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DEVELOPMENT OF NEW MATERIAL FORMULATIONS TO PRODUCE ACTIVE FILMS FOR FOOD PACKAGING

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Abstract

Packaging is one of the most important technological steps of food preservation. Microbial growth and oxidation reactions occurring on food surface are two of the main causes of deterioration of fresh and processed food products. Traditional food packaging generally protect foodstuff from external influences. Whereas active packaging systems interact with the food permitting the extension of their shelf-life and the maintenance, or even the improvement of their quality and sensorial features.

The main objective of this thesis is the attainment of knowledge related to the development of new material formulations for food packaging by using suitably modified inorganic nanoparticles in order to obtain a controlled release system of active substances and to improve active film physical and gas barrier performances.

As far as antioxidant active films is concerned, the aim of the work is the development of innovative films containing natural α-tocopherol adsorbed onto functionalised and not functionalised mesoporous silica particles (SBA-15; SBA-15+APTES) in order to protect it during LDPE film manufacture and to be able to control its release rate. The synthesized mesoporous powders were characterized by means of X-ray diffraction and N_2 adsorption/desorption at 77 K. Powders loaded with tocopherol were characterized by infrared spectroscopy and thermogravimetric analysis. Results show that the maximum of the pore size distribution reduces from 90 Å for purely siliceous SBA-15 to 73 Å for amino-functionalized SBA-15. Infrared analysis shows that tocopherol interacts with the amino groups of functionalized SBA-15. It has been also proven that circa 40% and 30% of tocopherol is loaded into SBA-15 and SBA-15+APTES respectively. Release tests performed using 96% v/v ethanol as fatty food stimulant show that the tocopherol diffusivity of films containing functionalized mesoporous silica decreased of about 50% with respect to films containing free tocopherol, as. This is due to the decrease in the pore size and to the increase in diffusion resistance caused by the functionalization of the internal pore walls with the amino groups. Moreover, the oxygen radical absorbing capacity (ORAC) assay of the produced active polymer films proved the antioxidant effectiveness of tocopherol released from samples after manufacturing process.

As far as antimicrobial activity is concerned, silver montmorillonite clays have been increasingly investigated as germicidal, bactericidal, antifungal, and antiseptic components in different food packaging formulation. The aim of this thesis is the development of a new class of antimicrobial systems in which the inorganic phyllosilicate clays (MMT) have been used as support for silver nanoparticles (AgNPs) . The Ag-MMT filler consists of nanometric metallic silver and oxides particles (size in the range 2-40nm) preferentially located on the surface of MMT single lamellae as the UV adsorption and FT-IR spectra showed. Antimicrobial bionanocomposites have been obtained by solution casting of AgMMT particles into chitosan, one of the most interesting biopolymers obtained from natural sources. The combined effect of glycerol and AgMMT particles on the thermal, structural and barrier properties of the obtained bionanocomposites was investigated. In fact, the plasticizer and the silver ions as well as the surface of metallic particles exert a combined effect which allows a reduction of the liquid water uptake and water permeability with respect to neat chitosan. Indeed, X.Ray results revealed that Ag-MMT particles result partially intercalated by chitosan macromolecules although it cannot be excluded in such an extent the exfoliation due to the collapse of MMT structure during the preparation of the active filler. Moreover, considering that the antibacterial mechanism of silver is mainly related to the action of silver ions and metallic AgNPs, the silver release kinetics from bionanocomposites in water at 25°C were also investigated.

In conclusion, samples of purely siliceous and amino-functionalized SBA-15 mesoporous silica were successfully used as α-tocopherol carriers for the production of active LDPE polymer films. In fact, active polymer films containing the functionalized carrier showed a slower tocopherol release when compared to samples containing free tocopherol and tocopherol loaded onto purely siliceous substrate. Whereas, for antimicrobial active film,. the silver supporting nanoparticles, Ag-MMT, contribute to modulate the release kinetics of silver ions from bionanocomposite films over a longer time interval (up to 20 days). This is of paramount importance for the production of active films to be used as food packaging materials or potentially as biomaterials.

Keywords:

Mesoporous Materials; Functionalization; Tocopherol; Active Polymer Films; Nanocomposites; Chitosan; Montmorillonite Clays; Food Packaging.

CHAPTER 1

INTRODUCTION

In this first chapter, the reader will be introduced to the background, the problem, the formulation and the purpose of the thesis.

1.1 INTRODUCTION

1.1.1 Food Packaging

The principal roles of food packaging are to protect food products from outside influences and damage, to contain the food, and to provide consumers with ingredient and nutritional information (Coles, 2003). The protection of packaged food contents for extended periods under any prolonged chilled or ambient-temperature conditions requires a series of interrelated actions:

1) The product itself must be processed in a manner that stabilizes it against enzymatic, microbiological, and biochemical activity, with the former two of concern primarily in foods packaged for short-term, chilled distribution.
2) The product must be protected against recontamination by active biological vectors of deterioration such as viable enzymes or microorganisms, usually by hermetic sealing.
3) The package generally should be a high gas-barrier structure that is hermetically closed, i.e., the seal is not a route for gas transmission into the package or for recontamination.
4) The environment within the hermetically sealed package should be made free of oxygen, which is not always an easy task since most foods contain occluded and dissolved air; finite quantities of reactable oxygen are usually present in the package headspace or product interstices whether or not a headspace is present, and the package material itself might contain oxygen that can be released into the food.

Traceability, convenience, and tamper indication are secondary functions of increasing importance. The goal of food packaging is to contain food in a cost-effective way that satisfies industry requirements and consumer desires, maintains food safety, and minimizes environmental impact (Marsh and Bugusu, 2007). Food packaging can retard product deterioration, retain the beneficial effects of processing, extend shelf-life, and maintain or increase the quality and safety of food. In doing so, packaging provides protection from 3 major classes of external influences: chemical, biological, and physical.

Chemical protection minimizes compositional changes triggered by environmental influences such as exposure to gases (typically oxygen), moisture (gain or loss), or light (visible, infrared, or

ultraviolet). Many different packaging materials can provide a chemical barrier. Examples of packaging material able to provide a nearly absolute barrier to chemical and other environmental agents are glass and metals, but for this packaging formulation, closure devices are added to facilitate both filling and emptying and may contain materials that allow minimal levels of permeability. Plastic packaging offers a large range of barrier properties but is generally more permeable than glass or metal.

Biological protection provides a barrier to microorganisms (pathogens and spoiling agents), insects, rodents, and other animals, thereby preventing disease and spoilage. In addition, biological barriers maintain conditions to control aging. This type of control can occur by means different mechanisms, for example preventing odor transmission, and maintaining the internal environment of the package.

Physical protection shields food from mechanical damage and includes cushioning against the shock and vibration encountered during distribution. Typically materials, able to resist impacts, abrasions, and crushing damage, are widely used as shipping containers and as packaging for a particular type of foods. Appropriate physical packaging also protects consumers from various hazards.

In addition to their typical protection function, packaging may contribute to the reduction of total solid waste. Significant food wastage has been reported in many countries, ranging from 25% for food grain to 50% for fruits and vegetables (FAO 1989). Inadequate preservation/protection, storage, and transportation have been cited as causes of food waste. Since packaging tend to extend the shelf-life of foods, prolonging their usability, reduces total waste

In a competitive environment a food package has also the role to exposure to consumers the product prior to purchase. Consequently, a distinctive or an innovative packaging can boost sales. The package may be designed to enhance the product image and/or to differentiate the product from the competition. Packaging also provides information to the consumer. For example, package labeling may be used to insert legal identification of product, nutritional value, ingredient, manufacturer information, etc.

Recently, The Codex Alimentarius Commission (2004) defines traceability as “the ability to follow the movement of a food through specified stage(s) of production, processing and distribution” (Codex Alimentarius Commission, 2004), so traceability represents an important packaging feature to considere during a new packaging form manufacturing. Traceability has 3

objectives: to improve supply management, to facilitate trace-back for food safety and quality purposes, and to differentiate and market foods with subtle or undetectable quality attributes. Incorporating a unique codes onto the package allows to track the products throughout the distribution process. Code format can be various (for example, printed barcodes or electronic radio frequency identification [RFID]).

Nowadays, the frenetic lifestyle influence anyway the food packaging features. Infact, consumers are always looking for a convenience food packaging formulation. As a consequence, packaging plays a vital role in minimizing the effort necessary to prepare and serve foods. Then, convenience features such as ease of access, handling, and disposal; product visibility; resealability; and microwavability are important and researched. Oven-safe trays, boil-in bags, and microwavable packaging enable consumers to cook an entire meal with virtually no preparation. New closure designs supply ease of opening, resealability, and special dispensing features. A membrane with a peelable seal covers the opening before sale and allows reclosure after opening. Advances in food packaging have facilitated the development of modern retail formats that offer consumers the convenience of 1-stop shopping and the availability of food from around the world.

Besides, to reduce or eliminate the risk of tampering and adulteration, willful tampering with food and pharmaceutical products has resulted in special packaging features. Although any package can be breached, tamper-evident features cannot easily be replaced. Tamper-evident features include banding, special membranes, breakaway closures, and special printing on bottle liners or composite cans such as graphics or text that irreversibly change upon opening. Special printing also includes holograms that cannot be easily duplicated. Tamper-evident packaging usually requires additional packaging materials, which exacerbates disposal issues, but the benefits generally outweigh any drawback (Marsh and Bugusu, 2007).

1.1.2 Food packaging materials

Different materials have traditionally been used in food packaging. It has always been important to select the right packaging material and technology to assure product quality and freshness during distribution and storage. As package design and construction play a significant role in determining the shelf life of a food product a wider variety of packaging materials have been

introduced with time. Today's food packages often combine several materials to exploit each material's functional or aesthetic properties. Marsh and Bugusu (2007) have classified food packaging materials on the basis of their category in:

Glass has an extremely long history in food packaging; the 1st glass objects for holding food are believed to have appeared around 3000 B.C. (Sacharow and Griffin, 1980). The production of glass containers involves heating a mixture of silica (the glass former), sodium carbonate (the melting agent), and limestone/calcium carbonate and alumina (stabilizers) to high temperatures until the materials melt into a thick liquid mass that is then poured into molds. Improved break resistance allows manufacturers to use thinner glass, which reduces weight and is better for disposal and transportation (McKown, 2000). Because it is odorless and chemically inert with virtually all food products, glass has several advantages for food-packaging applications. It is impermeable to gases and vapors, so it maintains product freshness for a long period of time without impairing taste or flavor. Glass is rigid, provides good insulation, and can be produced in numerous different shapes. Finally, glass packaging benefits the environment because it is reusable and recyclable.

Metal is the most versatile of all packaging forms. It offers a combination of excellent physical protection and barrier properties, formability and decorative potential, recyclability, and consumer acceptance. The 2 metals most predominantly used in packaging are aluminum and steel.

- *Aluminum.* Commonly used to make cans, foil, and laminated paper or plastic packaging, aluminum is a lightweight, silvery white metal derived from bauxite ore, where it exists in combination with oxygen as alumina. Magnesium and manganese are often added to aluminum to improve its strength properties (Page *et al.*, 2003). Unlike many metals, aluminum is highly resistant to most forms of corrosion; its natural coating of aluminum oxide provides a highly effective barrier to the effects of air, temperature, moisture, and chemical attack. Besides providing an excellent barrier to moisture, air, odors, light, and microorganisms, aluminum has good flexibility and surface resilience, excellent malleability and formability, and outstanding embossing potential. It is also an ideal material for recycling because it is easy to reclaim and process into new products. Pure aluminum is used for light packaging of primarily soft-drink cans, pet food, seafood, and prethreaded closures. The main disadvantages of aluminum are its high cost compared to other metals (for example, steel) and its inability to bewelded, which renders it useful only for making seamless containers.

- *Aluminum foil.* Aluminum foil is made by rolling pure aluminummetal into very thin sheets, followed by annealing to achieve dead-folding properties (a crease or fold made in the film will stay in place), which allows it to be folded tightly. Moreover, aluminum foil is available in a wide range of thicknesses, with thinner foils used to wrap food and thicker foils used for trays. Like all aluminum packaging, foil provides an excellent barrier to moisture, air, odors, light, and microorganisms. It is inert to acidic foods and does not require lacquer or other protection. Although aluminum is easily recyclable, foils cannot be made from recycled aluminum without pinhole formation in the thin sheets.
- *Laminates and metallized films.* Lamination of packaging involves the binding of aluminum foil to paper or plastic film to improve barrier properties. Thin gauges facilitate application. Although lamination to plastic enables heat sealability, the seal does not completely bar moisture and air. Because laminated aluminum is relatively expensive, it is typically used to package high value foods such as dried soups, herbs, and spices. A less expensive alternative to laminated packaging is metallized film. Metallized films are plastics containing a thin layer of aluminum metal (Fellows and Axtell, 2002). These films have improved barrier properties to moisture, oils, air, and odors, and the highly reflective surface of the aluminum is attractive to consumers. More flexible than laminated films, metalized films are mainly used to package snacks. Although the individual components of laminates and metalized films are technically recyclable, the difficulty in sorting and separating the material precludes economically feasible recycling.
- *Tinplate.* Produced from low-carbon steel (that is, blackplate), tinplate is the result of coating both sides of blackplate with thin layers of tin. The coating is achieved by dipping sheets of steel in molten tin (hot-dipped tinplate) or by the electro-deposition of tin on the steel sheet (electrolytic tinplate). Although tin provides steel with some corrosion resistance, tinplate containers are often lacquered to provide an inert barrier between the metal and the food product. Commonly used lacquers are materials in the epoxy phenolic and oleo resinous groups and vinyl resins. In addition to its excellent barrier properties to gases, water vapor, light, and odors, tinplate can be heat-treated and sealed hermetically, making it suitable for sterile products. Because it has good ductility and formability, tinplate can be used for containers of many different shapes. Thus, tinplate is widely used to form cans for drinks, processed foods, and aerosols; containers for powdered foods and sugar- or flour-

based confections; and as package closures. Tinplate is an excellent substrate for modern metal coating and litho-printing technology, enabling outstanding graphical decoration. Its relatively low weight and high mechanical strength make it easy to ship and store. Finally, tinplate is easily recycled many times without loss of quality and is significantly lower in cost than aluminum.

- *Tin-free steel.* Also known as electrolytic chromium or chrome oxide coated steel, tin-free steel requires a coating of organic material to provide complete corrosion resistance. Even though the chrome/chrome oxide makes tin-free steel unsuitable for welding, this property makes it excellent for adhesion of coatings such as paints, lacquers, and inks. Like tinplate, tin-free steel has good formability and strength, but it is marginally less expensive than tinplate. Food cans, can ends, trays, bottle caps, and closures can all be made from tin-free steel. In addition, it can also be used to make large containers (such as drums) for bulk sale and bulk storage of ingredients or finished goods (Fellows and Axtell, 2002).

The use of ***paper*** and ***paperboards*** for food packaging dates back to the 17th century with accelerated usage in the later part of the 19th century. Paper and paperboard are sheet materials made from an interlaced network of cellulose fibers derived from wood by using sulfate and sulfite. The fibers are then pulped and/or bleached and treated with chemicals such as slimicides and strengthening agents to produce the paper product. Paper and paperboards are commonly used in corrugated boxes, milk cartons, folding cartons, bags and sacks, and wrapping paper. Tissue paper, paper plates, and cups are other examples of paper and paperboard products.

- *Paper*. Plain paper is not used to protect foods for long periods of time because it has poor barrier properties and is not heat sealable. When used as primary packaging (that is, in contact with food), paper is almost always treated, coated, laminated, or impregnated with materials such as waxes, resins, or lacquers to improve functional and protective properties. The many different types of paper used in food packaging are as follows:
  - Kraft paper Produced by a sulfate treatment process, kraft paper is available in several forms: natural brown, unbleached, heavy duty, and bleached white. The natural kraft is the strongest of all paper and is commonly used for bags and wrapping. It is also used to package flour, sugar, and dried fruits and vegetables.
  - Sulfite paper Lighter and weaker than kraft paper, sulfite paper is glazed to improve its appearance and to increase its wet strength and oil resistance. It can be coated

for higher print quality and is also used in laminates with plastic or foil. It is used to make small bags or wrappers for packaging biscuits and confectionary.

- Greaseproof paper Greaseproof paper ismadethrough a process known as beating, in which the cellulose fibers undergo a longer than normal hydration period that causes the fibers to break up and become gelatinous. These fine fibers then pack densely to provide a surface that is resistant to oils but not wet agents. Greaseproof paper is used to wrap snack foods, cookies, candy bars, and other oily foods, a use that is being replaced by plastic films.
- Glassine Glassine is greaseproof paper taken to an extreme (further hydration) to produce a very dense sheet with a highly smooth and glossy finish. It is used as a liner for biscuits, cooking fats, fast foods, and baked goods.
- Parchment paper—Parchment paper is made from acid-treated pulp (passed through a sulfuric acid bath). The acid modifies the cellulose to make it smoother and impervious to water and oil, which adds some wet strength. It does not provide a good barrier to air and moisture, is not heat sealable, and is used to package fats such as butter and lard.

- *Paperboard.* Paperboard is thicker than paper with a higher weight per unit area and often made in multiple layers. It is commonly used to make containers for shipping—such as boxes, cartons, and trays—and seldom used for direct food contact. The various types of paperboard are as follows (Soroka, 1999):
  - White board Made fromseveral thin layers of bleachedchemical pulp, white board is typically used as the inner layer of a carton. White board may be coated with wax or laminated with polyethylene for heat sealability, and it is the only form of paperboard recommended for direct food contact.
  - Solid board Possessing strength and durability, solid board has multiple layers of bleached sulfate board. When laminated with polyethylene, it is used to create liquid cartons (known as milk board). Solid board is also used to package fruit juices and soft drinks.
  - Chipboard Chipboard is made from recycled paper and often contains blemishes and impurities from the original paper, which makes it unsuitable for direct contact with food, printing, and folding. It is often lined with white board to improve both appearance and

strength. The least expensive form of paperboard, chipboard is used to make the outer layers of cartons for foods such as tea and cereals.

  - Fiberboard Fiberboard can be solid or corrugated. The solid type has an inner white board layer and outer kraft layer and provides good protection against impact and compression. When laminated with plastics or aluminum, solid fiberboard can improve barrier propertiesandis used to package dry products such as coffee and milk powder. The corrugated type, also known as corrugated board, is made with 2 layers of kraft paper with a central corrugating (or fluting) material. Fiberboard's resistance to impact abrasion and crushing damage makes it widely used for shipping bulk food and case packing of retail food products.

- *Paper laminates.* Paper laminates are coated or uncoated papers based on kraft and sulfite pulp. They can be laminated with plastic or aluminum to improve various properties. For example, paper can be laminated with polyethylene to make it heat sealable and to improve gas and moisture barrier properties. However, lamination substantially increases the cost of paper. Laminated paper is used to package dried products such as soups, herbs, and spices.

Plastics are made by condensation polymerization (polycondensation) or addition polymerization (polyaddition) of monomer units. In polycondensation, the polymer chain grows by condensation reactions between molecules and is accompanied by formation of low molecular weight byproducts such as water and methanol. Polycondensation involves monomers with at least 2 functional groups such as alcohol, amine, or carboxylic groups. In polyaddition, polymer chains grow by addition reactions, in which 2 or more molecules combine to form a larger molecule without liberation of byproducts. Polyaddition involves unsaturated monomers; double or triple bonds are broken to link monomer chains. There are several advantages to using plastics for food packaging. Fluid and moldable, plastics can be made into sheets, shapes, and structures, offering considerable design flexibility. Because they are chemically resistant, plastics are inexpensive and lightweight with a wide range of physical and optical properties. In fact, many plastics are heat sealable, easy to print, and can be integrated into production processes where the package is formed, filled, and sealed in the same production line. The major disadvantage of plastics is their variable permeability to light, gases, vapors, and low molecular weight molecules.

There are 2 major categories of plastics: thermosets and thermoplastics. Thermosets are polymers that solidify or set irreversibly when heated and cannot be remolded. Because they are

strong and durable, they tend to be used primarily in automobiles and construction applications such as adhesives and coatings, not in food packaging applications. On the other hand, thermoplastics are polymers that soften upon exposure to heat and return to their original condition at room temperature. Because thermoplastics can easily be shaped and molded into various products such as bottles, jugs, and plastic films, they are ideal for food packaging. Moreover, virtually all thermoplastics are recyclable (melted and reused as raw materials for production of new products), although separation poses some practical limitations for certain products. There have been some health concerns regarding residual monomer and components in plastics, including stabilizers, plasticizers, and condensation components such as bisphenol A. To ensure public safety, FDA carefully reviews and regulates substances used to make plastics and other packaging materials. Any substance that can reasonably be expected to migrate into food is classified as an indirect food additive subject to FDA regulations. A threshold of regulation-defined as a specific level of dietary exposure that typically induces toxic effects and therefore poses negligible safety concerns may be used to exempt substances used in food contact materials from regulation as food additives. FDA revisits the threshold level if new scientific information raises concerns. Furthermore, FDA advises consumers to use plastics for intended purposes in accordance with the manufacturer's directions to avoid unintentional safety concerns. Despite these safety concerns, the use of plastics in food packaging has continued to increase due to the low cost of materials and functional advantages (such as thermosealability, microwavability, optical properties, and unlimited sizes and shapes) over traditional materials such as glass and tinplate (Lopez-Rubio *et al.*, 2004).

Multiple types of plastics are being used as materials for packaging food, including polyester, polyvinyl chloride, polyvinylidene chloride, polystyrene, polyamide, ethylene vinyl alcohol and polyolefins.

- *Polyesters*. Polyethylene terephthalate (PET or PETE), polycarbonate, and polyethylene naphthalate (PEN) are polyesters, which are condensation polymers formed from ester monomers that result from the reaction between carboxylic acid and alcohol. The most commonly used polyester in food packaging is PET.
  - Polyethylene terephthalate (PET). Formed when terephthalic acid reacts with ethylene glycol, PET provides a good barrier to gases (oxygen and carbon dioxide) and moisture. It also has good resistance to heat, mineral oils, solvents, and acids, but not to bases. Consequently, PET is becoming the packaging material of choice for many food

products, particularly beverages and mineral waters. The use of PET to make plastic bottles for carbonated drinks is increasing steadily (van Willige *et al.*, 2002). The main reasons, for its popularity are its glass-like transparency, adequate, gas barrier for retention of carbonation, light weight, and shatter resistance. The 3 major packaging applications of PET are containers (bottles, jars, and tubs), semi-rigid sheets for thermoforming (trays and blisters), and thin-oriented films (bags and snack food wrappers). PET exists both as an amorphous (transparent) and a semi-crystalline (opaque and white) thermoplastic material. Amorphous PET has better ductility but less stiffness and hardness than semi-crystalline PETE, which has good strength, ductility, stiffness, and hardness. Recycled PET from soda bottles is used as fibers, insulation, and other nonfood packaging applications.

- Polycarbonate. Polycarbonate is formed by polymerization of a sodium salt of bisphenol acid with carbonyl dichloride (phosgene). Clear, heat resistant, and durable, it is mainly used as a replacement for glass in items such as larger returnable/refillable water bottle sand sterilizable baby bottles. Care must be taken when cleaning polycarbonate because using harsh detergents such as sodium hypochlorite is not recommended because they catalyze the release of bisphenol A, a potential health hazard. An extensive literature analysis by vom Saal and Hughes (2005) suggests the need for a new risk assessment for the low-dose effects of this compound.
- Polyethylene naphthalate (PEN). PEN is a condensation polymer of dimethyl naphthalene dicarboxylate and ethylene glycol. It is a relatively new member of the polyester family with excellent performance because of its high glass transition temperature. PEN's barrier properties for carbon dioxide, oxygen, and water vapor are superior to those of PET, and PEN provides better performance at high temperatures, allowing hot refills, rewashing, and reuse. Because PEN provides protection against transfer of flavors and odors, it is well suited for manufacturing bottles for beverages such as beer.

- *Polyvinyl chloride.* Polyvinyl chloride (PVC), an addition polymer of vinyl chloride, is heavy, stiff, ductile, and a medium strong, amorphous, transparent material. It has excellent resistance to chemicals (acids and bases), grease, and oil; good flow characteristics; and stable electrical properties. Although PVC is primarily used in medical and other nonfood applications, its food uses include bottles and packaging films. Because it is easily

thermoformed, PVC sheets are widely used for blister packs such as those for meat products and unit dose pharmaceutical packaging. PVC can be transformed into materials with a wide range of flexibility with the addition of plasticizers such as phthalates, adipates, citrates, and phosphates. These alternative plasticizers also have the potential to leach into food but at lower levels than phthalates. Low levels of DEHA have shown no toxicity in animals. Finally, PVC is difficult to recycle because it is used for such a variety of products, which makes it difficult to identify and separate. In addition, incineration of PVC presents environmental problems because of its chlorine conten

- *Polyvinylidene chloride.* Polyvinylidene chloride (PVdC) is an addition polymer of vinylidene chloride. It is heat sealable and serves as an excellent barrier to water vapor, gases, and fatty and oily products. It is used in flexible packaging as a monolayer film, a coating, or part of a co-extruded product. Major applications include packaging of poultry, cured meats, cheese, snack foods, tea, coffee, and confectionary. It is also used in hot filling, retorting, low-temperature storage, and modified atmosphere packaging. PVdC contains twice the amount of chlorine as PVC and therefore also presents problems with incineration.
- *Polystyrene.* Polystyrene, an addition polymer of styrene, is clear, hard, and brittle with a relatively low melting point. It can be mono-extruded, co-extruded with other plastics, injection molded, foamed to produce a range of products. Foaming produces an opaque, rigid, lightweight material with impact protection and thermal insulation properties. Typical applications include protective packaging such as egg cartons, containers, disposable plastic silverware, lids, cups, plates, bottles, and food trays. In expanded form, polystyrene is used for nonfood packaging and cushioning, and it can be recycled or incinerated.
- *Polyamide.* Commonly known as nylon (a brand name for a range of products produced by DuPont), polyamides were originally used in textiles. Formed by a condensation reaction between diamine and diacid, polyamides are polymers in which the repeating units are held together by amide links. Different types of polyamides are characterized by a number that relates to the number of carbons in the originating monomer. For example, nylon-6 has 6 carbons and is typically used in packaging. It has mechanical and thermal properties similar to PET, so it has similar usefulness, such as boil-in bag packaging. Nylon also offers good chemical resistance, toughness, and low gas permeability.

- *Ethylene vinyl alcohol.* Ethylene vinyl alcohol (EVOH) is a copolymer of ethylene and vinyl alcohol. It is an excellent barrier to oil, fat, and oxygen. However, EVOH is moisture sensitive and is thus mostly used in multilayered co-extruded films in situation where it is not in direct contact with liquids.
- *Laminates and co-extrusions.* Plastic materials can be manufactured either as a single film or as a combination of more than 1 plastic. There are 2 ways of combining plastics: lamination and co-extrusion. Lamination involves bonding together 2 or more plastics or bonding plastic to another material such as paper or aluminum (as discussed in the section on metal). Bonding is commonly achieved by use of water-, solvent-, or solids-based adhesives. After the adhesives are applied to 1 film, 2 films are passed between rollers to pressure bond them together. Lamination using laser rather than adhesives has also been used for thermoplastics (Kirwan and Strawbridge, 2003). Lamination enables reverse printing, in which the printing is buried between layers and thus not subject to abrasion, and can add or enhance heat sealability. In co-extrusion, 2 or more layers of molten plastics are combined during the film manufacture. This process is more rapid (requires 1 step in comparison to multiple steps with lamination) but requires materials that have thermal characteristics that allow co-extrusion. Because co-extrusion and lamination combine multiple materials, recycling is complicated. However, combining materials results in the additive advantage of properties from each individual material and often reduces the total amount of packaging material required. Therefore, co-extrusion and lamination can be sources of packaging reduction.
- **Polyolefins.** Polyolefin is a collective term for polyethylene and polypropylene, the 2 most widely used plastics in food packaging, and other less popular olefin polymers. Polyethylene (PE) and polypropylene (PP) both possess a successful combination of properties, including flexibility, strength, lightness, stability, moisture and chemical resistance, and easy processability, and are well suited for recycling and reuse. The simplest and most inexpensive plastic made by addition polymerization of ethylene is polyethylene. There are 2 basic categories of polyethylene: high density and low density (HDPE and LDPE). HDPE is stiff, strong, tough, resistant to chemicals and moisture, permeable to gas, easy to process, and easy to form. It is used to make bottles for milk, juice, and water; cereal box liners; margarine tubs; and grocery, trash, and retail bags. LDPE is flexible, it is relatively transparent, it is predominately used in film applications and in applications where heat sealing is necessary.

Bread and frozen food bags, flexible lids, and squeezable food bottles are examples of low-density polyethylene. Polyethylene bags are sometimes reused (both for grocery and non grocery retail). Of the 2 categories of polyethylene, high-density polyethylene containers, especially milk bottles, are the most recycled among plastic packages. Harder, denser, and more transparent than polyethylene, polypropylene has good resistance to chemicals and is effective at barring water vapor. Its high melting point (160°C) makes it suitable for applications where thermal resistance is required, such as hot-filled and microwavable packaging. Popular uses include yogurt containers and margarine tubs. When used in combination with an oxygen barrier such as ethylene vinyl alcohol or polyvinylidene chloride, polypropylene provides the strength and moisture barrier for catsup and salad dressing bottles. Although more than 30 types of plastics have been used as packaging materials (Lau and Wong, 2000), polyesters and polyolefins are the most common.

1.1.3 Functionalized packaging materials

The so-called "functional packaging" is still today, the most significant innovation in the field of food packaging. The expression "functional packaging" refers, generally, to those packaging solutions in which is provided the use of a material, of a container or an accessory of packaging capable of perform an additional function than traditional containment and generic protection of the product. Since these possible "extra features" can have very different purposes, a classification has long been in use; according to it these new packaging formulations are divided into two categories, **intelligent** or **active**.

To understand what active and intelligent packaging have to offer the world of packaging, it is important to clarify what each phrase means. According to Huff (2008):

- Intelligent packaging can be defined as “packaging that contains an external or internal indicator to provide information about aspects of the history of the package and/or the quality of the food” (Robertson, 2006). Intelligent packaging is an extension of the communication function of traditional packaging, and communicates information to the consumer based on its ability to sense, detect, or record external or internal changes in the product's environment.

- Active packaging is accurately defined as "packaging in which subsidiary constituents have been deliberately included in or on either the packaging material or the package headspace to enhance the performance of the package system" (Robertson, 2006). This phrase emphasizes the importance of deliberately including a substance with the intention of enhancing the food product. Active packaging is an extension of the protection function of a package and is commonly used to protect against oxygen and moisture.

Intelligent packaging systems exist to monitor certain aspects of a food product and report information to the consumer. The purpose of the intelligent system could be to improve the quality or value of a product, to provide more convenience, or to provide tamper or theft resistance (Robertson, 2006). Intelligent packaging can report the conditions on the outside of the package, or directly measure the quality of the food product inside the package. In order to measure product quality within the package, there must be direct contact between the food product or headspace and the quality marker. In the end, an intelligent system should help the consumer in the decision making process to extend shelf life, enhance safety, improve quality, provide information, and warn of possible problems. Intelligent packaging is a great tool for monitoring possible abuse that has taken place during the food supply chain. Intelligent packaging may also be able to tell a consumer when a package has been tampered with. There is currently work being developed with labels or seals that are transparent until a package is opened. Once the package is tampered with, the label or seal will undergo a permanent color change and may even spell out "opened" or "stop". Perhaps intelligent packaging will be able to inform a consumer of an event that occurred such as package tampering that may save their life.

The intelligent packaging design that is leading the way in packaging technology is the time temperature indicator (TTI). The TTI is useful because it can tell the consumer when foods have been temperature abused. If a food is exposed to a higher temperature recommended, the quality of the food can deteriorate much quicker. A TTI can be placed on shipping containers or individual packages as a small self adhesive label, and an irreversible change, like a color change, will result when the TTI experiences abusive conditions. TTIs are particularly useful with chilled or frozen foods, where the cold storage during transportation and distribution are important for food quality and safety. TTIs are also used as freshness indicators for estimating the shelf life of perishable products. A TTI technology known as Time strip is currently being employed by Nestlè in their

food service products in the UK (Anonymous, 2007). The Time strip uses a steady diffusion of liquid through a membrane to measure the time that has elapsed at a particular temperature. This action can provide information about how long a product has been opened or in use. The Time strip is very useful for products like sauces that have to be refrigerated and used within a specific time period.

Food is a complicated material to package because it is capable of respiration and therefore may change its own atmosphere when inside a package. The gas composition within a package can easily change due to the interaction of food with its environment. Then, gas indicators are a helpful means of monitoring the composition of gases inside a package by producing a change in the color of the indicator though a chemical or enzymatic reaction (de Jong *et al.*, 2005). The indicators must be in direct contact with the gaseous environment directly surrounding the food in a package. Indicators are capable of signaling whether there is a gas leakage in the package, or they may be used to verify the efficiency of an oxygen scavenger. Gas indicators typically signal the presence or absence of oxygen and/or carbon dioxide. Oxygen in the air can cause oxidative rancidity, unwanted color changes in foods, and allow aerobic microbes to grow on foods. Oxygen indicators typically result in a color change when oxygen is present, and the presence of oxygen can indicate that the package has a leak or has been tampered with. Oxygen indicators can also indicate improper sealing of a package. Gas indicators are also being developed to detect water vapor, ethanol, and hydrogen sulfide.

Another examples of indicators used in an intelligent packaging solution for food preservation are inks. They are available that are temperature sensitive and can change colors based on temperature. These inks can be printed onto packages or labels such that a message can be conveyed to the consumer based on the color of the ink they are seeing. Thermochromic inks can let a consumer know whether a package is too hot to touch, or cold enough drink. Thermochromic inks are becoming a popular technology for beverages (Robertson, 2006). The inks used can be adversely affected by UV light and temperatures over 121°C, so consumers should not fully rely on the inks message when it comes to deciding the proper time to consume a food.

Active packaging systems are developed with the goal of extending shelf life for foods and increasing the period of time that the food is high quality. Active packaging technologies include some physical, chemical, or biological action which changes interactions between a package, product, and/or headspace of the package in order to get a desired outcome (Yam et al., 2005).

1.1.4 Active packaging

Active packaging has been considered a component of the packaging discipline for several decades or since the first inclusion of desiccants in dry product packages. Active packaging, sometimes referred to as interactive or "smart" packaging is intended to sense internal or external environmental change and to respond by changing its own properties or attributes and hence the internal package environment. In the moisture-permeable sachets, desiccants absorb water vapor from the contained product and from the package headspace, and absorb any water vapor that enters by permeation or transmission through the package structure. As separate entities within packages, active packaging sachets, pouches, patches, coupons, labels, etc., are not often integral to the package. Desiccant pouches are widely used in the packaging of hardware and metal goods (Brody *et al.*, 2002).

The best-known and most widely used active packaging technologies for foods today are those engineered to remove oxygen from the interior package environment. Oxygen scavengers reduce oxidative effects in the contained product. Most oxygen scavengers in commercial use today are gas-permeable, flexible sachets containing reduced iron (i.e., iron not in the fully oxidized state) particles inserted into food and other packages from which air is initially removed by vacuum or by flushing with inert gas. During the last two decades of the twentieth century, commercial incorporation of oxygen-removal materials directly into a package structure occurred with varying results. The goal of active packaging, in conjunction with other food processing and packaging, is to enhance preservation of contained food and beverage products (Brody *et al.*, 2002). For example, to optimize the effects of oxygen scavenging, oxygen should first be removed from the product during processing and packaging operations. The oxygen must also be thoroughly removed from the package interior and the package materials, and the package structure, including materials and closure, must be barriers to further oxygen entry. In other words, oxygen scavenging complements good oxygen-control practices. In addition, oxygen is certainly not the only vector that can influence the quality of the contained food. For example, moisture gain or loss, light, nonoxidative reactions, microbiological growth, and enzymatic activity may all, individually or collectively, be involved in food-product deterioration.

High-gas-permeability films, including some that increase their oxygen permeability with increasing temperature, are used for packaging fresh-cut produce. Use of these temperature-

sensitive package materials is expected to increase because the technology developer has acquired a fresh produce packager who, of course, uses the technology in its package materials. Carbon dioxide and ethylene scavengers for modified-atmosphere (MA) or, more precisely, controlled-atmosphere (CA) food preservation are common in large bulk shipments. Carbon dioxide emitters to suppress microbiological growth have experienced limited success in modified-atmosphere packaging (MAP). Ethylene scavengers are among the more successful commercial active packaging technologies in the fresh-fruit bulk-shipment category. Odors generated or captured within closed food packages are undesirable, and their obviation has been a research topic for years. Odor removers incorporated into packaging are increasingly important in some classes of food packaging.

Antioxidants and oxygen interceptors incorporated into package materials, such as tocopherols (vitamin E), have emerged in recent years and are increasingly employed to combat odors generated in plastic processing. Tocopherols, which are nonvolatile, have not replaced volatile butylated hydroxyanisole/ butylated hydroxytoluene (BHA/BHT) which migrate into foods in product antioxidant applications, but they appear to be new antioxidants of choice for mitigating the effects of oxygen. Entities such as oxygen scavengers/interceptors react with oxygen to form new compounds (Brody *et al.*, 2002). Oxygen absorbers may remove oxygen by any means, including physical. Antioxidants react with free radicals and peroxides to retard or block the actual oxidation reactions. Sequestering agents tie up inorganic catalysts that might otherwise accelerate adverse oxidative reactions.

Members of the food technology and packaging communities have long regarded package materials as an ideal reservoir and delivery vehicle for antimicrobial compounds. For many years, sorbic acid has been incorporated sparingly on the interior of package structures as an antimycotic in a limited number of dry food packages. The obvious benefits of sorbic acid as a mold and yeast inhibitor have been one foundation by which numerous other antimicrobial agents have found their way into food package materials. Unfortunately, most antimicrobial agents also exhibit toxicity when they enter the food from the package and would be consumed as part of the food. Thus, actual commercialization has been proceeding slowly, except in Japan where several compounds have been reported to function effectively as antimicrobials in commercial packages.

To this regard, the European Union's Regulation 1935/2004 offered for the first time the opportunity for active packaging to be used in Europe by allowing the application of materials with

agents that could migrate into foods. So far, the EU legislation on materials in contact with foodstuffs has protected the health of consumers by ensuring that no material in contact with foodstuffs can bring about a chemical reaction which would change the composition or organoleptic properties of these foodstuffs (taste, appearance, texture or even smell). Regulation 1935/2004/EC repeals this legislation in order to allow packaging to benefit from technological innovation. This was necessary in the EU because all packaging materials (including those that intentionally add substances to food) are subject to all requirements for food-contact materials, including the overall migration limits (OMLs) and specific migration limits (SMLs).

The Framework Regulation authorize the use of active and intelligent packaging, provided the packaging can be shown to enhance the safety, quality and shelf-life of the packaged foods. Article 1 notes that the purpose of the law is to secure a high level of protection of human health and protect the interests of consumers so that the Regulation is to be applied to all materials and articles (including active and intelligent packaging), which in their finished state are intended to contact food, or can reasonably be expected to contact food, or transfer their constituents to food under normal or foreseeable conditions of use. Article 3 entitled ‘‘general requirements” is particularly important because sets forth the proposition that manufacture of all materials or articles be in accordance with good manufacturing practice so that they do not transfer their constituents to food in any quantity that could endanger human health or bring about any organoleptic change or deterioration of the food. Releasing systems are however allowed to change the composition of the food, providing that the released substance is an authorized compound. Anyway, the main aspect of the new Regulation is that all new active and intelligent packaging systems initially need to be evaluated by the European Food Safety Authority (EFSA). Based on the outcome of that evaluation, the Commission (DG SANCO) will grant a petitioner authorisation for the submitted active and intelligent ingredients/systems, which will be entered in the Regulation (Figure 1). The authorisation is not ‘‘general” but is only for the petitioner (‘‘Authorisation holder”). The authorisation of active and intelligent components have to be granted in accordance with Articles 7–9 of Regulation 1935/2004/EC, upon submission of application. Application shall comprise a technical dossier containing specified information and EFSA shall give an opinion within 6 + 6 months providing an explanation for the delay (Restuccia et al., 2010).

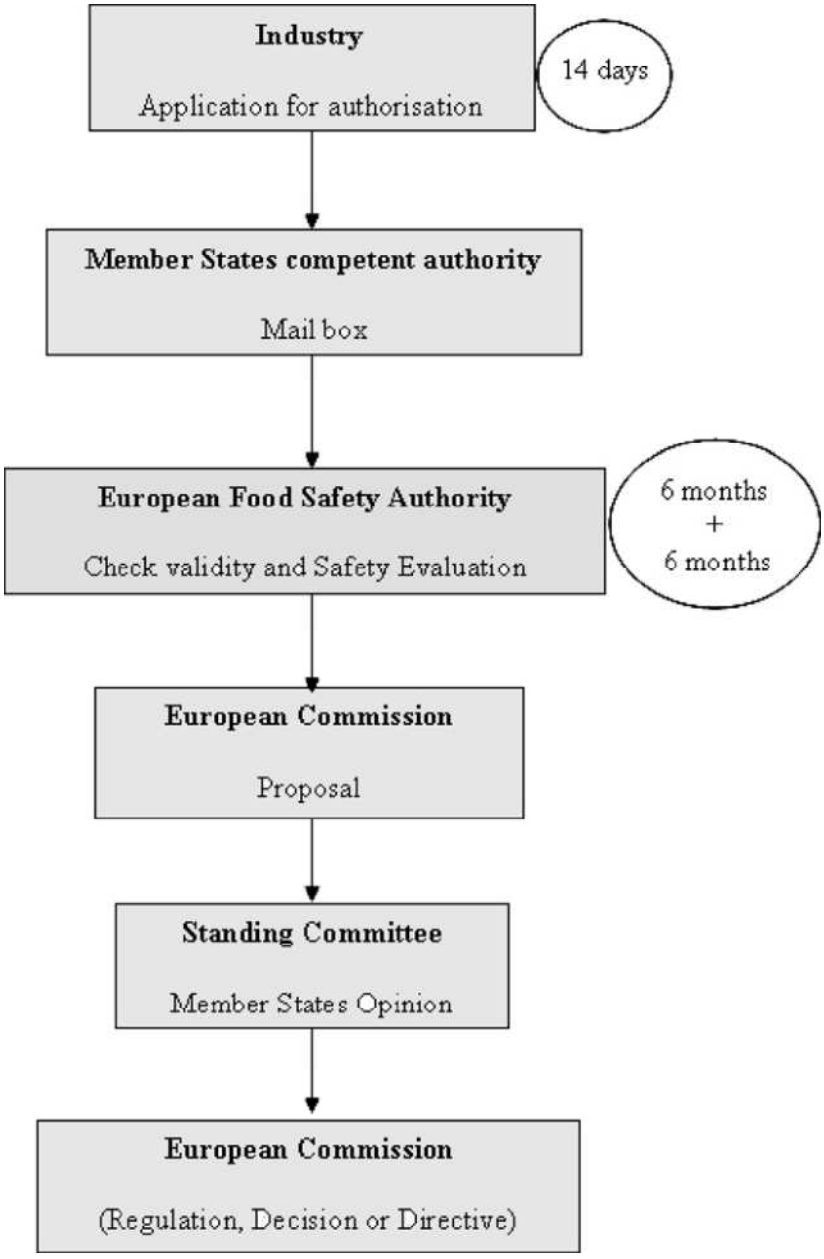


Figure 1: Authorisation procedure as defined by Reg. 1935/2004 EC (Restuccia et al., 2010).

1.2 ACTIVE COMPOUNDS WITH ANTIOXIDANT ACTIVITY

Ageing and stabilization polymers is a major part of materials science. Aging of polymers defined as a set of chemical and physical transformations, leads to the loss of their set of the desired properties. The main role in these transformations belong to chemical processes of degradation and crosslinking of macromolecules. Processes decomposition and structuring polymer conjugates include radical-chain, ionic and molecular reactions. Traditionally, the distinction is made between thermal, thermal-, photo-and radiation-chemical aging (Shybraeva, 2006).

Thermal oxidation and thermal-oxidative destruction are the most common and important processes in which polymer materials participate. It accompanies the posed for chemists experimenters and producers engaged in polymer materials creation is the problem of maintenance of high quality of output, prolongation of its service life at conditions of thermal oxidation influence and thermal-oxidative destruction. Thermal oxidation of polymers leads to a modification and functionalization of the polymer chains. At the same time thermal oxidation is accompanied by the destruction of bonds in the macromolecules and influence the destructive processes (Shybraeva, 2006). Thermal oxidation of polymers is a radical chain process with degenerate branching of kinetic chains of oxidation. The structure of polymer significantly influences on chain oxidation and destructive processes. Heterogeneity of polymers structures, the presence of regions differing in amplitudes of molecular motions, decrease of segment mobility, reduction of oxygen diffusion coefficient underlie this effect. These factors change kinetics and mechanism of process. As new methods and polymeric materials, researchers returned to the discussion of the induction period of oxidation of polymers. However, often in the literature there is confusion in the very concept and definition of the period induction. For example, when studying the thermal oxidative degradation of PP with different tacticity by thermogravimetric analysis (Chan and Balke, 1997; Nakatani *et al*, 2005) determine the induction period as a time corresponding to the onset of weight loss. Often thermal-oxidative degradation is identified with the thermal oxidation. However, in depending on the nature of the polymer, thermal oxidation process can take place without destruction chains, and with functionalization.

1.2.1 Oxidation

Shibryaeva (2006) have studied and outlined the reaction chain during oxidation process of polyolefins. According to him, three types reactions can be in the polymers in the presence of oxygen. 1) Separately occurring molecule reactions. 2) The radical - chain mechanism 3) The products of thermal decomposition and oxidation of polymers catalyze further decomposition of the polymer. The thermal oxidation of polyolefynes has been extensively investigated in various works. The investigation of the kinetics and mechanism of oxidation of solid polymers have shown convincingly that this process is a radical-chain with degenerate branching of kinetic chains. In the thermal degradation, thermooxidation and thermal oxidative degradation of polymers play a major role alkyd (R*), alkoxide (RO*) and peroxide (RO_2*) macroradicals and low molecular weight radicals (r*). The high reactivity of the past towards macromolecules strongly influences on aging processes. The chain reaction of the oxidation of a polymer includes alternate steps of the chain propagation proceeding either inside the same macromolecule or between two molecules. The investigation of kinetics of oxidation of the polymers, containing aliphatic groups (≡ C-H, -CH_2- or – CH_3), showed that this process was described by scheme, corresponding to the mechanism of chain oxidation of liquid phase (Denisov E.T. *et al.*, 1975).

For the oxidation of the polymer to the formation of macroradicals R*.

$$RH \rightarrow R^* + H^* \qquad (1)$$

where

RH: the monomer units of polymer.

Reaction can be triggered by physical factors such as ultraviolet and ionizing radiation, heat, ultrasound, or mechanical treatment chemical factors, such as catalysis, a direct reaction with molecular, singlet or atomic oxygen and ozone. However, initiation by direct interaction of molecular oxygen with the polymer, leads to detachment of a hydrogen atom, was unlikely, because it is endothermic reaction, enthalpy is 126-189 kJ/mol (Chan and Balke, 1997). Often, the birth of the chain portrayed as the bimolecular interaction of oxygen with the monomer units of polymer

$$RH + O_2 \rightarrow [RHO_2] \rightarrow R^* + HO_2{}^* \qquad (2)$$

HO_2* radicals, which formed, can enter on reaction with neighboring RH or on reaction of recombination with the primary radical R*

$$RH + O_2 \xrightarrow{k_0} [R^* + HO_2{}^*; RH] \rightarrow \sigma R^* \qquad (3)$$

Therefore, the radical yield (f) is: $0 < \sigma < 2$. RH- may be neighboring monomer units of one macromolecule or belong to different macromolecules. At the origin of the chain oxidation may participate impurities of transition metals, residues of catalysts or initiators, etc. These impurities get into the polymer as a result of receiving or processing the polymer.

The development of the kinetic chain by alternation of two reactions: the formation of peroxide radicals (RO_2*) and hydroperoxide (ROOH). Macroradicals R*, appeared in the initiation can easily react with oxygen molecules to give peroxide radicals RO_2* Peroxide radical can pull hydrogen from another polymer molecules to form polymeric hydroperoxides:

$$R^* + O_2 \xrightarrow{k_1} RO_2{}^* \qquad (4)$$

$$RO_2{}^* + RH \xrightarrow{k_2} \alpha ROOH + R^* \qquad (5)$$

where

k_2: the constant of continuation of kinetic chain rate.

α: the yield of hydroperoxide per mole of absorbed oxygen.

In the solid polymer free radical R* and hydroperoxide group, formed in reaction (5) cannot be away from each other. Part of ROOH is destroyed immediately after the formation of the reaction:

$$ROOH + R^* \rightarrow RO^* + ROH \qquad (6)$$

The reaction of (6) leads to a decrease in the yield of hydroperoxides during the oxidation of polymers in comparison with the oxidation of liquid hydrocarbon model. Their output ROOH is close to 100%. In the presence of oxygen even at low concentrations of the radicals R* are converted into RO_2* continue to ROOH. The concentration of the radicals R* is negligible compared to RO_2*, so oxidation rate (W_{O_2}) is determined (limited) reaction rate (5). In this case: $W_{O_2} = k_1 [R^*] [O_2] = k_2 [RO_2{}^*] [RH]$.

Branching of the kinetic chain of oxidation occurs in the decay of polymer hydroperoxides. Generally, consider a few basic mechanisms of decomposition of hydroperoxide

$$ROOH \xrightarrow{k'_d} RO^* + OH^* \qquad (7)$$

$$ROOH + RH \xrightarrow{k_d} RO^* + R^* + H_2O \qquad (8)$$

$$2\,ROOH \xrightarrow{k''_d} RO^* + H_2O + {RO_2}^* \qquad (9)$$

where kd, k'd, k"d: the constants of ROOH decomposition rate.

Monomolecular decay (7) comes with a large activation energy (140-160 kJ / mol). It occurs only in the oxidation of hydrocarbon fluids in the case of low concentrations of ROOH in solvents not containing weakly bound hydrogen atoms. Are more favorable reaction (8) and (9). Heat of reaction (9) is ~ 36 kJ / mol, and for reaction (8) varies widely depending on the binding energy of the R-H. Reaction (9) dominates at high concentrations of hydroperoxide, the reaction (8): in small quantities. In polymers containing weakly bound hydrogen atoms are predominant mechanism (8). As usual [ROOH] <<[RH], ROOH decay is described by a kinetic equation of first order.

The particularity of harden phase oxidation of polyolefyne is reaction of chain transfer – interaction of alkyl (R*) or alkoxy radical (RO*) with polymer competitive to its reaction with oxygen:

$$R^* + R'H \xrightarrow{k''_2} R'^* + RH \qquad (10)$$

$$[R'^*, R''^*, RO^*, OH^* + RH \rightarrow R^* + R'H, R''H, ROH, H_2O]$$

Break radical chain due to the interaction of free radicals with each other to form inactive products. There is quadratic termination of peroxide radicals at high pressure of oxygen:

$$ROR{O_2}^* + {RO_2}^* \xrightarrow{k_t} O_2 + \text{molecular products} \qquad (11)$$

Chain termination at low pressure of oxygen is quadratic termination of alkyl radicals

$$R^* + R^* \xrightarrow{k_4} R - R \qquad (12)$$

and alkyl with peroxide radicals:

$$RO_2^* + R^* \xrightarrow{k_5} ROOR \qquad (13)$$

where

k_t, k_4, k_5: the constants of chain termination rate.

Antioxidants, therefore, act to stop the propagation of radical reactions forming no-reactive species. The new radicals formed on the molecule of antioxidant are disabled through a process of resonance onto the aromatic ring.

1.2.2 Synthetic and natural antioxidant

Antioxidants generally are compounds that react with lipid or peroxide radicals or, in light, with singlet oxygen, and that are themselves oxidized to generate what are generally innocuous nontoxic compounds. Antioxidants are commonly incorporated into the food product itself as contrasted to being included as a part of a package material system. For many years, antioxidants were and still are fat-soluble compounds incorporated into fatty foods to preferentially react with intermediate oxidation products in the surrounding air or dissolved or occluded in the food product. In more recent years, the term antioxidant has been used more broadly to encompass compounds that react in non-lipid environments, such as, for example, in human body cells, which are water-based environments.

Synthetic antioxidants are chemically synthesized since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation. These antioxidants fall into two major categories depending on their mode of action: Primary antioxidants and Secondary antioxidants

The **primary antioxidants**, which prevent the formation of free radicals during oxidation, can further include three major categories:

1. *Free Radical terminators* - The radical terminators constitute the bulk of the synthetic antioxidants used as preservatives in food and these antioxidants prevent lipid oxidation by terminating the free radical chains. The important examples of radical terminators include Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Tertiary-butyl-

hydroquinone (TBHQ), and gallates such as propyl gallate (PG), dodecyl gallate (DG) and octyl gallate (OG).

2. *Oxygen scavengers* - The examples of oxygen scavengers which function as reducing agents, include sulphites, glucose oxidase and ascorbyl palmitate.
3. *Chelating agents* - The chelating agents prevent oxidation of lipids by binding the lipid oxidation catalysts such as heavy metals (iron, copper, etc). They do so by either precipitating the metal or by occupying all its coordination sites. Examples of such agents include Polyphosphatases and Ethylene diaminetetraacetic acid (EDTA).

Secondary antioxidants function is that breaking down hydroperoxides formed during lipid oxidation into stable end products. Thiodipropionic acid and Dilauryl theodipropionate are examples of secondary antioxidants.

BHT and BHA are the most prevalent synthetic antioxidants in food as reported by the National Research Council Food Additive Committee. Chemically, BHT and BHA are monohydric phenols with commercial Butyl hydroxyanisole (BHA) consisting of two isomers 3-tertiary butyl 4-hydroxyanisole and a 2-tertiary butyl 4-hydroxyanisole in the ratio of 9:1. BHA is available commercially as white waxy flakes, while BHT as a white crystalline solid, with both being extremely soluble in fats but not in water due to their phenolic structures with bulky hydrocarbon side chain. Both of these additives have good carry through properties, which determine their ability to withstand various processing steps such as baking and frying and maintain their functionality. BHA has slightly higher stability and thus is more effective especially in protecting the flavor and color of foods. Moreover, BHA is more effective in preserving foods containing animal fats with short chain fatty acids, such as those found in coconut and palm kernel oils used in confectionary products. Since, BHT and BHA are fairly volatile with their boiling points around 265°C and 268°C respectively, they are also used in the food packaging materials either by direct addition in the waxed inner linings or indirectly as emulsions, from which they migrate into the food. BHT and BHA have been shown to have synergistic effects when used in combination especially in nuts and nut products.

Tertiary buty hydroquinone (TBHQ) is another important synthetic antioxidant most often used in the preservation of food items containing frying oils. Chemically, TBHQ is a diphenol and is commercially available as beige colored solid, which is soluble in fats just like BHT and BHA

due to similar structural features. With its boiling point range of about 271.3°C - 311.3°C, it is less volatile as compared to BHT and BHA. TBHQ is an effective supplement to the expensive process of liquid oil hydrogenation to provide increased oxidative stability and color improvement, and provides good carry through properties to the fried product. In addition to its use as singular additive, TBHQ has been shown to have good synergistic effects when used in combination with BHT and/or BHA. Its stabilizing effects on the food lipids can also be enhanced when used in combination with chelating agents like citric acid, in substances such as vegetable oils, animal fats and particularly shortenings. But, the use of TBHQ with Propyl gallate (PG) is prohibited.

Propyl gallate (PG) is the most important and widely used antioxidant among all the gallates. Chemically, it is again a phenol and is prepared commercially by treatment of gallic acid with propyl alcohol and further distillation to get rid of the excess alcohol. PG is available as a white crystalline solid, which is sparingly soluble in water due to the presence of a number of hydroxyl groups in its structure. With a boiling point range of 161.3°C - 201.3°C, it is the most volatile among BHT, BHA and TBHQ. PG is particularly effective in stabilizing vegetable oils and animal fats in products such as meats, spices and snacks; but it is less effective as compared to TBHQ in preserving vegetable oils. PG has been shown to have good synergistic effects with BHA and/or BHA. In addition it is always used along with a chelating agent like citric acid, since in its absence PG chelates metal ions like iron forming an aesthetically unappealing blueablack complex.

Octyl gallate (OG) and dodecyl gallate (DG) are the other two gallates used as antioxidants in food. Both OG and DG are white to creamy white crystalline, odorless solids. OG is a diphenol like TBHQ, with similarities in their sizes and structures, while DG has more hydroxyl groups and a long hydrocarbon side chain in its ring structure. The increased solubility of DG in water due to the presence of a number of hydroxyl groups is counterbalanced by the decrease in solubility due to the long hydrocarbon side chain. As a result, both OG and DG are insoluble in water like TBHQ, BHT and BHA. With their boiling points ranging between 442.9°C - 522.9°C and 476.7°C - 566.7°C respectively, OG and DG are the least volatile among all the gallates.

Another important synthetic antioxidant Ethylenediaminetetraacetic acid (EDTA) falls under the category of chelating agents. It forms complexes with pro-oxidative metal ions like copper and iron, through n unshared pair of electrons in its molecular structure and thus lowers the amount of soluble reactive metals. It is mostly used in processed fruits and vegetables, soft drinks, salad dressings, margarine and canned shellfish. However, the use of EDTA in combination with certain

natural antioxidants is not advisable. For example, citric acid hampers the antioxidant capability of EDTA, since EDTA and citric acid combination forms catalytically active iron chelates that enhance oxidation reaction.

Natural antioxidant are those antioxidants that are found in natural sources, such as fruits, vegetables, and meats. There are several common natural antioxidants which are found in everyday foods, the most common of which being Vitamin C (ascorbic acid), Vitamin A (carotenoids), various polyphenols including flavonoids, anthyocyanins (a type of flavonoid), Lycopene (a type of carotenoid), Coenzyme Q_{10} also know as Ubiquitin which is a type of protein and Vitamin E (tocopherols).

Vitamin C, also know as ascorbic acid, is one of the most prevalent natural antioxidants in most everyday diets. It is a polar water soluble antioxidant and is found most commonly in fruit, particularly in citrus fruits such as oranges and lemons. Additionally, it is found in some vegetables, such as tomatoes. It has been observed in laboratory trials that in lower doses (less than 30 mg). absorption of dietary Vitamin C is fairly high (approximately 50%). However, the higher the dose, the lower the percent absorption. Thus, it is commonly recommended that in order to optimize Vitamin C absorption, smaller does should be consumed several times throughout the day, instead of consuming one large supplements.

β-carotene (which can be thought of interchangeably with Vitamin A) is a fat-soluble antioxidant member of the carotenoid family. Vitamin A is mostly found in vegetables such as kale, carrots, sweet potatoes, apricots, papayas, and other orange-hued produce items. Carotenoid bioavailability is considerably very low, approximately 10–30 % and just as with other antioxidants, absorption levels decrease as dose increases. Because of the low absorption levels of Vitamin A, excess is generally released from the body via solid waste. Addtionally, it should be noted that when Vitamin A is taken with high levels of dietary fiber, absorption decreases significantly.

Lycopene another fat- soluble antioxidant is also a member of the carotenoid family, but has one major difference: it cannot be converted into Vitamin A, as all other carotenoids can. Lycopene is found in tomatoes and tomato products. and interestingly, it has been found that lycopene levels in processed tomato products (such as tomato paste, pasta sauce, ketchup, etc.) are far greater than those in tomato fruits. As with other carotenoids, lycopene is best absorbed in the presence of dietary fats, and hindered by the presence of dietary fiber.

Polyphenols are another major class of natural antioxidants. They are most commonly found in products such as teas, particularly green tea and rooibos (red) tea, in addition to dark fruits such as Concord grapes. Bioavailability of polyphenols is approximately 15-20% of consumption, with absorption increasing when the consumed polyphenols do not have sugar molecules attached to them. Therefore, polyphenols from tea sources have superior absorptions to those from fruit sources, due to the relatively high sugar content of fruits.

Among polyphenols, flavonoids are one of the most common sub-categories found naturally in foods. Flavonoids have been seen to have particularly high concentrations in foods such as dark chocolate, potatoes, lettuce, wheat, red wine and black tea. Flavonoids have been seen to have much greater bioavailability when consumed from sources containing low quantities of sugar (approximately 50%) than when consuming flavonoids from sources with higher quantities of sugar such as fruits (approximately 15%). One type of flavonoid of particular interest are anthyocianins, which are typically found in dark fruit (such as blueberries, blackberries, Concord grapes, acai berries) and red wine. While anthocyanin absorption is very low, it has been show that the bioavailability of anthocyanins in darker fruits is much greater than that of lighter fruits (such as strawberries).

Another common type of natural antioxidant found in foods is the protein Coenzyme Q_{10} (CoQ_{10}) also known as Ubiquitin. CoQ_{10} is a small soluble protein used by animals as a natural antioxidant. Thus, it is found in substantial quantities in food sources such as fish and other meats, particularly organ meats (eg. chicken liver), as well as in high-protein plant sources such as wheat bran. The bioavailability of CoQ_{10} from meat sources and poultry has been seen to be approximately 60%.

Finally, Vitamin E, part of a family of antioxidants know as tocopherols, is a non polar, fat-soluble antioxidant. It is commonly found in several types of produce, such as cereal grains, broccoli and Brussels sprouts. It can also be found in more lipid–rich sources such as cooking oils like olive oil, sunflower oil, or safflower oil and nuts like almonds and hazelnuts. In general, bodily absorption of Vitamin E is tought to be rather inefficient, with the body absorbing only 20–40% of dietary intake. As with Vitamin C, it has been seen that the absorption levels of tocopherols are decreased as consumption levels increase. It has also been seen that when taken in conjunction with dietary fats, tocopherols have a higher bioavailability.

1.2.3 Tocopherol

α-Tocopherol is primarily recognized as a source of vitamin E and is available as a natural product (d-alpha-tocopherol) or a synthetic product (dl-alpha-tocopherol). Alpha-tocopherols exhibit some antioxidant potency, for this reason, the tocopherol concentrates are proving to be valuable ingredients where regulations do not permit the use of more effective synthetic antioxidants or where natural source antioxidants are simply preferred.

α-Tocopherol is a chain-breaking antioxidant that reacts with peroxyl radicals with a rate constant of about 106 $M^{-1}s^{-1}$, which is much faster than the reaction of peroxyl radicals with lipid RH (Wright *et al.*, 2001). The bond dissociation enthalpy (BDE) of phenolic antioxidants is an important factor in determining the antioxidant effectiveness, since the reaction rate with free radicals is faster with weak OH bonds. The RO_2• radical has a BDE on formation of the parent ROOH of about 88 kcal/mol, which will react rapidly in an exothermic reaction with α-tocopherol. The BDE of α-tocopherol is about 76 kcal/mol, while β-, γ-, and δ-tocopherol have slightly higher respective BDEs of 78, 78, and 80 kcal/mol (Wright *et al.*, 2001). These BDEs enable the tocopherols to function as effective chain-breaking antioxidants that prevent lipid peroxidation. The rate constant for the hydrogen atom transfer from α-tocopherol to cumylperoxyl radicals at 25 °C decreases by approximately 2 orders of magnitude upon transitioning from hexane to ethyl acetate as solvent (Valgimigli *et al.*, 1999). This solvent effect may be explained in terms of hydrogen bonding between α-tocopherol, which acts as hydrogen donor, and the solvent, which acts as hydrogen acceptor (Pedrielli *et al.*, 2001). The more solvating solvent induces added stability to the reactants relative to the transition state, which effectively increases the activation energy of the reaction.

Most vegetable oils contain tocopherols with the more unsaturated oils having higher concentrations of up to 1000 mg/kg or greater (Rossell, 1991). In the more saturated vegetable oils, such as coconut and palm kernel, tocopherols are almost completely lacking. Several types of tocopherol exist such as the α-, β-, γ-, and δ-forms, which differ from one another in the position and number of methyl groups on the phenol ring. A series of corresponding tocotrienols also exists in which the 16-carbon side chain is unsaturated. During the storage of unsaturated oils, tocopherols are consumed and their concentrations fall as oxidation proceeds. In coconut oil, α-tocopherol concentrations have been reported in the range 0.9–5 mg/kg (Slover, 1971; Rao and Perkins, 1972;

U.S. Department of Agriculture, 2007). α-Tocopherol exhibits the highest antioxidant activity of the tocopherols in vegetable oil with the least stability during storage (Player *et al.*, 2006). The degradation rate of α-tocopherol for 10 days of storage in soybean oil was 5.6% per day. α-Tocopherol degraded faster than both γ- and δ-tocopherol with its degradation rate 10 times faster than δ-tocopherol. α-Tocopherol easily donates a hydrogen atom to the peroxyl radical due to its relatively low BDE and is the most easily destroyed. α-Tocopherol is expected to function as a more potent hydrogen donor than γ- or δ-tocopherol due to its fully methylated structure (Player *et al.*, 2006).

The reported stability of α-tocopherol varies widely among researchers and appears strongly dependent upon factors of atmosphere, sample matrix, and analytical method. The stability of pure α-tocopherol upon storage was approximately halved with each 10 °C increase in temperature. The half-life of pure α-tocopherol at a temperature of 40 °C was 113 days, while at 120 °C the half-life was reduced to only 0.9 days. The degradation of α- tocopherol followed an induction type of curve with α-tocopherol having less stability in its pure form than when diluted in an oxidatively stable solvent. A complete loss of α-tocopherol is observed upon storage of red palm oil, groundnut oil, and their mixed blends (Lakshmi and Sarojini, 1996). α-Tocopherol is highly unstable to heat in red palm oil with 89% losses due to heating at 130 °C for 15 min during a frying process (Lakshmi and Sarojini, 1996). Significant decreases in tocopherols also occurred during the extrusion process of fish- and peanut containing half-products (Suknark *et al.*, 2001). Decreases in α-tocopherol content in fish and peanut extrudate were 23 and 18%, respectively, under extrusion conditions of around 100 °C and 250 rpm. These differences in α-tocopherol content are attributed to differences in fatty acid composition of the raw materials. Oxidative degradation of α-tocopherol has been studied by microcalorimetry and is clearly a temperature-dependent process between 50–80 °C (Otsuka *et al.*, 1994).

Thermal oxidation of α-tocopherol at 200 °C resulted in α-tocopherylquinone as a degradation product (Chung, 2004). Both tocopherol and tocopherylquinone were further degraded into fragments primarily at non-aromatic parts. The degradation products of α-tocopherol were combined with tocopherylquinone to produce thermal products through a dimerization process. Under an inert atmosphere that completely excludes oxygen, no degradation of α-tocopherol was observed at temperatures up to 240 °C, but the presence of any trace of oxygen will result in its immediate oxidation (Verleyen *et al.*, 2001). In monoacid triacylglycerols, α-tocopherol losses of

nearly 10% per hour were observed at 180 °C, leading to complete depletion after 10 h of heating (Barrera-Arellano *et al.*, 1999).

1.2.4 Santa Barbara Amorphous-15 (SBA-15)

In the 1960s, the modern view of drug delivery began in which drug performance was increasingly effective by specific targeting and timed delivery to control a therapeutic concentration for a longer duration than conventional dosing. Significant research on the incorporation of active compounds within food packaging and its subsequent controlled release to the packaged product began in earnest in the 1990s.

Controlled release packaging is well-suited by Potakamury *et al.*(1995) for controlling continuous food degradation reactions, such as microbial growth and lipid oxidation, because constant replenishment of active compounds can maintain safety and quality. Controlled release may be defined as a process by which one or more active ingredients are made available at a desired site and time at a specific rate.

So this release delivery system offers the following advantages:

a) active ingredients are released at controlled rates over prolonged periods of time,
b) ingredient loss during processing and cooking can be avoided or limited due to increased stability,
c) reactive or incompatible components can be separated (Potakamury *et al.*, 1995).

Until now, different materials have been intensively investigated in the last years as controlled release delivery system. Among all materials used as controlled release system matrices, both the chemical composition and the presence of porosity in them can influence the release pattern as literature has reported. However, the porosity of the conventional matrices is highly heterogeneous, owing to its complex chemical composition, and it often depends upon hazardous mixing and processing of materials that have different intrinsic capabilities for porosity generation. In general, the presence of micropores (pore diameter <2.0 nm) should be avoided in matrices designed for efficient and controlled drug delivery, as they produce generally desirable low diffusion rates and prevent the incorporation of interesting molecules due to size discrimination. M41S denoted a new family of ordered mesoporous materials which would allow overcoming these problems. These

materials contain a homogeneous distribution of mesopores (2 nm<d_p<50 nm) which are characterized by a very narrow pore size distribution. The pore size and pore volume of these materials make them suitable potential matrices for hosting and for further release of a large variety of molecules having therapeutic activity and not only one.

Since the discovery of MCM-41 in 1992, there has been much interest and research into mesoporous silicate materials. There are examples of the use of MCM-41 in catalysis, development of gas adsorption theory, and separation of large biological molecules, however these examples are limited and further work needs to be done to develop the industrial applications of mesoporous silicates. Indeed, it has been reported recently a new property of MCM-41, one of the members of the M41S family, for the controlled drug release (ibuprofen) under in vitro assays (Vallet-Regì *et al.*, 2001). The greatest advantage of these materials is their large surface area, uniform pore size, and controlled surface chemistry and hence their potential for absorption processes.

Among them, highly ordered hexagonal mesoporous silica structure SBA-15 has been synthesized by using commercially available block-copolymer surfactants in strong acid media (Figure 2) (Zhao *et al.*, 1998). SBA-15 possesses a hexagonal array of mesopores ~6.0 nm in diameter, which is much larger than the 3.0-nm pores characteristic of the MCM-41 structure reported so far for drug delivery. Therefore, SBA-15 is expected to have less restriction for the delivery of bulky molecules.

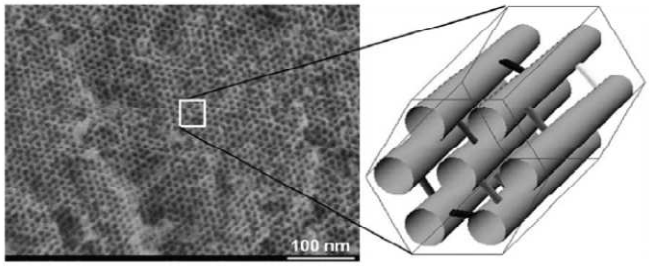


Figure 2: Highly ordered hexagonal mesoporous silica SBA-15 structure (Kleitz F., 2007).

The developed mesoporous silica SBA-15 offers new possibilities for incorporating biologically agents within silica hosts and for controlling their release kinetics from the matrix. In literature, in fact, a variety of studies about the use of SBA-15 silica as carrier for drug release can be found.

The unique mesoporous structure of these particles is the cause of the broad interest in their application in biotechnology. Their large internal volumes, high surface areas and straight narrow channels allow for the high adsorption of drugs into their structures. The straight channels allow for adsorbed drugs to diffuse out in a controlled manner over time frames that depend on the drug in question (size and chemical composition), the release medium, pore size, surface functionalization and particle size and morphology. Currently, the release of the drug from mesoporous materials is usually controlled by diffusion and so the release profile can be easily modified by changing the material properties such as, for example, pore diameter. In the case of hydrophobic and poorly water soluble drugs, the release from the ordered mesoporous SBA-15 silica matrix was enhanced compared to the direct dissolution of drug itself.

Enhanced release kinetics of drug/SBA-15 system can be explained in terms of displacement desorption of drug by the influx of water (Figure 3). Interactions of hydrophobic drug with the hydrophilic surface of SBA-15 are weaker than the interactions of water with silica walls. This is the reason why drug molecules effectively desorbed from the surface by competitive adsorption with water molecules. At loadings of active substances molecules where the monolayer capacity is exceeded, the release is controlled no longer by displacement desorption (no monolayer) but by the dissolution of crystalline or amorphous drug in the pores. After comparing SBA-15 with organic polymer based solid dispersions, we found that SBA-15 behaves differently. It is well known for organic polymer based solid dispersions that the dissolution rate decreases with increasing drug-to-polymer ratio while SBA-15 materials as carriers enable fast *in vitro* release kinetics even at high drug loadings.

Based on this considerations, the release or dissolution of the poorly water soluble drug could be enhanced by avoiding the breaking up of intermolecular interactions in the crystal structure. This may be accomplished by dispersing single drug molecules onto the walls of the SBA-15 matrix. Even when the monolayer capacity was exceeded, the release was still higher than the dissolution of the crystalline form because of the existence of nanoparticles inside the pores, whether crystalline or amorphous (Speybroeck *et al.*, 2008)

In the food packaging field, this SBA-15 ability could be much promising to realize new material formulation for the controlled release delivery.

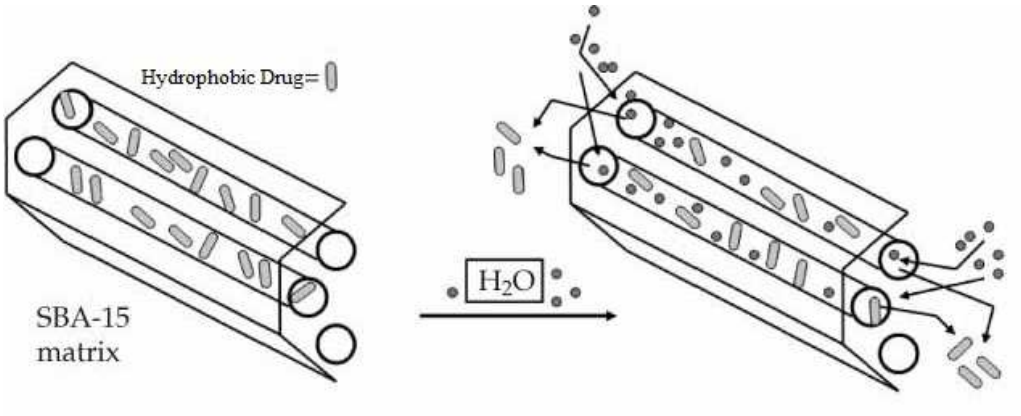


Figure 3: Schematic representation of the desorption mechanism (Speybroeck *et al.*, 2008).

1.2.5 State of Art of tocopherol based active films

Migration typically describes a diffusion process, which may be significantly affected by the interaction of food components with the packaging material (Arvanitoyannis and Bosnea, 2004). The majority of compounds commonly migrating from packaging materials to foods are those polymer additives, monomers, oligomers, and contaminants. Several types of additives are typically incorporated into polymers at concentrations of 0.01–1.0% (w/w) to limit the effects of oxidative degradation during processing and the lifetime of material (Calvert and Billingham, 1979). These additives may include plasticizers, antioxidants, light stabilizers, thermal stabilizers, lubricants, antistatic agents, antifog agents, and slip additives. Our attention was focalized on antioxidants and/or stabilizers.

Antioxidants can be classified into two groups: compounds that prevent formation of free radicals from hydroperoxides and compounds that inhibit the radical chain reaction (Scott, 1995). Antioxidants act sacrificially and are partially or completely converted to secondary products when they function to protect the polymer. Initially the majority of antioxidant substances used either as polymeric stabilizers or as active substances with an antioxidant function towards the foodstuff were synthetics. Tombesi and Freije (2002) found that the synthetic antioxidants was present in 46% of commercial drinking water bottled in polyethylene terphthalate (PET) in Argentina. They were considered more effective than the natural ones about antioxidant activity. In fact, the major

products formed when they were oxidized were electron acceptors and were much more effective than antioxidant itself as processing stabilizers (Scott, 1995).

However, the migratory nature of synthetic antioxidant into foods and food simulants has caused some concern regarding its continuous use as an antioxidant in food-packaging materials; therefore, α-tocopherol has been increasingly tested as a natural alternative. Natural antioxidants such, as α-tocopherol, are "generally recognized as safe" (GRAS) by FDA and its degradation products containing mostly tocopherol quinone compounds are harmless (Laermer and Zambetti, 1992). α-Tocopherol would be expected to have a slower migration rate from packaging into a food due to the larger molecular weight of α-tocopherol (431 g/mol) relative to BHT (220 g/mol). The thermal stability of α-tocopherol appears far superior to BHT by thermogravimetric analysis showing that α-tocopherol does not begin to volatilize until almost 300 °C (Laermer and Zambetti, 1992). α-Tocopherol was observed to be susceptible to thermo-oxidative degradation beginning at 165 °C as measured by non-isothermal DSC measurements (Giuffrida *et al.*, 2007). Lee *et al.* (2004) incorporated α-tocopherol and BHT into a heat seal layer (Surlyn/EVA®) coextruded with an HDPE layer at initial concentrations of 0.0073 and 0.1137% (w/w), respectively. They determined the first-order rate constants of α-tocopherol and BHT from the packaging film at 23°C to be 0.00115 h^{-1} and 0.0037 h^{-1}, respectively. After 18 weeks of storage at 23 °C, the relative percentage of antioxidant remaining in the packaging film was 36% of α-tocopherol and only 3% of BHT. After six days of accelerated testing at 45 °C, approximately 25% of α-tocopherol and no BHT remained in the film (Lee *et al.*, 2004). Wessling *et al.* (2000a) also found that BHT was depleted much more rapidly than α- tocopherol during storage at 20, 30, and 40 °C at 50% relative humidity. Importantly, their results indicate that the presence of a food product affects the loss of α-tocopherol from LDPE film. Oatmeal packaged in these LDPE pouches resulted in a loss of approximately 400 mg/g of α-tocopherol during four weeks of storage, but the film stored without the product had retained 900 mg/g of α-tocopherol during the same period (Wessling *et al.*, 2000a). Different characteristics of the food, such as contact phase properties, fat, alcohol, and acid content, influence the retention of α-tocopherol in LDPE films (Wessling *et al.*, 1999). α-Tocopherol is considered to be a more stable antioxidant than BHT when used in an LDPE film in contact with sunflower oil or 95% ethanol due to the much slower transfer from the polymer film to the fatty food simulants (Wessling *et al.*, 1998). Substantial loss of α-tocopherol from LDPE in contact with water has been reported but this was attributed to degradation caused by metal ions in the medium

(Wessling *et al.*, 1999). Higher retention of α-tocopherol was reported when citric acid, which has the ability to form complexes with metal ions, was added to the water in contact with the LDPE film. α-Tocopherol concentrations of 1000 and 10000 mg/kg have been used in LDPE films with the dual function of protecting the polymer from oxidative degradation during processing and delaying the onset of oxidation in the packaged food (Wessling *et al.*, 2000b). After processing the LDPE films, only about one-third (360 and 3400 mg/kg, respectively) of the α-tocopherol originally added to the polymer blend was retained with the remaining lost by manufacture and/or storage. These high levels of α-tocopherol showed a positive effect on the oxidation stability of linoleic acid emulsions at low temperatures, but may have detrimental effects on LDPE polymer characteristics (Wessling *et al.*, 2000b). Studies by Al-Malaika *et al.* (1994, 1999) report that the overall tocopherol retention level in PE and PP is generally greater than 80% of the original amount added with high initial concentrations of 1 –10% (w/w). Unstabilized LDPE loaded with lower initial concentrations of 0.2% (w/w) α-tocopherol leads to considerable loss of parent α-tocopherol during severe processing (Al-Malaika *et al.*, 1994). This extended LDPE processing resulted in approximately 97, 89, 84, and 66% α-tocopherol retention after each of four successive extrusion passes. The high antioxidant activity of α-tocopherol is at least partially the result of the transformation products formed during melt processing (Al-Malaika *et al.*, 1994). The major products formed are diastereoisomers of dimers and trimers in addition to aldehydes, whose relative concentrations are dependent upon processing severity and the initial α-tocopherol concentration.

A promising type of active packaging is antioxidative packaging in which an antioxidant is incorporated into the polymer material to be released into the packaged food product and as such extend their shelf-life. The principal action of this active packaging is based on the assure of a controlled release of the antioxidant from the film to the food product to provide a sufficient concentration of active compounds which prevents lipid oxidation and rancidity of the food product, with a continuous antioxidant effect in the packaging. If an antioxidant is added to the food as an ingredient, its antioxidant effect would be a onetime event and oxidation could persist after it is totally consumed. With controlled release, the antioxidant effect is prolonged to enhance the shelf-life.

Siró *et al.* (2006) produced films with retention of about 81% of the initial 2000 mg/kg α-tocopherol concentration in unstabilized LDPE. In fact, in this latter study the antioxidant was complexed into beta-cyclodextrin in order to give protection during extrusion and to ensure a

controlled release. Due to cyclodextrin polar hydrophilic outer shell and relatively hydrophobic cavity, they can build up host–guest complexes by inclusion of suitable hydrophobic molecules. Sirò et al. reported that complexation by beta-cyclodextrin was an effective tool for controlling the release of alpha-tocopherol from antioxidative active packaging because depletion of the antioxidant was completed in about 1000 h compared with about 3500 h in the cases of free and complexed alpha-tocopherol, respectively.

Recently, Heirlings *et al.* (2004) adsorbed α-tocopherol onto purely siliceous (non-functionalized) SBA-15, in an attempt to protect the antioxidant during extrusion and to ensure a controlled and sufficient release during the shelf-life of the food product. They considered that the pure SBA-15 porous network could serve as carrier for functional substances in new food active packaging solution, being a good reservoir for the accommodation of drug molecules due to its uniform pore size and highly ordered nanochannels.

Since a largely shared opinion is that the release of drugs from siliceous mesostructures depends on the pore architecture, the pore size and specific drug-silica pore wall interactions, with the latter phenomenon in turn depending on the presence of functionalizing groups on pore wall surfaces, a change in the pore size and the chemical functionality of the substrate with respect to its purely siliceous counterpart could allow a better performance in the controlled release.

In fact, when the interactions between desorbing molecules and silica pore walls are significantly strong and/or show some kind of specificity, the release also depends by the stability of the complex between the functional groups of the drug and those of the substrate. This phenomenon allows then to fine-tune the release of specific molecules from a given mesostructure by simply changing the functional groups that are attached to its pore walls during the synthesis process.

However, the approach proposed by Heirlings *et al.* (2004) had only a small influence on the release profile of α-tocopherol, which shows almost the same release kinetics as when the antioxidant is directly loaded into the film. With the aim to overcome this issue, in this work, a-tocopherol was adsorbed onto amine-functionalized SBA-15 in order to ensure a more controlled antioxidant release into a fatty food simulant by means of changes in the pore size and the chemical functionality of the substrate with respect to its purely siliceous counterpart.

1.3 ACTIVE COMPOUNDS WITH ANTIMICROBIAL ACTIVITY

When antimicrobial agents are incorporated into a polymer, the material limits or prevents microbial growth. This application could be used for foods effectively not only in the form of films but also as containers and utensils. Food package materials may obtain antimicrobial activity by common antimicrobial substances, radiation, or gas emission/flushing.

Radiation methods may include using radioactive materials, laser-excited materials, ultraviolet-exposed films, or far-infrared-emitting ceramic powders. However, universal irradiation sterilization of food package materials is not yet permitted by the FDA.

Gas emission/flushing controls mold growth. For examples, berries and grapes are stored in produce boxes, palletized, and stretch wrapped, then flushed with sulfite to prevent fungal spoilage. It is easy to use these types of bulk gas flushing and controlled-/modified-atmosphere technologies. However, there is no commercial material that contains or releases sterilizing gases such as sulfite. Sachet systems have been used to control the gas composition inside a package. An oxygen-scavenging system absorbs oxygen gas in the package and prevents growth of aerobic microorganisms, especially mold, as well as oxidation of food components.

Antimicrobial packaging materials have to extend the lag period and reduce the growth rate of microorganisms to prolong shelf life and maintain food safety. They have to reduce microbial growth of non sterile foods or maintain the stability of pasteurized foods without post-contamination. If the packaging materials have self-sterilizing ability because of their own antimicrobial activity, they may eliminate chemical sterilization of packages using peroxide and simplify the aseptic packaging process (Hotchkiss, 1977). The self-sterilizing materials could be widely applied for clinical uses in hospitals, biological labware, biotechnology equipment, and biomedical devices, as well as food packaging.

Commercial antimicrobial food package materials and structures still appear to be a few years in the future, but efforts of the 1990s underscored the continued development of new functional roles for packaging materials (Rice, 1995). Hotchkiss (1995) enumerated alternative antimicrobial packaging and summarized the issues relating to antimicrobials in package structures and materials. In most, but not all, solid or semisolid foods, microbial growth occurs primarily at the surface. In prepared or mixed foods, microbiological growth can occur anywhere in the mass. Antimycotic

agents are not uncommonly incorporated into waxes and other edible coatings used to package hard-skin fresh produce items such as oranges and apples. More recently, the concept of incorporating antimicrobial agents directly into package, films that contact the surface of the food has been developed, particularly in Japan, but also in other geographic regions.

1.3.1 Meccanism

Antimicrobial package materials may be classified into two types:

- those containing antimicrobial agents that migrate to the surface of the package material and thus can contact the food,
- those that are effective against food surface microbiological growth without migration of the active agent(s) to the food.

In the first case, a preservative is found either within the matrix or on the surface of the food-packaging material. The corresponding substance can be released completely or in a specific amount on the food surface to perform its biocide action.

Bastarrachea (2011) have studied four different migration mechanism of antimicrobial agents from packaging surface to the food. Figure 4 shows a schematic representation for the first case in which

(A) represents a packaging system that incorporates the antimicrobial agent in a single layer and releases it gradually into the food matrix,

(B) represents the same concept but with an inner layer, which can be useful in controlling the release of the antimicrobial compound,

(C) consists of a layer of food-packaging material coated with a formulation containing an antimicrobial substance.

In the second case, as it is shown in Fig.4, the scheme (D) represents a packaging system in which antimicrobial activity occurs only when microorganisms get in contact with the surface of the packaging material. For both types of systems, direct contact with the food is necessary, making these technologies a suitable option for vacuum-packed foods such as cheese, meat, fish, or poultry.

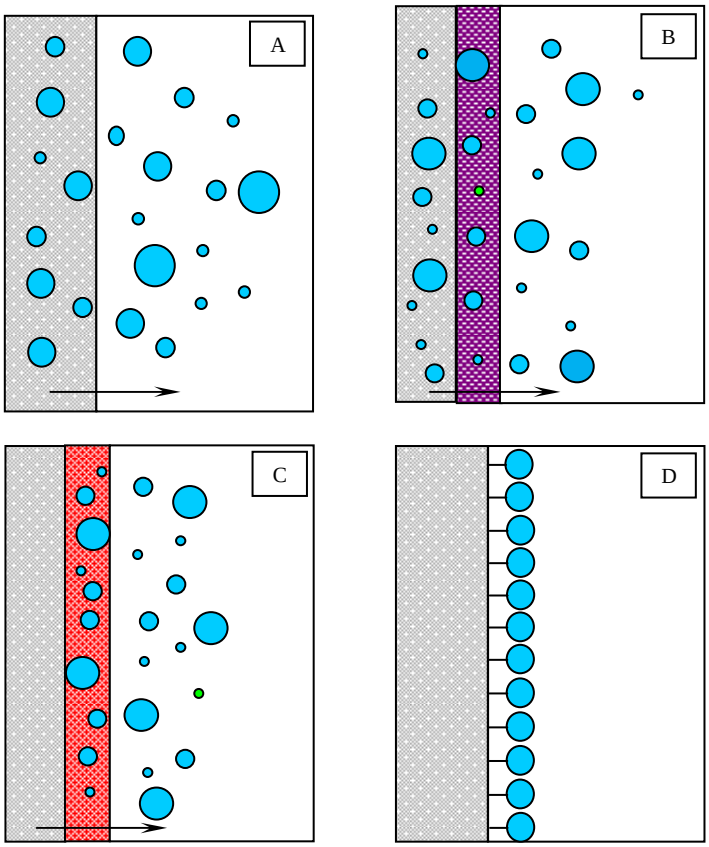


Figure 4: Antimicrobial food-packaging systems (adapted from Bastarrachea *et al*., 2011)

Several studies have investigated the effectiveness of antimicrobial films against microbial growth. Nevertheless, some attempts to produce films with antimicrobial activity have failed, since many factors affect their ability to suppress microbial growth. The interaction of the antimicrobial agent with the corresponding packaging material may adversely affect the release of such an agent, or the film production procedure can diminish activity of the antimicrobial agent to levels that make it ineffective for its purpose. Processing operations used during the manufacture of packaging film, such as extrusion, printing, drying, or lamination, may significantly affect the activity of the antimicrobial compounds due to phenomena such as degradation and evaporation. It is also important to consider the activity of the antimicrobial substance once it gets in contact with the food matrix. The interaction between the antimicrobial substance and food components may be strong enough for the antimicrobial agent to become ineffective against the microorganisms it is intended to suppress. This may occur even when the effectiveness of the packaging material has been tested and confirmed in vitro conditions (Bastarrachea *et al*., 2011).

Antimicrobial food-packaging films have been shown to influence the engineering properties of the film, such as the mass transfer properties of gases through the films, and the tensile, thermal, and morphological properties of these films (Bastarrachea *et al*., 2011). The level of influence depends on the type of film material, the film preparation procedure, and the antimicrobial agent used. These properties play an important role in determining the application of the film and the shelf life of the packaged food product. Modifications in the original properties of the film may be detrimental or even beneficial in the applicability of the packaging film, depending on the nature and extent of modification (Bastarrachea *et al*., 2011**)**. However, to date, there has been no comparative analysis of the engineering properties of food-packaging films after the incorporation of antimicrobials.

1.3.2 Antimicrobial packaging film

According to Appendini and Hotchkiss (2002) antimicrobial packaging can take several forms including:

1. Addition of sachets-pads containing volatile antimicrobial agents into packages.
2. Incorporation of volatile and non-volatile antimicrobial agents directly into polymers.
3. Coating or adsorbing antimicrobials onto polymer surfaces.
4. Immobilization of antimicrobials to polymers by ion or covalent linkages.
5. Use of polymers that are inherently antimicrobial.

1. The most successful commercial application of antimicrobial packaging has been sachets that are enclosed loose or attached to the interior of a package. Three forms have predominated: oxygen absorbers, moisture absorbers and ethanol vapor generators. Oxygen absorbers may reduce the oxygen inlet, inhibiting the aerobes (molds) growth as an antimicrobial. Moisture absorbers can reduce a_w, also indirectly affecting microbial growth. Ethanol vapor generators are usually used in products with reduced water activity (a_w<0.92) since the amount of ethanol generated is relatively small. These systems consist of ethanol absorbed or encapsulated in carrier materials and enclosed in polymer packets. The ethanol permeates the selective barrier

and is released into the headspace within the package. One of the drawbacks is the characteristic off flavor of ethanol.

2. As far as incorporation of bioactive agents including antimicrobial into polymers concerned, the research in this field for food application has more than doubled in the past 5 year. Until now, the different antimicrobial packaging form have been mainly applied in drug and pesticide delivery, textiles, surgical implants and other biomedical devices. GRAS, non-GRAS and 'natural' antimicrobials have been incorporated into different packaging materials, and have been tested against a variety of microorganisms. Of all the antimicrobials, silver-loaded micro- and mesoporous structures are the most widely used as polymer additives for food applications. Silver ion substitute functional ions present in these porous systems, which are antimicrobial against a wide range of bacteria and molds. Silver ions act inside microbial cells disrupting the cells' enzymatic activity. As in nature different substances present an antimicrobial activity, a combinations of more than one antimicrobial incorporated into packaging have also been investigated. In fact, it could be hypothesized that compounds active against Gram-positive bacteria (i.e. lysozyme) combined with chelating agents (i.e. EDTA) can target Gram-negative bacteria. The rationale for incorporating antimicrobials into the packaging is to prevent surface growth in foods where a large portion of spoilage and contamination occurs. This approach can reduce the addition of larger quantities of antimicrobials that are usually incorporated into the bulk of the food. The gradual release of an antimicrobial from a packaging film to the food surface may have an advantage over dipping and spraying. In the latter processes, antimicrobial activity may be rapidly lost due to inactivation of the antimicrobials by food components or dilution below active concentration due to migration into the bulk food matrix. Films with low diffusion rates were desirable since they maintained higher surface concentrations of sorbate for longer periods. Thermal polymer processing methods such as extrusion and injection molding may be used with thermally stable antimicrobials. In solvent compounding, instead, both the antimicrobial and the polymer need to be soluble in the same solvent. Biopolymers are good candidates for this type of film forming process, due to the wide variety of proteins, carbohydrates and lipids (which act as plasticizers) that form films and coatings. These polymers as well as their combinations are soluble in water, ethanol and many other solvents compatible with antimicrobials. Antimicrobial packaging materials must contact the surface of the food if they are non-volatile, so the antimicrobial agents can diffuse to the surface,

therefore, surface characteristics and diffusion kinetics become crucial. Then, packaging systems that release volatile antimicrobials have also been developed. These include chlorine dioxide, sulfur dioxide, carbon dioxide and allylisothiocyanate release systems. The theoretical advantage of volatile antimicrobials is that they can penetrate the bulk matrix of the food and that the polymer need not necessarily directly contact the product.

3. Antimicrobials that cannot tolerate the temperatures used in polymer processing are often coated onto the material after forming or are added to cast films. Cast edible films, for example, have been used as carriers for antimicrobials and applied as coatings onto packaging materials and/or foods.
4. A few examples of ionic and covalent immobilization of antimicrobials onto polymers or other materials have been achieved. This type of immobilization requires the presence of functional groups on both the antimicrobial and the polymer. Examples of antimicrobials with functional groups are peptides, enzymes, polyamines and organic acids. In addition to functional antimicrobials and polymer supports, immobilization may require the use of 'spacer' molecules that link the polymer surface to the bioactive agent. These spacers allow sufficient freedom of motion so the active portion of the agent can contact microorganisms on the food surface(Appendini and Hotchkiss, 2002). Spacers that could potentially be used for food antimicrobial packaging include dextrans, polyethylene glycol (PEG), ethylenediamine and polyethyleneimine, due their low toxicity and common use in foods. The potential reduction in antimicrobial activity due to immobilization must be considered. For proteins and peptides, changes in conformation and denaturization by solvents may result in low activity per unit area. Other antimicrobial enzymes that could potentially be covalently immobilized for packaging applications. A major challenge, however, is the incorporation of substrates into the system as well as managing undesirable products from the reactions.
5. Some polymers are inherently antimicrobial and have been used in films and coatings. Cationic polymers such as chitosan has been used as a coating and appears to protect fresh vegetables and fruits from fungal degradation. Physical modification of polymers has been investigated as means to render surfaces antimicrobial. Addition of nutrients could also potentially prevent cell membrane damage and bacterial recovery and/or inhibit the adhesion of the cells to the surface due to the interaction of salts and other cations with the surfaces.

1.3.3 Synthetic and natural antimicrobial

Antimicrobial agents can be divided into two categories according to their sources. These are chemical antimicrobial agents and natural antimicrobial agents.

Any chemical that when added to food tends to prevent or delay deterioration, but does not include common salt, sugars, vinegars, spices, or oils extracted from spices, or chemicals applied for their respective insecticidal or herbicidal properties are defined as chemical preservatives (Davidson and Branen 1993).

Benzoic acid is one of the oldest chemical preservatives used in the cosmetic, drug, and food industries. Sodium benzoate was the first chemical preservative approved for use in foods by the U.S. Food and Drug Administration. The structural formulas of benzoic acid is C_6H_5COOH and of sodium benzoate is C_6H_5COONa. Benzoic acid (molecular weight 122.1) occurs in pure form as colorless or white needles or leaflets. It is soluble in water. Sodium benzoate (molecular wieght 144.1) is a white granular or crystalline powder. It is much more soluble in water than benzoic acid (Davidson and Branen 1993).

In recent years, sorbic acid and its more water soluble salts, especially potassium sorbate known as sorbates are used widely throught the world as preservatives for various foods, animal feeds, pharmaceuticals, cosmetics and in other applications. They are very good preservatives because they inhibit or delay the growth of many microorganisms, including yeasts, molds, and bacteria. Under certain conditions, sorbates cannot inhibit some microbial strains. In general, however, they are considered effective food preservatives when used under sanitary conditions and in products processed using good manufacturing practices (Davidson and Branen, 1993).

Recently, due to health concerns and increased awareness, people preferred not to consume the food including chemical preservatives. Because of this condition, natural antimicrobial agents have gained a major importance. The most popular antimicrobial agents are enzymes such as lysozyme, lactoperoxidase, etc.

Nisin is an antibacterial polypeptide (molecular weight 3,510) produced by *Lactococcus lactis* subspecies *lactis* that broadly inhibits gram-positive bacteria and sporeformers (Padgett *et al.*, 1998) but when combined with a chelator, nisin also can inhibit growth of some gram-negative bacteria (Dawson *et al.*, 2005). Generally all proteins have absorbance at 280 nm but nisin has no absorbance at this wavelength, since it contains no aromatic acids. The solubility and stability of

nisin depend on the pH of the solution. In dilute HCl solutions at pH 2.5 its solubility is 12%. The solubility decreases to 4% at pH 5.0. Nisin is insoluble and irreversible inactivation occurs even at room temperature while pH are neutral and alkaline values (Davidson and Branen 1993).

Lysozyme (EC3.2.1.17), found in different sources including plants, animals and microorganisms, is a single peptide enzyme (Ibrahim *et al.*, 1996). Its molecular weight is 14400 and it contains 129 amino acid residues (Takahashi *et al.*, 2000, Ibrahim *et al.*, 1991). Its lytic activity on bacteria is occurred by hydrolyzing glycosidic β-linkages between N-acetylhexosamines of pepdidolycan (PG) in their cell walls. Because of that reason, many researchers have studied lysozyme for bio-preservation of foods. Most of the studies related with lysozyme have been focused on hen egg white lysozyme because the enzyme is commercially purified from hen egg white (Chang *et al.*, 2000). The antimicrobial activity of the enzyme is mainly against gram-positive bacteria but because of the PG layer surrounded by a protective lipopolysaccharide (LPS) membrane, it is ineffective against gram-negative bacteria (Nakamura *et al.*, 1991, Ibrahim *et al*,. 1991). So, many researchers have interested in increasing the antimicrobial spectrum of lysozyme. For instance, combination of lysozyme with EDTA makes lysozyme highly effective on gram-negative bacteria (Mecitoglu *et al.*, 2006), and conjugates of lysozym with dextran, galactomannan or xyloglucan have good antimicrobial activity against both gram-positive and gram-negative bacteria when applied in combination with mild heating at 50 °C (Nakamura *et al.*, 1992). In several studies, lysozyme was incorporated as a preservative in many packaging materials to extend the shelf life of the foods (Appendini and Hotchkiss 1997).

In addition to natural antibacterial agents, inorganic ones, typically silver, are now attracting attention in review of their safety from toxicity. In recent years, antibacterial goods, having an inorganic and/or antibacterial agent incorporated therein or applied, are becoming available in the market; such as used in wall carpeting, rugs, antibacterial fabric and fiber production, antibacterial film and the like. For examples:

- ✓ Ethyl alcohol – Ethyl alcohol adsorbed on silica or zeolite is emitted by evaporation and is somewhat effective but leaves a secondary odor.
- ✓ Chlorine dioxide – Chlorine dioxide is a gas that permeates through the packaged product. It is broadly effective against microorganisms but has adverse secondary effects such as darkening meat color and bleaching green vegetables.

- ✓ Allyl isothiocyanate – Allyl isothiocyanate, an active component in wasabi, mustard, and horseradish, is an effective broad spectrum antimicrobial and antimycotic. However, it has strong adverse secondary odor effects in food.
- ✓ Spice-based essential oils – Spice-based essential oils have been studied for antimicrobial effects.
- ✓ Metal oxides – Nanoscale levels of metal oxides such as magnesium oxide and zinc oxide are being explored as antimicrobial materials for use in food packaging (Garland, 2004). and, finally
- ✓ Among metallic ions, the silver ion has the strongest antimicrobial activity. Metallic silver does not release the ion easily, compared with copper, and so its antimicrobial activity is not quite as strong in its metallic state. Silver is a safe and relatively inert metal and therefore often used in direct human contact as dishes, plates, forks, spoons, knives, and tooth fillings. Silver is used as an antimicrobial agent in the form of medicine and water treatment agent. Silver ions can be dissolved in water, but they easily become insoluble by reacting with halogens, which is why silver's distribution in nature is limited. Silver is strongly absorbed by magnesium oxide, clay materials, and organic metal compounds. No reports exist with regard to carcinogenicity and mutagenicity of silver. In the United States, the standard for silver content in drinking water has been set at less than 50 ppb on the basis of a silver-containing medicine that causes angina symptoms.
- ✓ Silver ions – Silver nitrate that forms silver ions in water solution has strong antimicrobial activity. Activity at much lower concentration than the antimicrobial level causes protein denaturation. Consequently, silver nitrate has a history of use as a therapeutic for bacterial infection and as an antiseptic in hospital environments. Silver is considered to interfere with metabolic functions of respiratory and electron-transport systems of microorganisms and mass transfer across cell membranes. Silver salts function on direct contact, but they migrate slowly and react preferentially with organics. Research on the use of silver nanoparticles as antimicrobials in food packaging is ongoing, but one of the 1st product has already emerged: Fresher Longer ™ storage containers allegedly contain silver nanoparticles infused into polypropylene base material for inhibition of growth of microorganisms (NSTI 2006).

1.3.4 Silver based Nanoparticles

Silver has a broad spectrum of antimicrobial activities, being active against Gram-negative and Gram-positive bacteria, fungi, and certain viruses. Moreover, silver presents some important processing-related advantages such as high temperature stability and low volatility.

Effective antimicrobial nanocomposite packaging materials based on silver nanoparticles (AgNPs) have been presented. Thanks to their larger surface area-to-volume ratio, AgNPs present a more effective antimicrobial activity. On the other hand, the smaller size of nanoparticles may increase the possibility of human cell penetration and cytotoxicity (Su *et al.*, 2009).

The mechanism of the antimicrobial activity of AgNPs has not been well understood, but there are basically three common proposed mechanisms, as described in a review by Dallas *et al.* (2011): (i) gradual release of Ag^+ ions, resulting in inhibition of ATP production and DNA replication, (ii) direct damage to cell membranes by AgNPs, and (iii) generation of reactive oxygen species (ROS) by AgNPs and Ag^+ ions. Some authors have described the antimicrobial activity of AgNPs as being dependent on release of Ag^+ ions (Kumar and Münstedt, 2005). Ag^+ binds to electron donor groups in biological molecules containing sulfur, oxygen or nitrogen, such as thiols, phosphates, hydroxyls, and amines (Dallas *et al.*, 2011; Kumar and Münstedt, 2005). Feng *et al.* (2000) suggested that interactions of Ag^+ ions with thiol groups in proteins may induce inactivation of bacterial enzymes. As a reaction to the protein denaturation promoted by Ag^+ ions, DNA molecules may become condensed and unable to replicate.

Nanoparticles are important materials for fundamental studies and diversified technical applications, because of their size-dependent properties or highly active performance due to the large surface areas; but when pure nanoparticles are used alone, they present some common problems, eg, agglomeration between nanoparticles (Zhu *et al.*, 2002) To overcome agglomeration, preparation of nanoparticles based on clay compounds, in which nanoparticles are supported within the interlamellar spaces of clay and/or on its external surfaces, is one of the most effective solutions (Choy *et al.*, 1998, Miao *et al.*, 2006). Synthesis of metal nanoparticles on solid supports such as smectite clays is a suitable way to prepare practically applicable supported particles as well as to control the particle size. Smectite clays have excellent swelling and adsorption ability, which is especially interesting for the impregnation of antibacterial-active nano-size metals in the interlamellar space of clay (Rasal *et al.* 2010, and Paap *et al.* 2001). Montmorillonites (MMT) are

layered silicate belonging to the structural family of the 2:1 phyllosilicates. The presence of inorganic cations on the planar surface of MMT layers makes them hydrophilic and hence ineffective in hydrophobic polymers. However, cation–exchange reactions have been used to replace these inorganic cations with organic cationic surfactants which intercalate into the clay gallery, producing organically modified montmorillonites (OMMT) with increased interlayer spacing, hydrophobic surface and improved interactions with organic polymers.

Ag–MMT antimicrobial nanoparticles may be obtained by bringing Na–MMT in contact with $AgNO_3$ solutions in order to replace exchangeable Na^+ ions with Ag^+ ions (Costa *et al.*, 2011; Incoronato *et al.*,2010). Ag–MMT nanoparticles were incorporated by Incoronato *et al.* (2010) into three different polymeric matrices (agar, zein, and polycaprolactone), but only the agar nanocomposites presented antimicrobial activity, which was ascribed to the highest water content (thus highest level of macromolecular mobility) of the agar hydrogel. The release kinetics of Ag^+ ions was controlled because of the weak electrostatic interactions established with surface platelets of MMT. In a further study (Incoronato *et al.*, 2011), the group evaluated the effectiveness of an antimicrobial packaging system consisting of agar and Ag–MMT nanoparticles on cheese stability. The nanocomposite packaging system markedly increased the shelf life of the cheese, without affecting its functional dairy microbiota and sensory quality.

Costa *et al.* (2011) used Ag–MMT nanoparticles (in powder form) to extend stability of a kiwi–pineapple salad. The Ag–MMT powder (10, 15, or 20 mg) was left on the bottom of boxes containing 50 g of cut fruits and 70mL of 25% fructose syrup. The growth of mesophilic and psychrotrophic bacteria and yeasts was reduced by the nanoparticles. The overall sensory quality was assessed by using a five-point scale (from 1=“very poor” to 5=“excellent”), and the sensorial acceptability limit (SAL) was defined as the mean time for the product to achieve the overall quality threshold (score 3). The SAL was increased from 11.45 for the control sample (without nanoparticles) to 15.67 for the salad packaged in the box containing 20mg of Ag–MMT. The same group (Costa *et al.*, 2012) applied calcium alginate coatings loaded with Ag–MMT nanoparticles to fresh-cut carrots which were packaged in oriented polypropylene bags. The spread plate count technique was used for total bacteria, psychrotrophic bacteria, yeasts and *Pseudomonas* spp, and the plate count technique for Enterobacteriaceae. The increases in microbial population for the carrots with active coating were lower than for the uncoated carrots or those with a plain coating (without

Ag–MMT). When packaged in oriented polypropylene, the carrots with the active coating achieved a refrigerated shelf life of about 70 days against 4 days of the uncoated samples.

Su *e tal.* (2009) suggested that suspended AgNP/clay nanohybrids show electrostatic attraction to bacteria and form highly concentrated AgNP clusters on the bacterial cell wall. The authors attributed the antimicrobial activity of AgNP/clay nanohybrids to membrane disruption through the generation of intracellular reactive oxygen species (ROS). Su *et al.* (2011) observed that, although high electrostatic activity of MMT can disturb the cellular membrane integrity, MMT alone did not have significant biocidal activity, while AgNP/MMT promoted significant microbial growth reduction, indicating that AgNP is really effective antimicrobial agent in AgNP/MMT nanohybrids.

1.3.5 State of art of antimicrobial chitosan film

Although unmodified nanoclays are not antimicrobial by themselves, they may adsorb bacteria from a solution enabling better interaction with antimicrobial polymers such as chitosan (Wang *et al.*, 2006). Han *et al.* (2010) observed that chitosan–MMT nanocomposites were significantly more effective against *S. aureus* and *E. coli* than both pure chitosan and Na–MMT. Since the antimicrobial activity of chitosan has been ascribed to its cationic character, the increased antimicrobial activity of the nanocomposites seems contradictory, because the cations of chitosan are neutralized *via* electrostatic interactions with anionic silicate layers. The authors concluded that the nanocomposites exhibited synergistic effects between the components, because the chitosan molecules were evenly distributed through the inorganic matrix.

Wang *et al.* (2006, 2007, 2009) have carried out, instead, a series of studies on chitosan/rectorite intercalated nanocomposites with antimicrobial properties. In their first study (Wang *et al.*, 2006), chitosan/rectorite nanocomposites with different ratios were prepared *via* solution-mixing technique. Unmodified Ca^{2+}-rectorite (REC) and organic rectorite modified by cetyltrimethyl ammonium bromide (OREC) were used. The antibacterial activity of the nanocomposites was measured by the MIC against Gram-positive (*S. aureus* and *Bacillus subtilis*) and Gram-negative (*E. coli* and *Pseudomonas aeruginosa*) bacteria. Solutions of the nanocomposites, chitosan, REC and OREC were added to petri-dishes with nutrient agar to several

final concentrations, and the microorganism suspensions were inoculated on the nutrient agar, and incubated at 37°C for 24h. REC hardly inhibited the bacterial growth, and OREC only slightly inhibited it, but the nanocomposites showed better inhibitory effect than pure chitosan, REC and OREC, particularly against Gram-positive bacteria. In a further study (Wang *et al.,* 2007), the authors suggested that the antibacterial action of the nanocomposites were divided into two stages – first, the adsorption of the bacteria from solution and immobilization on the clay surface, and second, chitosan accumulation on the clay surface, inhibiting bacterial growth. Finally, in a more recent study (Wang *et al.*, 2009), N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (HTCC) was added into an OREC suspension under stirring at 80°C, at HTCC/OREC weight ratios of 1:1, 2:1, and 4:1, and the nanocomposites were freeze-dried and ground to powder. The MIC values of the nanocomposites were up to 32 times lower than those of HTCC against Gram-positive bacteria, up to 8 times lower against *E. coli*, and up to 16 times lower against *Aspergillus niveus*, indicating that the nanocomposites were more effective against Gram-positive bacteria. The excellent antimicrobial activity of the nanocomposites was attributed to the high affinity and strong interaction between quaternized chitosan and OREC, combining the adsorption and immobilization capacity of OREC with the antimicrobial activity of quaternized chitosan. In the three studies of the group (Wang *et al.*, 2006, 2007, 2009), the antimicrobial activity was reported to be directly proportional to the amount or the interlayer distance of the nanoclay, thanks to an increase in effective layers per unit weight, causing more bacteria to be adsorbed and immobilized on the clay surface.

Shameli e*t al.* synthesized silver nanoparticles (Ag-NPs) using a green physical synthetic route into the lamellar space of montmorillonite (MMT)/chitosan (Cts) utilizing the ultraviolet (UV) irradiation reduction method in the absence of any reducing agent or heat treatment. Cts, MMT, and $AgNO_3$ were used as the natural polymeric stabilizer, solid support, and silver precursor, respectively. They investigated the antibacterial activity of Ag-NPs in MMT/Cts against Gram-positive bacteria, ie, *Staphylococcus aureus* and methicillin-resistant *S. aureus* and Gram-negative bacteria (ie, *Escherichia coli*) by the disk diffusion method on Muller–Hinton Agar at different sizes of Ag-NPs. All of the synthesized Ag/MMT/Cts bionanocomposites were found to have high antibacterial activity. These results show that Ag/ MMT/Cts BNCs can be useful in different biologic research and biomedical applications, such as surgical devices and active substance delivery vehicles.

The antibacterial activity of silver nanoparticles loaded in chitosan agaist *S.aureus* were widely studied in literature. The goal was to achieve by Regiel *et al.* (with complete elimination of antibiotic resistant and biofilm forming strains of *Staphylococcus aureus* after short contact times. They have applied an innovative approach: the influence of the chitosan molecular weight and its deacetylation degree (DD) were analyzed together with the influence of the bacterial concentration and contact time. The best results were obtained with high DD chitosan where a fast reduction was favored; leading to smaller nanoparticles (nucleation is promoted), and a sufficiently high polymer viscosity prevented the resulting nanoparticles from undesired agglomeration. Instead, Soares *et al.* created a suitable method to produce reinforced antibacterial paper packaging using the antimicrobial triclosan (TC) and organically modified montmorillonite (OMMT) as "model" compounds. They were able to incorporate directly TC and OMMT into papers via coating process, and the resulting materials were characterized by in vitro antimicrobial assays. So the TC presence in the coated papers exhibited inhibitory effects against *S. auereus* and *Escherichia coli* but the OMMT did not improve the paper's antimicrobial efficiency.. Instead the incorporation of OMMT had increased the tensile strength of approximately 30% and had enhanced the water barrier property and thermal stability of paper.

The development of stabilized and densely dispersed chitosan-silver nanoparticles using a green approach based on electrochemical oxidation/complexation process followed by UV irradiation reduction was studied by Reicha *et al.*. The obtained nanoparticles were uniform and spherical. It was found. that increasing the Ag content in the chitosan-Ag based films tends to decrease their equilibrium swelling values. This reduction in swelling values may be attributed to the acting of the Ag as crosslinkers between chitosan chains. These crosslinking points increase with increasing the Ag content in the matrices which reduces the mobility of the chitosan chains and consequently reduces the swelling extent of the films. Reicha *et al.* carried also out antimicrobial activity assays against *Bacillus thuringiensis* and *Pseudomonas aeruginosa* bacteria. The nanoparticles demonstrated a relatively high antibacterial activity as compared to that of pure chitosan and the antibacterial activity increased with increasing the nanoparticle concentration.

Materials based on chitosan are applied in membrane separation, water treatment, material engineering, and in various application fields. These materials are characterized by good biocompatibility, biodegradability and accessibility, however, low mechanical properties as well as poor water stability can be a barrier for their applications. For this reason, in this work active

nanoparticlea have been obtained and embedded into chitosan matrix. This approach will bring the twofold advantage to improve the performances of the obtained nanocomposite material and to impart it an additional functionality such as the possibility to prolong the shelf-life of the packaged products Chemical structure of chitosan containing multiple functional groups (hydroxyl, carbonyl, carboxyl, amine, amid) creates possibility for new bonding between chitosan chain and nano-filler particles, like clay as montmorillonite (MMT). Moreover, as reported above, montmorillonite (MMT) is one of the layered silicates currently most widely employed in the production of polymer-clay nanocomposites with significant improvement in mechanical properties (Changa *et al*., 2003; Chang *et al*., 2003). MMT possesses a 2-to-1 layered structure with a single octahedral aluminum layer located between two layers of tetrahedral silicon. Furthermore, MMT as lamellar clay has intercalation, swelling, and ion exchange properties. It can be delaminated into elemental sheets without difficulty, so it is tempting to utilize these sheets as the substrate for preparation of nanoscale. Indeed, the MMT interlayer space has been used for the synthesis of material and biomaterial nanoparticles, as support for anchoring transition metal complexes and as adsorbents for cationic ions. With its cation exchange capacity and swelling property montmorillonite clays can encapsulate various protonated and hydrophilic organic molecules into the interlayer space, which can be released in controlled manners by replacement with other kinds of cations in the release media. Therefore, this clays are suggested to be good delivery carriers of hydrophilic molecules.

Given the above scenario, in this research work, organic-inorganic hybrid materials were developed by intercalating chitosan macromolecules into active montmorillonite clays (AgMMT) obtained by means a ionic change reaction. The MMT clay and silver ions were employed as carrier for controlled release and active guest molecules, respectively. Whereby the resulting bionanocomposites, composed by Chitosan/Ag-MMT, show interesting structural and functional properties.

1.4 AIM OF THESIS

The main objective of this thesis is the attainment of knowledge related to the development of new nanocomposite systems loaded with suitably modified inorganic nanoparticles and to the improvement of their production processes and performances for the obtainment of active films intended for food packaging applications. The general aim is the development of a methodology for the design and production of new packaging systems able to successfully answer to the evolutions of food market.

This purpose is strictly related to the need of the industrial food and food packaging sectors and takes into account new demands of the packaged food distribution. As a matter of fact, this field is highly influenced by new social habits: people do the shopping at always longer time intervals, for this reason packages able to maintain characteristics of fresh food for longer time are greatly desired. On the basis of these considerations, the development of new active packaging materials can represent useful innovation necessary to face the highlighted problems.

In the development of the proposed new active packaging systems, the attention has been also focused on the environmental impact considering that waste disposal is becoming a problem of enormous relevance. In particular, the use of recyclible polymers such as LDPE and eco-friendly polymeric materials from natural sources such as chitosan have been proposed. In fact, chitosan is nontoxic, biodegradable and biocompatible. It is derived via the deacetylation of chitin, which is the most abundant biopolymer found in nature, with the exception of cellulose. Chitin is a natural biopolymer found in the exoskeleton of crustaceans and insects as well as in the cell walls of fungi and microorganism. Chitosan has a potential for use as a packaging polymer due to its excellent film forming ability. Therefore, chitosan has been used in edible film or coating in order to extend the shelf life of foodstuffs. Actually, the poor thermal, barrier and mechanical properties are the main reasons of their limited diffusion. The inherent hydrophilic nature of chitosan limits its application because it causes the resulting film to exhibit a low water vapor barrier and poor mechanical properties in the presence of water and in humid environments. Many research studies have focused on improving the physical properties of chitosan–based biodegradable packaging films by decreasing its hydrophilicity and improving its mechanical properties (Rhim *et al*., 1998; Park *et al*., 2002; Chung *et al*., 2005). Others strategies have been explored including the addition of plasticizers such as glycerol which increase flexibility of the final product (Srinivasa *et al*.,

2007). The addition of other biodegradable aliphatic polyesters, such as poly-caprolactone (PCL), poly(butylene succinate) (PBS), poly(lactic acid) (PLA), poly(butylene terephthalate adipate) (PBTA), and poly(butylenes succinate adipate) (PBSA) (Correlo *et al*., 2005; Olabarrietaa *et al*., 2001), has also been investigated to produce materials with properties intermediate between the two components.

The use of nanometric particles appear extremely interesting both to confer new functionalities to the polymeric material and to improve some of their properties. Moreover one of the purposes of the research activity is to conveniently modify the nanometric particles to confer them active properties and, thus allowing the obtainment of new materials having improved properties to be used for prolonging the shelf life of packaged foodstuf. In a period characterized by high-tech packaging the nanotechnology could be used to reach consumer satisfaction, guarantee hygienic safety and food quality, rationalize packaging and reduce waste. An exhaustive research must be planned in order to achieve the necessary knowledge to understand the relationship between structure, process and properties of active nanocomposite materials and their efficacy in improving the hygienic, nutritional and sensory quality of packaged goods. By using this approach it will be possible to apply significant innovations that represent and confirm the developments of the scientific research in this specific topic.

In conclusion, the overall objective of this thesis is to study and to develop of an active packaging with specific functional properties, such as antioxidant and antimicrobial activities.

As far as antioxidant activity is concerned, there has been a growing interest in the manufacturing of active films for food packaging in which an antioxidant compound is added carring out a dual function: the first one is to protect and stabilize the polymer from oxidative degradation during processing and the second one is to delay the onset of oxidation of packaged foodstuffs during storage. To extent the shelf life of protected foods, the packaging formulation must assure a controlled release of antioxidant substances because an antioxidant added directly into the food as ingredient exhibits a more and more low functional activity in the time. One of the possible approaches chosen within the research activity of this PhD Course to control the release kinetics of antioxidants into fatty food simulants is the use of a particular molecular system as functionalised and not functionalised mesoporous silica particles.

As far as antimicrobial activity is concerned, germicidal, bactericidal, antifungal, and antiseptic components has been increasingly investigated as active compounds in different food

packaging formulation. A new approach is bsed on the incorporation of substances in the inorganic compound, such as clays or zeolites, to be used as carriers to ensure the release of the active compounds over time. In this way, the obtained active inorganic compound can be used to protect and prolong the shelf-life of packaged foods.The aim of the research activity will be reached throught the following steps:

1. **Choice of the polymeric matrices suitable for the selected application.**

   For antioxidant films, low density poly ethylene (LDPE) was chosen as film material. It is widely used for heavy duty materials like packaging, trash bags, food wraps, cable sheathing, greenhouse films, buckets, etc. .

   For antimicrobial one, the chitosan was chosen as film material. It is a linear aminopolysaccharide obtained by deacetylation of chitin, representing one of the most abundant natural biopolymer. Chitosan is a very promising biopolymer because it is environmentally friendly due to its biodegradability, and it exhibits good film-forming properties.

2. **Choice of active compounds suitable for the selected application.**

   As antioxidant substance, α-Tocopherol has proved to be very stable under processing conditions, it is favorable migration characteristics and excellent solubility in the polyolefins. Furthermore the use of α-tocopherol is cost-efficient compared with other antioxidant since the required effective concentration is considerably lower than that most of other commercial anitoxidant. Because of the stabilization mechanism of the radicals and its reactivity in the polyolefin oxidation process this additive get a particularly effective antioxidant.

   As antimicrobial agent, silver is well known for its strong toxicity to a wide range of microorganisms, with high temperature stability and low volatility. Some mechanisms have been proposed for the antimicrobial property of silver nanoparticles (Ag-NPs): adhesion to the cell surface, degrading lipopolysaccharides and forming ‘‘pits’’ in the membranes, largely increasing permeability; penetration inside bacterial cell and releasing antimicrobial Ag^+ ions by Ag-NPs dissolution.

3. **Study on the technique to control the release of the active substances in the food.**

   Recent breakthroughs in the synthesis of mesoporous silica materials with controlled particle size, morphology, and porosity, along with their chemical stability, has made Santa

Barbara Amorphous-15 silica matrix (SBA-15) highly attractive as the structural basis for a formulation of antioxidant active film in this research work. In addition, the possibility to functionalize mesoporous silica nanoparticle surface allow to design a new generation of controlled release delivery systems.

Moreover, montmorillonite clays have attracted great interest from researchers since they exhibit good adsorb ability, cation exchange capacity, and drug-carrying capability. These features added to intercalation capacity of organic species into its layered structure to prepare organic–inorganic hybrids, make montmorillonite clays both a good filler and carrier for active ompounds into antimicrobial films.

4. **Active nanoparticles.**

   As far as antioxidant films is concerned, functionalized and not-functionalized mesoporous silica nanoparticles (SBA-15 and SBA-15+APTES),were loaded with the antioxidant compounds by impregnation.

   As far as antimicrobial films is concerned, silver-montmorillonite particles have been obtained by ionic exchange reaction.

5. **Characterization of active nanoparticles.**

   Neat and tocopherol-loaded SBA-15 and SBA-15+APTES have been characterized in order to determine the verify the effectiveness of the impregnation process with tocopherol. In particular, X-Ray Diffraction (XRD), Fourier Transform-Infrared Ray (FT-IR), N_2 Adsorption/Desorption and Thermo-Gravimetric Analysis (TGA) have been performed.

   Ag-MMT nanoparticles have been characterized by UV/Vis observation tests and FT-IR.

6. **Active film manufacturing and optimization of processing conditions**

   Antioxidant films were obtaining loading pure tocopherol and tocopherol adsorbed into SBA -15 and SBA-15+APTES into unstabilized LDPE matrix trhough a three step process: mixing, press and extrusion

   Sodium Montmorillonite and silver montmorillonite nanoparticles were embedded by solution casting technique into chitosan polymer to produce antimicrobial film.

7. **Characterization of the obtained active films**

   Several studies were performed on the obtained active films in order to investigate the influence of the resence of the active particels on their structural and functional proprties

Migration tests.

The kinetic release of tocopherol from unfunctionalized and functionalized SBA/LDPE system in a fatty food stimulant, as 96% v/v ethanol,were studied.

Release tests of silver ions from chitosan bionanocomposites in a aqueus solution at ambient temperature were carried out.

8. **Study on the effectiveness of the obtained active packaging films.**

   In order to verify the effectiveness of the obtained active films, tests in model systems have been performed.

CHAPTER 2

MATERIALS AND METHODS

In this chapter the reader will be introduced to the research techniques and the research tools used in the thesis.

2.1 MATERIALS

2.1.1 Antioxidant film

Antioxidant packaging films (AO) have been developed to slow down or minimize oxidative deterioration of packaged products during storage. These active packaging, including the concept of the release of packaging components to foodstuffs, has received considerable attention recently. The migration of antioxidants from a packaging material to a foodstuff may result in prolonged shelf-life and preservation of quality of the product. In this thesis the following matrices and active compoinds were chosen

2.1.1.1 Polymer Matrix

Low-density polyethylene (LDPE) is commonly used in packaging applications involving contact with food. One of the reason of its widespread use is its versatility. LDPE is relatively inert chemically and almost insoluble in all solvents at room temperature, although some softening and swelling may occur due to sorption of food components. The permeability of water vapor through LDPE is low, but many organic vapors and oils pass rapidly through the material. It has a fairly high permeability towards oxygen and is therefore not suitable when oxidation is likely to be a problem (Paine, 1992). However, LDPE can be combined with other materials exhibiting better barrier properties towards oxygen. LDPE is a thermoplastic made from the monomer ethylene. It was the first grade of polyethylene, produced in 1933 by Imperial Chemical Industries (ICI) using a high pressure process via free radical polymerization. Its manufacture employs the same method today.

The polyethylene used in the experimentation is unstabilized LDPE granules provided by "Riblene®"; the low density polyethylene, a thermoplastic resin obtained by polymerization of ethylene, consists of highly branched polymer chains and characterized by a low level of

crystallinity and low density. It is characterized by a melting temperature of 130 °C and a density of 0.91/0.96 g/cm^3, has an operating temperature between -50°C and +70 °C.

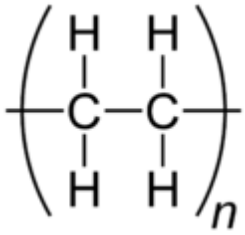


Figure 5: Monomer of Low density Poly ethylene (LDPE).

The structure of the ethylene monomer is show in figure 5. The physical properties (Table 1) of the polyolefin are governed primarily by chain entanglement and non-covalent van der Waals forces, allowing them to be softened into a fluid melt state by the application of heat (Lang, 2009).

The incorporation of an antioxidant into the packaging material to help prevent oxidation of the foodstuff contained therein could increase the use of LDPE in food packaging. One antioxidant which could be used in LDPE is α-tocopherol, which has proven to be an excellent stabilizer of the material (Laermer, 1990). The main purpose of incorporating an antioxidant into a polymer is to protect the polymer from degradation during processing.

Strandeberg and Albertsson studied the efficiency of α-tocopherol as a long-term and process antioxidant stabilizer in film-blown LDPE in comparison with a commercial phenolic antioxidant (Irganox 1076). According to the chromatographic analysis, there was less α-tocopherol than commercial phenolic antioxidant in the film materials after processing. They explained this result as consequence of the fact that α-tocopherol is more volatile and less soluble in the polymer matrix than Irganox 1076 because of its lower molecular weight, shorter aliphatic tail, and lower melting temperature. The process-stabilizing efficiency was nevertheless higher for the material containing α-tocopherol, as the oxygen induction time (OIT) measurements showed. The high OIT value of LDPE-Toc, despite the low residual content, presumably depends on a higher diffusion rate and mobility in the polymer matrix of α-tocopherol due to the small size and on its low bond dissociation energy for H-donation (Denivson). Another possible explanation for the high efficiency of α-tocopherol is its oxidation products, which are chain-breaking antioxidants and deactivate both

alkyl peroxyl and alkyl radicals. The oxidation products are said to have an even higher efficiency as stabilizers than α-tocopherol (Al Malaika *et al.*, 1999).

After processing, however, certain amounts of antioxidant often remain in the plastic and at this stage the antioxidant could possibly be released from the material in contact with foodstuffs.

Table 1: Some physical properties of LDPE (Lang, 2009).

PROPERTY	LDPE
Molecular weight	$1 – 3 \cdot 10^4$
Cristallinity (%)	55 – 70
Density (g/cm^3)	0.915 – 0.935
Softening Point °C	86
Melting Point °C	112
Tensile Strenght (kg/m^2) $\cdot 10^{-3}$	0.06 – 0.14
Glass transiton Temeprature T_g °C	-20

2.1.1.2 Active Coumpound

The natural antioxidant **tocopherol**, also know as Vitamin E, was one of the first active compounds selected for inclusion in antioxidant packaging materials. As an antioxidant for food systems, tocopherol as several advantages including good heat stability, high effectiveness against lipid oxidation and being natural; perhaps most importantly, it provides a clean label preferred by consumers. Tocopherol has also been used in extruded polymers as a polymer antioxidant, indicating its suitability for such applications. Tocopherol occurs naturally as a mixture of four isomers, differing in the number and position of methyl groups on the ring. In this study I chose to use only α-tocopherol, the most bioactive form.

L 'α-tocopherol (Figure 6) used is provided by the "Sigma Aldrich", and is extracted from vegetable oils.

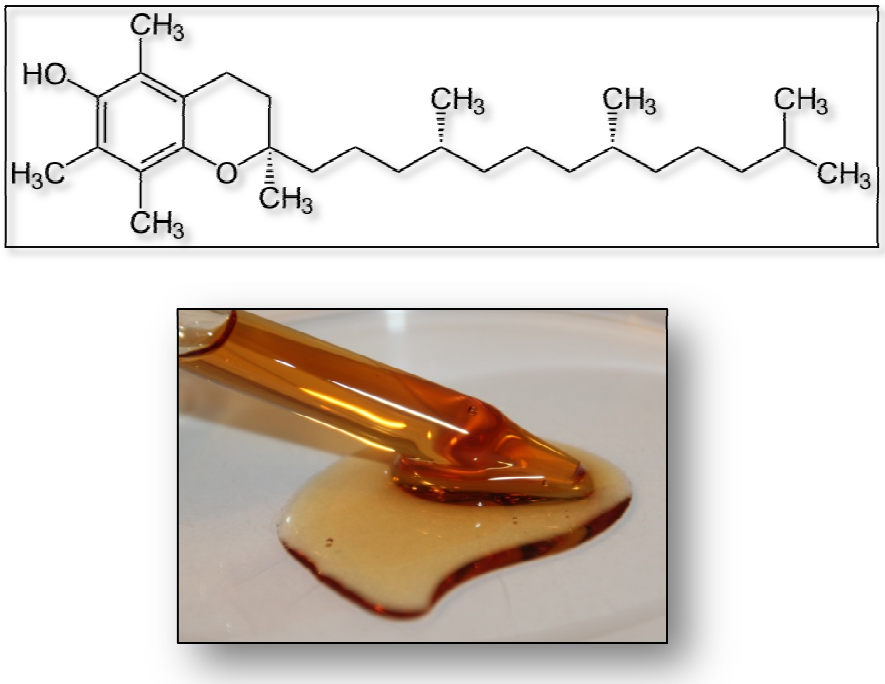


Figure 6: Structure of Alpha-tocopherol, extracted from vegetable oil.

It is characterized by a density of 0.95g/ml at 25 °C. It looks like a colored oily viscous liquid clear pale yellow.

The types of vitamin E are 8:

- tocotrienols (α, β, γ and δ, in a first time known as vitamin T)
- tocopherols (α, β, γ and δ)

There are, in nature, eight compounds, derivatives of 6-chromanol with four methyl groups bound to the aromatic ring and with a side chain isoprenoid to 16 carbon atoms, saturated or unsaturated, in position 2, with common chemical structure, having the 'biological activity of vitamin E. Depending on the presence of a saturated or unsaturated chain, these compounds are divided into two groups: the tocopherols (α, β, γ, δ) and tocotrienols (α, β, γ, δ). The latter, in fact, have three double bonds on the isoprenoid chain. The methyl groups arrangement allow to distinguish the individual compounds of the two classes. Biologically the α-tocopherol (Figure ...) is the most potent and active form.

The tocopherols have three centers of chirality (on C2, C4 and C8) and tocotrienols one (C2). The natural tocopherol, used as a term of comparison for the evaluation of the other substances

biological activity, being the most active, presents the three chiral atoms in the R configuration (to which is also called RRR-tocopherol). Tocopherols are oily compounds, insoluble in water and soluble in polar solvents. They are easily degraded by oxygen and UV rays and are quite resistant to heat.

2.1.1.3 Inorganic Carrier

According to the International Union of Pure and Applied Chemistry (IUPAC), porous materials are classified into three categories: microporous with pore diameters less than 2 nm, mesoporous having pore diameters between 2 and 50 nm, and macroporous with pore sizes larger than 50 nm. Highly ordered mesoporous silicates such as MCM (**M**obil **C**omposition of **M**atter No. 41, 48,...) and SBA (**S**anta **B**arbara **A**morphous material No. 1, 3, 15,...) have been long recognized as very promising materials with a rich variety of possible applications (Wan *et al.*, 2007). A wide variety of SBA materials has been prepared, such as SBA-1 (cubic), SBA-11 (cubic), SBA-12 (3D hexagonal network), SBA-14 (lamellar), SBA-15 (2D hexagonal) and SBA-16 (cubic cage-structured). They show ordered arrangements of channels and cavities of different geometry confined between walls built up from SiO_2 units (Ukmar and Planinšek, 2010).

Mesoporous silica nanoparticles are synthesized by reacting tetraethyl orthosilicate with a template made of micellar rods. The result is a collection of nano-sized spheres or rods that are filled with a regular arrangement of pores. The template can then be removed by washing with a solvent adjusted to the proper pH. In another technique, the mesoporous particle could be synthesized using a simple sol-gel method or a spray drying method. Tetraethyl orthosilicate is also used with an additional polymer monomer (as a template).

Then the resulted structure of mesoporous silica SBA-15 is composed by hexagonally packed one-dimensional nanochannels (Figure 7).

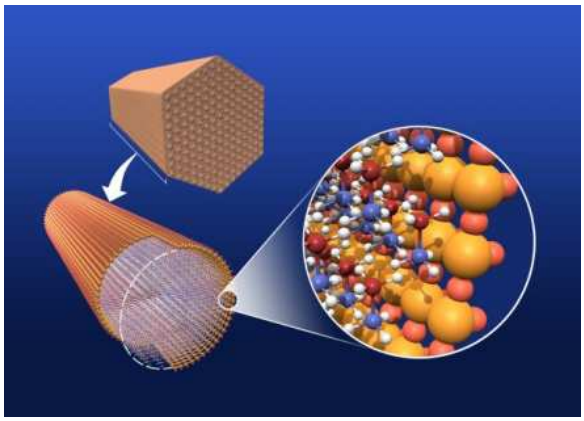


Figure 7: Internal structure of one–dimensional nanochannels of SBA-15 (Source:Pacifical Northwest National Laboratory).

2.1.2 Antimicrobial film

There has been a growing interest in recent times to develop materials with film-forming capacity and having antimicrobial properties which help improve food safety and shelf life. Antimicrobial packaging is one of the most promising active packaging systems that have been found highly effective in killing or inhibiting spoilage and pathogenic microorganisms that contaminate foods (Salleh *et al.*, 2007). It is well-known that microbial alternations are responsible for the enormous losses in food and hence, over the years, various chemical and physical processes have been developed to extend shelf-life of foods. Among such processes adequate packaging of food products is a fundamental factor in their conservation and marketing phases. Thus, packaging is not only crucial but actually preponderant for food quality preservation. The antimicrobial packagings have been used to control microbial growth in a food ingredient using packaging materials and edible films and coatings that contain antimicrobial agents and sometime by using techniques that modify the atmosphere within the package. Because of the increase in consumer demand for minimally processed, preservative-free products, the preservative agents must be applied to packaging in such a way that only low levels of preservatives come into contact with the food. In order to meet this demand, the film or coating technique is considered to be more effective though little cumbersome to apply. A greater emphasis on safety features associated with the

addition of antimicrobial agents is gaining ground as one of the emerging areas for development in packaging technology and it has likely to play a major role in the next generation of 'active' packaging systems (Brody, 2001). Active packaging is the packaging system possessing attributes beyond basic barrier properties that are achieved by adding active ingredients in the packaging system and /or using functionally active polymers. In this context, chitosan films have shown great promise for their application in food preservation.

2.1.2.1 Polymeric matrix

Chitosan, a linear polysaccharide consisting of (1,4)-linked 2- amino-deoxy-b-D-glucan, is a deacetylated derivative of chitin, which is the second most abundant polysaccharide found in nature after cellulose (Figure 8). Chitosan has been found to be nontoxic, biodegradable, biofunctional, biocompatible in addition to having antimicrobial characteristics (Darmadji and Izumimoto, 1994)

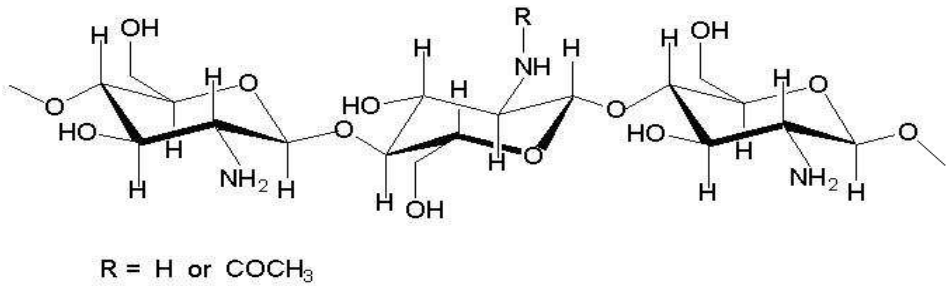


Figure 8: Chitosan Structure.

Jayakumar *et al.*, 2007; Jayakumar *et al.*, 2005; Jayakumar *et al.*, 2006; Jongrittiporn *et al.*,2001; Wang, 1992). As compared with other bio-based food packaging materials, chitosan has the advantage of being able to incorporate functional substances such as minerals or vitamins and possesses antibacterial activity (Chen *et al.*, 2002; Jeon *et al.*, 2002; Möller *et al.*, 2004). In view of these qualities, chitosan films have been used as a packaging material for the quality preservation of a variety of food (Park & Zhao, 2004; Suyatma *et al.*, 2005; Tsai & Su, 1999; Wu *et al.*, 2005).

2.1.2.2 Active Compound

It is well known that silver ions and silver-based compounds are highly toxic to microorganisms (Zhao *et al.*, 1998) showing strong biocidal effects on as many as 16 species of bacteria. **Silver** is a metallic chemical element with the chemical symbol **Ag** and atomic number 47. A soft, white, lustrous transition metal, it has the highest electrical conductivity of any element and the highest thermal conductivity of any metal. The metal occurs naturally in its pure, free form (native silver), as an alloy with gold and other metals, and in minerals such as argentite and chlorargyrite. Most silver is produced as a byproduct of copper, gold, lead, and zinc refining.

Silver has long been valued as a precious metal, and is used as an investment, to make ornaments, jewelry, high-value tableware, utensils (hence the term silverware), and currency coins. Today, silver metal is also used in electrical contacts and conductors, in mirrors and in catalysis of chemical reactions. Its compounds are used in photographic film, and dilute silver nitrate solutions and other silver compounds are used as disinfectants and microbiocides (oligodynamic effect). While many medical antimicrobial uses of silver have been supplanted by antibiotics, further research into clinical potential continues. Silver metal dissolves readily in nitric acid (HNO_3) to produce silver nitrate ($AgNO_3$), a transparent crystalline solid that is photosensitive and readily soluble in water. Silver nitrate is used as the starting point for the synthesis of many other silver compounds (Figure 9).

Figure 9: Silver ion and tridimensional structure silver nitrate.

2.1.2.3 Inorganic Carrier

Nanoparticles used as fillers or additives in polymers for various desired effects are receiving an increased interest for research and development. Various types of nanoparticles, including nanocarbon, carbon nanotubes, nanoclays, and metal oxides, are currently used to modify the polymer performance. The essential nanoclay raw material is montmorillonite. **Montmorillonite**, talc, pyrophyllite, saponite, and nontronite are a few members of the larger smectite clay group. The general formula for the chemical structure of this group is $(Ca,Na,H)(Al,Mg,Fe,Zn)_2(Si,Al)_4O_{10}(OH)_2XH_2O$. The important difference among the members of this group is seen in the chemical characteristics. These are used as fillers in paints, rubbers, as plasticizer in molding sands, in drilling muds, and as electrical, heat, and acid-resistant porcelain. The structure of clay particles is perceived in layers; each layer is composed of two types of structural sheets: octahedral and tetrahedral. The tetrahedral sheet is composed of silicon-oxygen tetrahedral linked to neighboring tetrahedra by sharing three corners, resulting in a hexagonal network. The remaining fourth corner of each tetrahedron forms a part to adjacent octahedral sheet. The octahedral sheet is usually composed of aluminum or magnesium in six-fold coordination with oxygen from the tetrahedral sheet and with hydroxyl. The two sheets together form a layer, and several layers may be joined in a clay crystallite by interlayer cations, Van der Waals force, electrostatic force, or by hydrogen bonding. Theoretical formula and structure are shown in Figure 10. As we can see, montmorillonite is a 2:1 clay, meaning that it has 2 tetrahedral sheets sandwiching a central octahedral sheet. The particles are plate-shaped with an average diameter of approximately one micrometre.

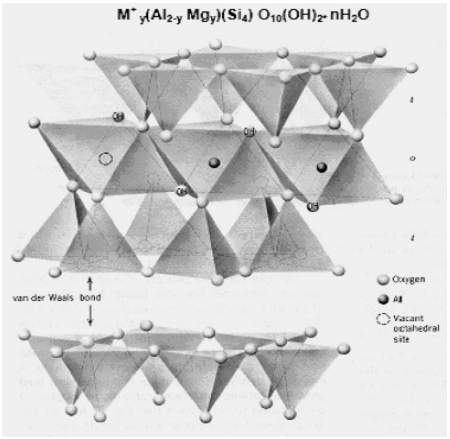


Figure 10: Theoretical formula and structure of Montmorillonite (MMT).

2.2 METHODS

2.2.1 Antioxidant film

2.2.1.1 Synthesis and functionalization of nanoparticles

Typical synthesis of SBA-15 requires triblock copolymer, typically non-ionic triblock copolymer (Zhao *et al.*, 1998b) as structure directing agent and Tetramethyl Orthosilicate (TMOS), Tetraethyl Orthosilicate (TEOS) or Tetrapropyl Orthosilicate (TPOS), as silica source. According to Zhao *et al*. (2000) the formation of ordered hexagonal SBA-15 with uniform pores up to 30 nm was synthesized using amphiphilic triblock copolymer in strong acidic media i.e., pH ~1. If pH is more than the isoelectric of silica i.e., at pH 2-6, no precipitation or formation of silica gel would occur. Disordered or amorphous silica would likely to be happened at neutral pH of 7. Nevertheless, Cui *et al*. (2005) have proved to synthesize SBA-15 at pH 2-5, which is above isoelectric point of silica. They suggested that the prehydrolyzed of TEOS at pH<3 would interact with template agent to form a mesophase under weak acidic condition. The most recent study is the synthesis of highly siliceous SBA-15 from low silica inorganic precursor with the presence of impurities of industrial waste product which typically consist of metal oxide containing 42 wt% SiO_2. Template removal is one of the crucial aspects in ordered mesoporous silica synthesis in which this procedure could modify the final properties of desired porous structure. The usual method of removing template is calcination. According to Zhao *et al*. (1998a) the calcination of mesoporous structure SBA-15 at 500°C would produce final properties of porous structure with interlattice d spacing of 74.5-320 Å between the (100) planes, pore volume fraction up to 0.85 and silica wall thickness of 31-64 Å (Zhao *et al.*, 1998a). Besides mesoporosity structure, SBA-15 was found to contain micropores which indicated the hierarchical template of material structure (Schmidt-Winkel *et al.*, 1998). These microporous structures are generally disordered and provide interconnectivity between ordered mesopores (Brady *et al.*, 2008).

In this work purely siliceous and functionalized SBA-15 were synthesized and then impregnated with tocopherol by the research group of Prof. Caputo and Dr. N. Gargiulo at Department of Materials and Procudction Engeneering , University of Naples according to a recipe

reported in our common paper (Gargiulo *et al.*, 2011). Two grams of Pluronic P123 (Aldrich) were dissolved in 15g of ultra-purified water and 60g of 2M HCl solution with stirring at room temperature. Then, 4.25g of tetraethylorthosilicate (TEOS, Aldrich) was added into that solution with stirring at room temperature for 20h. The mixture was aged at 80 C for 24h without stirring. The solid product was filtered, washed, and air-dried at room temperature. Calcination was carried out at 500°C for 6h (heating rate: 1°C/min).

The mesoporous silica structure allow a chemical function allocation at nanometer level. We have already seen how these materials are obtained by a sol-gel polycondensation in the presence of surfactant micellar aggregates, which act as structuring (Gargiulo N., PhD Thesis) (Figure 11).

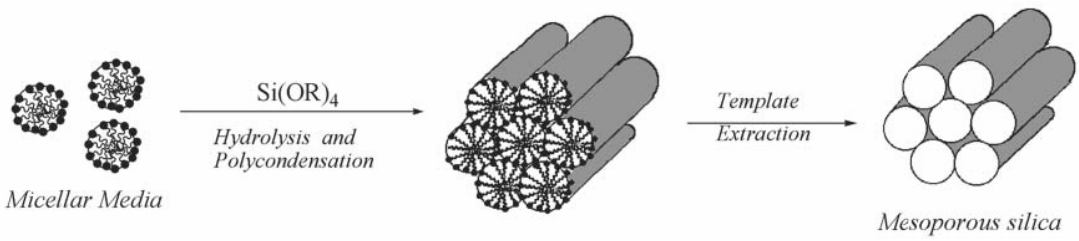


Figure 11: Mesoporous silica synthesis scheme (Gargiulo N., PhD Thesis).

As reported in Dr. Gargiulo N., Ph D thesis, after the elimination of the surfactant, the materials are characterized by a long-range order and exhibit a high surface area with a narrow pore-size distribution, which is controlled by the surfactant. Many scientists have studied the functionalization of these materials, in order to make them suitable for applications such as catalysis (Maschmeyer *et al.*, 1995), separation (Salesch *et al.*, 2002), chemical detection (Mercier *et al.*, 1997), housing compounds included, molecular sieve (Zhang *et al.*, 1996) and controlled release as well as for the exploitation of physical properties in the fields of optics, magnetism, electrical conductivity, etc…

The different type of modifications have been grouped into three classes:

1. Functionalization of the porous system channels;
2. Functionalization of the structure;
3. Functionalization of the porous system and the structure channels.

The first one consists of a "direct synthesis" by means of co-hydrolysis and polycondensation of tetraethylorthosilicate (TEOS) with a organictrhietoxysilane $RSi(OEt)_3$ in the presence of a structuring agent (Corriu *et al.*, 2001) This single stage method allows a control of the organic groups load, as well as their regular distribution inside the the porous system channels (Corriu *et al.*, 2000). The characteristics of these materials are very similar to those of purely silica SBA-15 (Margolese et al., 2000). In any case, the diameters of the pores are smaller due to the functionalization of the porous system channels.

In this PhD thesis the amino-functionalized SBA-15 (SBA-15+APTES) synthesized according to a recipe reported in literature has been used (Gargiulo *et al.*, 2011). The functionalization was carried out in a similar way of purely silica SBA-15 synthesis (Wang *et al.*, 2005). 2.0g of Pluronic P123 was dissolved in 62.5g of 2.0M HCl solution at room temperature. After TEOS was added, the resultant solution was equilibrated at 40 C for prehydrolysis, and then aminopropyltriethoxysilane (APTES, Aldrich) was slowly added into the solution. The molar composition of the mixture was 0.9 TEOS:0.1 APTES:6.1 HCl:0.017 P123:165 H_2O. The resulting mixture was stirred at 40°C for 20h and then aged at 90°C for 24h without stirring. The solid product was recovered by filtration and dried at room-temperature overnight. The template was removed from the as-synthesized material by refluxing in 95% ethanol for 24h. Finally, the material was filtered, washed several times with water and ethanol, and dried at 50°C.

2.2.1.2 Impregnation

Different techniques are used to impregnate various mesoporous structure with metallic oxides or other active substances in order to produce nanocomposite materials used as molecular sieve, adsorbents and heterogeneous catalysts.

Wet Impregnation (WI) and Incipient-Wetness Impregnation (IWI) are more commonly used ”one solvent” techniques, while denominated “two solvent” one is an innovative method.

In the WI technique support is put in contact with a high amount of solution containing the precursor/active substance. The incipient wetness technique (IWI) is similar to dry impregnation, since during the impregnating process the solution containing the substance is added in such quantity as soon as the wet powder, equal to the pore volume of the support. The impregnation

method called "Two-Solvents"derived from the traditional IWI method, and is based on the combination of a hydrophobic solvent and water to impregnate the mesoporous material(Xu *et al.*, 2005).First the support is suspended in a hydrophobic solvent (n-hexane or cyclohexane), poorly miscible with water, and then is put in contact with a quantity of aqueous solution containing the precursor/active substance equal to the host material pore volume.

As reported by Gargiulo *et al*., 2012, for our samples of SBA-15 and SBA-15+APTES the impregnation with α-tocopherol was carried out using a slightly modified version of the method reported by Xu *et al.* (2005), similar to IWI technique. Typically, the desired α-tocopherol amount was dissolved in ethanol (8mL per gram of substrate); then mesostructured silica was added to the solution. The solid powder was recovered through drying at 35°C for 16h under reduced pressure. The tocopherol/silica weight ratio was 0.73 for purely siliceous SBA-15 and 0.42 for SBA-15+APTES. Both of them were chosen with the aim of having the amount of tocopherol approximately capable of filling the total pore volume of the hosting systems.

2.2.1.3 Active film preparation

The obtained films are coded as follows: LDPE/TOC for films containing 1% wt/wt pure tocopherol, LDPE/SBA-15/TOC and LDPE/SBA-15+APTES/TOC for films containing approximately 3% wt/wt of SBA-15 and SBA-15+APTES loaded with tocopherol corresponding to a tocopherol amount of about 1% wt/wt.v

The unstabilized LDPE films were prepared through several steps: mixing, compression, pelletization and extrusion. Each step will be hereafter described in detail.

1) Mixing - The mixer is an instrument equipped with a mixing chamber, the two rotors, co-rotating or counter-rotating, and a feed hopper; the best mixing is obtained with co-rotating rotors enterprising, ie whose threads enter one inside the other (Figure 12). Through the hopper mixing chamber is fed with polymeric materials and additives in the form of granules, chopped, powders and liquids.

   Thanks to the action of electric heating elements and thermocouples is possible to increase and control the temperature for the plastification of the material and impose a thermal history. For the mixing of LDPE with α-tocopherol has been used a Haake Rheomix 600 mixer

produced by Thermo Scientific (Figure 13); the mixing chamber consists in three temperature-controlled heads and two counter-rotating rotors, and has a volume of about 50 cm3.

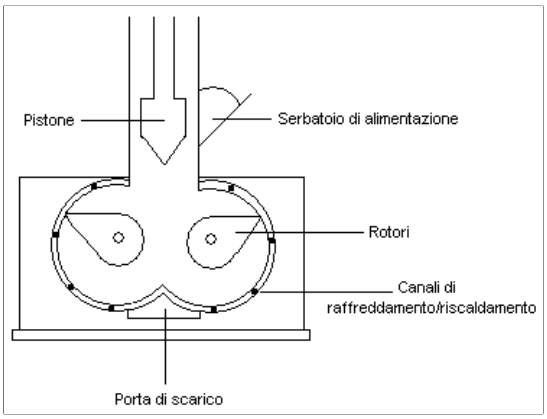


Figure 12: Mixer scheme.

The temperature set for the three heads of the mixer is 140°C, and the mixing of the components lasts 6 minutes and the rotation speed of the rotors is 20 revolutions/min.
The temperature control of the three heads and the mixing time has a fundamental importance, because temperatures and melting times too low or too high may cause, respectively, inefficient mixing or degradation of the material.

Figure 13: Thermo Scientific Haake Reomix Mixer.

2) Press - The obtained material was pressed in a press Collin - P300P (Figure 14) in order to obtain sheets of a weight of approximately 5-10 g and a thickness of one millimeters.The action of the press takes place through the following method:

1. For 180'', temperature is 140 °C and no pressure exerted.
2. For 120'' temperature remains at 140 ° C and pressure is 50 bar.
3. For 10', the pressure is brought to 10 bars.
4. The temperature gradually decreases to 30 ° C.

During cooling the material is maintained under pressure in order to avoid the occurrence of the thermal shrinkage phenomenon. The sheets thus obtained are cut into thin strips and then pelletized. The pellets fed into the extruder.

Figure 14: Collin P300P Press.

3) Extrusion - The extruder is a machine used for molding polymeric materials. It consists in a screw inside a hollow cylinder placed in rotation by a motor; the cylinder walls are temperature-controlled, and thanks to the joint action of the rotating screw and heat, the fed material is plasticized, pressurized, and is pushed up to "die" which gives it the shape (Figure 15).

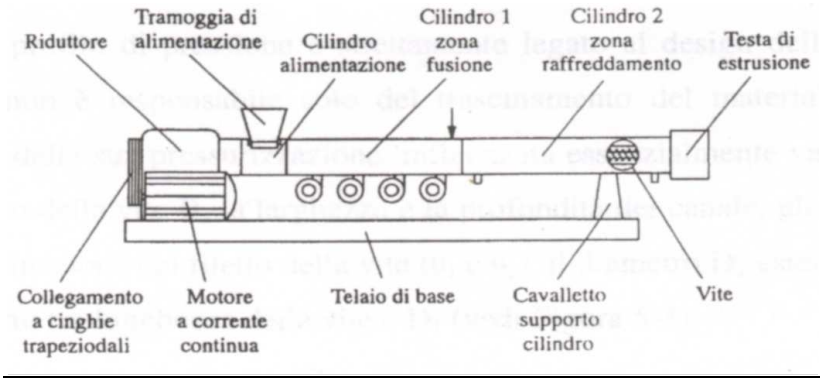


Figure 15: Schematic representation of an extruder.

Polymeric materials and additives in the form of granules, chopped, powders and liquids, can also be fed in the extruder, through the hopper, as in the mixer. The fusion process of the material takes place in the extruder section which follows the supply section; overcome this phase, the material proceeds toward the die driven by the screw rotation and subjected to a pressure variable and dependent on the profile of the screw itself. The profile of the screw is made as a function of the pressure profile obtained in the extruder; the final screw section (metering), for example, is designed to reach the value of higher pressure, this to ensure the complete melting of the material (Figure 16).

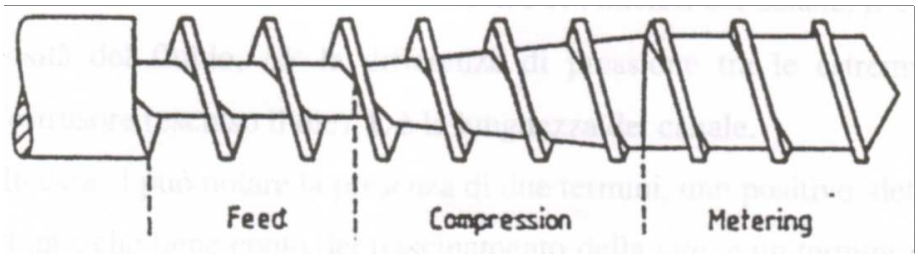


Figure 16: Screw section of a extruder.

At the die output swelling phenomena occurs, in other words the polymeric material swelled due to the action of gravity force and the entanglements deformation. To minimize this effect the die must be lengthened and the melt speed decreases. Then the polymer is pressed and stretched by a calender constituted by two cooled rotating rollers (Figure 17).

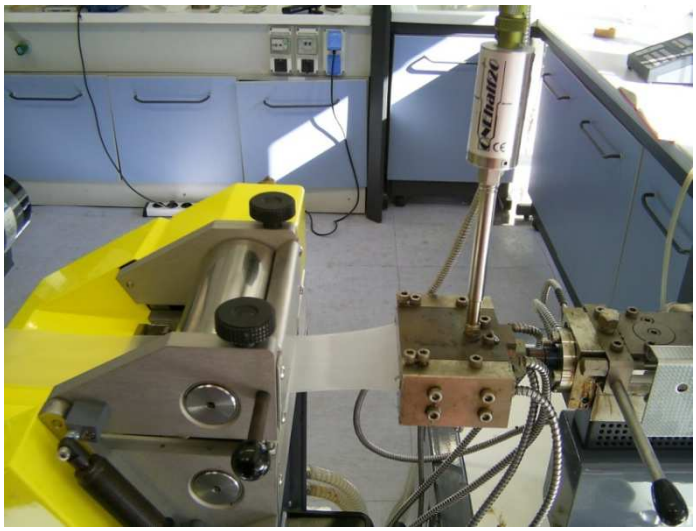


Figure 17: Calendering of LDPE polymeric film.

The extruder used is the PRISM Eurolab 16 Twin Screw Extruder manufactured by Thermo Electron Corporation (Figure 18).

Figure 18: Prism Eurolab 16 Twin Screw Extruder.

It consists in seven temperature-controlled sections, inside the rotors are co - rotating twin screw, 40cm long, with a ratio L/D equal to 24; the useful volume of the extruder is 70 cm^3. For active film production was used the temperature profile indicated in Table 2.

Die-head machine was used in order to realize thin films of the maximum thickness of 300 μm. We used the following process parameters:

- feeding hopper screw speed = 7rpm.
- extruder screw speed = 30rpm (the material protrudes from the head after a residence time about 15 minutes).
- speed of the rollers of the calender which is stretched film 2.7rpm.
- imbobinazione speed varies between 1.9 and 2.5rpm
- the distance between the die and the rollers is of 11cm.

In order to obtain a good film is often necessary to control the pressure inside the extruder, which can oscillate between 25 and 40 bar.

Table 2: Temperature profile setting in the extruder.

Section 1	Section 2	Section 3	Section 4	Section 5	Section 6	Die
135°C	145°C	150°C	150°C	150°C	150°C	145°C

2.2.2 Antimicrobial film

2.2.2.1 Ion Exchange Mechanism

Ion exchange basically is a chemical reaction between ions in solution and ions in an insoluble phase. In ion exchange, certain ions are removed by the ion exchange solid, since electro neutrality must be maintained, the solid releases ions to the solution. Ion exchangers, by common definition, are insoluble solid materials, which carry exchangeable cations or anions. These ions can be exchanged for a stoichiometrically equivalent amount of other ions of the same sign when the ion exchanger is in contact with an electrolyte solution. Carriers of exchangeable cations are called cation exchangers, and carriers of anion exchangers, anion exchangers. Certain materials are capable of both cation and anion exchange. These are called amphoteric ion exchangers.

A typical cation exchange is:

$$2\ \underline{NaX} + CaCl_2(aq) \leftrightarrows \underline{CaX_2} + 2NaCl(aq)$$

A typical anion exchange is:

$$2\ \underline{XCl} + Na_2SO_4(aq) \leftrightarrows \underline{X_2SO_4} + 2NaCl\ (aq)$$

X represents a structural unit of the ion exchanger; solid phases are underlined; aq indicates that the electrolyte is in aqueous solution.

Ion exchange resembles sorption in that, in both cases a dissolved species is taken up by a solid. The characteristic difference between the two phenomena is that ion exchange, in contrast to sorption, is a stoichiometric process. Each ion, which is removed from the solution, is replaced by an equivalent amount of another ionic species of the same sign. In sorption, on the other hand, a solute (an electrolyte or nonelectrolyte) is taken up without being replaced by another species. That is, adsorption is the enrichment of one or more components in an interfacial layer (adsorbent surface + adsorption space), and it may also be used to denote the process in which adsorptive molecules are transferred to and accumulated in the interfacial layer (Helfferich, 1962). Ion exchangers owe their characteristic properties to a peculiar feature of their structure. They consist of a framework, which is held together by chemical bonds or lattice energy. This framework carries a (+) or (-) electric surplus charge which is compensated by ions of opposite sign, the counter ions. They are free to move within the framework and can be replaced by other ions of the same sign. The counter

- ion content of the ion exchanger - the so-called ion exchange capacity - is a constant, which is given solely by the magnitude of the framework charge and is independent of the nature of the counter- ion.

As a rule the pores are occupied not only by counter ions but also by solvent and solutes, which can enter the pores when the ion exchanger is in contact with a solution.

2.2.2.2 Ion Exchange in Montmorillonite

Various clays, zeolites and alumosilicate minerals with cation–exchange properties are known. Among the different alternative ion exchangers; montmorillonite have some advantages compared with the conventional organic resin types. They have a 3 dimensional framework structure with cavities in which the counter ions can move.

The primary features of montmorillonite can be listed as:

1. They consist of uniform molecular sized cavities through which cations diffuse in order to undergo exchange in sites, within the crystal.
2. Most clays do not undergo any appreciable dimensional change with ion exchange due to their 3 dimensional framework structure.
3. The aluminum content, that is the number of tetrahedrally oriented aluminum atoms per unit cell of framework, defines the maximum number of (-) charges available to cations.
4. Ion selectivity shown by specific clays for one cation over another is unusual and does not follow the typical rules that can be shown by other inorganic and organic exchangers.

The cation exchange behavior of montmorillonite depends upon:

1. Nature of cation species, cation size, and cation charge
2. Temperature
3. Concentration of cation species in solution
4. Anion species associated with the cation in solution
5. The solvent
6. The structural characteristics

The capacity of ion exchangers is defined in terms of the number of inorganic groups in the material and is usually given in milliequivalents per gram of dry H^+ form or Cl^- form of the resin, or

per milliliter of swollen resin bed. The capacity, when defined in this way, is a characteristic constant of the material. The common ion-exchange resins have capacities between 2 and 10 meq. /g (Helfferrich, 1962).

Ion exchangers can distinguish between different counter ions. When counter ions are exchanged, the ion exchanger usually takes up or retains counter ions in preference to others. This selectivity can arise from one or several of the following physical causes. The Donnan potential, a purely electrostatic effect, results in a preference for the counter ion of higher valence (electroselectivity), particularly when the ion–exchange capacity is high and the external solution is dilute. Specific interactions between a counter ion and the fixed ionic groups, formation of ion pairs or strong complexes, result in a preference for this ion. In resins and other gels, the tendency of the elastic matrix to contract results in a preference for the smaller ion, which causes less swelling. The selectivity of mesostrusture silica, is chiefly determined by lattice forces and by steric effects such as sieve action and space requirements of the (nonsolvated) counter ions. The selectivity of ion exchangers is also affected by interactions in the external solution, particularly by complex formation of the counter ions with the co–ion. The counter ion, which forms the weaker complex, is preferred. Thus, by addition of a complexing agent to the solution, the selectivity of a given ion exchanger can be enhanced or varied.

Ion exchange is a diffusion process. Its mechanism is a redistribution of the counter ions by diffusion. The co – ion has relatively little effect on the kinetics and the rate of ion exchange. The rate determining step in ion – exchange is interdiffusion of the exchanging counter ions either within the ion exchanger itself (particle diffusion) or in an adherent liquid, ‘film’ which is not affected by agitation of the solution (film diffusion).

Film diffusion control is favored by high capacity, low degree of cross linking, and small particle size of the ion exchanger; by low concentration and weak agitation of the solution; and by preference of the ion exchanger for the counter ion which is taken up from the solution. A simple criterion can be used for predicting whether particle or film diffusion will be rate–controlling under a given set of conditions.

Particle diffusion controlled exchange is more rapid when the counter ion, which is initially in the ion exchanger, is the faster one. For film diffusion controlled exchange, the opposite holds. The counter ion, which is preferred by the ion exchanger, is taken up at the higher rate and released at the lower rate. Factors which favor high rates are high counter ion mobilities, small particle size and

low degree of cross linking of the ion exchanger, presence of the ion exchanger for the counter ion which is taken up, high concentration and efficient agitation of the solution, and elevated temperatures.

2.2.2.3 Antimicrobial Particles by Ion Exchange Process

It has been known that certain materials such as silver, copper, and zinc or their compounds are effective as antimicrobial agents. Silver is one of the most commonly used metal possessing the highest antimicrobial activity.

Some studies describe the antimicrobial compositions in which montmorillonite particles are supports for antimicrobial metal ions (Malachova *et al.*, 2009, Magaña *et al.*,2008, Praus *et al.*, 2008, Darroudi *et al.*, 2009). By treating montmorillonite with solutions of metal ions, a desired antimicrobial metal ion can be substituted in the clays structure.

In fact, silver-montmorillonite (Ag-MMT) nanoparticles were prepared by ion exchanged reaction. Firstly an amount of 5 g of Na-MMT were dispersed in 100 ml of a 0.2 M NaCl solution for 4 h while stirring. The solid was then separated by centrifugation at a speed of 10000 rpm for about 15 min and then washed with deionized water for three times. The washed Na-MMT was brought in contact with silver nitrate ($AgNO_3$) solutions at different concentration. In particular, Na-MMT was dispersed firstly in a 500 ppm $AgNO_3$ solution, at 70°C for 3 hours under stirring, covering the top and side of the beaker in order to prevent the UV room light exposure. The solid and liquid parts of the slurry were separated by centrifugation at a speed of 10000 rpm for 15 min. Afterwards, the collected solid part was brought in contact with 1000 and 5000 ppm $AgNO_3$ solutions following the procedure previously described. Finally, the collected sediment was washed with deionized water for three times and then allowed to dry overnight in a vacuum oven at 80°C. Dried samples were ground until a homogeneous powder was obtained.

2.2.2.4 Active film preparation

The chitosan film for casting were produced through thefollowing steps:

- A chitosan solution was prepared by dissolving 2 g of CS powder in 100 ml of aqueous acetic acid solution (1%, v/v), using a magnetic stirring plate at 90°C and 150rpm for 20 min and then cooled to room temperature.
- Both free chitosan and nanocomposite films were plasticized with glycerol by adding glycerol (25% (wt/wt) on solid CS) to the CS solution, while stirring for 20 min at 60 ◦C.
- Nanocomposite samples were obtained by dispersing selected amounts of clay (3 and 10% (wt/wt) on solid CS) in 100 ml of 1% (v/v) aqueous acetic acid solution for 1 h at room temperature.
- This dispersion was added to the CS solution, stirred for 1 h at room temperature and then for 30 min at 25°C in an ultrasonic bath.
- The dispersion was then poured onto glass discs (diameter = 14 cm) and dried at ambient conditions (*T* = 22°C and RH= 53%) for 3 days, until the water was completely evaporated.
- The cast films were then dried overnight in a vacuum oven at 25°C.

The chitosan and nanocomposite films (Figure 19) produced (average thickness 120±5mm) were respectively coded as follows:

- CS for free chitosan film ,
- CS/3MMT for nanocomposite film containing 3%wt/wt of Ag MMT powder
- CS/10MMT/GLY for nanocomposite film containing 10%wt/wt of Ag MMT powder .

Figure 19: Chitosan casting film.

2.3 PARTICLE AND FILM CHARACTERIZATION

2.3.1 Particle Characterization

2.3.1.1 Fourier transform infrared (FT-IR)

FT-IR stands for Fourier Transform InfraRed, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful to:

• identify unknown materials

• determine the quality or consistency of a sample

The normal instrumental process is as follows:

1. **The Source:** Infrared energy is emitted from a glowing black-body source. This beam passes through an aperture which controls the amount of energy presented to the sample (and, ultimately, to the detector).
2. **The Interferometer:** The beam enters the interferometer where the “spectral encoding” takes place. The resulting interferogram signal then exits the interferometer.
3. **The Sample:** The beam enters the sample compartment where it is transmitted through or reflected off of the surface of the sample, depending on the type of analysis being accomplished. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed.
4. **The Detector:** The beam finally passes to the detector for final measurement. The detectors used are specially designed to measure the special interferogram signal.

5. **The Computer:** The measured signal is digitized and sent to the computer where the Fourier transformation takes place. The final infrared spectrum is then presented to the user for interpretation and any further manipulation.

Because there needs to be a relative scale for the absorption intensity, a **background** spectrum must also be measured. This is normally a measurement with no sample in the beam. This can be compared to the measurement with the sample in the beam to determine the "percent transmittance." This technique results in a spectrum which has all of the instrumental characteristics removed. Thus, all spectral features which are present are strictly due to the sample. A single background measurement can be used for many sample measurements because this spectrum is characteristic of
the **instrument** itself.

Fourier transform infrared (FT-IR) spectra were performed by using a Nicolet FT-IR spectrometer (Figure 20)

Figure 20: Nicolet FT-IR spectrophotometer.

2.3.1.2 Thermo-Gravimetric Analysis (TGA)

The thermo-Gravimetric analysis (TGA) is a measurement method in which the product is continuous record of sample mass changes, in a controlled atmosphere and as a function of temperature or time. The result of the analysis is usually expressed with a graph, which shows on the abscissa the temperature or the time and on the ordinate the variation of mass as an absolute value or percentage; this chart is also defined curve of thermal decomposition.

The instrumentation used for the thermogravimetry is composed of four parts:

- a thermal balance;
- an oven;
- a purge gas system that ensures an inert or reactive environment, depending on the case;
- a computer for controlling the instrument.

The scales available for those types of analysis have operating range of between 5-20 mg. Thermobalance in the sample is placed in the oven, while the rest is thermally insulated. A variation of mass of the sample produces a deflection yoke that goes to interpose a shutter between the lamp and one of the two photodiodes. The consequent variation of the current of the photodiode is amplified and sent to a coil located between the poles of a permanent magnet. The magnetic field generated by the current in the coil shows the yoke in its original position. The current is amplified by the photodiodes is measured and translated into a mass or mass loss through the processing system.

The oven operates normally in the range between room temperature and 1500°C, with a heating rate that can be varied from little more than zero up to 200°C per minute.

Usually nitrogen or argon are used to purge the oven and prevent oxidation of the sample. In other cases it can also feed oxygen if you want to study oxidation. The presence, inside the oven, of an inert atmosphere favors the dispersion of the gas decomposition of the sample, which would otherwise be hampered by the saturation of the environment.

The information obtainable with the thermogravimetric method are limited compared to other thermal methods such as differential thermal analysis (Differential Thermal Analysis or DTA) or differential scanning calorimetry (Differential Scanning Calorimetry or DSC). This type of analysis is therefore limited to the study of phenomena of decomposition, oxidation, loss of the

crystallization solvent, The thermogravimetric chromatogram provide information on the molecules decomposition mechanisms and kinetics, so that they can be used for the substances recognition.

The thermo gravimetric analysis of the films produced has been performed with using the sponge platinum (Figure 21); the measurement was performed using the following method:

- with the aid of the instrument thermobalance were weighed approximately 8mg of films
- the sample has been equilibrated at 40°C
- the sample was heated from 40°C to 700°C with a speed of 10°C / min.

Thermo-Gravimetric analysis (TGA) was carried out on a TGA 2950 thermobalance (TA Instruments) (Figure 21).

Figure 21: TGA 2950 thermobalance, TA Instruments (Source: TA Instrument, website).

2.3.1.3 N_2 Adsorption/Desorption Analysis

Gas adsorption manometry is the method generally used for the determination of adsorption isotherms of nitrogen at the temperature of liquid nitrogen (~77 K). This type of approach was known as a 'volumetric determination' (or alternatively as the 'BET volumetric method') since it originally involved the measurement of gas volumes, before and after adsorption. However, it has been pointed out (Roquerol *et al.*, 1999) that it is no longer appropriate to use the term 'volumetric'

since the amount adsorbed is now generally evaluated by measuring the change of gas pressure, rather than a change in gas volume.

Two different operational procedures can be used for the determination of the adsorption isotherm. The conventional technique makes use of a discontinuous, point-by-point procedure. Successive amounts of the adsorptive are introduced and at each stage the system is allowed sufficient time to attain equilibrium, which of course corresponds to a series of single points on the adsorption isotherm. The continuous approach is more recent and is dependent on the principle of 'quasi-equilibrium' (Roquerol, 1972). In this case, the introduction of the adsorptive must be slow enough to provide a continuous 'equilibrium' isotherm (i.e. with an infinite number of points). If properly used, the continuous manometric procedure has the great advantage that it is possible to reveal inconspicuous features (e.g. sub-steps), which may not be detectable by the discontinuous method (Roquerol, 1972).

In general, commercial manometric equipment has not been designed for measurements at very low relative pressures. Since the filling of micropores of molecular dimensions (i.e. ultramicropores) takes place at $p/p^0<10^{-4}$, it is necessary to undertake high-resolution adsorption (HRADS) measurements (Roquerol *et al.*, 1999; Kelly *et al.* and Llewellyn *et al.*, 2000) to investigate 'primary micropore filling'.

As is well known, the BET theory (Brunauer et al.) is based on an over-simplified model of physisorption (Gregg *et al.*, 1982). As in the Langmuir theory, the adsorbent surface is pictured as an array of equivalent sites on which molecules are adsorbed in a random manner. It is assumed that the occupation probability of a site is independent of the occupancy of neighbouring sites and that there are no lateral interactions between the adsorbed molecules (i.e. the ideal localised monolayer). The molecules in the first layer act as sites for molecules in the second layer; these in turn are sites for molecules in the third layer and so on for molecules in the higher layers. Although no lateral interactions are allowed, all layers above the first are assumed to have liquid-like properties.

In view of the artificial nature of the BET theory, it is not surprising to find that the range of applicability of the BET equation is always limited to a part of the nitrogen isotherm. The best fit rarely extends above $p/p^0\sim0.30$ and with some adsorbents (e.g. graphitised carbons) the upper limit is at $p/p^0<0.1$. It is evident that the location and extent of the linear region of a BET plot is dependent on the adsorption system (both adsorbent and adsorptive) and the operational temperature. In view of this situation, it is strongly recommended (Roquel *et al*., 1999) that the BET

monolayer capacity, n_m, should be derived from the best linear fit for that part of the isotherm which includes Point B.

A high value of the parameter C, which is associated with a sharp Point B, is an indication of strong adsorbent–adsorbate interactions. Typical C values in the range 80–150 for nitrogen at 77 K are consistent with the formation of well-defined monolayers on many non-porous and mesoporous adsorbents (Roquel *et al.*, 1999; Gregg *et al.*, 1982).

The second stage in the application of the BET method is the calculation of the specific surface area, a(BET), from n_m. The evaluation of a(BET) is, of course, dependent on the average area, σ, occupied by each molecule in the completed monolayer. In the case of nitrogen at 77 K, $\sigma(N_2)$ is usually taken as 0.162 nm^2, this value was originally proposed by Emmett and Brunuaer and was based on the assumption that the monolayer had the liquid form of close-packed structure. However, other investigators have proposed various alternative values, extending over the range $\sigma(N_2)$=0.13−0.20 nm^2 (Gregg *et al.*, 1982; Roquel *et al.*, 1999) and we are left with an apparently confused picture.

The following are inherent difficulties of the BET-method — (a) the validity of n_m is questionable; (b) the monolayer structure is not the same on all surfaces; (c) strong adsorption at very low p/p^0 may involve localised monolayer coverage and/or primary micropore filling (i.e. in pores of molecular dimensions). In principle, it is not difficult to establish whether there is a significant micropore filling contribution, but the true value of $\sigma(N_2)$ is usually unknown, especially when the adsorbent surface is heterogeneous.

Figure 22: Micromeritics ASAP 2020 (Source: LabCommerce, Inc., website).

N_2 adsorption/desorption analysis were carried out at 77K using a Micromeritics ASAP 2020 (Figure 22).

2.3.1.4 UV/Vis Spectroscopy

Ultra**V**iolet–**Vis**ible **S**pectroscopy or **U**ltra**V**iolet-**Vis**ible **S**pectrophotometry (**UV-Vis** or **UV/Vis**) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state. The principle by means UV/Vis Spectroscopy act is the following: molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons, the longer the wavelength of light it can absorb.

UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied.

- Solutions of transition metal ions can be colored (i.e., absorb visible light) because d electrons within the metal atoms can be excited from one electronic state to another. The colour of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For instance, the colour of a dilute solution of copper sulfate is a very light blue; adding ammonia intensifies the colour and changes the wavelength of maximum absorption (λ_{max}).
- Organic compounds, especially those with a high degree of conjugation (e.g. DNA, RNA, protein), also absorb light in the UV or visible regions of the electromagnetic spectrum. The solvents for these determinations are often water for water-soluble compounds, or ethanol

for organic-soluble compounds. (Organic solvents may have significant UV absorption; not all solvents are suitable for use in UV spectroscopy. Ethanol absorbs very weakly at most wavelengths.) Solvent polarity and pH can affect the absorption spectrum of an organic compound. Tyrosine, for example, increases in absorption maxima and molar extinction coefficient when pH increases from 6 to 13 or when solvent polarity decreases.

- While charge transfer complexes also give rise to colours, the colours are often too intense to be used for quantitative measurement.

The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how quickly the absorbance changes with concentration. This can be taken from references (tables of molar extinction coefficients), or more accurately, determined from a calibration curve.

A UV/Vis spectrophotometer may be used as a detector for HPLC. The presence of an analyte gives a response assumed to be proportional to the concentration. For accurate results, the instrument's response to the analyte in the unknown should be compared with the response to a standard; this is very similar to the use of calibration curves. The response (e.g., peak height) for a particular concentration is known as the response factor.

The wavelengths of absorption peaks can be correlated with the types of bonds in a given molecule and are valuable in determining the functional groups within a molecule. The Woodward-Fieser rules, for instance, are a set of empirical observations used to predict λ_{max}, the wavelength of the most intense UV/Vis absorption, for conjugated organic compounds such as dienes and ketones. The spectrum alone is not, however, a specific test for any given sample. The nature of the solvent, the pH of the solution, temperature, high electrolyte concentrations, and the presence of interfering substances can influence the absorption spectrum. Experimental variations such as the slit width (effective bandwidth) of the spectrophotometer will also alter the spectrum. To apply UV/Vis spectroscopy to analysis, these variables must be controlled or accounted for in order to identify the substances present.

The instrument used in ultraviolet-visible spectroscopy is called a UV/Vis spectrophotometer. It measures the intensity of light passing through a sample (*I*), and compares it to the intensity of

light before it passes through the sample (I_0). The ratio I/I_0 is called the *transmittance*, and is usually expressed as a percentage (*%T*). The absorbance, *A*, is based on the transmittance:

$$A = -log\left(\frac{\%T}{100\%}\right)$$

The UV-visible spectrophotometer can also be configured to measure reflectance. In this case, the spectrophotometer measures the intensity of light reflected from a sample (*I*), and compares it to the intensity of light reflected from a reference material (I_0) (such as a white tile). The ratio is called the *reflectance*, and is usually expressed as a percentage (*%R*).

The basic parts of a spectrophotometer are a light source, a holder for the sample, a diffraction grating in a monochromator or a prism to separate the different wavelengths of light, and a detector. The radiation source is often a Tungsten filament (300-2500nm), a deuterium arc lamp, which is continuous over the ultraviolet region (190-400nm), Xenon arc lamp, which is continuous from 160-2000nm; or more recently, light emitting diodes (LED) for the visible wavelengths. The detector is typically a photomultiplier tube, a photodiode, a photodiode array or a charge-coupled device (CCD). Single photodiode detectors and photomultiplier tubes are used with scanning monochromators, which filter the light so that only light of a single wavelength reaches the detector at one time. The scanning monochromator moves the diffraction grating to "step-through" each wavelength so that its intensity may be measured as a function of wavelength. Fixed monochromators are used with CCDs and photodiode arrays. As both of these devices consist of many detectors grouped into one or two dimensional arrays, they are able to collect light of different wavelengths on different pixels or groups of pixels simultaneously.

A spectrophotometer can be either *single beam* or *double beam*. In a single beam instrument, all of the light passes through the sample cell. I_0 must be measured by removing the sample (Figure 23). This was the earliest design and is still in common use in both teaching and industrial labs. In a double-beam instrument, the light is split into two beams before it reaches the sample. One beam is used as the reference; the other beam passes through the sample. The reference beam intensity is taken as 100% Transmission (or 0 Absorbance), and the measurement displayed is the ratio of the two beam intensities. Some double-beam instruments have two detectors (photodiodes), and the sample and reference beam are measured at the same time. In other instruments, the two beams pass through a beam chopper, which blocks one beam at a time. The detector alternates between measuring the sample beam and the reference beam in synchronism with the chopper. There may

also be one or more dark intervals in the chopper cycle. In this case, the measured beam intensities may be corrected by subtracting the intensity measured in the dark interval before the ratio is taken.

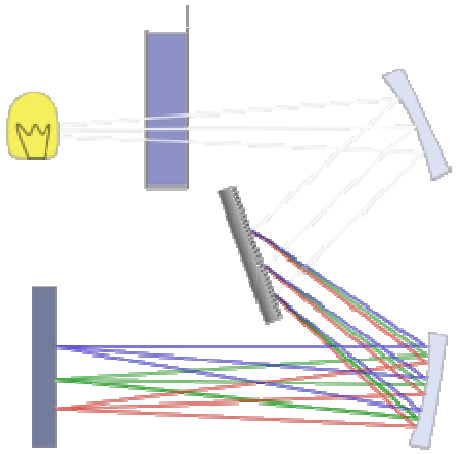


Figure 23: Diagram of a single-beam UV/Vis spectrophotometer (Source: Wikipedia).

Samples for UV/Vis spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, known as a cuvette. Cuvettes are typically rectangular in shape, commonly with an internal width of 1cm. (This width becomes the path length, *L*, in the Beer-Lambert law). Test tubes can also be used as cuvettes in some instruments. The type of sample container used must allow radiation to pass over the spectral region of interest. The most widely applicable cuvettes are made of high quality fused silica or quartz glass because these are transparent throughout the UV, visible and near infrared regions. Glass and plastic cuvettes are also common, although glass and most plastics absorb in the UV, which limits their usefulness to visible wavelengths.

Specialized instruments have also been made. These include attaching spectrophotometers to telescopes to measure the spectra of astronomical features. UV-visible microspectrophotometers consist of a UV-visible microscope integrated with a UV-visible spectrophotometer.

A complete spectrum of the absorption at all wavelengths of interest can often be produced directly by a more sophisticated spectrophotometer. In simpler instruments the absorption is determined one wavelength at a time and then compiled into a spectrum by the operator. By removing the concentration dependence, the extinction coefficient (ε) can be determined as a function of wavelength.

UV-visible absorption spectra were measured with a Lambda 850 spectrometer (Perkin Elmer) (Figure 24).

Figure 24: Perkin Elmer Lambda 850 spectrometer (Source: University of Leicester, website).

2.3.1.4 X-Ray Analysis

Small-angle X-ray scattering (SAXS) is a small-angle scattering (SAS) technique where the elastic scattering of X-rays (wavelength 0.1…0.2 nm) by a sample which has inhomogeneities in the nm-range, is recorded at very low angles (typically 0.1-10°). This angular range contains information about the shape and size of macromolecules, characteristic distances of partially ordered materials, pore sizes, and other data. SAXS is capable of delivering structural information of macromolecules between 5 and 25nm, of repeat distances in partially ordered systems of up to 150nm. USAXS (ultra-small angle X-ray scattering) can resolve even larger dimensions.SAXS and USAXS belong to a family of X-ray scattering techniques that are used in the characterization of materials. In the case of biological macromolecules such as proteins, the advantage of SAXS over crystallography is that a crystalline sample is not needed. Nuclear magnetic resonance spectroscopy methods encounter problems with macromolecules of higher molecular mass (> 30-40 kDa). However, owing to the random orientation of dissolved or partially ordered molecules, the spatial averaging leads to a loss of information in SAXS compared to crystallography.

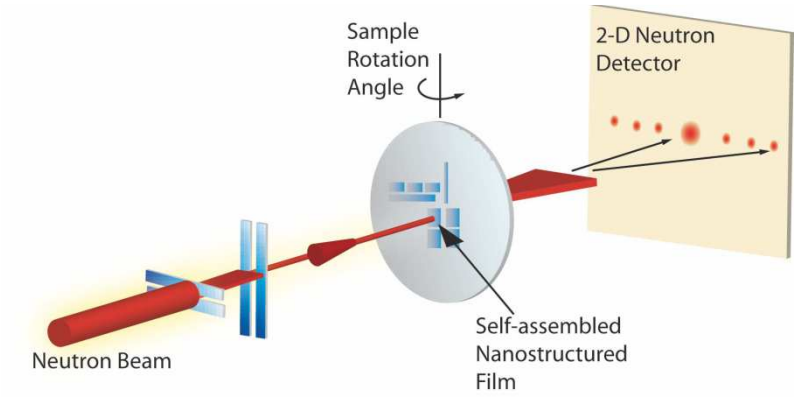


Figure 25: Diagram of monochromatic beam of X-Ray (Source: NIST, website).

In an SAXS instrument a monochromatic beam of X-rays is brought to a sample from which some of the X-rays scatter, while most simply go through the sample without interacting with it. The scattered X-rays form a scattering pattern which is then detected at a detector which is typically a 2-dimensional flat X-ray detector situated behind the sample perpendicular to the direction of the primary beam that initially hit the sample. The scattering pattern contains the information on the structure of the sample (Figure 25). The major problem that must be overcome in SAXS instrumentation is the separation of the weak scattered intensity from the strong main beam. The smaller the desired angle, the more difficult this becomes. The problem is comparable to one encountered when trying to observe a weakly radiant object close to the sun, like the sun's corona. Only if the moon blocks out the main light source does the corona become visible. Likewise, in SAXS the non-scattered beam that merely travels through the sample must be blocked, *without* blocking the closely adjacent scattered radiation. Most available X-ray sources produce *divergent* beams and this compounds the problem. In principle the problem could be overcome by *focusing* the beam, but this is not easy when dealing with X-rays and was previously not done except on synchrotrons where large bent mirrors can be used. This is why most laboratory small angle devices rely on collimation instead. Laboratory SAXS instruments can be divided into two main groups: point-collimation and line-collimation instruments:

1. **Point-collimation instruments** have pinholes that shape the X-ray beam to a small circular or elliptical spot that illuminates the sample. Thus the scattering is centro-symmetrically

distributed around the primary X-ray beam and the scattering pattern in the detection plane consists of circles around the primary beam. Owing to the small illuminated sample volume and the wastefulness of the collimation process — only those photons are allowed to pass that happen to fly in the right direction — the scattered intensity is small and therefore the measurement time is in the order of hours or days in case of very weak scatterers. If focusing optics like bent mirrors or bent monochromator crystals or collimating and monochromating optics like multilayers are used, measurement time can be greatly reduced. Point-collimation allows the orientation of non-isotropic systems (fibres, sheared liquids) to be determined.

2. **Line-collimation instruments** confine the beam only in one dimension so that the beam profile is a long but narrow line. The illuminated sample volume is much larger compared to point-collimation and the scattered intensity at the same flux density is proportionally larger. Thus measuring times with line-collimation SAXS instruments are much shorter compared to point-collimation and are in the range of minutes. A disadvantage is that the recorded pattern is essentially an integrated superposition (a self-convolution) of many pinhole adjacent pinhole patterns. The resulting smearing can be easily removed using model-free algorithms or deconvolution methods based on Fourier transformation, but only if the system is isotropic. Line collimation is of great benefit for any isotropic nanostructured materials, e.g. proteins, surfactants, particle dispersion and emulsions.

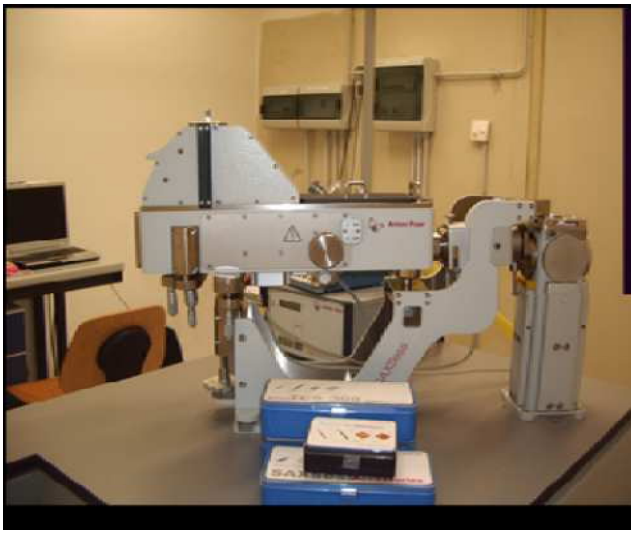


Figure 26: SAXSess apparatus by Anton Paar.

During the experiment step, the structural characterization of the inorganic samples were carried out by means of Small Angle X-ray Scattering (SAXS) analysis. SAXS spectra were performed using an Anton Paar SAXSess camera equipped with a 2D imaging plate detector. CuKα X-Rays with 1.5418 Å wavelength were generated by a Philips PW3830 sealed tube source (40 kV, 50 mA) and slit collimated (Figure 26).

As concerned antimicrobial substances the structures of Na-MMT and and Ag-MMT clays were evaluated using wide angle X-ray diffraction (WAXD) measurements. Wide-angle X-ray scattering is the same technique as small-angle X-ray scattering (SAXS) only the distance from sample to the detector is shorter and thus diffraction maxima at larger angles are observed. Depending on the measurement instrument used it is possible to do WAXS and SAXS in a single run (small- and wide-angle scattering, SWAXS). The spectra were collected in the transmission mode (i.e., the X-ray beam moves through the sample) by scanning the 2θ (θ is the diffraction peak angle) range between 1.5 and 40°.

All scattering data were dark current and background subtracted, and normalized for the primary beam intensity. In order to remove the inelastic scattering from the data, the SAXS profiles were additionally corrected for both the Porod constant and desmearing effect.

2.3.1.5 Trasmission Electron Microscopy

Transmission **E**lectron **M**icroscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera.

TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small de Broglie wavelength of electrons. This enables the instrument's user to examine fine detail-even as small as a single column of atoms, which is tens of thousands times smaller than the smallest resolvable object in a light microscope. TEM forms a major analysis method in a range of scientific fields, in both physical and biological sciences. TEMs find

application in cancer research, virology, materials science as well as pollution, nanotechnology, and semiconductor research.

At smaller magnifications TEM image contrast is due to absorption of electrons in the material, due to the thickness and composition of the material. At higher magnifications complex wave interactions modulate the intensity of the image, requiring expert analysis of observed images. Alternate modes of use allow for the TEM to observe modulations in chemical identity, crystal orientation, electronic structure and sample induced electron phase shift as well as the regular absorption based imaging.

Figure 27: FEI Tecnai G12 Spirit Twin microscope (Source: FEI, website).

Transmission electron microscopy analysis was carried out by using a FEI Tecnai G12 Spirit Twin microscope operated at 120 kV (LaB6 cathode, point resolution 0.35 nm) (Figure 27). Images were recorded on a CCD camera with the resolution of 4096 × 4096 pixels. Powder samples of Ag-MMT were dispersed in distilled water and the suspension was treated with ultrasound for 2 min. A drop of a very dilute suspension was placed on a holey-carbon coated copper grid and allowed to dry by evaporation at room temperature before observation.

2.3.2 Film Charatcterization

2.3.2.1 Water Vapor Permeability

The permeability of pure gases in the various films was investigated by using a system whose operation diagram is shown in Figure 28

As observed in Figure 28, the flat film sample material is placed in a test cell. Test cells are divided into two chambers separated by the sample material. The inner chamber is filled with nitrogen (carrier gas) and the outer chamber with water vapor (test gas). Molecules of water (delivered to the outer chamber by the test gas) diffuse through the film to the inside chamber and are conveyed to the sensor by the carrier gas. This system uses a patented modulated infrared sensor to detect water vapor transmission through flat materials. This high performance sensor provides parts-per-million sensitivity.

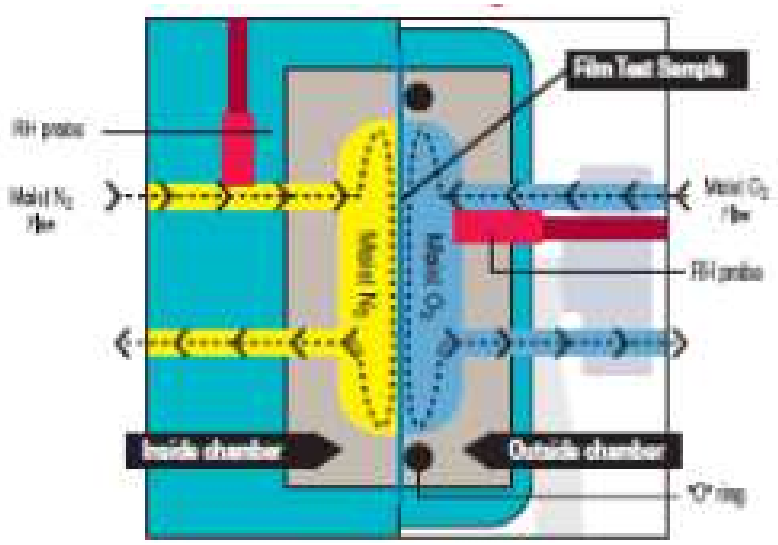


Figure 28: Operation diagram of water vapor permeability apparatus.

The computer monitors the increase in water vapor concentration in the carrier gas and it reports that value on the screen as the water vapor transmission rate. The RH of test cells is monitored by RH probes inserted into the outside chamber. When absorbent material is saturated with distilled water the system provides a test gas atmosphere of 100% RH.

Then, water vapor permeability was determined by means of Permatran, Mocon, Model W 3/31 (Figure 29).

Figure 29: Permatran, Mocon, Model W 3/31 (Source: Mocon, website).

2.3.2.2 Oxygen permeability

For oxygen permeability test the apparatus is similar to water vapor permeability one. In fact the test cell is also divided in two chambers, inside and outside one. So flat film samples are clamped into the diffusion cell, which is then purged of residual oxygen using an oxygen-free carrier gas. The carrier gas is routed to the sensor until a stable zero has been established. In this system the used sensor is a patented coulometric sensor to detect oxygen transmission through flat materials. This high performance sensor (three models available, each with distinct ranges for higher accuracy) provides parts per- billion sensitivity even in the presence of water vapor. This is an intrinsic or absolute sensor that does not require calibration. Calibration films are provided to ensure the entire system is performing to the highest precision and accuracy standards.

Pure (99.9%) oxygen is then introduced into the outside chamber of the diffusion cell. Molecules of oxygen diffusing through the film to the inside chamber are conveyed to the sensor by the carrier gas.

On the basis of this principles of operation, oxygen permeability was determined by means of Ox-Tran (Mocon, Model 2/20).

2.3.2.3 Water sorption

Water absorption is a physical phenomenon or a process in which water molecules enter some bulk phase, in this case in a solid material. This phenomenon is characterized by a swelling of the polymer matrix because when the polymer network comes in contact with aqueous solutions, the thermodynamic compatibility of the polymer chains and water causes the polymer to swell. As water penetrates inside the glassy network, the glass transition temperature of the polymer decreases and the material becomes rubbery.

For this reason chitosan films were cut in small pieces (1.2cm×1.2 cm), desiccated overnight under vacuum and weighed to determine their dry mass. The weighed films were placed in closed beakers containing 30 ml of water (pH=7) and stored at *T*=25°C. The swelling kinetics were evaluated by periodically measuring the weight increment of the samples with respect to dry films with a balance accurate to 0.0001 g, after gently bottling the surface with a tissue, until equilibrium was reached. The water gain (W.G.) was calculated as follows:

$$\mathrm{W.G.}\,(\%) = \frac{\left(m_{\mathrm{WetFilm}} - m_{\mathrm{DryFilm}}\right)}{m_{\mathrm{DryFilm}}} \times 100$$

where m_{WetFilm} and m_{DryFilm} are the weight of the wet and dry film, respectively.

2.3.2.4 Release Tests

As concerned the antioxidant film, the release test were conducted analyzing the different samples with the high pressure liquid chromatography. (HPLC).

High **P**erformance **L**iquid **C**hromatography or **H**igh **P**ressure **L**iquid **C**hromatography, (HPLC) is a type of chromatography (Figure 30) that allows to separate two or more compounds present in a solvent by exploiting the balance of affinity between a "stationary phase" placed inside of the chromatographic column is a "mobile phase" flowing through it. A substance closer to the stationary phase compared to the mobile phase takes a longer time to walk the chromatographic column (retention time), respect to a substance with a low affinity for the stationary phase and high for the mobile phase.

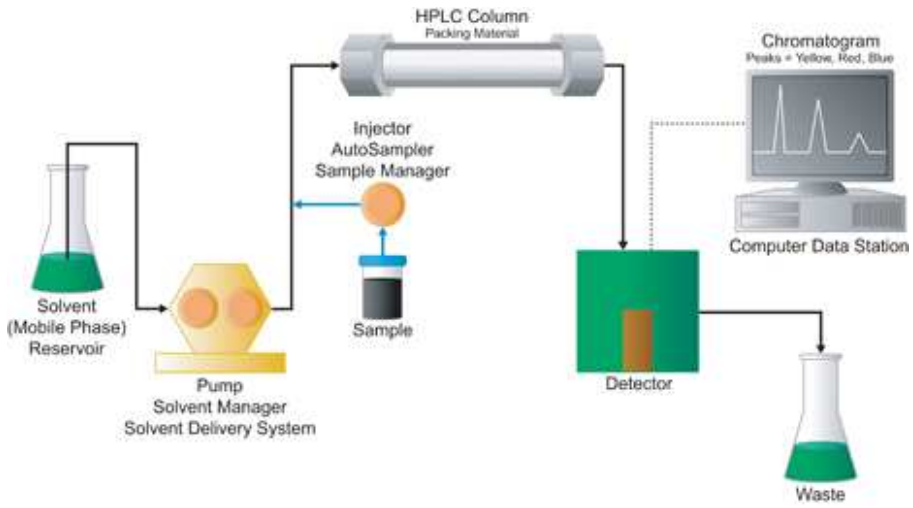


Figure 30: High Performance Liquid Chromatography apparatus (Source: Comsol, website).

Chromatography can be described as a mass transfer process involving adsorption. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with a sorbent, leading to the separation of the sample components. The active component of the column, the sorbent, is typically a granular material made of solid particles (e.g. silica, polymers, etc.), 2-50 micrometers in size. The components of the sample mixture are separated from each other due to their different degrees of interaction with the sorbent particles. The pressurized liquid is typically a mixture of solvents and is referred to as "mobile phase". HPLC is distinguished from traditional ("low pressure") liquid chromatography because operational pressures are significantly higher (50-350 bar), while ordinary liquid chromatography typically relies on the force of gravity to pass the mobile phase through the column. Due to the small sample amount separated in analytical HPLC typical column dimensions are 2.1 - 4.6 mm diameter, and 30-250 mm length. Also HPLC columns are made with smaller sorbent particles (2-5 micrometer in average particle size). This gives HPLC superior resolving power when separating mixtures, which is why it is a popular chromatographic technique.

The sample to be analyzed is injected at the beginning of the chromatographic column where it is "pushed" through the stationary phase by the mobile phase thanks to a pressures of the order of hundreds of atmospheres. To obtain high efficiency separation particle size of column filling have to be very small (usually have diameters ranging from 3 to 10 μm). For this reason, it is important to apply a high pressure to maintain an eluent reasonable flow rate and an appropriate time analysis.

At the end of the column a detector (IR, UV-VIS, spectrofluorometry, mass spectrometer) and a computer are positioned to allow a continuous analysis at the column output and to quantify and/or to identify the injected substances.

The main advantages of this technique are: the column reduced size which avoids longitudinal deviations (longitudinal movement of the mobile phase) and alternative routes problems; constant and adjustable elution rate (mobile phase through the column); decreased test speed; small compound amount required to the analysis (ranged between 5-10 μg of sample) and a greater accuracy and precision. The tool used for the analysis and the Agilent 1100 series of Hevelett Packard (Figure 31).

Figure 31: Hevelett Packard Agilent 1100 series (Source: Test Equipment Connection, website).

In these experiments, 96% v/v ethanol was used as fatty food stimulant and small pieces (~ 1,6g–1 cm^2) of α-tocopherol film were immersed in beaker containing 80 mL of chosen stimulant at 25°C. At specific intervals of time the amount of tocopherol released from the polymer films into the ethanol was monitored as a function of time until the attainmentof an asymptotic value. In detail, α- tocopherol concentration in ethanol was determined using an HPLC (Agilent Model 1100, Milan, Italy) following the chromatographic method proposed by Sirò *et al.* (2006). Before the analysis started, the instrument was calibrated injecting different known concentrations solutions of

α-tocopherol; thus it was possible to calculate the height of the peak areas and to express as a function of the concentration. The calibration curve was constructed for peak area against α-tocopherol concentration of standard solutions from 10 to 100 ppm.

As concerned the antimicrobial film, the concentration of silver released in an aqueous solution from the nanocomposite films was measured by inductively coupled plasma atomic emission spectroscopy (ICP/AES).

Inductively **C**oupled **P**lasma **A**tomic **E**mission **S**pectroscopy (ICP-AES), also referred to as **I**nductively **C**oupled **P**lasma **O**ptical **E**mission **S**pectrometry (ICP-OES), is an analytical technique used for the detection of trace metals (Figure 32). It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element (Stefánsson *et al.*, 2007; Mermet *et al.*, 2005). The intensity of this emission is indicative of the concentration of the element within the sample.

The ICP-AES is composed of two parts: the ICP and the optical spectrometer. The ICP torch consists of 3 concentric quartz glass tubes. The output or "work" coil of the radio frequency (RF) generator surrounds part of this quartz torch. Argon gas is typically used to create the plasma. When the torch is turned on, an intense electromagnetic field is created within the coil by the high power radio frequency signal flowing in the coil. This RF signal is created by the RF generator which is, effectively, a high power radio transmitter driving the "work coil" the same way a typical radio transmitter drives a transmitting antenna. The argon gas flowing through the torch is ignited with a Tesla unit that creates a brief discharge arc through the argon flow to initiate the ionization process. Once the plasma is "ignited", the Tesla unit is turned off. The argon gas is ionized in the intense electromagnetic field and flows in a particular rotationally symmetrical pattern towards the magnetic field of the RF coil. A stable, high temperature plasma of about 7000 K is then generated as the result of the inelastic collisions created between the neutral argon atoms and the charged particles. A peristaltic pump delivers an aqueous or organic sample into an analytical nebulizer where it is changed into mist and introduced directly inside the plasma flame. The sample immediately collides with the electrons and charged ions in the plasma and is itself broken down into charged ions. The various molecules break up into their respective atoms which then lose electrons and recombine repeatedly in the plasma, giving off radiation at the characteristic wavelengths of the elements involved. In some designs, a shear gas, typically nitrogen or dry compressed air is used to 'cut' the plasma at a specific spot. One or two transfer lenses are then used

to focus the emitted light on a diffraction grating where it is separated into its component wavelengths in the optical spectrometer. In other designs, the plasma impinges directly upon an optical interface which consists of an orifice from which a constant flow of argon emerges, deflecting the plasma and providing cooling while allowing the emitted light from the plasma to enter the optical chamber. Still other designs use optical fibers to convey some of the light to separate optical chambers. Within the optical chamber(s), after the light is separated into its different wavelengths (colors), the light intensity is measured with a photomultiplier tube or tubes physically positioned to "view" the specific wavelength(s) for each element line involved, or, in more modern units, the separated colors fall upon an array of semiconductor photodetectors such as charge coupled devices (CCDs). In units using these detector arrays, the intensities of all wavelengths (within the system's range) can be measured simultaneously, allowing the instrument to analyze for every element to which the unit is sensitive all at once. Thus, samples can be analyzed very quickly. The intensity of each line is then compared to previously measured intensities of known concentrations of the elements, and their concentrations are then computed by interpolation along the calibration lines. In addition, special software generally corrects for interferences caused by the presence of different elements within a given sample matrix (Figure 32).

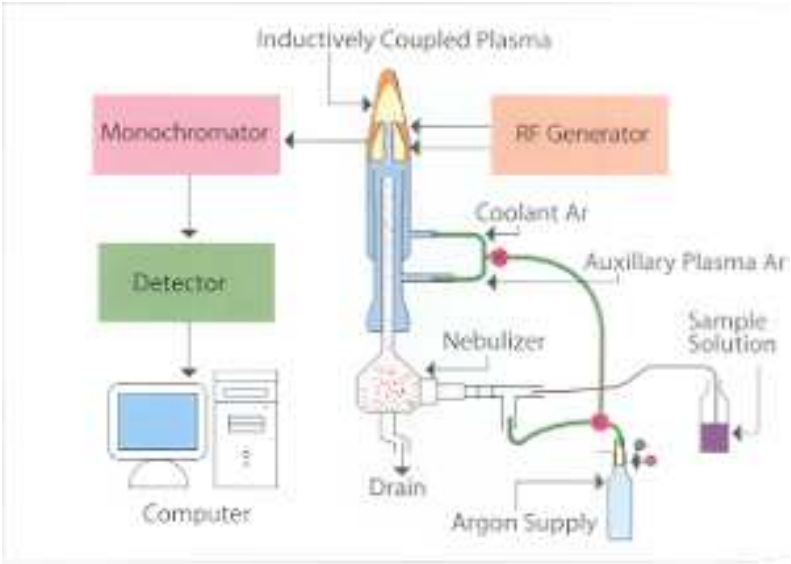


Figure 32: Inductively Coupled Plasma Atomic Emission Spectroscopy apparatus (Source: Indian Istitute of Technology, Bombay, website).

2.3.2.5 Oxygen radical adsorbance capacity (ORAC)

Oxygen **R**adical **A**bsorbance **C**apacity (ORAC) is a method of measuring antioxidant capacities in biological samples *in vitro* (Ou *et al.*, 2001; Cao *et al.*, 1993).

The assay measures the oxidative degradation of the fluorescent molecule (either beta-phycoerythrin or fluorescein) after being mixed with free radical generators such as azo-initiator compounds. Azo-initiators are considered to produce the peroxyl radical by heating, which damages the fluorescent molecule, resulting in the loss of fluorescence. Antioxidants are considered to protect the fluorescent molecule from the oxidative degeneration. The degree of protection is quantified using a fluorometer (Figure 33).

The fluorescent intensity decreases as the oxidative degeneration proceeds, and this intensity is typically recorded for 35 minutes after the addition of the azo-initiator (free radical generator). So far, AAPH (2,2'-azobis(2-amidino-propane) dihydrochloride) is the sole free-radical generator used. The degeneration (or decomposition) of fluorescein is measured as the presence of the antioxidant slows the fluorescence decay. Decay curves (fluorescence intensity vs. time) are recorded and the area between the two decay curves (with or without antioxidant) is calculated. Subsequently, the degree of antioxidant-mediated protection is quantified using the antioxidant trolox (a vitamin E analogue) as a standard. Different concentrations of trolox are used to make a standard curve, and test samples are compared to this. Results for test samples (foods) have been published as "trolox equivalents" or TEs (Huang *at al.*, 2010; Garret *et al.*, 2005).

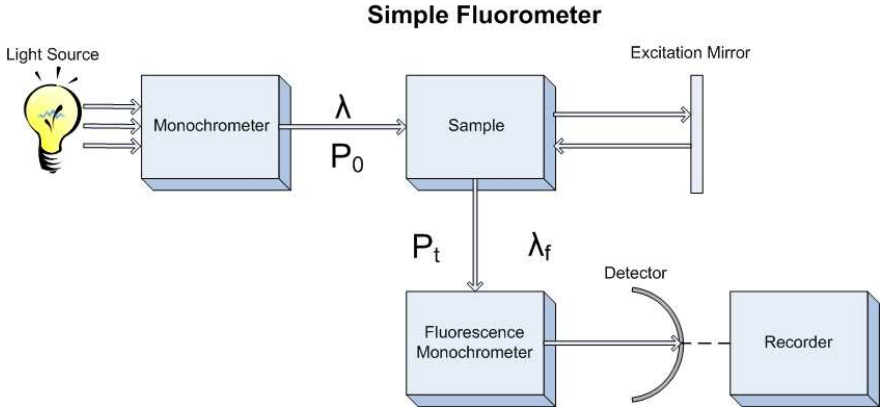


Figure 33: Diagram of fluorometer (Source: ChemWiki).

One benefit of using the ORAC method to evaluate substances' antioxidant capacities is that it takes into account samples with and without lag phases of their antioxidant capacities. This is especially beneficial when measuring foods and supplements that contain complex ingredients with various slow- and fast-acting antioxidants, as well as ingredients with combined effects that cannot be precalculated.

In this work, ORAC measurements have been performed by Dr. M. Lavorgna at Dipartimento di Scienze della Vita, Seconda Università degli Studi di Napoli as reported in our common paper (Gargiulo et al., 2012). In detail, 25 μL of tocopherol released in ethanol at 4 and 25 °C for various time (1, 3, 6, 24, 72, 168 h) by the three investigated active films were added to 25 μL of sodium phosphate buffer (0.2 M, pH 7.4) with 150 μL of fluorescein sodium salt (4 μM). The microplates (96 wells) were incubated in the fluorescent plate reader for 30 min at 37 °C. Immediately before starting the measurements at 535 nm (485 nm excitation filter, Spectra Fluor Tecan Group Ltd., Männedorf, Switzerland), 25 μL of the AAPH radical generator (153 mM) were placed in the reaction mixture. The analyzer was programmed to record the fluorescence of fluorescein every 5 min, after the addition of AAPH, for a total of 55 min. A 100 μM solution of Trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid) was utilized as standard and sodium phosphate buffer as positive control. The samples were tested three times (three independent assays) and the data reported are the average of these assays. The results, expressed as μmol Trolox Equivalents (TE)·100 mL-1 of sample (ORAC value), were calculated as follows:

$$ORAC\ values = V_{sample}\left(\frac{AUC_{sample} - AUC_{solvent\ control}}{AUC_{trolox} - AUC_{solvent\ control}}\right)$$

where V_{sample} is the volume of the sample (25 μL) and *AUC* is the area under the fluorescence decay curve.

CHAPTER 3

EXPERIMENTAL RESULTS

In this chapter the reader will be introduced to the knowledge and understanding the mechanism and the interaction occurring inside the material composites used in the thesis.

ANTIOXIDANT ACTIVE POLYMERIC FILM

3.1 NANOPARTICLES CHARACTERIZATION

Neat and tocopherol-loaded SBA-15 and SBA-15+APTES were characterized in order to verify the effectiveness of the impregnation process with tocopherol. In particular, X-Ray Diffraction (XRD), Fourier Transform-Infrared Ray (FT-IR), N_2 Adsorption/Desorption and Thermo-Gravimetric Analysis (TGA) have been performed.

3.1.1 X Ray Diffraction (XRD)

X-ray diffraction is a versatile, non-destructive analytical technique for identification and quantitative determination of the various crystalline compounds, known as 'phases', present in solid materials and powders.

Figure 34 shows the XRD patterns of non-impregnated and tocopherol-loaded SBA-15 and SBA-15+APTES mesoporous silicas. In brackets there are the Miller indices of significant lattice planes.

The spectrum of non-impregnated SBA-15 sample, reported in Fig. 34 (a), reports, as typical for this material, an intense diffraction peak corresponding to the (100) plane and two weak, well-resolved diffraction peaks corresponding to (110) and (200) planes. On the contrary, only one very broad (100) peak is presented by the amino-functionalized SBA-15+APTES samples, as reported in Fig. 34 (b). The disappearance of the other two peaks in the XRD pattern of this product demonstrates a disordered mesostructure, indicating a strongly adverse effect of APTES on the formation of SBA-15. Moreover, for both SBA-15 and SBA-15+APTES powders, the impregnation with α-tocopherol gave rise to a strong decrease in the intensity of the XRD peaks: this should be attributed to the pore-filling effects that can reduce the scattering contrast between the pores and the silica walls.

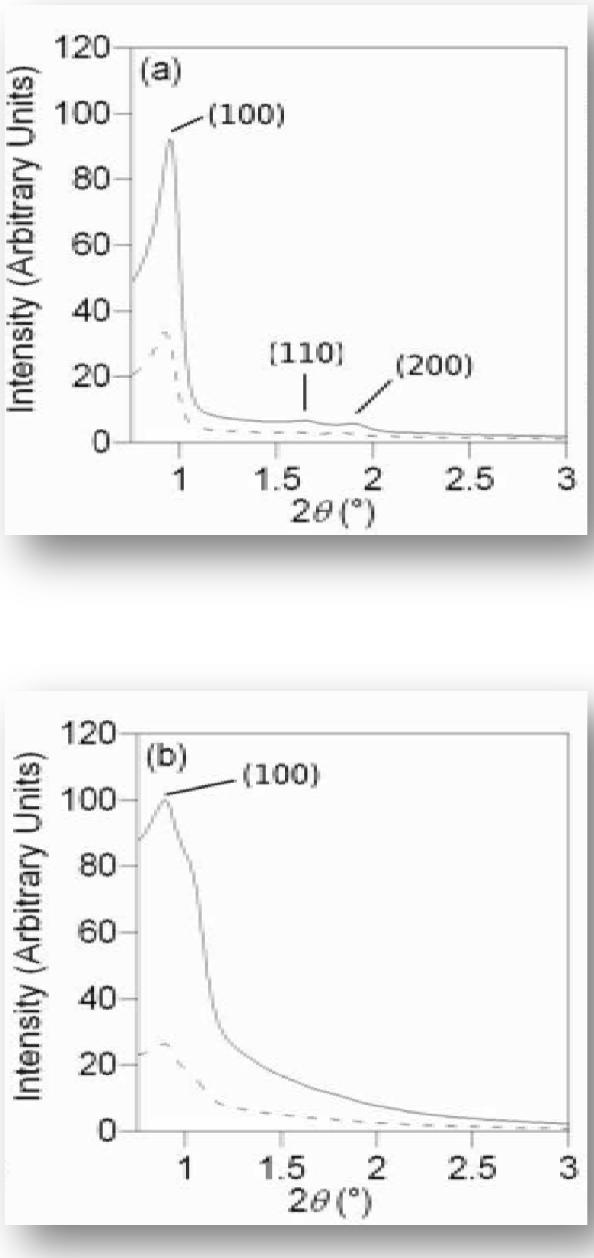


Figure 34: X-ray diffraction (XRD) patterns of: (a) non-impregnated and tocopherol-loaded SBA-15; (b) non-impregnated and tocopherol-loaded SBA-15+APTES. Solid lines: non-impregnated samples. Dashed lines: tocopherol-loaded samples.

3.1.2 Temo-gravimetric Analisys (TGA)

Thermogravimetric Analysis (TGA) measures the amount and rate of change in the weight of a material as a function of temperature or time in a controlled atmosphere. Measurements are used primarily to determine the composition of materials and to predict their thermal stability at temperatures up to 1000°C. The technique can characterize materials that exhibit weight loss or gain due to decomposition, oxidation, or dehydration.

Thermogravimetric analysis (TGA) was carried out on a thermobalance on which powder samples were heated from 100 to 1000 °C at a heating rate of 10 °C/min under air flow.

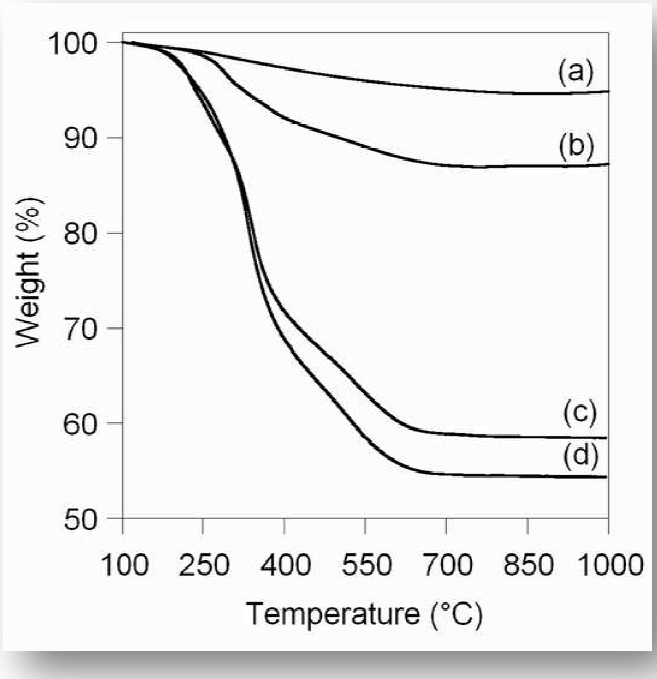


Figure 35: Thermogravimetric (TG) analyses of: (a) non-impregnated SBA-15, (b) non-impregnated SBA15+APTES, (c) tocopherol-loaded SBA-15+APTES, (d) tocopherol-loaded SBA-15.

The comparison between the TG curves of the non-impregnated materials allows to highlight the weight loss of the amino-functionalized sample due to the thermal decomposition of the organic groups that are anchored on the walls of the pore channels. Moreover, by comparing the TG residues at 1000 °C of the non-impregnated samples, *i.e.*, (a) and (b) curves in Figure 35, with

those of their respective tocopherol-loaded counterparts, *i.e.*, (c) and (d) curves in Figure 35, it is possible to evaluate the antioxidant content, that turned out to be about 40% wt/wt for purely siliceous SBA-15 and 28% wt/wt for SBA-15+APTES. Such results correspond to tocopherol/silica weight ratios that are very similar to those used during the impregnation process, which then proves to be very effective in loading antioxidant molecules within the pores of the selected mesostructures.

3.1.3 N_2 adsorption/desorption analysis

The knowledge of the pore-network structure based on physical adsorption/desorption analyses is fundamental to the characterization of nanometric powders. The BET and BJH methods has been used to study pore dimensions and surface areas of neat and α-tocopherol-loaded SBA-15 functionalized and unfunctionalized nanopowders.

The purely siliceous SBA-15 was degassed at 150°C for 15 h, while the amino-functionalized one was activated for the same time interval at 100 °C. Specific surface areas were evaluated using the BET method, while pore size distributions were determined by applying the BJH method on the adsorption branches of the isotherms (Figure 36).

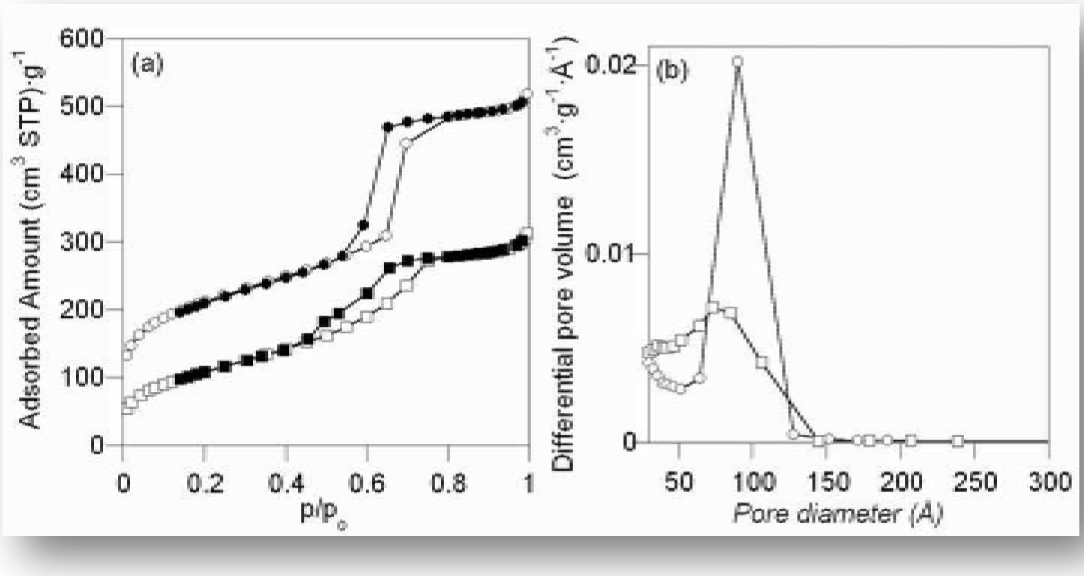


Figure 36: N_2 adsorption/desorption isotherms and the BJH pore size distributions of non-impregnated SBA-15 and SBA-15+APTES mesoporous silicas.

Both samples give type IV isotherms with two sharp increases of N_2 adsorbed amount, one for relative pressures >0.5, and another one at very low relative pressures. The first increase corresponds to the capillary condensation of the adsorptive, while the second one corresponds to micropore filling: the coexistence of these two phenomena is typical of bimodal porous materials that possess both meso- and microporosity. The specific surface area turns out to be about 750 m^2/g for purely siliceous SBA-15, and 400 m^2/g for SBA-15+APTES. The maximum of the pore size distribution is registered at 90Å for purely siliceous SBA-15 and at 73Å for SBA-15+APTES; moreover, the distribution of purely siliceous SBA-15 is significantly sharper than that of SBA-15+APTES. The total pore volume of purely siliceous SBA-15 is about 0.77 cm^3/g: if this volume is completely filled with tocopherol, it is possible to load about 0.73g of the antioxidant per gram of hosting mesoporous silica (about 40%wt/wt). The total pore volume of SBA-15+APTES is about 0.44 cm^3/g, and allows a loading of about 0.42g of tocopherol per gram of silica (about 29%wt/wt). The difference in porosity between purely siliceous and amino-functionalized SBA-15 may be related to the structure-distorting effect of APTES inside the synthesis mixture.

3.1.4 Fourier Transform Infrared (FT-IR)

Fourier transform infrared (FT-IR) spectra were performed on KBr pressed disks containing 1% w/w of inorganic samples. FT-IR spectra were collected over the range 400–4000 cm^{-1} with a resolution of 4 cm^{-1}. The samples were dried at 80 °C overnight before testing.

FT-IR measurements have been performed to demonstrate the effectiveness of the incorporation of amine groups in the mesoporous silicate by co-condensation of TEOS and APTES, as well as the molecular interaction between tocopherol and the amino-functionalized walls of the mesoporous channels.

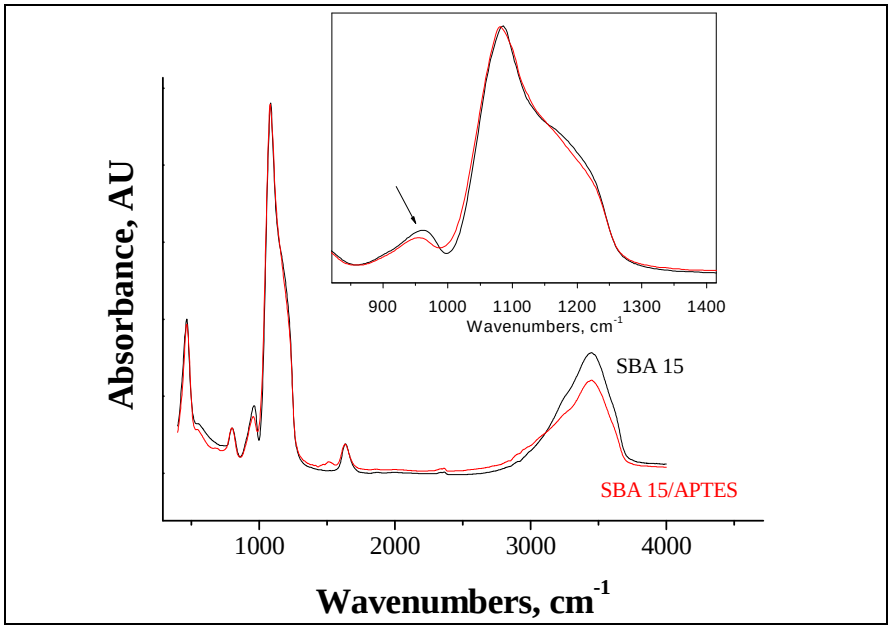


Figure 37: Fourier transform infra-red (FTIR) spectra of non-impregnated SBA-15 and non- impregnated SBA-15+APTES.

Purely siliceous SBA-15 exhibits the characteristic absorption bands at 465, 801, 1080 and about 1200 cm^{-1} corresponding to the vibration stretching and bending modes of the Si-O-Si bonds along with the absorption bands at 950 and 3640 cm^{-1} corresponding to the vibration modes of silanol Si-OH groups (see inset graph of Fig. 37). Furthermore, the peak at 1633 cm^{-1} and the broad band at 3438 cm^{-1} correspond respectively to the bending and stretching mode of adsorbed or hydrogen-bonded water molecules. After incorporation of amino-groups (see SBA-15+APTES spectrum), the intensity band at 950 cm^{-1} decreases, whereas two new absorption bands at 673 and 1510 cm^{-1}, indicated by arrows in Fig. 37 and ascribed, respectively, to the bending of –N-H amine bonds and the symmetrical-NH_3^+ bending mode, appear. This spectroscopic result indicates that the modification of SBA-15 by APTES brings a reduction of the amount of silanol groups on the channel walls. Furthermore, in acidic conditions, some amine groups protonate leading to the formation of zwitterions with silanol groups (-NH_3^+….-OSi-) (Walcarius. *et al.*, 2003). The stretching mode of C-N bonds in the anchored APTES moieties is not resolved due to the overlay with the infrared absorption of Si-O-Si and –Si-CH_2-R groups in the range 1000 to 1250 cm^{-1}. In addition, the broadening of the peak at 3450 cm^{-1} for the sample with APTES is ascribed to the symmetric stretching modes of N-H amine groups which are reported at around 3346 cm^{-1} for free amine and around 3305 cm^{-1} for terminal amine groups cross-linked with the silanol group through

zwitterions interactions. Finally, in the sample SBA-15+APTES the peak at 1633 cm^{-1} is assigned to the overlay between the bending of –N-H amine groups and the –OH bending of adsorbed water molecules.

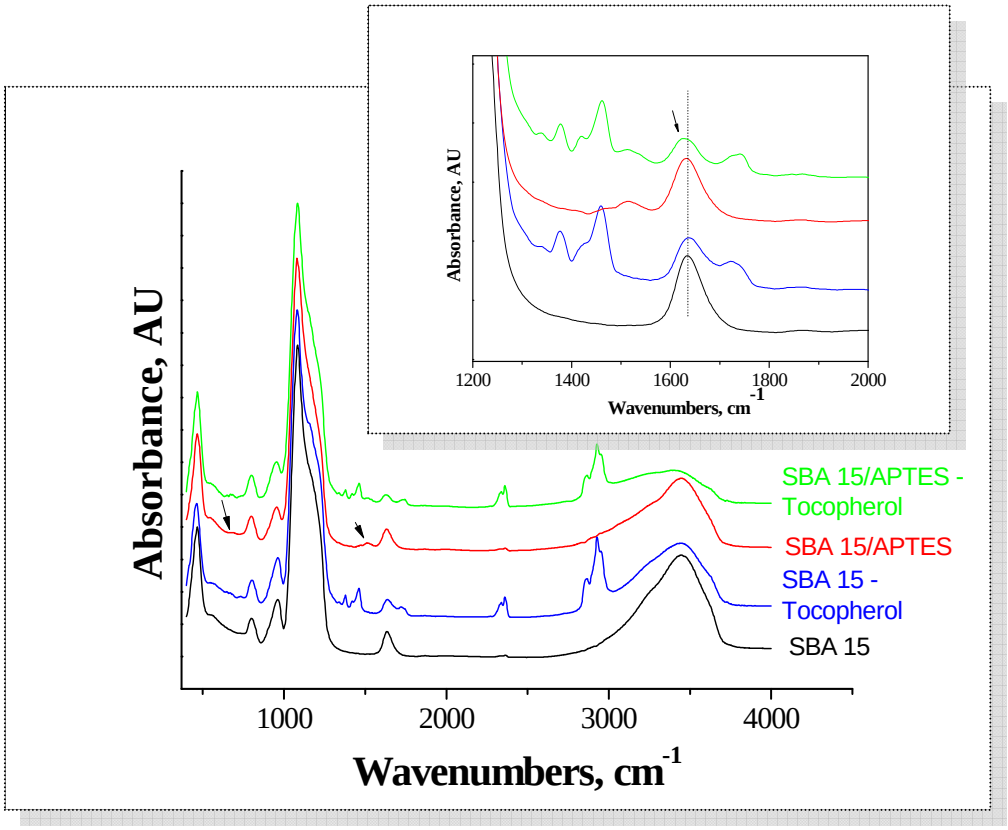


Figure 38: FT-IR spectra of non-impregnated SBA-15, tocopherol-loaded SBA-15, non- impregnated SBA-15+APTES, tocopherol-loaded SBA-15+APTES.

When the mesoporous silicas were filled with tocopherol, additional absorption bands appear in the spectra (Figure 38). The band at 1465 cm^{-1} is for phenyl, skeletal and methyl asymmetric bending and the band at 1388 cm^{-1} is associated with methyl symmetric bending. Comparing the spectra of non-impregnated and tocopherol-loaded SBA-15+APTES (see inset graph of Fig. 38) it is possible to observe that the peak at 1633 cm^{-1}, which is due to the overlapping of the bending vibration modes of adsorbed water and primary amine groups, shows a defined component at 1620 cm^{-1}, indicated by an arrow in the inset of Fig. 38 and most probably arising from the interaction between the -NH_2 groups of APTES and the -OH groups of tocopherol molecules. The FT-IR results confirm that the mesoporous silica channels are filled with the tocopherol and weak interaction between -NH_2 groups of APTES and -OH groups of α-tocopherol has been occurred.

3.2 ACTIVE FILM CHARACTERIZATION

3.2.1 UV Adsorption

To obtain absorption information, a sample is placed in the spectrophotometer and ultraviolet and/or visible light at a certain wavelength (or range of wavelengths) is shone through the sample. The spectrophotometer measures how much of the light is absorbed by the sample. Indeed, when white light passes through or is reflected by a coloured substance, a characteristic portion of the mixed wavelengths is absorbed. For films, the lower the absorbance value, the clearer the film and vice versa.

The optical properties of films which contain unfucntionalized and functionalized silica materials with adsorbed tocopherol, were determined and compared to a reference LDPE film and LDPE film with pure tocoperol. The results are shown in Figure 39.

The increase in adsorbance value can be due to surface imperfections or due to the incorporation of silica materials and/ or tocopherol in the film.

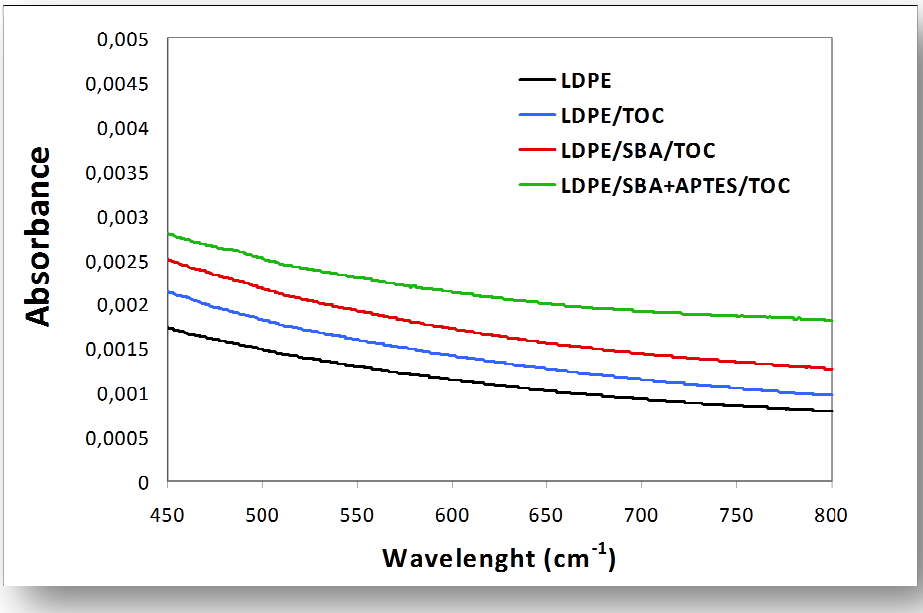


Figure 39: UV/Vis adsorption spectra of reference LDPE film, LDPE/TOC film and films containing SBA-15/TOC and SBA-15+APTES/TOC.

In particular, the higher reduction in transparency is observed in the case of tocopherol adsorbed into SBA-15+APTES. This can be due to yellow colour of tocopherol and to the precense of silica particles. However, films result homogenus and aggregates cannot be detected, as shown in the figure 40

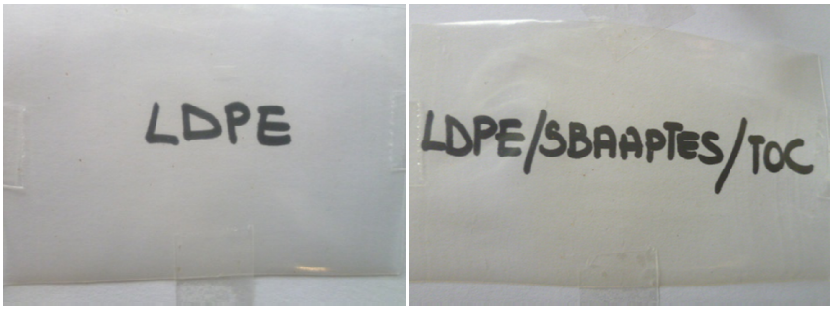
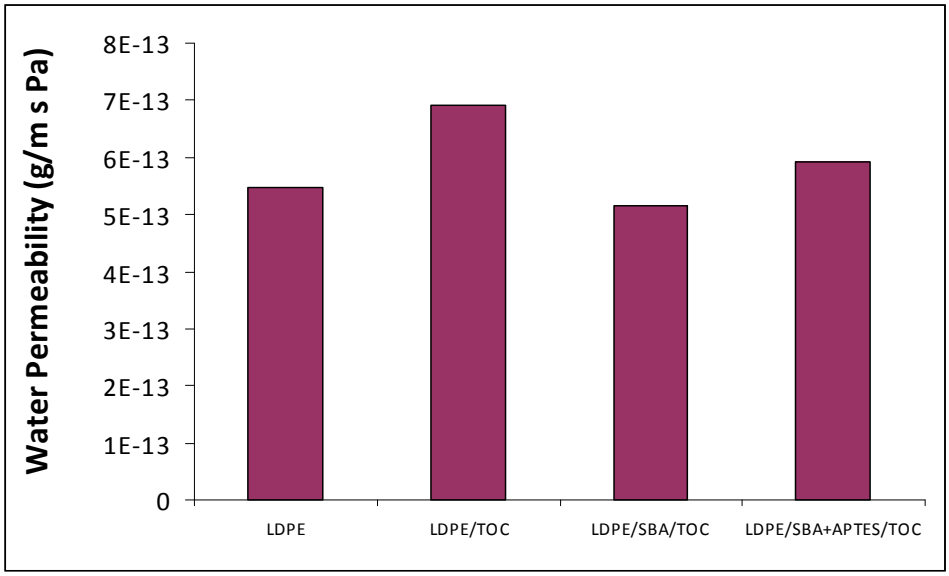


Figure 40: Small pieces photo of LDPE film and LDPE loaded with α-tocopherol in SBA-15+APTES.

3.2.2 Water vapor and Oxygen Permeability

Permeability is defined as transmission of a permeate through a resisting material. In absence of cracks, pinholes, or other flaws, the primary mechanism for gas and water vapour flow through a film or coating is by activated diffusion, i.e., the permeate dissolves in the film matrix at the higher concentration side, diffuses through the film driven by a concentration gradient and evaporates from the other surface.

On this principle basis, samples with a surface area of 5 cm^2 were tested at 25°C. The water permeation tests were conducted by keeping the water activity on the downstream side of the film equal to 0, and keeping the water activity at the upstream side of the film at the constant value of 0.5 whereas . the oxygen permeability of the investigated film was determined setting the water activity at the downstream and upstream side of the film at 0.5. Each test was made in duplicate.



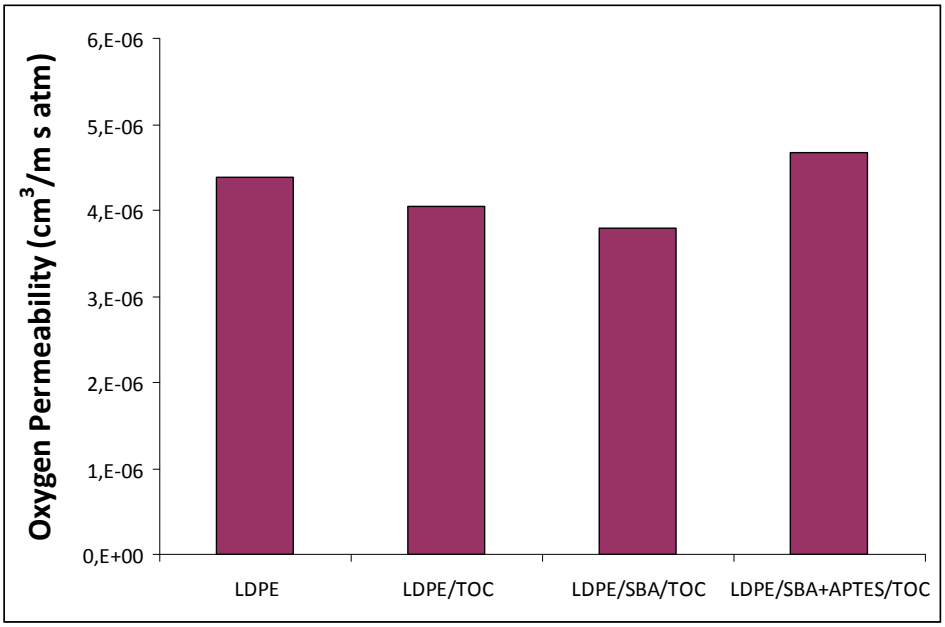


Figure 41: Water vapor (a) and oxygen permeability values of LDPE, LDPE/TOC, LDPE/SBA/TOC and LDPE/SBA+APTES/TOC active polymeric film samples.

As far as water vapor and oxygen barrier properties are concerned, no significant differences are observed among the neat LDPE and the active films (Figure 41). This can be related to the similar degree of cristallinity observed for these films (data not shown). These data provide a positive result since an increase in the oxygen transmission rate could result in an acceleration in the

oxidation of the packaged product and, thus, counteract the positive shelf-life extension effect due to the presence of the residual antioxidant into the active film.

3.2.3 Release Tests

EU directives propose to use olive oil as fatty food simulant for migration tests, however, in order to avoid quantification problems arising from some analytical restriction and analytical interference, alternative simulants have been proposed. In these experiments, 96% v/v ethanol was used and the amount of tocopherol released from the polymer films (~1.6 g) in the chosen food simulant (80 ml) at 25 °C was monitored as a function of time until the attainment of an asymptotic value. In detail, α-tocopherol concentration in ethanol was determined using an HPLC (Agilent Model 1100, Milan, Italy) following the chromatographic method proposed by Sirò *et al.* (2006). The calibration curve was constructed for peak area against α-tocopherol concentration of standard solutions from 10 to 100 ppm. Migration tests were performed in order to investigate the effect of the adsorption of tocopherol onto the functionalized and non-functionalized silica material to control the release kinetics of the antioxidant from the polymer film to the food simulant. Results are reported in Figure 42.

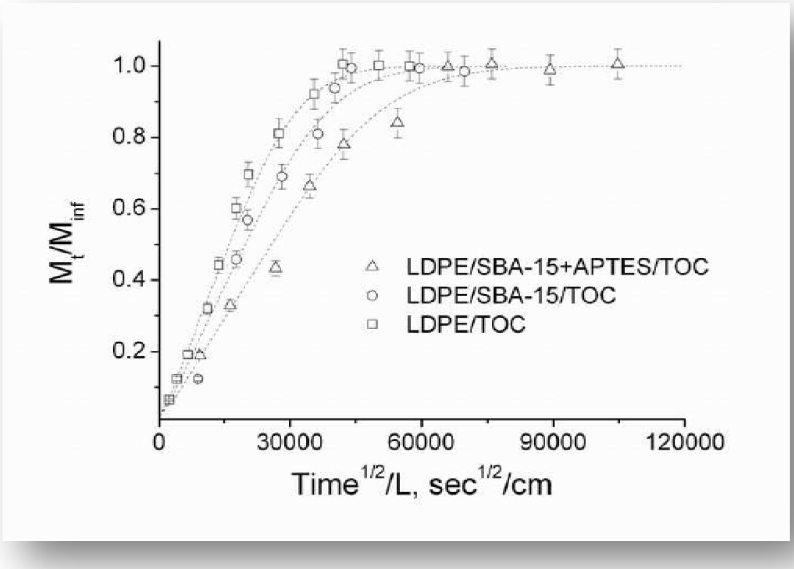


Figure 42: Tocopherol release profiles of LDPE/TOC, LDPE/SBA-15/TOC and LDPE/SBA-15+APTES/TOC active polymer film samples.

Mathematical models generally based on Fick's second law are often used to describe migration of active compounds from polymer films. The analytical solution of Fick's second diffusion equation for one-dimensional diffusion and limited volumes of food simulants given by Crank is reported in the following:

$$\frac{M_t}{M_{inf}} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} exp\left[-\frac{(2n+1)^2\pi^2}{4l^2} D\right] \quad (1)$$

where M_t and M_{inf} are the amount of tocopherol in the food simulant at a particular time t and at the equilibrium, respectively, l is the thickness of the film and D is the diffusion coefficient of tocopherol. Eq. (1) was fitted to the experimental data. The diffusion coefficient was determined by minimizing the sum of squares in errors between the estimated and measured values by using a Matlab Code.

Diffusion coefficients, obtained by fitting Eq. (1) to the experimental data, are $1.9\ 10^{-10}$, $1.3\ 10^{-10}$ and $0.7\ 10^{-11}$ cm^2s^{-1} for films embedding neat tocopherol, tocopherol loaded into SBA-15 and tocopherol loaded into SBA-15+APTES, respectively. It is possible note the tocopherol diffusion was reduced about 30% when it impregnated on SBA-15 and about 60% when it impregnated on SBA-15+APTES.

They show that the use of non-functionalized SBA-15 delays only to a small extent the migration of the antioxidant, as also reported by Herilings *et al.*(2004). As for functionalized mesoporous silica, the tocopherol diffusivity decreased significantly. This could be attributed to the decrease in the pore size and to the increase in diffusion resistance caused by the functionalization of the internal pore walls with the amino groups. The release of pre-adsorbed molecules from the pores of mesoporous silica involves two processes: first, the solvent diffuses into the pores to dissolve the molecules, and second, the dissolved molecules diffuse out of the pores. Thus, in the present work, the solvent ethanol takes more time to diffuse into the pores of the amino-modified system because of the increased hydrophobicity of the pore surfaces which, in addition to pore size and interaction between tocopherol and APTES amino groups, may be another factor to delay the antioxidant release of the functionalized samples. Therefore, the changes in the tocopherol release rate are caused by the changes in the host-guest interactions induced by functionalization.

3.2.4 Oxygen radical absorbing capacity (ORAC) assay

The antioxidant properties of the samples was measured according to the method described by Cao *et al.*. The ORAC assay is based on the inhibition of the peroxylradical oxidation of fluorescein sodium salt (substrate compound) induced by heat decomposition of 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH).

Antioxidant activity detected by ORAC assay was investigated in order to verify the effectiveness of tocopherol released into the simulant food solution after the obtainment of the active films trough the high temperature melting process. Results reported in Table 3 show that for all the investigated films and regardless the temperature at which release tests were carried out, antioxidant activity increases as a function of time, thus confirming the release kinetics previously determined. It is worth noting that the effectiveness of tocopherol released slightly depends on the temperature, resulting higher at 25 °C. Moreover, the activity depends on the method used to introduce the antioxidant in the film. Tocopherol relased at 25 °C from functionalized mesoporosus silica exhibits the highest activity probably because of its better preservation during film manufacturing due to the inclusion into the functionalized silica material.

Table 3: Results of the oxygen radical absorbing capacity (ORAC) assay on LDPE/TOC, LDPE/SBA-15/TOC and LDPE/SBA-15+APTES/TOC active polymer film samples.

Time (days)	ORAC VALUES					
	T=4°C			**T=25°C**		
	LDPE/ TOC	LDPE/SBA-15/ TOC	LDPE/SBA-15 +APTES/TOC	LDPE/ TOC	LDPE/SBA-15/ TOC	LDPE/SBA-15 +APTES/TOC
0.04	10.02	9.698	9.91	12.63	11.05	12.32
0.25	17.25	13.71	15.32	17.25	13.57	15.40
1	19.53	15.81	16.76	19.69	16.58	23.10
3	21.11	16.26	17.67	20.77	16.89	23.52
7	21.21	16.71	19.45	21.18	17.22	23.66

ANTIMICROBIAL ACTIVE POLYMERIC FILM

3.3 NANOPARTICLES CHARACTERIZATION

Ag-MMT has been chemical characterized through UV-visible observation tests. X-Ray diffraction analyses of Na-MMT and Ag-MMT clays were conducted to highlight the MMT structural modifications that take place when Ag ions replace Na ions and silver nanoparticles locate onto the MMT layered structure. Using this approach, it is possible to roughly estimate the location of the silver within the MMT structure (i.e., in the galleries or on the surface of MMT platelets).

3.3.1 UV/Vis absorption

UV-visible absorption spectra were recorded using quartz cuvettes within the range of 200 to 800 nm. The cuvettes were filled with a 500-ppm shaken water dispersion of Na-MMT and Ag-MMT, and their spectra were recorded. The Ag spectrum was isolated by subtracting the Na-MMT spectrum from the Ag-MMT spectrum.

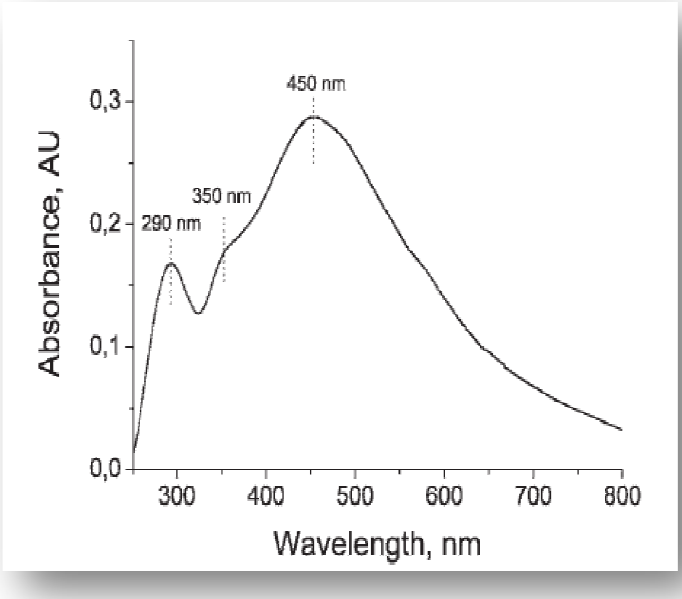


Figure 43: UV-visible absorption spectra of silver Na-MMT and XRD spectra of Na-MMT and Ag-MMT clays.

The UV-visible absorption spectrum related to silver is shown in Figure 43. Characteristic silver surface Plasmon bands were detected at 290, 350, and 450 nm, and these bands were tentatively ascribed to the presence of residual silver ions (Wang *et al.*, 2005) or with the existence of silver nanoparticles smaller than 2 nm (Shao *et al.*, 2006), smaller than 10 (Huang *et al.*, 2008), and smaller than 40 nm (Sharma *et al.*, 2009), respectively. The broad peak at 450 nm is indicative of the presence of silver nanoparticle aggregates.

3.3.2 X Ray diffraction test

The structures of Na-MMTand Ag-MMT clays were evaluated using wide angle X-ray diffraction (WAXD) measurements. The spectra were collected in the transmission mode (i.e., the X-ray beam moves through the sample) by scanning the 2θ (θ is the diffraction peak angle) range between 1.5 and 40°. All spectra were corrected only for the dark current and the empty holder background.

X-ray diffraction (XRD) analyses of Na-MMT and Ag-MMT clays were conducted to highlight the MMT structural modifications that take place when Ag ions replace Na ions (along with K, Mg, and Ca ions) and silver nanoparticles locate onto the MMT layered structure. Using this approach, it is possible to roughly estimate the location of the silver within the MMT structure (i.e., in the galleries or on the surface of MMT platelets).

The XRD spectra of Na-MMT and Ag-MMT powders are compared in Figure 44. Unmodified Na-MMT clay has an intense diffraction peak at around 2θ = 7.2°, corresponding to a basal d_{001} spacing between the silicate platelets of about 1.2 nm. The MMT modified with Ag also had a distinctive diffraction peak at 2θ = 7.2°, but the peak shape and intensity was markedly different from that of the Na-MMT clay. These features reflect the structural modifications that take place when Ag ions replace Na ions inside the layered structure of MMT. MMT clay probably loses its layered structure, giving rise to nanoparticles with collapsed or exfoliated structure (Praus *et al.*, 2008). The occurrence of structural modifications also was indicated by the disappearance of the Na-MMT peak at 2θ around 29°. An additional small peak with maximum at 2θ = 37.9°, in the XRD pattern of Ag-MMT corresponds to the (111) reflection of Ag, thus proving the presence of metallic silver. The diffraction peak at 2θ around 32° can be assigned to AgO-Ag_2O crystalline

domains, which are generated as a result of a limited reduction of Ag ions under basic conditions (Sharma *et al.*, 2009).

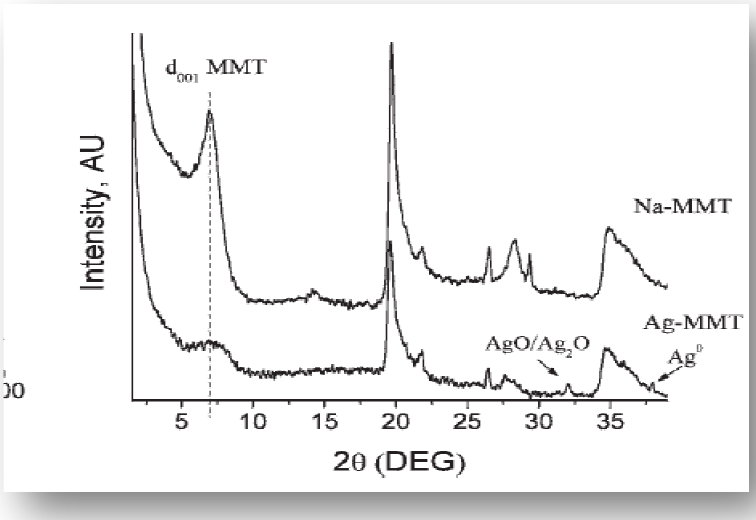


Figure 44: XRD spectra of Na-MMT and Ag-MMT clays.

Based on the analysis results of WAXD, and taking into account that the basal spacing $d_{(001)}$ of MMT is around 1.2 nm, the metallic and oxide silver nanoparticles as well as the silver ions are mainly located on the surface of both single MMT platelet and MMT tactoids as schematized in Figure 45.

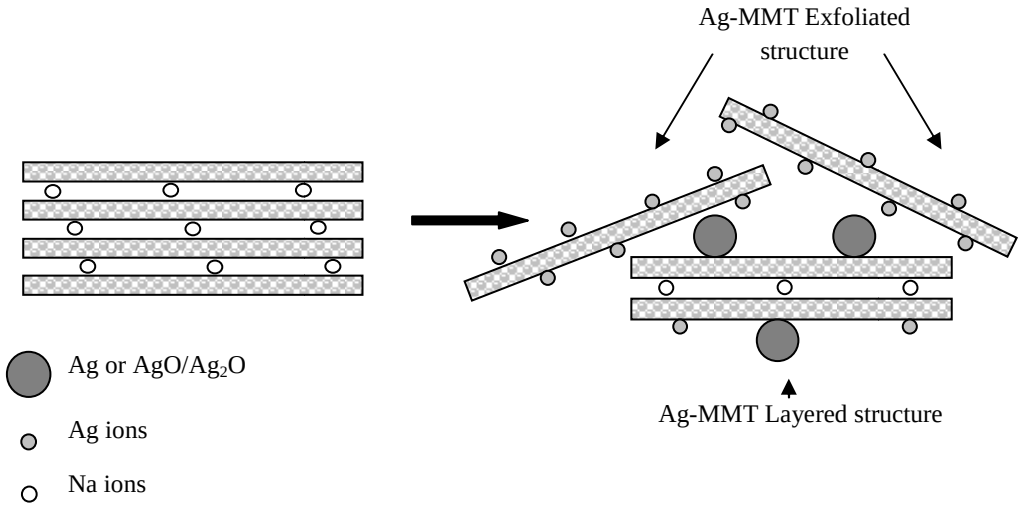


Figure 45: Schematic representation of the Na-MMT and Ag-MMT structures.

3.3.3 Transmission Electron Microscope (TEM)

TEM micrographs of Ag-MMT particles are shown in Figure 46. The Ag and Ag_2O nanoparticles are deposited on the MMT lamellae with a preferential location on the edges. The particles are well-separated from each other and show a size in the range from 2 to 30nm. Most of silver nanoparticles have been detached from silicate platelets by sonication process and result homogeneously dispersed around the MMT lamellae. In the insert image of Figure 46 it is observed the presence of Ag or Ag2O nanoparticles with average size of about 2-3nm alongside the presence of MMT platelet fragments likely originated by the ion exchange process. The presence of these fragments confirms the destroying of MMT layered morphology as already shown by XRD analysis.

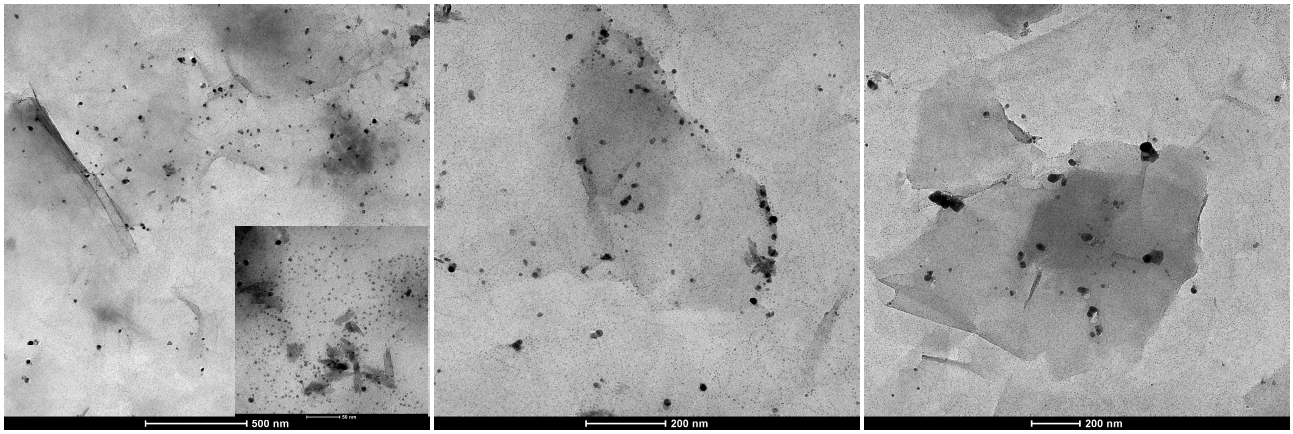


Figure 46: TEM images of Ag-MM at different magnifications.

3.4 FILM CHARACTERIZATION

3.4.1 X Ray Diffraction

The crystalline structure of chitosan polymer was also evaluated by WAXD measurements. The spectra were collected in the transmission mode by scanning the 2θ range between 1,5 and 40° as well as for the morphology of Ag-MMT and Na-MMT in the polymer.

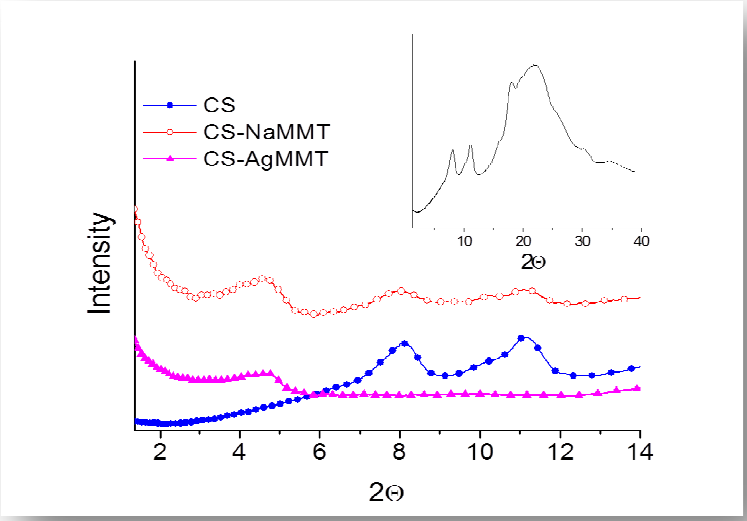


Figure 47: X-ray diffraction spectra of neat CS, CS/NaMMT and CS/AgMMT active films.

Figure 47 reports the WAXD spectra for neat chitosan and nanocomposite films with NaMMT and AgMMT, with glycerol as plasticizer. Neat chitosan films show characteristic crystallinity peaks at 2θ= 8°, 11.2° and 18° as well as a broad peak corresponding to the amorphous structure at 23°(see the inset graph of the Figure 47) (Wang *et al.*, 2005). The crystalline structure of chitosan is strongly dependent on its processing treatment, as well as its origin and molecular constitution, such as degree of deacetylation and molecular weight (Rhim *et al.*, 2006). However, the data reported in Fig. 47 show that the crystallinity of chitosan is slightly reduced by the addition of the NaMMT clay. As the unmodified NaMMT clay presents a distinctive diffraction peak at around 2θ = 7.2°, corresponding to a d_{001} spacing between the silicate platelets of about 1.2 nm, it

can be observed that for chitosan/clay nanocomposites the NaMMT diffraction peak shifts to 4.5° (i.e. d_{001} spacing equal to 1.8nm corresponding to a thickness expansion of the original clay of about 50%). This shows that the chitosan macromolecules are able to intercalate the NaMMT stacks. The obtained results indicate that the intercalation of chitosan chains within the silicate galleries takes place in different regimes of dispersions as well as to a different extent. An analogue result were reached for chitosan/AgMMt clay nanocomposites because of basal (001) MMT diffraction peak shift; so in the bionanocomposites chitosan macromolecules are intercalated in the MMT silicate galleries. Indeed in CS-AgMMT sample, the intensity of the reflection was lower, whereas its half-width was larger than this of undoped clay minerals, whereby the highly ordered parallel lamellar structure of the mineral was disrupted by metal nanoparticle formation .

The crystalline structure of chitosan is not affected by both glycerol and Na-MMT clay addition whereas it is significantly modified by the presence of the Ag-MMT filler. Probably the Ag ions and metallic particles interact with the chitosan macromolecules acting as additional cross-linkers which strengthen the polymeric network.

3.4.2 Water vapor Permeability

Water vapor transmission rate for silver nanoclays chitosan film was recorded by keeping film samples in contact with a water activity gradient between downstream and upstream side of material.

In table 4 are reported the value of water vapor permeability (WVP) of pure chitosan film, montmorillonite loaded chitosan and silver montmorillonite loaded chitosan.

WVP decreases when chitosan film is loaded with both nanoclays systems, Na-MMT and AgMMT, and it slightly changes as the amount of AgMMT increase up to 10% (Table 4). Chitosan has a poor barrier property against water vapor because of its hydrophilicity (Mao *et al.*, 2003; Liu *et al.*, 2007), so the presence of Ag-MMT particles allows a reduction of the water vapor permeability of about 30% with respect to neat chitosan.

As X-ray spectra showed, NaMMT nanoclays resulted in the coexistence of both intercalated and exfoliated structures in the matrix of nanocomposites. With adding AgMMT clays, it was clear that an intercalated morphology with additional crosslinking was present. A tortuous pathway

formed by well-dispersed layers of AgMMT (Figure 48) acted as a barrier against gas transmission because of the increased path length (Rhim, 2006). Since negatively charged clay acts as an ionic crosslinker, the addition of clay will strongly affect the low-swelling ratio as well as the high cross-linking density of chitosan films (Liu *et al.*, 2007). The resulting CS/AgMMT nanocomposite shrunk in the interlayer space, resulting in pore blocking that inhibited the passage of gas molecules.

Table 4: Water vapor permeability values of CS, CS/3%NaMMT, CS/10%naMMT, CS/3%AgMMT and CS/10%AgMMT active polymeric film samples.

SAMPLE	WATER VAPOR PERMEABILITY [g/m s Pa]
CS	$3{,}02 \cdot 10^{-11}$
CS/3%NaMMT	$2{,}50 \cdot 10^{-11}$
CS/10%NaMMT	$2{,}05 \cdot 10^{-11}$
CS/3% AgMMT	$2{,}21 \cdot 10^{-11}$
Cs/10%AgMMT	$2{,}21 \cdot 10^{-11}$

The observed decrease in WVP is of great importance in evaluating the nanocomposites films for use in food packaging, protective coatings and other application where efficient polymer barriers are needed.

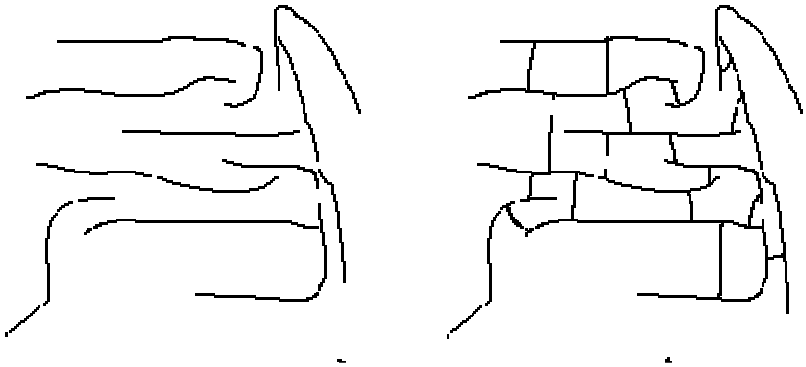


Figure 48: Cross-linking phenomena in a polymer.

3.4.3 Water Adsorption

Water uptake is a measure of the resistance of a film sample to water. Though the water solubility of all nanocomposites films was not significaly different than that of neat chitosan film (Table 5). This results clearly indicates that the water absorption of nanocomposite films was not affect by mixing rather with unmodified NaMMT or Ag nanoparticles-loaded MMT.

Table 5: Results of water sorption tests for CS, CS/10%NaMMT and CS/10%AgMMT.

SAMPLE	WATER ADSORPTION [%]
CS	136
CS/10%NaMMT	190
Cs/10%AgMMT	148

The established interaction between Ag ions or Ag metallic surface with chitosan macromolecules and the extent of intercalation confer to the obtained material a higher stability in liquid water, as confirmed by WAXD analysis

The water uptake of CS, CS/10%NaMMT and CS/10%AgMMT nanocomposites at various pHs is shown in Figure 49. As we observe, the swelling of CS matrix was pH-dependent. CS/10%AgMMT nanocomposite swelled greatly in the acidic medium compared to the neutral or basic one. Chitosan with AgMMT nanocomposites swelled higher at a much faster rate in a lower pH medium than in a higher pH medium. The effect of pH on the water uptake of the chitosan-containing AgMMT nanoparticle is ascribed to the hydrolysis of amide linkages in the crosslinked chitosan network by acid and the regeneration of amine groups in networks (Huang *et al.*, 1998; Sung *et al.*, 1999). Because the amino groups reformed in the network could be protonated in acidic medium, the water gain at equilibrium of chitosan-containing Ag nanoclays in the acidic solution was larger than that in the neutral one. The electrostatic repulsion of the protonated NH^{3+} groups along the chitosan chain could lead to an expansion of the network and hence a higher swelling.

The presence of glycerol also confers a higher dimensional stability to the samples. This behaviour is most likely to be due both to the formation of the crosslink network induced by the

hydrogen bonds between the chitosan and glycerol and to an enhanced intercalation of chitosan molecules into the silicate galleries (Lavorgna *et al.*, 2010).

The samples containing Na-MMT filler show an increment of the water liquid uptake compared to neat chitosan which is attributed to the high water affinity of the phyllosilicate particles or platelets. However, it can be inferred from the data at pH 3.56 and 6 that the presence of Ag-MMT particles allows a reduction of the water uptake in comparison to the Na-MMT bionanocomposite (Figure 49). This result is extremely significant because one of the limitations to chitosan commercialization is mainly related to its high water liquid uptake which compromises the sample dimensional stability and consequently the applicative properties. It is likely that the Ag ions or Ag metallic surface increase the network extent of chemical and physical cross-linking between the chitosan macromolecules conferring to the obtained material a higher stability in liquid water.

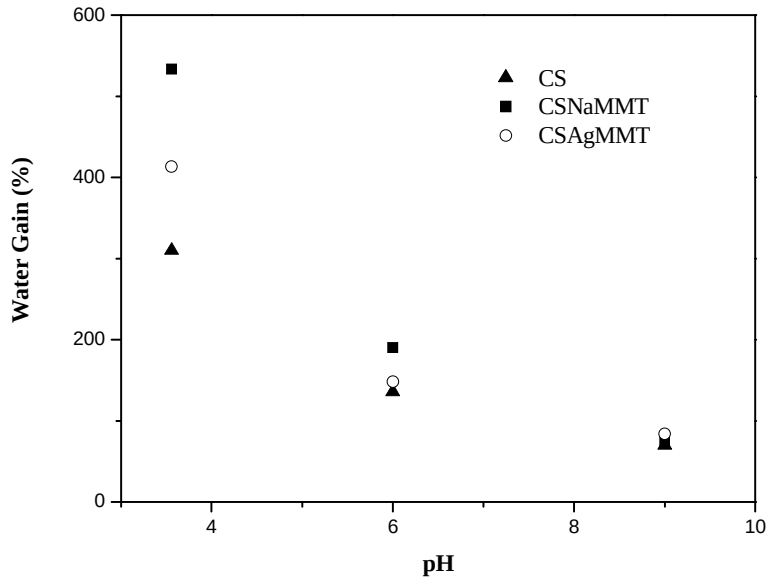


Figure 49: Water sorption values at equilibrium at 25 ◦C of film (▲) CS, (■) CS/NaMMT and (○) CS/AgMMT at various pHs.

3.4.4 Release Test

For migration tests, a known amount of films were immersed in an aqueous solution (10 ml) at ambient temperature. Liquid samples were withdrawn from the solution at different times and analyzed by ICP/AES. A commercial ICP multistandard solution was used for calibration. In Figure 50 is reported the release kinetics after 20 days of immersion for both two different concentration of Ag-MMT chitosan films.

Silver ions are released in a steady and prolonged manner, thus showing that most of Ag^+ released comes from the oxidation of the metallic surface as the water diffuses through the structure determined by the interactions between inorganic platelets and organic chitosan macromolecules. It can be noticed that the amount of silver ions released after a given immersion time increases with growing concentration of the silver particles in the polymer. Moreover, after an immersion time of about 20 days, an increase of the ions release is still measurable indicating that the silver reservoir is not yet depleted.

The steady Ag release can be an useful property for tailoring the antimicrobial activity of the bionanocomposite films and to design materials which explicit their function in preventing food degradation over a longer time operating window.

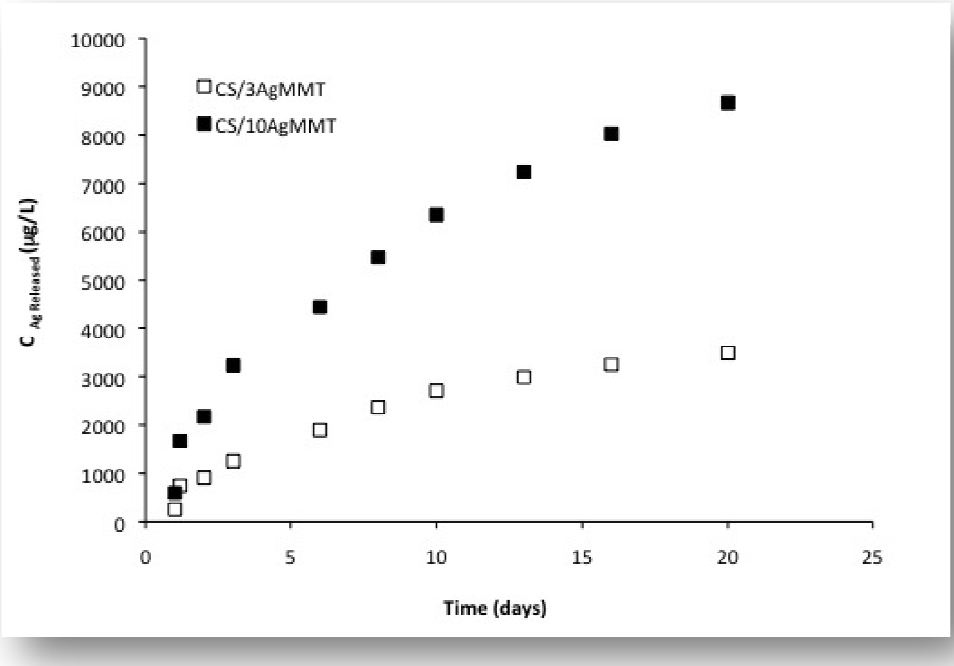


Figure 50: Silver release profiles of CS/3AgMMT and CS/10%AgMMT active polymer film samples.

CHAPTER 4

CONCLUSION

4.1 CONCLUSION

4.1.1 Antioxidant active Film

In this thesis, samples of purely siliceous and amino-functionalized SBA-15 mesoporous silica were successfully used as α-tocopherol carriers for the production of active LDPE polymer films. X-ray diffraction and microporosimetric measurements revealed the strong influence of adding the functionalizing agent (aminopropyltriethoxysilane) to the mesophase synthesis mixture on the structure and the pore size of the final product. Fourier transform infrared spectroscopy tests proved that the interaction between tocopherol and the amino groups of functionalized SBA-15 occurred. Migration tests were performed on active polymer films containing tocopherol-loaded mesoporous silica particles in order to investigate the effect of the amino functionalization on controlling the release kinetics of the antioxidant to a food simulant. Active polymer films containing the functionalized carrier showed a slower tocopherol release when compared to samples containing free tocopherol and tocopherol loaded onto purely siliceous substrate. In fact, the antioxidant diffusivity of films containing functionalized mesoporous silica decreased of about 50% with respect to films containing free tocopherol. Such a result is attributed to the decrease in the pore size of the carrier with respect to the non-functionalized one, and to the increase in diffusion resistance caused by the functionalization of the internal pore walls with the amino groups. Finally, the oxygen radical absorbing capacity (ORAC) assay of the produced active polymer films proved the antioxidant effectiveness of tocopherol released from samples after manufacturing process.

4.1.2 Antimicrobial active Film

In this work of thesis, a new bionanocomposite film exhibiting antimicrobial activity was produced. Ag-MMT nanoparticles were obtained throught a ion exchange reaction and inserted in the characteristic lamellar structure of montmorillonite. Chitosan, chosen ass matrix for the active films, was successfully loaded with these new antimicrobial silver-based nanoparticles.

Characterization of AgMMT nanoparticles show that it consists of nanometric metallic silver and oxides particles (size in the range 2-40nm) preferentially located on the surface of MMT single lamellae. Moreover, X-ray diffraction spectra shows that Ag-MMT particles result partially intercalated by chitosan macromolecules although it cannot be excluded in such an extent the exfoliation due to the collapse of MMT structure during the preparation of the active filler.

The crystalline structure of chitosan is not affected by both glycerol and Na-MMT clay addition as shown WAXD spectra, but the glycerol plasticizer and the silver ions as well as the surface of metallic particles exert a combined effect which allows a reduction of the liquid water uptake and water permeability with respect to neat chitosan. In fact, the presence of Ag-MMT particles allows a reduction of the water uptake of about 50% with respect to either neat chitosan and Na-MMT nanocomposite.

Finally, by means of release tests it is possible to observe how the silver supporting nanoparticles, Ag-MMT have contributed to modulate the release kinetics of silver ions from bionanocomposite films over a longer time interval (up to 20 days).

Results obtained in this work suggest that chitosan films loaded with montmorillonite clays containing antimicrobial agents (silver ions) exhibith improved water absorption properties and can be used as novel food packaging materials with controlled release properties. This is of paramount importance for the production of active films to be used as food packaging materials or potentially as biomaterials.

4.2 FUTURE DEVELOPMENT

The field of food packaging can be considered one of the emerging applications of stimuli-responsive polymer materials. They are an interesting, innovative and challenging class of materials that can adapt to surrounding environments and can regulate the transport of molecules as a reaction to external stimuli. To sustain life and maintain biological function, nature requires selectively tailored molecular assemblies and interfaces that provide a specific chemical function and structure as well as a change in their environment. Synthetic polymer systems with very similar attributes are often prepared for a broad range of applications such as controlled- release systems. Recently, stimuli-responsive macromolecular nanostructures have been developed; they are capable of conformational and chemical changes upon reception of external signals such as change in temperature, pH or chemical composition. These materials will permit triggering the release of active compounds only when strictly needed by the system, thus avoiding waste (Stuart *et al.*, 2010).

Mother Nature shows us abundant examples of stimuli responsive (or smart) materials which have triggered the interest of researchers for a long time: leaves of *Mimosa pudica* collapse suddenly when touched; leaves of the Venus flytrap snap shut on doomed insect prey; leaflets of *Codariocalyx motorius* rotate under exposure to sunlight; sunflowers turn toward the sun; chameleons change color according to the environmental situation; and so on. Mimicking the functions of such organisms, scientists have made great efforts to synthesize stimuli-responsive polymers which have scientific significance and promising applications (Hu *et al.*, 2012) .

Stimuli-responsive polymers (SRPs) rapidly change their configuration, dimension or physical properties with small changes in the appropriate stimuli such as heat, moisture/water, pH value, electricity, light, magnetic field, and solvent (Hu *et al.*, 2012).

Recently, the use of polymeric nanocarriers to transport active compounds like small-molecular, peptides, or others found an increased attention throughout the different fields of industrial and material engineering. Not only that these nanocarriers enhance the properties of already existing material in terms of bioavailability, barrier and mechanical properties, furthermore they can be tailor-made in such a manner that they selectively release their cargo at the desired time of action.

Fleige *et al.*, (2012) have noticed that the nano-structured carriers have been fabricated from a practically limitless variety of organic and inorganic materials including, but not limited to, polymers and dendrimers, lipids, amphiphiles, carbon nanotubes, DNA-like scaffolds, elemental nanocrystals, quantum dots and mesoporous materials. The hierarchical assemblies of these materials, architectures and particulate systems such as polymeric micelles, conjugates and complexes of dendrimers and hyperbranched polymers, inorganic nanoparticles, polyplexes, liposomes and vesicles, caged architectures and many others, which share the dimensional feature of nanometer scale-range and are used for delivery of bioactive agents, are termed as nanocarriers (Fleige *et al.*, 2012).

From the above, it is possible imagine to combine the particular efficiency of active composites with the innovative feature of stimuli responsive materials. In fact , stimuli-responsive macromolecular nanostructures could be able of conformational and chemical changes on receiving an external signal. These changes are accompanied by variations in the physical properties of the polymer. The signal is derived from changes in the materials' environment, such as a change in temperature, chemical composition or applied mechanical force, or that can be triggered exogenously by irradiation with light or exposure to an electrical and magnetic field.

This new point of view can open the doors to a new material formulation research to be used also in the food packaging field.

CHAPTER 5

REFERENCES

5 REFERENCES

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