As mentioned in the introduction, active packaging systems are based on the incorporation into the system of a substance, or mixture of substances, which by themselves or by chemical reaction perform an activity that results in a beneficial effect on the packaging content; in particular, in antimicrobial packaging systems, an inhibitory or lethal effect on microorganisms. In this chapter, the methods of incorporation are listed and briefly described, including the steps for preparing the active agents and the techniques employed for their incorporation into the packaging system.

3.1 Active Component Preparation

As described in Chapter 2, there are numerous pure compounds and mixtures that have been described in the literature and reviewed in books which possess antimicrobial activity. These substances exhibit very different chemical and biochemical properties, and therefore the mode of action and conditions of activity need to be known in order to use them efficiently in any type of application, including antimicrobial packaging systems.

Organic acids such as acetic, lactic, propionic, sorbic and benzoic acids and their salts are commonly used as food preservatives, and for decades they have been, and currently are, incorporated into food formulations to increase food safety and shelf life. They are, in general, thermally stable and can be incorporated into polymers by melt blending during the manufacture of packaging materials. This is also the case with several metal oxides and salts which have been used in active packaging.

Many plant essential oils are well-known antimicrobial substances and have traditionally been used as such in cooking and home preserves, and as flavourings. This latter use is due to the volatility of their constituents and the very low concentration threshold of detection for the majority of consumers; characteristics which are responsible for their valuable aroma. However, when considering their use in active
antimicrobial packaging systems, this volatility must be controlled in order to facilitate incorporation of the active substances and to reduce loss of the substances during packaging production and storage. High losses may mean not only economic loss but also low or zero efficiency in the protection of the packaged product.

However, volatility is not the only reason for manufactured antimicrobial materials, with pure or blended agents, exhibiting low efficiency. Degradation caused by reactions with other packaging components, thermal abuse and mechanical stress are other potential hazards that may result in loss of the active molecule and subsequent reduction of antimicrobial activity. Enzymes and bacteriocins are polypeptide macromolecules whose antimicrobial activity is highly dependent on their three-dimensional (3D) structure. In many cases, exposure of these substances to conventional thermomechanical procedures would lead to breakage of the molecule or at least conformational changes that result in loss of antimicrobial characteristics.

Consequently, various procedures have been developed and are used to protect the active component during packaging manufacture, reducing the stresses it would be exposed to in the case of direct melt blending with the polymer or evaporation during the drying processes involved in impregnation, coating or casting techniques. In some cases the active agent is absorbed onto the surface of porous substrates (generally inorganic), reducing its volatility and providing partial protection from the aforementioned stresses. In others, the agent is encapsulated in an organic environment via incorporation into the organic matrix or inside an empty particle.

### 3.1.1 Use of an Inorganic Substrate

Inorganic substances are commonly added to polymeric matrices. Metal oxides, salts, zeolites, clays and so on have been used to modify the functional properties of polymers, including food packaging materials. Titanium dioxide is commonly used in polymer applications as a white pigment and also as an ultraviolet (UV) radiation shield. Calcium stearate is a lubricant used to improve the flowability of powders and is largely used in polymers for food applications because of its approval as a food additive (E470).

Clays broken down into microsized fillers are often incorporated into polymer matrices to enhance their mechanical properties. These microcomposites generally exhibit modulus improvement compared with the pure polymer, however the elongation at break and toughness of the polymer are reduced. More recently, clay nanocomposites have been developed with smectite-type clays, such as hectorite, montmorillonite and synthetic mica.
A nanocomposite is defined as a composite in which at least one of the phases has one or more dimensions of the nanometre order, whereas a polymer nanocomposite consists of a polymer or copolymer possessing nanofillers dispersed in the matrix. In the food packaging sector, nanofillers are added to polymer films in an attempt to develop plastics for food packaging with greater performance with regard to thermal stability, mechanical strength and gas barrier properties. The difference between these two fillers, i.e., nanofiller and microfiller, is the particle dimension. The surface-to-volume ratio increases exponentially as the particle diameter decreases, and the effect of this large increase modifies the final properties of the composites. In the case of smectite clays, which have a layered structure, the original microparticle structure is modified by separating the constituting layers to different degrees. The final composites exhibit enhanced mechanical properties at a low smectite clay loading percentage without compromising toughness, and they have improved heat resistance, barrier properties and so on.

Mesoporous silica is a highly porous form of silica which is used in many applications because of the large surface-to-volume ratio and the high free volume percentage, which can be used to protect compounds and/or to deliver substances. Apart from the obvious applications in biomedicine, pharmacology, biosensing and so on, mesoporous silica is also used in food and food packaging applications.

Other reinforcing materials commonly present in polymer composites are carbon fibres and glass fibres, which have wide applications in industry (vehicles, sports items and so on), including food containers, but with rare applications in the packaging sector. However, carbon nanotubes are being explored as reinforcing materials because they have high flexibility, low mass density and a large aspect ratio, and there are ongoing studies to use them in food packaging.

Inorganic/polymer composites can be prepared via numerous manufacturing processes, including sheet forming, fabric thermostamping, compression moulding, injection moulding and extrusion moulding. Since most polymer packaging materials are produced via injection or extrusion processes, the preparation of filler/polymer composites for packaging applications is typically carried out by melt blending in extruders or similar equipment. In some cases they are combined with a polymer at a high concentration to produce a masterbatch that can then be diluted with virgin polymer to achieve the required concentration.

Other procedures commonly used in film manufacturing, such as film coating and printing technologies (gravure, flexography), are also available for composite manufacture. In these procedures, as described below, a solution or dispersion of the components is produced and the final composite is obtained by solvent evaporation.
As a step prior to film extrusion or packaging injection moulding, these inorganic substances can be used to protect the antimicrobial agents from evaporation or degradation. Liquid antimicrobial substances such as plant extracts or essential oils can be exposed to absorbent materials. The oil components can wet the surface of the inorganic filler or can enter the pores of these materials, reducing their vapour pressure which facilitates the feeding of film manufacturing equipment. Since this effect takes place mainly at the surface of the filler, materials with a high surface-to-volume ratio are preferred. In this way, the final load of the filler in the polymer can be reduced. Nanoparticulate materials, clay platelets, mesoporous silica and carbon nanotubes are particularly interesting in this context.

### 3.1.1.1 Clay Nanocomposites

Nanocomposites based on the incorporation of nanometric inorganic materials loaded with active molecules into a polymer film is a promising area of research with potential applications in the development of active food packaging. Nanostructured polymer matrices could be employed in the design of antimicrobial food packages capable of releasing antimicrobials into the food matrix or to the package headspace surrounding the food. Antimicrobials are usually introduced during the processing of the polymer, but this step has several potential drawbacks because the active molecule may be inactivated, degraded or lost by evaporation during processing. Nanotechnology can deal with these deficiencies by loading the nanofiller with the active molecule before it is incorporated into the polymer film. In addition, nanostructured polymers could also modulate the release of the active compounds in order to enhance their effectiveness when they reach the food product. It is expected that these possibilities will be exploited in the near future.

In general, nanomaterials are classified as nanolayers, nanofibres or nanoparticles (NP) in accordance with their dimensional structure. Owing to their large aspect ratio, nanofibrous and nanolayered materials are usually employed as reinforcing fillers in most polymer nanocomposites. Layered clays such as montmorillonite are currently the most important for the production of polymer nanocomposites and a large number of commercial developments have been derived from intense research in this area.

Various considerations should be taken into account when using naturally occurring clays as systems for the delivery of active molecules; in particular, the kind of clay and the physico-chemical properties of the active agent, such as the presence of functional groups, polarity, size and chemical structure. Depending on the presence and type of functional groups and the polarity of the molecule, the sorption of the active molecule to the clay may be chemical (cationic exchange, ligand exchange, protonation) or physical (van der Waals forces). Thus, active molecule-clay interactions will determine
the release pattern of the former [1], whereas the size and molecular geometry of the active molecule will determine its ability to intercalate into layered structures or to be entrapped in hollow tubular structures.

However, little work is currently focused on the incorporation of active molecule-clay systems in polymer matrices for use in antimicrobial food packaging. Recently, Liu [2] studied the intercalation of benzoate and gallate anions in layered double hydroxides and their incorporation into polyhydroxyalkanoate polymer films for antimicrobial food packaging applications. Intercalation was achieved by immersion and stirring the solid in an aqueous solution of sodium salts. Halloysite clay nanotubes are used for encapsulation and sustained release of active agents. Furthermore, it has been demonstrated that the sustained delivery efficiency of halloysite is better than other encapsulating materials, such as mesoporous silica NP or polymer microcapsules [3]. The empty lumen of nanotubes can be loaded by immersion of the nanotubes in a concentrated solution of the agent in a solvent compatible with the clay (polar), making a vacuum to remove the trapped air and re-establishing pressure so that the agent enters the tube lumen, after which the particles are washed and dried.

Although there are a considerable number of studies regarding its use to improve the mechanical strength, barrier properties and adhesion of polymer films and coatings, more studies need to be performed regarding the incorporation of halloysite loaded with active agents into polymer matrices to modulate and extend their release. For pharmaceutical purposes, tetracycline-loaded halloysite has been incorporated into polyvinyl alcohol (PVA) and polymethyl methacrylate films and the release of the active agent was studied [4]; however only a few studies have investigated the use of halloysite-polymer nanocomposites for active food packaging applications.

Besides acting as carrier systems, inorganic materials have also been incorporated into polymer matrices for the purpose of modifying the release of active compounds. In this regard, bentonite clay, an aluminium phyllosilicate consisting mostly of montmorillonite, has been incorporated into polymer and biopolymer matrices to modify the release of antimicrobial molecules. It has been shown that bentonite can slow the release of carvacrol from ethylene-vinyl alcohol (EVOH) copolymer films [5], or herbicides from alginate and other synthetic polymer matrices [6, 7]. Halloysite has also been employed to control the release of opioids added to microcrystalline cellulose pellets and antiseptic brilliant blue incorporated into poly(ε-caprolactone) [3].

It is worth noting that the great capacity of smectites for cation exchange, in particular montmorillonite and saponite, has been used to replace Na⁺ cations with metallic ions that have antimicrobial capacity, such as Ag⁺, and embed them in biopolymer matrices for application in antimicrobial food packaging [1]. Zeolites are naturally occurring hydrated aluminosilicate minerals that have a porous structure. Zeolite ions
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exchanged with silver, copper and zinc cations, which possess antimicrobial activity against a wide spectrum of microorganisms, are capable of slowing the release of these metal ions into a liquid medium and have been incorporated into materials where antimicrobial properties are required [8]. Other inorganic materials, such as zirconium phosphate and glass, have been tested as carriers and release systems for antimicrobial metal ions. Silver nanorods have been synthesised inside the lumen of halloysite nanotubes, by the thermal decomposition of silver acetate embedded in the particles from an aqueous solution, and then incorporated into polyurethane paint; the resulting inhibition of bacterial growth was due to the slow release of silver ions from the nanotubes [9].

3.1.1.2 Metal Nanoparticles

NP are widely studied and employed in the manufacture of polymer nanocomposites for the development of novel materials with specific properties (optical, conductive, antimicrobial and so on). With respect to the design of antimicrobial nanocomposites for active food packaging, it is worth mentioning that metal NP exhibit antimicrobial properties via various mechanisms of action. In this regard, silver metal NP have been proved to be a reservoir of metal ions; the silver metal of the NP surface is oxidised to silver ions in an aqueous medium, and over time the NP dissolve, releasing antimicrobial ions into the liquid environment. Thus, immobilising NP in a polymer matrix capable of releasing oxidised silver ions is another way of creating antimicrobial polymer nanocomposites. There are many studies, patents and commercial applications regarding silver NP embedded in polymer matrices. However, their application in the development of antimicrobial packaging for food is limited owing to legislative issues regarding the amount of silver that migrates into food.

It is worth pointing out that the antimicrobial activity of polymer nanocomposite films incorporating antimicrobial ions will depend on several factors which affect their release, such as polymer crystallinity and water swelling capacity, as the polymer needs to be in an aqueous medium for it to become plasticised and facilitate diffusion. Other factors are related to the type of nanomaterial used to hold the ions, i.e., the release of the ions from zeolites is different to release from metal NP; for example, silver NP offer more stability and slower silver ion release rates than silver-impregnated zeolites, whereas the latter have a superior antimicrobial effect for short periods. The size and shape of the particle also affect the antimicrobial activity. Smaller particles have a larger relative surface area for silver ion release and spherical silver NP are highly reactive, owing to their high atomic density, which allows the faster release of ions [10]. There are several procedures for obtaining silver NP. In most cases, solutions of silver salt are used to impregnate polymer films in the presence of a reducing agent. Stable and uniformly distributed silver NP have been obtained via the in situ reduction
of silver salts in the presence of sodium borohydride as a reducing agent and using hydrogel networks as nanoreactors. In other cases, aqueous solutions containing both the polymer and silver salts are used and the formation of silver NP is due to the ability of the polymer to act as a reducing agent, as occurs with PVA or chitosan (CS) [10, 11]. In some of these procedures, temperature treatments or irradiation with UV or ultrasound are used to start the reaction. Evaporation of the solvent results in films containing the NP, as shown in Figure 3.1. Another procedure for obtaining antimicrobial materials based on silver is by the use of silver-impregnated zeolites [12]. Silver ions are incorporated into zeolite pores by ion exchange and then zeolites can be incorporated as a solid in any matrix.

Similarly, copper NP act as a reservoir for the sustained release of copper ions generated by the oxidation of copper metal on the NP surface. Copper NP, copper oxide NP and copper ion-impregnated nanofillers have been incorporated into various polymers of both synthetic and natural origin [13, 14]. Cioffi and co-workers [14] electrochemically manufactured colloidal copper NP and the NP were then dispersed in a polyvinyl chloride (PVC) solution.

Zinc oxide NP have been added to several polymers, including polypropylene (PP). Their mechanism of action relies on the generation of hydrogen peroxide when stimulated by visible light in conjunction with the release of zinc ions. Titanium oxide NP are used in food processing industries to decontaminate food contact surfaces and to prevent biofilm formation. The antimicrobial activity of these NP is related to the photocatalytic generation of reactive oxygen species when illuminated with UV light, but also with visible light irradiation. Recent research has been devoted to studying the viability of using these NP for antimicrobial packaging applications. For this purpose they have been incorporated into the polymer matrix or applied as a coating on the polymer film surface to inhibit or reduce microbial growth on the
Surface of solid foods. Recently, zinc nanocrystals have been embedded into polymer matrices for antimicrobial packaging purposes. Other metal and metal oxide NP with potential applications in the development of antimicrobial food packaging include gold, aluminium oxide, cadmium selenide/telluride and magnesium oxide [15, 17].

3.1.1.3 Mesoporous Silica

Mesoporous silica NP are characterised by a large surface area and pore volume, with a pore size range of 2 to 50 nm. The applications of these materials include catalysis, adsorption, sensing and polymer reinforcement, and they are also good candidates for encapsulation purposes [18]. They can accommodate a great volume of molecules, increasing the solubility of poor water-soluble molecules, protecting them against external factors or working as carriers and controlled release systems for active compounds. Mesoporous silica NP are widely studied as controlled release systems.

Research on mesoporous NP is focused on their morphology, pore size and chemical surface functionalisation. These nanomaterials can be synthesised with different pore morphologies and shapes (sphere, rod, fibre, doughnut and so on), and can be functionalised to modify their interaction with encapsulated molecules and their release pattern [19].

A great amount of research is focused on the application of mesoporous silica NP as carriers and controlled release drug-delivery systems [20, 21]; however, only a small amount of investigation has been devoted to the study of bioactives [22], naturally occurring antimicrobials allyl isothiocyanate and menthol [23, 24], herbicides [25], antimicrobial silver ion-exchanged mesoporous silica [26] and silver nanocrystals encapsulated in mesoporous silica as antimicrobials whose activity is induced through their oxidative dissolution [27].

The use of mesoporous silica NP for the design of polymer nanocomposites is currently receiving attention, and many properties of novel nanocomposites have yet to be elucidated [28]. In this context, studies regarding the use of these materials as active agent carriers and release systems for the development of active food packaging are scarce. Heirlings and co-workers [29] used mesoporous silica SBA-15 for the development of antioxidant films for active food packaging applications; in order to achieve this the antioxidant α-tocopherol was adsorbed in the material and incorporated into low-density polyethylene (LDPE) and ethylene-vinyl acetate (EVA) to protect it from polymer extrusion and facilitate extended agent release. Recently, Buonocore and co-workers [30] compared the sustained release properties of SBA-15 and amino-functionalised SBA-15 loaded with tocopherol and incorporated into a LDPE matrix.
Besides silica NP, other mesoporous inorganic materials, such as aluminium oxide functionalised with hydrophilic and hydrophobic groups, titanium dioxide nanospheres and nanosized hydroxycarbonate apatite, are being studied as carriers and release systems for drugs [19].

Hollow silica and mesoporous hollow silica have been synthesised and their potential in catalysis, as supports for immobilising molecules, as carriers and controlled release drug-delivery systems is being studied. Their application in the development of polymer composites and active packaging is expected to increase over the next few years.

### 3.1.2 Encapsulation in an Organic Substrate

Encapsulation enables the immobilisation of a compound or mixture of compounds in a material that coats it or in which it is dispersed. The substance to be encapsulated can be a solid or liquid compound or even a gas. Using encapsulation it is possible to achieve various interesting effects, such as: isolating compounds that might react, absorbing liquids into solids to facilitate their handling, protecting the encapsulate from the environment around it, improving product manipulation, masking odours, colours and flavours, and also increasing the effectiveness of active compounds through controlled release or release sustained over time. Encapsulation processes now occupy an important position in various areas including medicine, pharmaceuticals, cosmetics, textiles, agrochemicals, food and, of course, packaging.

The first studies involving encapsulation and its possible application in the pharmaceutical area began with the preparation of microcapsules of gelatin by means of coacervation and it subsequently spread to other fields, such as inks in the manufacture of tracing paper, and aromas and flavours in the food industry. In the 1950s the American Chicle company asked the Atlantic Gelatin Division of General Foods to develop a method to prolong the mint flavour in its chewing gum, for which purpose they encapsulated tiny droplets of mint oil in gelatin capsules; thus the mint flavour was released slowly. Since then, microencapsulation has found interesting applications in a variety of fields.

Pharmaceutical companies have led the applications of microencapsulation [31], pursuing objectives such as masking unpleasant flavours in various drugs, protecting them against pH in their passage through the stomach, controlling the rate at which drugs are released and reducing differences in the drug concentration in an organism between doses.

Some enzymes, animal and plant cells and microorganisms have been immobilised in microcapsules. The substrates pass through the coatings and are processed by the