony counting were converted to colony forming units per milliliter (CFU/ml) and used to calculate the percentage of bacterial reduction. Two other flasks, one containing
untreated fabric sample (without microcapsules), and another flask containing only the working bacterial dilution (without sample addition), both submitted to the same procedure of colony counting and percentage of bacteria reduction determination, were used as control.

After the first 15 minutes of testing, the inoculum solution of the treated fabric samples and the blank control was renewed and the sampling was repeated for bacteria counting at 30, 45, 60, 75, 90, 105 and 120 minute time periods. Each time before renewing the inoculum solution of the fabric sample, the sample was washed thoroughly with sterile deionized water before being introduced into the fresh inoculum solution. It is noteworthy to mention that the step of the inoculum renovation (every 15 minutes) is a modification of the original E 2149-01 standard method, and was introduced by Fernandes et al.,\textsuperscript{22} to evaluate the effective antibacterial activity for immobilized antimicrobials on leather coatings. Using a fresh bacterial inoculum at given specific contact times gives a better idea about the real amount of inhibition after that time of exposure.\textsuperscript{22} The percent of bacterial reduction upon contact with the fabric samples was calculated using the following equation:\textsuperscript{21}

\[
\text{Reduction (\%)} = (A - B/A) \times 100
\]

where B is the CFU/ml for the flask containing the treated fabric sample after the specified contact time and A is the CFU/ml for the flask containing the inoculum before the addition of the treated fabric.

**6.3. Results and discussion**

**6.3.1. Characterization of the produced microcapsules**

**6.3.1.1. Optical microscopy**

The micrographs of the optical microscopy revealed that all the microcapsules were spherical in shape. The images of formulations 4, 6 and 8 were included in the preceding chapter, section (5.3.1), with sample codes D, C and F, respectively. The optical microscope images of formulations 1, 2, 3, 5, 7 and 9 are shown in Figure 6.3, 6.4, 6.5, 6.6, 6.7 and 6.8, respectively.
Figure 6.3 Optical microscope images of formulation 1. Magnification: a) 400 x and b) 1000 x.

Figure 6.4 Optical microscope images of formulation 2. Magnification: a) 400 x and b) 1000 x.
6.3.1.2. Particle size evaluation

The differential and cumulative particle size distribution (relative to both the total volume and number of the microcapsules) of the nine formulations was investigated using laser particle size analyzer. The mean size of the microcapsules of the 9 formulations is shown in Table 6.4.

As it was mentioned in the previous chapters, the type of the emulsifier has an influence on the size of the produced microcapsules. It can be observed that the use of the emulsifier Tween 20 with sodium tripolyphosphate as hardening agent, resulted in the production of microcapsules with the smallest mean diameters, regardless of the amount of the oil or wall material used. It could be also noticed that for the same amount and type of core material; limonene formulations 4 and 6, and vanillin formulations 7 and 9, PGPR emulsifier produced microcapsules with larger average size than Span 85.
Figure 6.6 Optical microscope images of limonene microcapsules of formulation 5; produced by PGPR emulsifier. Magnification: a) 100 x and b) 200 x.

Figure 6.7 Optical microscope images of vanillin microcapsules of formulation 7; (a) after hardening (b) before hardening. Magnification: a) 200 x and b) 400 x.


Figure 6.8 Optical microscope images of vanillin microcapsules of formulation 9 produced by Span 85 emulsifier. Magnification: a) 400 x and b) 1000 x.

Table 6.4 Mean diameters per volume of microcapsules, EE %, solid content % and the emulsifier used in each formulation.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Type of Emulsifier</th>
<th>Mean particle size (µm)</th>
<th>EE %</th>
<th>Solid Content %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tween 20</td>
<td>11.2</td>
<td>64.2%</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>Tween 20</td>
<td>3.9</td>
<td>92.3%</td>
<td>24.9</td>
</tr>
<tr>
<td>3</td>
<td>Tween 20</td>
<td>8.0</td>
<td>98.4%</td>
<td>20.4</td>
</tr>
<tr>
<td>4</td>
<td>PGPR</td>
<td>18.4</td>
<td>90.4%</td>
<td>27.8</td>
</tr>
<tr>
<td>5</td>
<td>PGPR</td>
<td>39.0</td>
<td>94.1%</td>
<td>28.8</td>
</tr>
<tr>
<td>6</td>
<td>Span 85</td>
<td>11.1</td>
<td>98.6%</td>
<td>25.3</td>
</tr>
<tr>
<td>7</td>
<td>PGPR</td>
<td>15.7</td>
<td>95.7%</td>
<td>28.3</td>
</tr>
<tr>
<td>8</td>
<td>PGPR</td>
<td>38.3</td>
<td>98.3%</td>
<td>29.7</td>
</tr>
<tr>
<td>9</td>
<td>Span 85</td>
<td>10.4</td>
<td>100 %</td>
<td>25.4</td>
</tr>
</tbody>
</table>

The size distribution charts for all the studied formulations are included in the Appendix of this chapter, except for formulations 3, 4, 8 and 6 which were formerly presented in chapter 5.
6.3.1.3. Encapsulation efficiency of microcapsules

The percentage of the encapsulated core material in each formulation is reported in Table 6.4. The values of EE% ranged from 64.2% to 100%. This variation was discussed before in detail in chapter 4.

6.3.1.4. Effect of heat on the morphology of microcapsules

Limonene microcapsules resulting from formulations 3, 4, 5 and 6 were all subjected to temperatures of 50°C, 120°C, and 160°C for 5, 2 and 2 minutes, respectively. The optical microscope images of the remaining microcapsules (microcapsules not destroyed during the heating process) revealed that the highest decline in the number of the microcapsules was observed for formulations 3 and 5 (Figure 6.9 (a) and (c)). It was also observed that the multinuclear microcapsules of formulation 5 maintained their morphology in spite of their decreased number, unlike the microcapsules of formulation 4 which did not retain the multinuclear morphology after the thermal treatment but did not show significant decrease in number. This may be due to the higher amount of the emulsifier PGPR and limonene in formulation 5, in comparison with formulation 4, helped the microcapsules to maintain their original structural features.

6.3.1.5. Solid content

The solid content of the formulations was determined prior to the grafting process and without washing the microcapsules according to the procedure described in chapter 3 (Section 3.2.2.6). The values extended from 6.1% to 29.7% as shown in Table 4. It was found that the microcapsules prepared with tannic acid as the hardening agent had higher solid content % than the ones hardened with sodium tripolyphosphate. Furthermore, it was noticed that microcapsules prepared with the emulsifier PGPR and hardened with tannic acid had a higher solid content % than those produced with Span 85, using the same hardening agent.
6.3.2. Characterization of the treated fabrics

6.3.2.1. Simple coating method

In this procedure the microcapsules were not chemically fixed onto the fabrics, but rather applied by immersing the fabric in the microcapsules suspension and allowing it to be air-dried. These physically attached microcapsules would not be retained if the fabric is washed.\textsuperscript{19} However, this method might be suitable for disposable fabrics, such as surgical clothing and gowns and some other healthcare textiles that would not need to be washed and thus, no chemical reaction is needed to fix the microcapsules onto the fabric. SEM images of the surface of cotton fabrics treated with vanillin microcapsules of formulation 1 by this method are shown in Figure 6.10. The SEM images reveal that the deposited microcapsules have spherical shape and rough surface and were found mostly in aggregates positioned in the spaces present between the fabric fibers.

![SEM images of the surface of cotton fabrics treated with vanillin microcapsules of formulation 1 by the simple coating method.](image)

**Figure 6.9** Optical microscope images of limonene microcapsules, after exposure to temperatures of 50ºC, 120ºC, and 160º C for 5, 2, and 2 minutes, successively. (a) Formulation 3, (b) Formulation 4, (c) Formulation 5 and (d) Formulation 6.
Figure 6.10 SEM images of cotton fabrics with vanillin microcapsules of formulation 1 applied with simple coating method.
The elemental analysis of the wall material composition of the vanillin microcapsules deposited on the fabrics with quantification is presented in Figure 6.11. The presence of carbon, nitrogen and oxygen with percentages similar to the originally present in the structure of gum Arabic and chitosan was detected, which confirms the presence of both polymers in the shell of the microcapsules.

![Figure 6.11 Results of the elemental analysis of the wall material composition of the microcapsules on the treated fabrics (obtained by SEM).](image)

**Figure 6.11** Results of the elemental analysis of the wall material composition of the microcapsules on the treated fabrics (obtained by SEM).

### 6.3.2.2. Fixation of microcapsules with Baypret USV® binder

Baypret USV® is a polymeric binder based on polyether polyurethane that is frequently used to immobilize the microcapsules on the fabrics. It could be observed from the SEM images (Figure 6.12) of the fabrics impregnated with vanillin microcapsules of formulations 1 and 2 by using this polymeric binder that the morphological features of the microcapsules were significantly vanished becoming completely covered with a thick film of the binder. The drawbacks of using polymeric binders, as reported in the literature, include the change of the textiles properties, such as the decrease of the air permeability and breathability, softness, increased fabric drape, and change in the tensile strength.\(^{23}\)

However, the use of polyurethane binders to successfully bind microcapsules to textiles substrates has been reported, such as the example of polyurethane-urea microcapsules\(^1,16\) and melamine–formaldehyde microcapsules.\(^23\) Since it has been also reported that the fixation of the microcapsules with a polymeric binder depends on the chemical nature of the microcapsules, as well as the textile substrates,\(^23\) in this context, we can conclude that polyurethane binder is most likely inadequate to be used with the microcapsules wall materials and/or the fabric used in this study.
Figure 6.12 SEM micrographs of cotton fabrics impregnated with vanillin microcapsules and Baypret USV® binder: (a) Formulation 1 (b) Formulation 2 (c) Control fabric.
6.3.2.3. Grafting with citric acid and thermofixation

Cotton fabrics impregnated with microcapsules of different formulations and grafted thermally with citric acid were examined with SEM. It was observed that only small amount of the limonene microcapsules of formulation 3, the one prepared with Tween 20 emulsifier and hardened with sodium tripolyphosphate could be grafted onto the fabrics by drying at 90°C for 2 min and curing at 120°C for 3 min (Figure 6.13). The grafted microcapsules of this formulation were spherical in shape and some had a noticeable rough surface. A very similar observation was reported by Butstraen et al., who encapsulated Miglyol by chitosan and gum Arabic using sodium tripolyphosphate as the hardening agent. Butstraen et al reported that the rough surface of the shell consisted of smaller coacervated particles. Apparently, this rough surface is a result of using sodium tripolyphosphate as the hardening agent; since the surface of the microcapsules has become smooth when tannic acid was used to harden the microcapsules instead of sodium tripolyphosphate, as presented in the succeeding sections. The small number of grafted microcapsules could be due to the thermal instability of the microcapsules and their inability to tolerate the high temperature of the treatment.

Fabrics impregnated with formulations 6 and 9 that were produced with Span 85 emulsifier did not have any attached microcapsules after the drying and curing step. However, some remnants of the microcapsules could be observed in spaces between the fabric fibers as shown in Figure 6.14. This suggests that the formulations obtained from Span 85 emulsifier could not survive the conditions of the thermofixation and curing at 120°C.
Figure 6.13 Limonene microcapsules of formulation 3 grafted on cotton fabrics by chemical grafting method followed by drying at 90°C and curing at 120°C. Grafting was done by citric acid and sodium phosphate monobasic monohydrate as a catalyst.
Figure 6.14 SEM images of fabrics impregnated with (a) limonene microcapsules of formulation 6 and (b) vanillin microcapsules of formulation 9. Both formulations could not sustain the treatment and remnants of microcapsules could be observed.
SEM images of the fabrics treated with limonene and vanillin microcapsules obtained from formulations 4 and 7 are shown in Figure 6.15 and 6.16, respectively. Despite the fact that the two formulations were prepared with the same amounts of PGPR emulsifier (0.35 g) and the hardening agent tannic acid, and undergone the same conditions of drying at 90ºC and curing at 120ºC, it could be observed that higher number of vanillin microcapsules could be grafted (Figure 6.16) than the limonene ones (Figure 6.15). This suggests that the type of the core material may have an influence on the stability and thus, on the amount of the grafted microcapsules; since the two formulations experienced the same conditions during the preparation and grafting and contained the same amounts and types of all the ingredients, except for the encapsulated core. Taking into consideration that the vanillin was dissolved in corn oil, it might have helped in keeping the integrity of the microcapsules during the treatment more than the other formulation in which the core phase was limonene itself.

It was also observed that the grafted microcapsules in the both formulations seemed to be covered with a thin film-like layer. This film was considerably less evident when the curing temperature was elevated to 150ºC for 2 minutes. SEM micrographs of grafted vanillin microcapsules of formulation 7 (Figure 6.17) show that the microcapsules have become more visible than the grafted vanillin microcapsules (Figure 6.16) after raising the curing temperature of the samples to 150ºC.

The highest amount of microcapsules that was successfully grafted onto the fabrics resulted from the limonene microcapsules of formulation 5 (Figure 6.18 and 6.19) and vanillin microcapsules of formulation 8 (Figure 6.20) using a curing temperature of 120ºC for 3 minutes. This is probably because of their relatively higher solid content (w/w %) when compared with the other formulations, as shown in Table 6.4 (Formulation 5= 28.8%; Formulation 8= 29.7%). Perhaps the high amount of PGPR and the presence of tannic acid as the hardening agent resulted in improved thermal stability of the microcapsules during the thermofixation and curing steps. It was also noticed that the increasing of the amount of emulsifier to 0.6 g, together with increasing the amount of core (limonene oil and vanillin dissolved in corn oil) made the limonene formulation endure the treatment better than the one with vanillin; on the contrary to what was observed previously in formulations 4 and 7 with lower core and emulsifier amounts, where the vanillin formulation was more stable than that with limonene. The impact was obvious in the
amount and distribution of the fixed microcapsules. The film that covered the microcapsules in the previous formulations did not appear in this case, revealing the smooth appearance of the microcapsules surface, although the curing was performed at the same temperature.

Compared with the mean average size of microcapsules in both formulation 5 and 8 (39.0µm and 38.3µm, respectively), it was observed that only microcapsules of smaller diameter could be grafted and retained on the textiles after curing. This is perhaps due to the removal of the bigger microcapsules in the washing step that was performed directly after the reaction with citric acid and before the thermofixation process. Monllor et al. reported a similar observation and concluded that the smaller microcapsules tend to remain on the fabrics after several washing cycles, whereas the larger ones are usually lost faster.

Interestingly, the optical microscope images of fabrics impregnated with these two formulations indicated that the microcapsules seemingly kept their polynuclear morphology of their internal phase after the thermal fixation and curing reaction at 120ºC (Figure 6.21).

Figure 6.22 shows that increasing the curing temperature applied to limonene microcapsules of formulation 5 to 150ºC resulted in lowering the number of the grafted limonene microcapsules. Accordingly, it can be concluded that curing at 120ºC for 3 minutes was more favorable for this formulation.

### 6.3.2.4. Effect of washing

SEM images of fabrics impregnated with limonene microcapsules of formulation 5 that was subjected to a washing process with commercial soap and acid, process repeated for three times according to the method described previously in Section 6.2.4.4, are shown in Figure 6.23. It can be seen that the acidic washing solution affected the appearance of the fabrics; nevertheless, it showed that the microcapsules are still firmly attached onto them.
Figure 6.15 SEM images of fabrics impregnated with limonene microcapsules of formulation 4 cured at 120°C.
Figure 6.16 SEM images of fabrics impregnated with vanillin microcapsules of formulation 7 cured at 120°C for 3 minutes.
Figure 6.17 SEM images of fabrics impregnated with vanillin microcapsules of formulation 7 cured at 150°C for 2 minutes.
Figure 6.18 SEM images of fabrics impregnated with limonene microcapsules of formulation 5 cured at 120°C for 3 minutes.
Figure 6.19 SEM images of fabrics impregnated with limonene microcapsules of formulation 5 cured at 120°C for 3 minutes.
Figure 6.20 Vanillin microcapsules of formulation 8 grafted on cotton fabrics by chemical grafting method followed by thermal drying and curing at 120°C for 3 minutes.
Figure 6.21 Optical microscope images of (a) limonene microcapsules of formulation 5 and (b) vanillin microcapsules of formulation 8, grafted on cotton fabrics by chemical grafting method followed by thermal drying and curing at 120°C for 3 minutes.

6.3.2.5. Fixation with citric acid and microwave

Using the microwave technology to adhere and cure the microcapsules seemed to be destructive, since the SEM images of fabrics impregnated with formulation 5 and 8 and treated with the microwave for one minute showed almost no microcapsules except few scattered ones which are oval in shape (Figure 6.24). This perhaps suggests that the curing time or/and the microwave power level should have been decreased to avoid the loss of the microcapsules or that this method is not suitable for these formulations.
Figure 6.22 SEM images of fabrics impregnated with limonene microcapsules of formulation 5 cured at 150°C for 2 minutes.
Figure 6.23 SEM micrographs of cotton fabrics impregnated with limonene microcapsules (formulation 5) after being washed with 2% commercial soap and 0.1N acetic acid.
Figure 6.24 SEM images of fabrics impregnated with (a) limonene microcapsules of formulation 5 and (b) vanillin microcapsules of formulation 8; both cured using a home-use microwave.

6.3.2.6. FTIR Spectra

The FTIR spectra of tannic acid (the hardening agent), the freeze-dried limonene microcapsules (formulation 5), citric acid (the cross-linker), control cotton fabric, and the treated cotton fabric (with formulation 5, cured at 120°C for 3 minutes) are shown in Figure 6.25. The FTIR spectrum of tannic acid (Figure 6.25.A) showed a broad band from about 3000 cm⁻¹ to 3350 cm⁻¹ centered at 3326 cm⁻¹; which represents the stretching vibrations of –OH groups in the phenolic structure of tannic acid.²⁶ The two sharp peaks present at 1698 cm⁻¹ and 1607 cm⁻¹ can be assigned to the C=O group stretching and the aromatic C-O stretching vibrations, respectively. Similar characteristic sharp peaks for tannic acid are reported at 1713 cm⁻¹ and 1613 cm⁻¹ in the literature.²⁶,²⁷

The spectrum of the chitosan/gum Arabic microcapsules loaded with limonene (Figure 6.25.B) showed the presence of an important peak at 2855 cm⁻¹. This same peak was reported in the literature as an indication of the occurrence of complex coacervation between chitosan and gum Arabic; and it was also reported that such peak does not appear in the individual FTIR spectra of chitosan and gum Arabic.²⁸ Additionally, the broad band at about 3300 cm⁻¹ is attributed to the –OH groups of both chitosan and gum Arabic along with the overlapping stretching band of the –NH of chitosan. This band can also represent the hydrogen bonds established between gum Arabic and chitosan.²⁴ It can also reflect the hydrogen bonding with tannic acid that was used as
the hardening agent; as a similar broad band was seen in its individual spectrum as previously mentioned. The peak that appears at 1731 cm\(^{-1}\) indicates the chemical combination between chitosan and gum Arabic that formed the shell of the microcapsules; since a similar peak (at 1720 cm\(^{-1}\)) was reported by Yang et al.\(^{28}\) The peak appearing at 2924 cm\(^{-1}\) is due to C-H stretching vibration, while the one present at 1610 cm\(^{-1}\) is related to chitosan structure and represents –NH angular deformation.\(^{24}\)

The spectrum of citric acid (Figure 6.25.C) has shown peaks of stretching vibrations absorption of –OH groups at about 3492 cm\(^{-1}\) and 3280 cm\(^{-1}\). The two peaks of strong intensity which appeared at 1742 cm\(^{-1}\) and 1693 cm\(^{-1}\) represent the characteristic stretching C=O of the -COOH group of acids. These values are very close to those reported by Yang et al.\(^{10}\)

The FTIR spectrum of the untreated (control) cotton fabric sample (Figure 6.25.D) has shown peaks at 3332 cm\(^{-1}\), 2899 cm\(^{-1}\) and 1029 cm\(^{-1}\) that correspond to the stretching vibrations of the groups –OH, -CH and –C-O-C- present in the cellulosic structure of cotton, respectively.\(^{10,19}\) The peak that appears at 1645 cm\(^{-1}\) is probably due to the presence of interstitial water in the cellulosic structure.\(^{29}\)

The spectrum of cotton fabric impregnated with limonene microcapsules (Figure 6.25.E) has shown the disappearance of the sharp peaks at 1742 cm\(^{-1}\) and 1693 cm\(^{-1}\) that previously appeared in the spectrum of the cross-linker citric acid, which indicates that they had become involved in bonding, i.e., the esterification reaction between the carboxylic group of citric acid and the –OH group of the cotton cellulose.\(^{10}\) The spectrum also revealed the appearance of a new peak of C=O ester stretching at 1729 cm\(^{-1}\), which was not present in the control cotton fabric sample. This peak confirms the covalent attachment between the polymeric shell of the microcapsules (of chitosan and gum Arabic) and cotton cellulose via citric acid through ester bond formation.\(^{12}\) Additionally, the presence of a peak at 1637 cm\(^{-1}\) with small intensity, which is assigned to the bending vibration of the –NH group, points out to the chemical reaction between the residual free –NH\(_2\) groups of chitosan in the microcapsules shells and the –COOH groups of citric acid.\(^{10}\)
Figure 6.25 FTIR spectra of: A) tannic acid; B) microcapsules; C) citric acid; D) untreated cotton fabric and E) cotton fabric treated with microcapsules.
6.3.3. Antibacterial activity

6.3.3.1. Agar diffusion

This assay was conducted to investigate the antibacterial activity of the free microcapsules before being grafted onto the fabrics. Figures 6.26 and 6.27 show the results of the agar diffusion assay for different formulations of limonene and vanillin, respectively. The results indicated that all the formulations exhibited bacterial growth inhibition against both *S. aureus* and *E. coli*. Table 6.5 lists the values of the measured diameters of the inhibition zones of the formulations after incubating the plates for 24 hours and for 4 days, the assay was performed in duplicate for each sample. Since the antibacterial effect of chitosan mainly depends on the presence of its positively charged amino groups freely to interact with the negative charges of the bacterial wall, it is important to mention that the antibacterial effect exhibited by the microcapsules is predominantly due to the encapsulated vanillin and limonene during their release through the microcapsules wall (chitosan and gum Arabic), and not from the chitosan itself. This is because during the microcapsules preparation process by the complex coacervation method most of the positively amino groups of chitosan have been complexed with negative carboxylic groups of the gum Arabic to form the shell of the microcapsules.

The higher initial antibacterial effect that was exhibited by the non-encapsulated limonene oil dissolved in DMSO, and manifested in the bigger zone of inhibition (as shown in Table 6.5) might be related to the antibacterial activity of DMSO along with the limonene. Furthermore, it has been observed for the previously obtained inhibition zones, that after 4 days of incubation, they have become covered with bacteria. In contrast, the bacterial effect of the encapsulated oil was maintained after 4 days of incubation under the same conditions. This sustainable antibacterial effect of the encapsulated limonene and vanillin in the examined microcapsules formulations is acquired as a result of the achieved controlled release (previously investigated in chapter 5), and demonstrates the enhanced stability and prolonged antibacterial effect of the encapsulated core materials, which would extend the potential use of vanillin and limonene microcapsules in different applications.
Table 6.5 Average diameters of inhibition zones (cm) of limonene and vanillin microcapsules suspensions and free oils in the plate test with E. coli and S. aureus.

<table>
<thead>
<tr>
<th>Formulation/Sample</th>
<th>E. coli After 24 hours of incubation</th>
<th>E. coli After 4 days of incubation</th>
<th>S. aureus After 24 hours of incubation</th>
<th>S. aureus After 4 days of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core material</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limonene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.25±0.080</td>
<td>1.25±0.080</td>
<td>1.50±0.133</td>
<td>1.50±0.133</td>
</tr>
<tr>
<td>5</td>
<td>1.5±0.000</td>
<td>1.5±0.000</td>
<td>1.45±0.069</td>
<td>1.45±0.069</td>
</tr>
<tr>
<td>6</td>
<td>1.25±0.400</td>
<td>1.25±0.400</td>
<td>1.35±0.074</td>
<td>1.35±0.074</td>
</tr>
<tr>
<td>Vanillin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.45±0.207</td>
<td>1.45±0.207</td>
<td>1.45±0.069</td>
<td>1.45±0.069</td>
</tr>
<tr>
<td>8</td>
<td>1.50±0.000</td>
<td>1.55±0.065</td>
<td>1.50±0.000</td>
<td>1.55±0.065</td>
</tr>
<tr>
<td>9</td>
<td>0.80±0.000</td>
<td>1.20±000</td>
<td>1.55±0.065</td>
<td>1.55±0.065</td>
</tr>
<tr>
<td>Limonene oil/DMSO</td>
<td>3.30±0.303</td>
<td>- (*)</td>
<td>3.30±0.181</td>
<td>- (*)</td>
</tr>
<tr>
<td>Vanillin /corn oil</td>
<td>0.95±0.316</td>
<td>- (*)</td>
<td>1.00±0.200</td>
<td>- (*)</td>
</tr>
</tbody>
</table>

- (*) After 4 days of incubation, the bacteria grown up in the inhibition zone initially formed.

6.3.3.2. Standard test method under dynamic contact conditions

This assay was conducted on cotton fabrics impregnated with limonene microcapsules of formulation 5 and vanillin microcapsules of formulation 8 (cured at 120° C for 3 minutes); as they gave good grafting outcome. The assay was performed after washing the samples by the method previously described in Section 6.2.4.4. The results of the assay for formulation 5 and 8 are shown in Table 6.6 and Table 6.7, respectively.
Figure 6.26 Zone of inhibitions of limonene microcapsules of formulations: a (4), b (5) and c (6) against *S. aureus*, and of formulations: d (4), e (5) and f (6) against *E.coli* after 24 hours of incubation.

Figure 6.27 Zone of inhibitions of vanillin microcapsules of formulations: a (7), b (8) and c (9) against *S. aureus*, and of formulations: d (7), e (8) and f (9) against *E.coli* after 24 hours of incubation.
Table 6.6 Results of the bacterial reduction % in the dynamic test of the fabric impregnated with limonene microcapsules formulation 5.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Sample of bacteria – number of bacteria – inoculum solution (CFU/ml) (A)</th>
<th>Number of bacteria – control (fabric without microcapsules) (CFU/ml)</th>
<th>Number of bacteria – sample (fabric with microcapsules) (CFU/ml) (B)</th>
<th>Bacterial reduction * (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.00x10^5</td>
<td>3.00x10^5</td>
<td>1.50x10^4</td>
<td>49.00</td>
</tr>
<tr>
<td>15</td>
<td>3.00x10^5</td>
<td>2.50x10^5</td>
<td>1.24x10^4</td>
<td>95.90</td>
</tr>
<tr>
<td>30</td>
<td>3.00x10^5</td>
<td>2.96x10^5</td>
<td>1.42x10^4</td>
<td>52.72</td>
</tr>
<tr>
<td>45</td>
<td>3.00x10^5</td>
<td>2.81x10^5</td>
<td>1.69x10^4</td>
<td>43.70</td>
</tr>
<tr>
<td>60</td>
<td>3.00x10^5</td>
<td>2.94x10^5</td>
<td>1.91x10^4</td>
<td>36.33</td>
</tr>
<tr>
<td>75</td>
<td>2.98x10^5</td>
<td>2.96x10^5</td>
<td>1.91x10^4</td>
<td>35.92</td>
</tr>
<tr>
<td>90</td>
<td>2.90x10^5</td>
<td>2.86x10^5</td>
<td>1.93x10^4</td>
<td>33.44</td>
</tr>
<tr>
<td>105</td>
<td>3.00x10^5</td>
<td>2.88x10^5</td>
<td>2.20x10^4</td>
<td>33.03</td>
</tr>
<tr>
<td>120</td>
<td>3.00x10^5</td>
<td>3.00x10^5</td>
<td>2.20x10^4</td>
<td>26.72</td>
</tr>
</tbody>
</table>

(*) – reduction of the bacteria number = (A-B)/A*100

It can be observed, that both fabric samples exhibited an antibacterial activity against *E. coli*, whereby the fabric treated with limonene microcapsules showed 95.90% of bacterial reduction and the one impregnated with vanillin microcapsules showed 98.17 % after 15 minutes of contact. A bacteriostatic activity is generally regarded if a reduction percentage between 90% and 99.9% of the total bacteria count (CFU/mL) in the original inoculum is obtained. As was mentioned previously, this assay involved the renewal of the bacterial inoculum at each sampling. In other words, every 15 minutes the fabric sample was withdrawn, washed thoroughly with sterilized water and placed in contact with a new/fresh bacterial inoculum in order to take samples for colony counting. It is obvious from the results obtained that although the bacterial reduction percentage decreased with time, nevertheless, it was maintained throughout the 8 renewal cycles for both fabric samples, which is consistent with a gradual release and a long lasting effect.
Table 6.7 Results of the bacterial reduction % in the dynamic test of the fabric impregnated with vanillin microcapsules of formulation 8.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Sample</th>
<th>Number of bacteria – inoculum solution (CFU/ml) (A)</th>
<th>Number of bacteria – control (fabric without microcapsules) (CFU/ml)</th>
<th>Number of bacteria – sample (fabric with vanillin microcapsules) (CFU/ml) (B)</th>
<th>Bacterial reduction * (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.00x10^5</td>
<td>2.94x10^5</td>
<td>1.34x10^4</td>
<td>55.30</td>
<td></td>
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<tr>
<td>15</td>
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<td>2.74x10^5</td>
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<td>30</td>
<td>2.50x10^5</td>
<td>2.69x10^5</td>
<td>1.41x10^4</td>
<td>43.60</td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>2.45x10^5</td>
<td>2.93x10^5</td>
<td>1.58x10^4</td>
<td>35.51</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>2.36x10^5</td>
<td>2.95x10^5</td>
<td>1.54x10^4</td>
<td>34.80</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>2.71x10^5</td>
<td>2.94x10^5</td>
<td>1.88x10^4</td>
<td>30.63</td>
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<td>90</td>
<td>2.82x10^5</td>
<td>2.92x10^5</td>
<td>1.98x10^4</td>
<td>29.80</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>2.78x10^5</td>
<td>2.94x10^5</td>
<td>1.96x10^4</td>
<td>29.50</td>
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<tr>
<td>120</td>
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<td>2.96x10^5</td>
<td>1.99x10^4</td>
<td>23.46</td>
<td></td>
</tr>
</tbody>
</table>

(*) – reduction of the bacteria number = (A-B)/A*100

6.4. Conclusion

In this chapter, the possibility of imparting a durable antimicrobial finish to cotton fabrics by the impregnation of the limonene and vanillin microcapsules was successfully achieved using green and non-toxic materials. Nine formulations of microcapsules and several methods for their grafting onto the cotton fabrics were investigated. Considering the optimum fixation conditions, it was found that the use of citric acid as a polycarboxylic acid cross-linker followed by the thermofixation and thermal curing (at 120ºC) provided higher yield of attached microcapsules without affecting their morphology.
6.5. References


Appendix 6. Particle size distribution charts of the formulations investigated in Chapter 6

(a) Particle size distribution of vanillin microcapsules of formulation 1; (a) distribution in volume (b) in number.

Figure 6.A Particle size distribution of vanillin microcapsules of formulation 1; (a) distribution in volume (b) in number.
Figure 6.B Particle size distribution of vanillin microcapsules of formulation 2; (a) distribution in volume (b) in number.
Figure 6. C Particle size distribution of limonene microcapsules of formulation 5; (a) distribution in volume (b) in number.
Figure 6.D Particle size distribution of vanillin microcapsules of formulation 7; (a) distribution in volume (b) in number.
Figure 6.E Particle size distribution of vanillin microcapsules of formulation 9; (a) distribution in volume (b) in number.
Chapter 7: General Conclusions and Future Prospects

In light of the proposed objectives and the obtained results of this thesis project, it could be concluded that the production of microcapsules with fragrant and antimicrobial properties and their application onto textile substrate using eco-friendly materials was successfully achieved.

In summary, the main findings of the work are:

- The production of limonene and vanillin microcapsules was accomplished by means of the complex coacervation using gum Arabic and chitosan as shell materials and green hardening agents (tannic acid and sodium TPP). To our knowledge, this is the first successful encapsulation of the cargo using this method; as the available literature on complex coacervation to date did not refer to the encapsulation of limonene and vanillin (in pure form and not vanilla oil) by the usage of chitosan and gum Arabic as the wall material pair.

- The type of the emulsifier used in the microcapsule preparation was found to have a significant influence on their size, morphology (being mononuclear or polynuclear), EE% and the release pattern of the core material through the wall.

- The encapsulation efficiencies of the prepared microcapsule formulations was determined by GC-FID and ranged between 62% and 100% and the values highly depended on the emulsifier used. Span 85 emulsifier resulted in the highest EE %.

- The release profile was affected by the concentration of the used biopolymers, type of core material and the morphology of the microcapsules. Increasing the amount of polymeric wall materials resulted in slower release rate. Additionally, it was found that lower amount of vanillin than limonene was released over the same release duration and conditions. This might be related to the lower volatility and also diffusivity of the former. It was also noted that the limonene multinuclear microcapsules demonstrated slower release rate than the mononuclear ones.

- Among all the different formulations that were prepared, it was confirmed by SEM and FTIR that the multinuclear limonene and vanillin microcapsules obtained by the PGPR emulsifier and hardened with tannic acid are the ones that tolerated the thermofixation conditions and were successfully grafted on the cotton fabrics by citric acid. This highlights the fact that some formulations, regardless of their high EE% and uniform
release profiles were not suitable for the grafting reaction and could not survive its high temperature.

- The antibacterial assays of both the free microcapsules and the treated cotton fabrics have shown that they exhibited a sustained antibacterial activity. The average diameters of the inhibition zones of the agar diffusion tests ranged between 0.8-1.5 cm against *E. coli*, and 1.35-1.55 cm against *S. aureus*, and did not show a significant increase after being incubated for 4 days. However, this inhibitory effect of the microcapsules was maintained, unlike the non-encapsulated limonene and vanillin, which displayed bacterial growth in the inhibition zones that were initially formed; indicating un-sustained bacterial inhibition. The cotton fabrics impregnated with limonene and vanillin microcapsules have shown 95.9 % and 98.2 % of bacterial reduction after 15 minutes of exposure, respectively. This percentage significantly decreased but the bacterial reduction was maintained to more than 20% after 8 renewable cycles of the bacterial inoculum.

**Suggestions for future research:**

- It is recommended that future work on the impregnated fabrics produced here would focus on the washing durability and maintaining the fragrant and the antimicrobial effect of the treated fabrics after several washing cycles. This would in turn promote the extension of the production to a large scale.

- Another important area for future research is working on narrowing the size distribution of the produced microcapsules; as this is expected to affect the release behavior and uniformity of the grafted microcapsules.

- It is also important in the next step to study the release of the active agents from the microcapsules after being fixed on the fabrics. This would help in determining whether the fixed microcapsules would maintain the same release profiles as the free ones or the grafting affects their release pattern.

- More investigations are needed to be conducted on the formulations of vanillin and limonene microcapsules that were produced by Span 85 emulsifier and have shown the highest EE% (ranging between 98.6% and 100 %) but failed to be grafted on cotton fabrics by citric acid. These microcapsules exhibited a controlled release profile and sustained antibacterial activity against *S. aureus* and *E. coli*. Therefore, it is desirable to study their fixation on the fabrics by other means or their incorporation in other possible
applications, such as cosmetics or food products that do not need high processing temperature range.