

The use of nanotechnology as an approach for essential oil-based formulations with antimicrobial activity

A. São Pedro^{1,*}, I. Espírito Santo¹, C. V. Silva¹, C. Detoni² and E. Albuquerque¹

¹PEI, Escola Politécnica, Universidade Federal da Bahia, Rua Aristides Novis, 2, Federação, 40210-630, Salvador, Bahia, Brazil

²Departamento de Saúde, Universidade Estadual de Feira de Santana, Av. Universitária, s/n Km 03 da BR 116, VI Módulo, Campus Universitário, 44031-460, Feira de Santana, Bahia, Brazil

The resistance of the bacteria and fungi to the innumerable antimicrobial agents constitutes one of the great challenges in the treatment of infections, conditioning to the necessity of searching and finding new sources of substances with antimicrobial properties. Essential oils are aromatic oily liquids that can be obtained from several parts of the plants. Known for their medicinal properties, mainly antimicrobial, these kind of compounds can easily undergo oxidation reactions resulting in allergenic products and/or products with less biological activity. The nanoencapsulation of these oils in drug delivery systems have been proposed due to their capability of improving the solubility, stability and efficacy of essential oil-based formulations, by maintenance of therapeutic drug blood levels. Among them, polymeric nanoparticulate formulations have been extensively studied with significant improve of the essential oil antimicrobial activity. Similar results also were obtained on studies focusing on lipid carriers, including liposomes, solid lipid nanoparticles, as well as nanoemulsions. Furthermore, other innovative carriers have been applied providing interesting results.

Keywords essential oils; antimicrobial; nanotechnology

1. Essential Oils

Essential oils (EOs) are volatile, natural, aromatic oily liquids that can be obtained from several parts of the plants especially the aerial ones as leaves and flowers. They are derived from complex metabolic pathways in order to protect the plant organism from diverse pathogenic microorganisms, to repel insects that act as plague vectors, to reduce the appetite of some herbivorous by conferring unpleased taste do the plant. Taking into account the strong sensitivity of insects to smells, the EOs generally facilitate the plant reproduction by attraction of specific insects that favour the dispersion of pollens and seeds [1]. Due to the aromatic feature of EOs, they have been widely used in industries for the production of soaps, perfumes and toiletries. On the other hand is remarkable their prospection and application as therapeutic products by folk medicine since middle ages. In observance to the popular faith oh the medicinal properties of EOs, a series of ethnopharmacological investigations has been evidenced their application for treatment of a wide range of pathological conditions [2, 3]. In fact, the large bioactivity of EOs has being confirmed by several studies, including antibacterial, antiviral, anti-inflammatory, antifungal, antimutagenic, anticarcinogenic, and antioxidant as well as other miscellaneous activities [4].

The extensive panel of biological activities of EOs can be linked to the complexity and variability of their chemical composition. Several factors can affect the final composition of this natural product. Different parts of the plant are composed by different systems and cell lines with different and extensive metabolic pathways involved on the production these oils. Beyond leaves and flowers, the extraction of EOs can be carried out from fruits, seeds, stems, barks, roots, rhizomes, gums and oleoresin exudations. Taking into account the seasonal variation of environmental conditions of the nature during the year, it is also highly important the period of the year when is harvested the plant material for oil extraction. The nutrients available for plant growth have a crucial role on the definition the biochemical reactions on plant organelles, as well as, the exposition to light, the environmental temperature and water supply. Aware of these factors, the artificial cultivation of the plants for EO production must take in consideration a large list of variables [5]. All of these incognitos justify the existence of EOs with different compositions from the same plant material, i.e. there are several chemotypes for the same oil.

Different methods of preparation can also provide different EOs. Some of the oldest techniques for EO production are steam and hydrodistillation [6]. The first documents describing distillation are dated from the 9th century. Imported into Europe by the Arabs, the concept of EOs was limited to distilled aromatic waters obtained from plants treat with alcohol. In the 16th century the notions of fatty oils and EOs, as well as the methods for separating essences from aromatic waters, became established. In these methods the EO released from the plant material by heating are carried by aqueous vapour and after mutual condensation occurs the separation between aqueous and oily drops. Extraction methods mediated by organic solvents are also applied, however considering the high toxic potential of this kind solvents, alternatives have been pursued, such as inert gases and innocuous supercritical fluids.

From a general point of view, the plant metabolites present in the EOs are comprised among terpenes and terpene derivatives formed via acetate-mevalonic acid pathway, aromatic compounds via shikimic-phenylpropanoid route and others molecules derived from miscellaneous origin. Oxygenated constituents comprising various moieties are mainly

responsible for providing taste and odour to essential oil. Among terpenes, the monoterpenes, sesquiterpene and diterpenes represent the major constituents of EOs which can be found accumulate in cell cytosol, vacuoles of epidermal cells, mesophyll cells and glandular cells of the plant. Due to the typical small size and apolar nature of monoterpene molecules they readily diffuse through cell membranes, as well as skin layers which enables their use as permeation enhancers for active pharmaceutical ingredients [7]. The monoterpenes are composed of two branched, unsaturated C₅ units (isoprene), having a molecular weight of 136amu. The sesquiterpenes are composed of three isoprene units, counting a molecular weight of 204amu. There are more than 10,000 kinds of sesquiterpenes with a broad range of biological activities. The terpenoids, derived from oxygenation reaction routes of terpenes, including aromatic and aliphatic acid esters, alcohol and phenolic compounds, are associated to a large list of biological activities [8, 9].

1.1. Antimicrobial Profile of Essential Oils

The literature demonstrates a plenty of studies overviewing the remarkable performance of EOs as antimicrobials. *In vitro* and *in vivo* studies have been confirming what ethnopharmacological studies have been found in the folk knowledge: an enormous number of EOs exhibits exceptionally good activity against bacteria, yeasts and filamentous fungi. These works are encouraged by the emergence of drug-resistant pathogenic microorganisms due to abundant and in some cases abusive use of anti-infective agents [10]. Community and hospital acquired strains of diverse pathogens, including *Staphylococcus aureus*, *Salmonella*, *Shigella*, *coagulase-negative Staphylococcus*, *Enterococcus sp.*, *Escherichia coli*, and *Pseudomonas aeruginosa* represent the main multi-drug resistant bacteria [11]. An uncountable number of scientific papers have been cited EOs from varied origin with clear inhibitory profile against pathogenic bacteria and fungi. Several works describe even broadly known EOs, such as lemongrass [12], oregano [12, 13], peppermint [14], rosemary [13], thyme [15], cinnamon and clove oil [16] as large spectrum antimicrobial agents. Taking into account that the world flora still holds millions of novel vegetal species not studied yet, several possibilities can be drawn for the future of new strategies on infective diseases control.

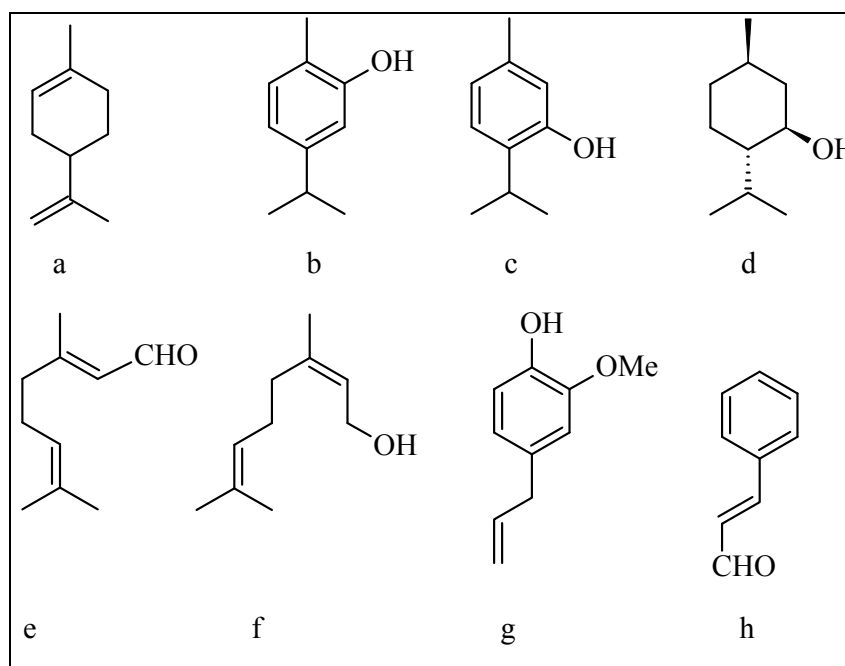


Fig. 1 Structures of classical phytochemicals derived from EOs with known antimicrobial activity: the terpene a) limonene and the terpenoids b) carvacrol, c) thymol, d) menthol, e) citral, f) geraniol, g) eugenol, h) cinnamaldehyde.

In a general form, the high hydrophobicity of the EO constituents, as well as the short extension of their carbon chains enable their tight interaction with lipid cell membranes. The partition of terpenes with the lipids of microbial cells affects the fluidity of external membrane, as well as, mitochondrial membrane leading to their disruption with subsequent death of the pathogen [11, 17, 18]. The oil metabolites can also act by crossing the cell membrane and bind for inhibit specific proteins. Their adherence to or intercalation into DNA or RNA is reported as well. The Figure 1 depicts some of the EO metabolites with recognized antimicrobial activity. In comparison to the traditional pool of synthetic antibiotics commercial available, the EOs have some advantages on combat against microorganisms. Known as phyto-synergic interactions, the constituents of EOs act synergistically by different and until not fully understood mechanisms which significantly reduce the possibility of occurrence of resistance [19-21]. The assumption of only active phytochemicals are involved on antimicrobial mechanism is already overcome. Interaction among compounds in the EO can involve the protection of an active substance from degradation by enzymes from the pathogen; modification

of transport across enabling the circumvention of innumerable multi-drug resistance mechanisms based on expulsion of antibiotic molecules ionic bomb-mediated [20].

The multi-target mechanisms of phytochemicals from EOs explain their synergistic behaviour with commercial antibiotics. Several works have been performed showing the significant improve of antibiotics activity against drug resistant microorganism strains. These findings assist the decrease of medicine intake leading to safer treatment by reduction of adverse effects [22-24]. Yap and others [25] highlighted a pronounced synergistic relationship between piperacillin/cinnamon bark oil, piperacillin/lavender oil, piperacillin/peppermint oil as well as meropenem/peppermint oil against two bacteria strains that carries plasmids encoding beta-lactamases. Ahmad and colleagues [26] identified the ability of thymol and carvacrol in restoring the fluconazole susceptibility in resistant clinical isolates of *Candida* genus. The combination of oregano EO with amoxicillin, polymycin, and lincomycin showed an additive effect against extended-spectrum β -lactamase-producing *Escherichia coli*, while the combination with fluoroquinolones, doxycycline, lincomycin, and maquinox florfenicol displayed synergistic behaviour [27]. de Sousa and others [28] described the significant reduction of MIC values of aminoglycosides antibiotics against multiresistant strains of *Escherichia coli* and *Staphylococcus aureus* after their association with *Lantana montevidensis* Briq. essential oil. Similar results were also obtained for *Lantana camara* Linn EO[29]. Other group demonstrated significant improvement of amoxicillin activity against methicillin-resistant *Staphylococcus aureus* post association with lemon grass oil [30].

1.2. Essential Oil-Based Formulations

The cosmetic and pharmaceutical formulations based upon EOs are usually tailored by dispersing them on semi solid media. In order to stabilize the formation of emulsions a wide range of emulsifiers is used on this formulations. Taking into account the high hydrophobic nature of EOs, various fat-like excipients have been applied on development of creams, ointments, foams, cosmetic butters and lotions. Most of preparations are made for topical usage, and among them O/W types emulsions are preferred due to their better spreadability on the skin, as well as to avoid the awkward residual oily sensation after product application. Taking into account the natural antimicrobial profile of several EOs their use can lead to a reduced demand of synthetic preservative excipients like parabens which possess known allergenic potential.

Meantime, the preparations based on EOs have some important limitations. It is not recommended to expose the skin directly to EOs because of potential allergic reactions. Considering that the main constituents of EOs have unsaturated carbon chains, it is well known their susceptibility to oxidation mediated by light or heat [31]. The oxidation products of terpenes, specially oxidated sesquiterpenes with lacone rings, terpenoids and other plant metabolites have been demonstrated high allergenic activity, mainly potent contact reactions [5, 32, 33]. This justifies the need of use a small quantity on cosmetic formulations, in order to protect the site of application, as well as the integrity of the oil. The high volatility of EO components also discourages their free use, *i.e.* without a pharmaceutical vehicle. Furthermore, the low aqueous solubility also limits the possible routes of administration. The neglectable solubility of EOs in biological fluids impedes their absorption leading to a very low bioavailability. All these factors limit the application of EOs as candidates for pharmacotherapeutic treatments what demands solutions from pharmaceutical technology studies. So, the nanoencapsulation technology has been quoted as an alternative for overcoming these problems.

2. Nanoencapsulation Technology

For several years, the research on development of drug formulations has focused on tailoring of delivery systems which are capable of delay and sustain the drug release after administration [34]. These kinds of formulations, commonly known as modified drug delivery systems, take several advantages when compared with the conventional preparations. Their capability of controlling EO release confers to them improved efficacy, reduced toxicity, improved patient compliance and convenience [35]. In addition, intending the optimization of the delayed drug release from these formulations, controlled release systems have been widely studied. Controlled release may be defined as a method which allows controlling time and the site of drug release at a specific rate [36]. Among the controlled drug release systems, colloidal systems have played a prominent role on pharmaceutical research field. Colloidal particles possess average size ranging on nanometric scale, also known as nanoparticles [37]. Taking in consideration the concept of controlled release, the major aims on designing nanoparticles have included the control of particle size, morphology and release of bioactive chemicals in order to achieve site-specific action of the drug at a therapeutically rate and dose regimen [38].

Nanoparticles were first developed around 1970 for carrying of vaccines and anti-cancer drugs [35]. Besides controlling the release of drug molecule, nanocarriers also protect EOs against possible thermal or photo degradation which assures increased stability, flavor and function, consequently extending the final product shelf life [39]. Considering these features, these systems can actually represent an interesting approach for overcoming essential oils limitations. Nanocarriers can potentially protect the essential oil from oxidation or evaporation, as well as facilitate their antimicrobial activity by providing diverse diffusion properties trough biological membranes due to nanoscale particles dimension, *i.e.* new routes of administration can be considered.

Nanocarriers can be structured by a great variety of material and designs. This chapter is focused on the main organic nanocarrier systems, which are lipid based particles, nanoemulsions and biocompatible polymer based particles, as depicted on Figure 2. Antimicrobial EOs can also be associated with inorganic nanocarriers such as metal oxide base particles [40] and nanoclay [41], even though these carriers represent a promising EO delivery strategy they have been less studied.

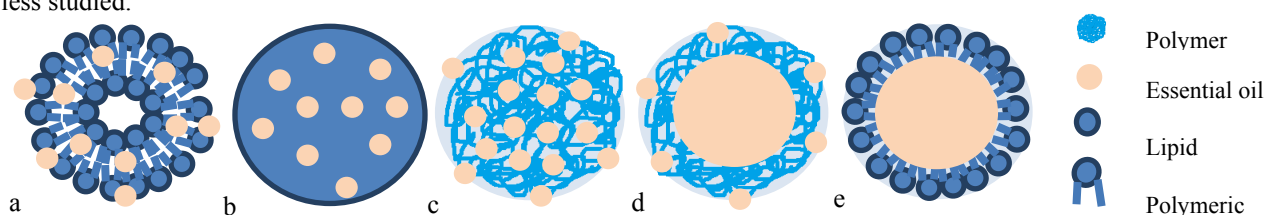


Fig. 2 Schematic illustration of nanoparticle platforms for antimicrobial essential oils: a) liposomes, b) solid lipid nanoparticles, c) polymeric nanospheres, d) polymeric nanocapsules, and e) nanoemulsions

2.1. Polymeric Nanoparticles

Polymeric nanoparticles are classified as nanocapsules and nanospheres. Nanocapsules have two compartments; a polymeric wall and a core, which is commonly oily. Nanospheres are matrix systems. The antimicrobial essential oil may be conjugated with the polymer (matrix or wall) or in the oily core. When considering an antimicrobial activity in medicine and as preservatives for food and cosmetics, these particles must be constituted of biocompatible materials. Biocompatible polymers may be of synthetic origins, such as poly (DL-lactide-co-glycolide) (PLGA), or natural origins, such as chitosan [42].

These particles are prepared by different techniques. One of the most used methods is based on an antisolvent procedure and may be referred to as nanoprecipitation or solvent displacement [42]. The polymer and antimicrobial EO are solubilized in an organic solvent or mixture of solvents. The organic solution is then added to water, with or without a hydrophilic surfactant. This step of the procedure occurs under constant stirring and requires either a laminar flux [43] or high shear rates [44]. The organic solvent is subsequently removed by evaporation techniques. The water may also be partially [43] or completely removed; rendering films [44] or powders [45].

The association of antimicrobial EO with polymeric nanoparticles presents several advantages; controlled release [44-47], enhanced apparent water solubility [48], reduced cytotoxicity [49] and equal or enhanced antimicrobial activity (Table 1) [43-46, 48-50].

Table 1 Associations of essential oils and polymeric particles.

Formulation	Drug	Targeted Microorganism	Activity	References
PCL nanocapsules	Tea tree oil	<i>Trichophyton rubrum</i>	Increased the EO effectiveness against fungal cells infecting nails	[43]
Zein–Sodium Caseinate (SC) Nanoparticles	Thymol	<i>E. coli</i> and <i>Salmonella</i>	Presented efficient antimicrobial activity and two-phase release profile	[44]
PLGA nanoparticles	Cinnamaldehyde and Eugenol	<i>Salmonella spp.</i> and <i>Listeria spp.</i>	Presented efficient antimicrobial activity and controlled release	[45]
Chitosan nanoparticles	Carvacrol	<i>E. coli</i> , <i>S. aureus</i> and <i>B. cereus</i>	Enhanced antimicrobial activity in comparison to chitosan NP	[46]
Chitosan nanoparticles	Oregano essential oil	-	Controlled release	[47]
Zein nanoparticles	Thymol and Carvacrol	<i>E. coli</i>	Presented efficient antimicrobial activity and enhanced solubility	[48]
Chitosan nanoparticles	Eugenol and Carvacrol	<i>S. aureus</i> and <i>E. coli</i>	Decreased cytotoxicity towards mice cells at MIC in comparison to free EOs	[49]
PLGA nanoparticles	Carvacrol	<i>S. epidermidis</i> biofilms	Reduced the elasticity and mechanical stability of preformed biofilms	[50]
Methyl and Ethylcellulose nanoparticles	Thymol	<i>S. aureus</i> , <i>E. coli</i> and <i>P. aeruginosa</i>	Presented efficient antimicrobial activity and is an effective preventive for cosmetic lotions creams and gels	[51]

A known advantage of nanoencapsulating antimicrobials is improving their apparent water solubility [52]. Zein nanoparticles containing carvacrol or thymol both presented higher apparent water solubility. The nanocapsule suspensions were prepared in different pH, 4, 6.5 and 10. EO-loaded nanocapsules increased solubility of the EOs in all tested pH conditions. The different pH conditions had influence on the degree of solubility enhancement. The data suggest that solubility of EOs was less improved at pH 10 and better improved at pH 4. A possible explanation is the deprotonation of the phenolichydroxyl group of EOs under high pH [48].

EO controlled release is desirable in medicine and in the prevention of food spoilage. In addition to maintaining the antimicrobial concentration at MIC, controlled release systems reduce the EO's organoleptic characteristics [45]. Polymeric nanocapsules containing EO in its core usually present a two phase release profile; a burst release followed by a prolong release phase [44, 45]. PLGA nanocapsules containing eugenol or *trans*-cinnamaldehyde both presented a two phase EO release. The first phase was rapid (under 30 minutes) and approximately 20% of the EO load was detected, the second release phase was prolonged and after 72 hours 64% of eugenol and 87% of *trans*-cinnamaldehyde were detected [45]. Considering that PLGA has a low degradation rate, the release was governed mostly by diffusion with a possible influence of polymer swelling and bulk erosion. The first release phase may be attributed to the molecules that are adsorbed to the polymeric wall, while the second release phase represents the EO present in the core of the nanocapsules which diffuses through the polymeric wall [45].

Zein nanoparticles containing thymol, used to form a film, also presented a two phase release profile of the EO. The particles were obtained by an antisolvent procedure, after the organic solvent was evaporated glycerol was added to the aqueous suspension and the water was evaporated in a thermostatic and humidistatic chamber [25 ± 1 °C, $50 \pm 5\%$ relative humidity (RH)] for 48 h, in order to form a film [44]. The film was easily dissolved in water and the release profile was assayed using the dialyses bag method. The authors believe that the rapid first phase represents the portion of thymol that was in the external phase of the film, the slower second phase represents thymol that was contained in the zein particles [44]. Chitosan nanoparticles containing oregano EO presented a similar two phase release profile [47]. Thus, the release assays of antimicrobial EOs in polymeric nanoparticles performed up to now indicate that these actives are usually present in the core of the particle and adsorbed to its surface resulting in a two phase release profile; burst release and prolonged release. This release profile was also observed for PLGA nanoparticles containing carvacrol. Since carvacrol antimicrobial activity occurs in the first 3 hours of exposure, a "burst" effect in the drug release kinetic could ensure adequate EO concentration right after the administration assuring its antimicrobial effect [50].

Thymol cumulative release was dependent on the encapsulated concentration; in lower concentrations (10% and 20%) thymol released a lower proportion of the total EO load than when in higher concentrations (30% and 40%). Atomic force micrographs showed that nanoparticles containing 10% and 20% of thymol were larger than the particles containing 30% and 40% of thymol when in the forming the films [44]. Oregano EO cumulative release from chitosan nanoparticles was also dependent on the drug load, but the opposite relation was observed. Higher concentrations of oregano EO lead to a lower percentile release from chitosan nanoparticles. In this case, the particle size was smaller in lower concentrations of EO [47]. Considering these two studies, it is possible that the release of EO from polymeric nanocapsules is linked to particle size, since smaller particles have higher surface-to-volume ratio which results in a greater release of the EO.

Most researches comparing antimicrobial activity of free and encapsulated EO find that the encapsulation dose not reduce inhibitory doses and betters other EO characteristic such as control release and cytotoxicity [46]. Considering mammalian use of polymeric nanoparticles loaded with antimicrobial EO, an important advantage is the reduction of EO cytotoxicity. Chitosan nanoparticles loaded with carvacrol or eugenol presented reduced cytotoxicity of T3T mice fibroblasts at MIC when compared to the free EOs. Even though the MIC found for free and encapsulated EO were similar, the advantages of the carvacrol and eugenol nanoparticles over eugenol and carvacrol can be seen by comparing their respective MIC against bacteria and the cytotoxicity values for 3T3 mouse fibroblasts. Based on previews researches and on their experimental data Chen and co-workers [49] were able to observe that free eugenol and carvacrol MIC values were three orders of magnitude higher than the cytotoxic concentration of the free EOs, while eugenol and carvacrol nanocapsules had MIC values under the cytotoxic concentration for these nanocapsules [49].

EO nanocapsulation in polymeric particles is also able to enhance the EO antimicrobial activity. PLGA nanocapsules containing carvacrol significantly altered rheological characteristic of bacterial biofilms potentially facilitating the action of antimicrobial agents [50]. Biofilms are organized bacterial communities constituted of cells embedded in a matrix of self-produced polymeric substances, which usually have gel-like mechanical properties. PLGA/carcacrol nanoparticles altered the architecture of the biofilm leading to a fluidification of the extracellular matrix that could enhance the penetration of antimicrobial agents into the deep core of the biofilm. The core of biofilms contain cells that have a reduced metabolism which makes them tolerant of the action of the majority of the antimicrobial agents, this represent an obstacle to the effective eradication of biofilms. Therefore it is important that antimicrobial agents with mechanism of action towards structural and functional properties of the cell membranes, such as carvacrol, reach the core of biofilm [50].

Another example of enhanced EO antimicrobial activity is PCL nanocapsules containing tea tree oil, which were capable of reducing dermatophyte fungus growth on human nails [43]. The antimicrobial activity observed for the

polymeric capsules was higher than the observed for the conventional and nanometric emulsions. Nanometric formulations have larger surface area, potentially providing greater contact of the active substance with microbial cell surface [53]. Furthermore, the controlled release of the EO would promote continuous diffusion of antimicrobial to the fungal membrane, site of action of tea tree oil. The better results obtained for the polymeric nanocapsules in comparison with the nanoemulsions may be linked to an interaction between the polymer and lipophilic biological surfaces [43].

Polymeric nanocapsules are easily incorporated in creams and gels [53], which facilitates the use of EOs as cosmetic and pharmaceutical preservatives. Methyl cellulose/ethyl cellulose polymeric particles containing thymol were able to reduce and preserve levels of *E. coli* in an oil/water lotion and in a hydrophilic gel, of *P. aeruginosa* in an oil/water lotion and of *S. aureus* in an oil/water lotion and in a water/oil cream. Interestingly, free thymol was also capable of reducing microbiologic levels in these formulations, but the preservation period was shorter except for the *S. aureus* in an oil/water lotion where free thymol maintained low microbial levels for the same period as the nanocapsules. The better performance of encapsulated thymol in comparison to the free EO is due to volatilization and degradation of the free EO in some formulations. Another interesting observation is that thymol nanocapsules present a late antimicrobial activity but functioned well as a preservative. It is possible that the late antimicrobial activity occurs because the polymeric gel network retards the contact between the particles and the bacterial surface [51].

2.2. Lipid nanoparticles

Lipid nanoparticles can be roughly divided in liposomes, niosomes, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC). Liposomes and niosomes are colloidal association of amphiphilic lipids that organize themselves spontaneously in bilayer vesicles and that are suitable with hydrophilic and hydrophobic compounds. SLN and NLC are solid particles at room and human body temperatures that present lipid core, which makes these carriers a proper medium for entrapment of lipophilic compounds, as EO.

Several methods can be used to produce lipid nanoparticles. Liposomes are mainly produced by thin lipid film hydration method [54] and ethanol injection [55]. SLN are produced by solvent-in-water emulsion diffusion technique, replacing liquid lipid of an o/w emulsion for a solid or a blend of solid lipids [56].

As these nanoparticles are composed by lipids and/or phospholipids, they have the ability of interaction with several cell types [57]. So, these carriers can be seen as alternatives for treatment of microbial infections, due to their capacity of interaction with infected cells [58, 59]. Furthermore, the association of EO with lipid nanoparticles has different objectives, but the main aims are enhance the stability and the solubility in aqueous media of EO, maintenance or even enhance their biological activity, and drug targeting [60, 61]. Since SLN have been developed very recently, most of the papers published so far involving lipid nanoparticles and EO are related to liposomes. So, Table 2 summarizes some of these works described in the literature.

Table 2 Associations of essential oils and lipid nanoparticles.

Formulation	Drug	Targeted Microorganism	Reference
Hydrogenated PC* liposomes	soy Thymol, carvacrol, <i>p</i> -cymene, γ -terpinene and <i>Santolina insularis</i> EO	Herpes Simplex Virus 1 (HSV-1)	[60]
Liposomes	<i>Origanum dictamnus</i> EO	Gram-positive and gram-negative bacteria, and fungi (<i>Listeria monocytogenes</i>)	[62]
PC* + cholesterol liposomes	EO from four <i>Thymus</i> species	Gram-positive and gram-negative bacteria, human pathogenic fungi and food-pathogen bacteria.	[63]
Hydrogenated soy PC liposomes	<i>Artemisia arborecens</i> EO	Herpes Simplex Virus 1 (HSV-1)	[64]
Liposomes	Thymol, carvacrol, <i>p</i> -cymene, and γ -terpinene	<i>S. aureus</i> and <i>E. coli</i>	[65]
PC, PC/PS, PC/SA, PG/CL* liposomes	Linalyl acetate, menthol and thymol	<i>S. aureus</i> and <i>E. coli</i>	[66]
PC* liposomes	<i>Zanthoxylum tingoassuiba</i> EO	Dermatophyte fungi and gram-positive bacteria	[67]
Compritol 888 ATO SLN	<i>Artemisia arborescences</i> EO	Ecological pesticides	[68]
Comprisol 888 ATO SLN	<i>Artemisia arborescences</i> EO	Herpes Simplex Virus 1 (HSV-1)	[69]

*PC: Phosphatidylcholine, PS: Phosphatidylserine, SA: Stearylamine, PG: Phosphatidylglycerol, CA: Cardiolipin

Encapsulated essential oil presented high stability, and in certain studies for more than one year [60, 69], improving the fact that the nanoparticle can protect entrapped compounds.

S. aureus and *E. coli* are symbiotic bacteria for human beings. However, in large amounts, they can be virulent species due to the presence of toxins. As infections with *S. aureus* have pathogenic forms resistant to some antibiotics, it is extremely important to discover therapeutic alternatives for this bacteria. Cristani *et al.* [65] and Trombetta *et al.* [66] studied the antimicrobial activities of different encapsulated terpenes. It was demonstrated that liposome entrapped thymol and menthol were more toxic to *S. aureus* than the other terpenes, while carvacrol and p-cymene inhibited more intensively *E. coli* growth.

Cristani and co-workers [65] also studied several parameters in order to describe the mechanism of action antibacterial activity of EO. It was suggested that these terpenes presented antimicrobial activity due to their capability of disturb lipid fraction of bilayer membrane of the microorganism. Furthermore, after crossing the membrane, the terpenes disturbed some important site inside of the cells, leading to the antibacterial activity.

As aforementioned, entrapment of compounds can increase their chemical and biological activity. An example of the increase of activity is the comparison of free and encapsulated *Origanum dictamnus* EO activity [62]. α -tocopherol was used as standard for antimicrobial and antioxidant activities. It was show that *O. dictamnus* EO presented superior intensity of activity than α -tocopherol. However, entrapped EO presented even higher antioxidant and antimicrobial activities. This pattern was repeated for encapsulation of EO from different *Thymus ssp* species [63, 70].

Another example of enhanced of biological activity is liposomes containing EO from *Z. tingoassuiba* [67]. Antifungal activity observed for the entrapped EO was higher than free EO. It was also observed that the smaller the vesicle, the greater the activity. Furthermore, as α -bisabolol is one of the major constituents of this EO, the biological activity of this compound was also studied. It was demonstrated that the encapsulated terpene also presented higher antifungal activity, proving that the encapsulation of this EO was efficient in inhibiting fungal growth.

EOs do not have only antimicrobial and antifungal activity. Studies have been made demonstrating that some EOs possess also antiviral activity. *Santolina insularis* EO was tested against HSV-1 and presented satisfactory results, probably due to direct virucidal effects. When this EO was loaded into liposomes, there was still a certain antiviral activity. However, this activity was decreased [60]. As virus possess different organization, probable the EO activity was hampered by the presence of the virus capsule. A different behavior was found when EO from *Artemisia arborescences* was entrapped into liposomes [64] and SLN [69]. According to authors, the antiherpetic activity was not affected by the encapsulation. Actually, the results showed that the encapsulated EO activity was even better than the free EO. Nevertheless, the essential oil was able to inactivate the virus only when it was delivered from particles, since these particles acted like target carriers and delivered the EO direct to the action site.

EO from *A. arborescences* was also tested against ecological pesticides [68] and it was shown that the entrapment into SLN reduced the rapid evaporation of EO. This led to a targeted release of EO for pesticide attack, demonstrating that this type of drug carrier is also suitable in agriculture. It is important to notice that most researchers compared results of biological activity of free EO and loaded with lipid nanoparticles and the biological activity was, at least, maintained. In some cases, the activity was even greater, which demonstrate the suitability of these carriers encapsulating EO.

2.3. Micro and nanoemulsions

Over the past few decades, extensive research has been done on nanoparticles. The most formulations using this technology are effectively based on micro and nanometric-scaled emulsions and despite apparently being respected by droplet size, micro- and nanoemulsions have other differences as stability and source process [71, 72].

Microemulsions can be defined as homogeneous thermodynamically stable transparent dispersions of two immiscible liquids stabilized by an interfacial film of surfactants. They have droplet size above 500nm and require very less energy to formulate emulsion, since they form spontaneously when aqueous, oily and amphiphilic components are brought into contact, besides having a lower production cost compared to nanoemulsions. One major drawback to microemulsions is that formation requires high surfactant concentration, which causes toxicity when used in pharmaceutical applications. In contrast nanoemulsions can be prepared using lower surfactant concentrations [73-75].

Nanoemulsions are fine oil-in-water dispersions, non-equilibrium systems with a spontaneous tendency to separate into the constituent phases. Nevertheless, nanoemulsions may possess a relatively high kinetic stability even for several years, due to their very small size, essentially the consequence of significant steric stabilization between droplets. They have droplet covering the size range of 10 –500 nm and also referred to as mini-emulsions, ultrafine emulsions, emulsoids, unstable microemulsions and submicrometer emulsions. It has been demonstrated that nanoemulsion systems increased the retention of a drug in the body, hence enhanced the bioavailability and reducing amount of drug required for the therapeutic action [72, 76]. They can be prepared by sonication, phase inversion temperature and high pressure homogenizer. These techniques are expensive because require a special kind of equipment [72].

Many different applications of micro and nanoemulsions have been reported including preservatives in foods, delivering pesticides in agrochemical and antimicrobial agents in disinfectant formulas [77, 78]. Antimicrobial

properties of micro and nanoemulsion are believed to result from the small size of oil particles that have a high surface tension which can fuse with and subsequently disrupt the membrane of isolated prokaryotic cells, viruses, and eukaryotic cells of fungi but they do not affect eukaryotic cells of higher organisms. A synergistic antimicrobial effect could be afforded by including some substances which possess strong antimicrobial activity into the formula, reducing the amounts of active substances and detergents used for killing microorganisms by the conventional method and the cost of raw materials. Furthermore, irritation caused from detergents in the formula is not likely to happen when they are used in a low concentration [78].

Increased awareness of consumers on the ill-effects of synthetic preservatives and development of antibiotic resistant strains have allowed the use of naturally occurring compounds exhibiting antimicrobial activity is receiving considerable attention nowadays, both in food and pharmaceutical industry. Essential and fixed oils showed encouraging results with micro and nanoemulsions [79]. In food industry, studies with nanoemulsions prepared with sunflower oil showed promising results against strains of *Salmonella typhi*, *Listeria monocytogenes*, and *Staphylococcus aureus* and it also demonstrated a high fungicidal activity against *Rhizopus nigricans*, *Aspergillus niger* and *Penicillium* sp. and a greater sporicidal activity against *Bacillus cereus* and *Bacillus circulans*. In situ evaluation for antimicrobial activity on food products such as raw chicken, apple juice, milk and mixed vegetable showed a significant reduction in the native cultivable bacterial and fungal populations of these products [79]. Anjali and colleagues [80] demonstrated that microemulsions with sunflower oil inhibited the bacterial growth against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In spite of the results with fixed oils, essential oils, because of their compositions, have been shown to best effect when in the form of nano- and microemulsions according to the following table.

Table 3 Associations of essential oils and micro and nanoemulsions.

Formulation	Oil	Targeted Microorganism	Purposes	References
Nanoemulsion	Eucalyptus	<i>Proteus mirabilis</i>	Pharmaceuticals	[81]
Microemulsion	Clove and Eugenol	<i>E. coli</i> , <i>S. aureus</i> , <i>Salmonella typhi</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacillus cereus</i> and <i>Listeria monocytogenes</i>	Pharmaceuticals, Cosmetics, Foods and Desinfectants	[82]
Microemulsion	Ocimum	<i>Propionibacterium acnes</i>	Pharmaceuticals, Cosmetics	[83]
Nanoemulsion	Carvacrol, Limonene, Cinnamaldehyde, sunflower Oil	<i>E. coli</i> , <i>Lactobacillus delbrueckii</i> and <i>Saccharomyces cerevisiae</i>	Foods	[84]
Nanoemulsion	Limonene, terpenes from Melaleuca, sunflower oil and palm oil	<i>Lactobacillus delbrueckii</i> , <i>Saccharomyces cerevisiae</i> , <i>Escherichia coli</i>	Foods	[85]
Nanoemulsion	Andiroba oil and Aroeira oil	<i>T. evansi</i>	Pharmaceuticals	[86]
Nanoemulsion	Peppermint	<i>S. aureus</i> and <i>Listeria monocytogenes</i>	Foods	[39]
Nanoemulsion	Thymol	<i>Escherichia coli</i> <i>Listeria monocytogenes</i>	Foods	[87]
Nanoemulsion	Ocimum	<i>E. coli</i>	Foods	[73]
Nanoemulsion	Lemon myrtle oil	<i>E. coli</i> , <i>Listeria monocytogenes</i> , <i>Salmonella Typhimurium</i> , <i>P. aeruginosa</i> , and <i>B. cereus</i>	Pharmaceuticals, Cosmetics, Foods and Desinfectants	[78]
Microemulsion	Carvacrol and eugenol	<i>Escherichia coli</i> <i>Listeria monocytogenes</i>	Foods	[88]
Microemulsion	mustard oil	<i>E. coli</i>	Foods	[77]

3. Final remarks

Essential oils are important antimicrobials due to the synergism of their components and their capability of modulating antibiotic resistance. These natural products have conquered space in the food, drug and cosmetic commercial arena. Unfortunately, like many lipophilic and aromatic active substances, EO present some drawbacks such as, low water solubility, strong organoleptic characteristics (flavor and aroma), low stability and present limited administration routes.

Nanoencapsulation in liposomes, solid lipid nanoparticles, nanoemulsions and polymeric nanoparticles is a promising strategy to facilitate the applicability of essential oils as antimicrobials. Additionally, these particles are capable of controlling the release of the EO promoting a prolonged preservative effect in cosmetics and foods, as well as a potential advantage for pharmaceutical dosage forms. Regarding the antimicrobial effect, when the results of biological activity of free EO and EO-loaded nanoparticles the activity was, at least, maintained. In some cases, the activity was even greater, which demonstrate the suitability of these carriers encapsulating EO.

References

- [1] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils--a review. *Food Chem Toxicol.* 2008;46:446-475.
- [2] Hennebelle T, Sahnaz S, Joseph H, Bailleul F. Ethnopharmacology of *Lippia alba*. *Journal of Ethnopharmacology.* 2008;116:211-222.
- [3] Belay G, Tariku Y, Kebede T, Hymete A, Mekonnen Y. Ethnopharmacological investigations of essential oils isolated from five Ethiopian medicinal plants against eleven pathogenic bacterial strains. *Phytopharmacology.* 2011;1:133-143.
- [4] Shaaban HAE, El-Ghorab AH, Shibamoto T. Bioactivity of essential oils and their volatile aroma components: Review. *Journal of Essential Oil Research.* 2012;24:203-212.
- [5] Vigan M. Essential oils: renewal of interest and toxicity. *European Journal of Dermatology.* 2010;20:685-692.
- [6] Charles DJ, Simon JE. Comparison of Extraction Methods for the Rapid Determination of Essential Oil Content and Composition of Basil. *Journal of the American Society for Horticultural Science.* 1990;115:458-462.
- [7] Nokhodchi A, Sharabiani K, Rashidi MR, Ghafourian T. The effect of terpene concentrations on the skin penetration of diclofenac sodium. *International Journal of Pharmaceutics.* 2007;335:97-105.
- [8] Breitmaier E. *Terpenes.* Wiley; 2006.
- [9] Schütte H-R. *Secondary Plant Substances: Sesquiterpenes.* Springer Berlin Heidelberg; 1999.
- [10] Reichling J, Schnitzler P, Suschke U, Saller R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties--an overview. *Forsch Komplementmed.* 2009;16:79-90.
- [11] Solórzano-Santos F, Miranda-Novales MG. Essential oils from aromatic herbs as antimicrobial agents. *Current Opinion in Biotechnology.* 2012;23:136-141.
- [12] Choi J-Y, Damte D, Lee S-J, Kim J-C, Park S-C. Antimicrobial activity of lemongrass and oregano essential oil against standard antibiotic resistant *Staphylococcus aureus* and field isolates from chronic mastitis cow. *International Journal of Phytomedicine.* 2012;4:134-139.
- [13] Mathlouthi N, Bouzaienne T, Oueslati I, Recoquilly F, Hamdi M, Urdaci M, Bergaoui R. Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: in vitro antimicrobial activities and effects on growth performance. *Journal of Animal Science.* 2012;90:813-823.
- [14] Hamoud R, Sporer F, Reichling J, Wink M. Antimicrobial activity of a traditionally used complex essential oil distillate (Olbas(R) Tropfen) in comparison to its individual essential oil ingredients. *Phytomedicine.* 2012;19:969-976.
- [15] Sienkiewicz M, Lysakowska M, Denys P, Kowalczyk E. The antimicrobial activity of thyme essential oil against multidrug resistant clinical bacterial strains. *Microbial Drug Resistance.* 2012;18:137-148.
- [16] Cava R, Nowak E, Taboada A, Marin-Iniesta F. Antimicrobial activity of clove and cinnamon essential oils against *Listeria monocytogenes* in pasteurized milk. *Journal of Food Protection.* 2007;70:2757-2763.
- [17] Devi KP, Nisha SA, Sakthivel R, Pandian SK. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhiby* disrupting the cellular membrane. *Journal of Ethnopharmacology.* 2010;130:107-115.
- [18] Lv F, Liang H, Yuan Q, Li C. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International.* 2011;44:3057-3064.
- [19] Bassolé IHN, Juliani HR. Essential Oils in Combination and Their Antimicrobial Properties. *Molecules.* 2012;17:3989-4006.
- [20] Efferth T, Koch E. Complex Interactions between Phytochemicals. The Multi-Target Therapeutic Concept of Phytotherapy. *Current Drug Targets.* 2011;12:.
- [21] van Vuuren S, Viljoen A. Plant-based antimicrobial studies-methods and approaches to study the interaction between natural products. *Planta Medica.* 2011;77:1168-1182.
- [22] Langeveld WT, Veldhuizen EJ, Burt SA. Synergy between essential oil components and antibiotics: a review. *Critical Reviews in Microbiology.* 2013;in press.
- [23] Palaniappan K, Holley RA. Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. *International Journal of Food Microbiology.* 2010;140:164-168.
- [24] Hemaiswarya S, Kruthiventhi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine.* 2008;15:639-652.
- [25] Yap PSX, Lim SHE, Huc CP, Yiap BC. Combination of essential oils and antibiotics reduce antibiotic resistance in plasmid-conferred multidrug resistant bacteria. *Phytomedicine.* 2013;in press.
- [26] Ahmad A, Khan A, Manzoor N. Reversal of efflux mediated antifungal resistance underlies synergistic activity of two monoterpenes with fluconazole. *European Journal of Pharmaceutical Sciences.* 2013;48:80-86.
- [27] Si H, Hu J, Liu Z, Zeng Z-l. Antibacterial effect of oregano essential oil alone and in combination with antibiotics against extended-spectrum β -lactamase-producing *Escherichia coli*. *FEMS Immunology & Medical Microbiology.* 2008;53:190-194.
- [28] de Sousa EO, Rodrigues FFG, Campos AR, Lima SG, da Costa JGM. Chemical composition and synergistic interaction between aminoglycosides antibiotics and essential oil of *Lantana montevidensis* Briq. *Natural Product Research.* 2012;First:1-4.

- [29] Sousa EO, Silva NF, Rodrigues FFG, Campos AR, Lima SG, Costa JGM. Chemical composition and resistance-modifying effect of the essential oil of *Lantana camara* Linn. *Pharmacogn Mag*. 2010;6:79-82.
- [30] El-Kalek HHA, Mohamed EA. Synergistic effect of certain medicinal plants and amoxicillin against some clinical isolates of methicillin – resistant *Staphylococcus aureus* (MRSA). *International Journal of Pharmaceutical Applications*. 2012;3:387-398.
- [31] Neumann M, Garcia N. Kinetics and mechanism of the light-induced deterioration of lemon oil. *Journal of Agricultural and Food Chemistry*. 1992;40:957-960.
- [32] Hammer KA, Carson CF, Riley TV, Nielsen JB. A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil. *Food and Chemical Toxicology*. 2006;44:616-625.
- [33] Sköld M, Börje A, Matur M, Karlberg A-T. Studies on the autoxidation and sensitizing capacity of the fragrance chemical linalool, identifying a linalool hydroperoxide. *Contact Dermatitis*. 2002;46:267-272.
- [34] Maderuelo C, Zarzuelo A, Lanao JM. Critical factors in the release of drugs from sustained release hydrophilic matrices. *Journal of Controlled Release*. 2011;154:2-19.
- [35] Kumar MNVR. Nano and Microparticles as Controlled Drug Delivery Devices. *Journal of Pharmacy & Pharmaceutical Sciences* 2000;3:234-258.
- [36] Pothakamury UR, Barbosa-Canovas GV. Fundamental aspects of controlled release in foods. *Trends in Food Science and Technology*. 1995;6:397-406.
- [37] Kamble VA, Jagdale DM, Kadam VJ. Solid lipid nanoparticles as drug delivery system. *International Journal of Pharma and Bio Sciences*. 2010;1:1-9.
- [38] Mohanraj V, Chen Y. Nanoparticles – A Review. *Tropical Journal of Pharmaceutical Research*. 2006;5:561-573.
- [39] Liang R, Xu S, Shoemaker CF, Li Y, Zhong F, Huang Q. Physical and Antimicrobial Properties of Peppermint Oil Nanoemulsions. *Journal of Agricultural and Food Chemistry*. 2012;60:7548-7555.
- [40] Chifiriuc C, Grumezescu V, Grumezescu AM, Saviuc C, Lazăr V, Andronescu E. Hybrid magnetite nanoparticles/*Rosmarinus officinalis* essential oil nanobiosystem with antibiofilm activity. *Nanoscale Research Letters*. 2012;7:209-215.
- [41] Abdollahi M, Rezaei M, Farzi G. A novel active bionanocomposite film incorporating rosemary essential oil and nanoclay into chitosan. *Journal of Food Engineering*. 2012;111:343-350.
- [42] Pinto Reis C, Neufeld RJ, Ribeiro AJ, Veiga F. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2006;2:8-21.
- [43] Flores FC, Lima JA, Ribeiro RF, Alves SH, Rolim CMB, Beck RCR, Silva C. Antifungal Activity of Nanocapsule Suspensions Containing Tea Tree Oil on the Growth of *Trichophyton rubrum*. *Mycopathologia*. 2013;175:281-286.
- [44] Li K-K, Yin S-W, Yang X-Q, Tang C-H, Wei Z-H. Fabrication and Characterization of Novel Antimicrobial Films Derived from Thymol-Loaded Zein–Sodium Caseinate (SC) Nanoparticles. *Journal of Agricultural and Food Chemistry*. 2012;60:11592-11600.
- [45] Gomes C, Moreira RG, Castell-Perez E. Poly (DL-lactide-co-glycolide) (PLGA) Nanoparticles with Entrapped trans-Cinnamaldehyde and Eugenol for Antimicrobial Delivery Applications. *Journal of Food Science*. 2011;76:N16-N24.
- [46] Keawchaon L, Yoksan R. Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles. *Colloids and Surfaces B: Biointerfaces*. 2011;84:163-171.
- [47] Hosseini SF, Zandi M, Rezaei M, Farahmandghavi F. Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: Preparation, characterization and in vitro release study. *Carbohydrate Polymers*. 2013;95:50-56.
- [48] Wu Y, Luo Y, Wang Q. Antioxidant and antimicrobial properties of essential oils encapsulated in zein nanoparticles prepared by liquid–liquid dispersion method. *LWT - Food Science and Technology*. 2012;48:283-290.
- [49] Chen F, Shi Z, Neoh KG, Kang ET. Antioxidant and antibacterial activities of eugenol and carvacrol-grafted chitosan nanoparticles. *Biotechnology and Bioengineering*. 2009;104:30-39.
- [50] Iannitelli A, Grande R, Stefano AD, Giulio MD, Sozio P, Bessa LJ, Laserra S, Paolini C, Protasi F, Cellini L. Potential Antibacterial Activity of Carvacrol-Loaded Poly(DL-lactide-co-glycolide) (PLGA) Nanoparticles against Microbial Biofilm. *International Journal of Molecular Sciences*. 2011;12:5039-5051.
- [51] Wattanasatcha A, Rengpipat S, Wanichwecharungruang S. Thymol nanospheres as an effective anti-bacterial agent. *International Journal of Pharmaceutics*. 2012;434:360-365.
- [52] Zhang L, Pornpattananangkul D, Hu C-M, Huang C-M. Development of nanoparticles for antimicrobial drug delivery. *Current Medicinal Chemistry*. 2010;17:585-594.
- [53] Lboutounne H, Chaulet J-F, Ploton C, Falson F, Pirot F. Sustained ex vivo skin antiseptic activity of chlorhexidine in poly(ϵ -caprolactone) nanocapsule encapsulated form and as a digluconate. *Journal of Controlled Release*. 2002;82:319-334.
- [54] Bangham AD. Physical structure and behavior of lipids and lipid enzymes. *Advances in Lipid Research*. 1963;1:65-104.
- [55] Batzri S, Korn ED. Single bilayer liposomes prepared without sonication. *Biochimica et Biophysica Acta*. 1973;298:1015-1019.
- [56] Pardeike J, Hommoss A, Müller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. *International Journal of Pharmaceutics*. 2009;366:170-184.
- [57] Lasic DD. *Liposomes, from physics to application*. Amsterdam, Holland: Elsevier; 1993.
- [58] Allahverdiyev AM, Kon KV, Abamor ES, Bagirova M, Rafailovich M. Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents. *Expert Review of Anti-infective Therapy*. 2011;9:1035-1052.
- [59] Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *Journal of Controlled Release*. 2011;156:128-145.
- [60] Valenti D, Logu AD, Loy G, Sinico C, Bonsignore L, Cottiglia F, Garau D, Fadda AM. Liposome-incorporated *Santolina insularis* essential oil: preparation, characterization and in vitro antiviral activity. *Journal of Liposome Research*. 2001;11:73-90.
- [61] Liolios CC, Gortzi O, Lalas S, Tsaknis J, Chinou I. Liposomal incorporation of carvacol and thymol isolated from the essential oil of *Origanum dictamnus* L. and in vitro antimicrobial activity. *Food Chemistry*. 2009;112:77-83.
- [62] Gortzi O, Lalas S, Chinou I, Tsaknis J. Evaluation of the antimicrobial and antioxidant activities of *Origanum dictamnus* extracts before and after encapsulation in liposomes. *Molecules*. 2007;12:932-945.

- [63] Gortzi O, Lelas S, Chinou I, Tsaknis J. Reevaluation of antimicrobial and antioxidant activity of *Thymus* spp. extracts before and after encapsulation in liposomes. *Journal of Food Protection*. 2006;69:2998-3005.
- [64] Sinico C, Logu AD, Lai F, Valenti D, Manconi M, Loy G, Bonsignore L, Fadda AM. Liposomal incorporation of *Artemisia arborescens* L. essential oil and *in vitro* antiviral activity. *European Journal of Pharmaceutics and Biopharmaceutics*. 2005;59:161-168.
- [65] Cristani M, D'Arrigo M, Mandalari G, Castelli F, Sarpietro M, Micieli D, Venuti V, Bisignano G, Saija A, Trombetta D. Interaction of four monoterpenes contained in essential oil with model membranes: Implications for their antibacterial activity. *Journal of Agricultural and Food Chemistry*. 2007;55:6300-6308.
- [66] Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristiani M, Daniele C, Saija A, Mazzanti G, Bisignano G. Mechanisms of antibacterial action of three monoterpenes. *Antimicrobial Agents and Chemotherapy*. 2005;49:2474-2478.
- [67] Detoni CB, Cabral-Albuquerque EC, Holemwerger SV, Sampaio C, Barros TF, Velozo ES. Essential oil from *Zanthoxylum tingoassuiba* loaded into multilamellar liposomes useful as antimicrobial agents. *Journal of Microencapsulation*. 2009;26:684-691.
- [68] Lai F, Wissing SA, Muller RH, Fadda AM. *Artemisia arborescens* L. essential oil-loaded solid lipid nanoparticles for potential agricultural application: preparation and characterization. *AAPS PharmSciTech*. 2006;7:E10-E18.
- [69] Lai F, Sinico C, De Logu A, Zaru M, Muller RH, Fadda AM. SLN as a topical delivery system for *Artemisia arborescens* essential oil: *in vitro* activity and skin permeation study. *International Journal of Nanomedicine*. 2007;2:419-425.
- [70] Gortzi O, Lelas S, Chinou I, Tsaknis J. Evaluation of antimicrobial and antioxidant activity of *Thymus* spp. extracts before and after encapsulation in liposomes. *Molecules*. 2007;12:932-945.
- [71] Anton N, Benoit J-P, Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates—A review. *Journal of Controlled Release*. 2008;128:185-199.
- [72] Sharma N, Bansal M, Visht S, Sharma P, Kulkarni G. Nanoemulsion: A new concept of delivery system. *Chronicles of Young Scientists*. 2010;1:2-6.
- [73] Ghosh V, Mukherjee A, Chandrasekaran N. Ultrasonic emulsification of food-grade nanoemulsion formulation and Evaluation of its bactericidal activity. *Ultrasonics Sonochemistry*. 2012;4:497-500.
- [74] Peng L-C, Liu C-H, Kwan C-C, Huang K-F. Optimization of water-in-oil nanoemulsions by mixed surfactants. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2010;370:136-142.
- [75] Tadros T, Izquierdo P, Esquena J, Solans C. Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*. 2004;108:303-318.
- [76] Gutiérrez JM, González C, Maestro A, Solè I, Pey CM, Nolla J. Nano-emulsions: New applications and optimization of their preparation. *Current Opinion in Colloid & Interface Science*. 2008;13:245-251.
- [77] Ghosh V, Mukherjee A, Chandrasekaran N. Mustard oil microemulsion formulation and evaluation of bactericidal activity. *Ultrasonics Sonochemistry*. 2013;20:338-344.
- [78] Buranasuksombat U, Kwon Y, Turner M, Bhandari B. Influence of emulsion droplet size on antimicrobial properties. *Food Science and Biotechnology*. 2011;20:793-800.
- [79] Joe MM, Bradeeba K, Parthasarathi R, Sivakumar PK, Chauhan PS, Tipayno S, Benson A, Sa T. Development of surfactin based nanoemulsion formulation from selected cooking oils: Evaluation for antimicrobial activity against selected food associated microorganisms. *Journal of the Taiwan Institute of Chemical Engineers*. 2012;43:172-180.
- [80] Anjali CH, Madhusmita D, Chandrasekaran N, Amitava M. Anti-bacterial activity of sunflower oil microemulsion. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2010;2:123-128.
- [81] Saranya S, Chandrasekaran N, Mukherjee A. Antibacterial activity of eucalyptus oil nanoemulsion against *Proteus mirabilis*. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2012;4:668-671.
- [82] Hamed SF, Sadek Z, Edris A. Antioxidant and antimicrobial activities of clove bud essential oil and eugenol nanoparticles in alcohol-free microemulsion. *Journal of Oleo Science*. 2012;61:641-648.
- [83] Viyoch J, Pisutthanan N, Faikreua A, Nupangta K, Wangtorpol K, Ngokkuen J. Evaluation of *in vitro* antimicrobial activity of Thai basil oils and their micro-emulsion formulas against *Propionibacterium acnes*. *International Journal of Cosmetic Science*. 2006;28:125-133.
- [84] Donsi F, Annunziata M, Vincenzi M, Ferrari G. Design of nanoemulsion-based delivery systems of natural antimicrobials: Effect of the emulsifier. *Journal of Biotechnology*. 2012;159:342-350.
- [85] Donsi F, Annunziata M, Sessa M, Ferrari G. Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *LWT - Food Science and Technology*. 2011;44:1908-1914.
- [86] Baldissera MD, Da Silva AS, Oliveira CB, Zimmermann CEP, Vaucher RA, Santos RCV, Rech VC, Tonin AA, Giongo JL, Mattos CB, Koester L, Santurio JM, Monteiro SG. Trypanocidal activity of the essential oils in their conventional and nanoemulsion forms: *In vitro* tests. *Experimental Parasitology*. 2013;in press.
- [87] Shah B, Davidson PM, Zhong Q. Nanocapsular Dispersion of Thymol for Enhanced Dispersibility and Increased Antimicrobial Effectiveness against *Escherichia coli* O157: H7 and *Listeria monocytogenes* in Model Food Systems. *Applied and Environmental Microbiology*. 2012;78:8448-8453.
- [88] Gaysinsky S, Davidson PM, McClements DJ, Weiss J. Formulation and Characterization of Phyto-phenol-Carrying Antimicrobial Microemulsions. *Food Biophysics*. 2008;3:54-65.