Chapter

23

CHALLENGES IN DEVELOPMENT OF ESSENTIAL OIL NANODELIVERY SYSTEMS AND FUTURE PROSPECTS

Deborah Quintanilha Falcão*, Samanta Cardozo Mourão, Juliana Lopes de Araujo, Patricia Alice Knupp Pereira, Anne Caroline Andrade Cardoso, Kessiane Belshoff de Almeida, Fiorella Mollo Zibetti, and Barbara Gomes Lima

Department of Pharmaceutical Technology, Faculty of Pharmacy, Universidade Federal Fluminense, Rio de Janeiro, Brazil

*Corresponding author: deborah@vm.uff.br

Contents

23.1. INTRODUCTION

Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterisation, and application of materials and devices whose functional organisation is in the nanometer scale. Such substrates can be designed to display specific and controlled chemical and physical properties, which in medicine means to interact with cells and tissues in the subcellular level with a high degree of functional specificity, thereby providing the integration between technology and biological systems. Therefore, nanotechnology is not a natural emerging scientific discipline, but a multidisciplinary meeting of traditional sciences to provide the collective expertise necessary for the development of new technologies [1,2]. Despite the initial concept proposed by Feynman in 1959 to explore the possibility of a material handling scale of individual atoms and molecules, the term nanotechnology was not used until 1974, then being related to the electronics industry [3].

The application of nanotechnology in the pharmaceutical field is a topic that every day arouses more attention in the research and development (R & D) field and has been slowly transforming the landscape related to treatment, prevention, and diagnosis of many diseases, leading to the concept of "nanomedicine" in 2005, which was introduced by the National Institutes of Health in the United States. Nanomedicine is thus a wide field of study that includes nanoparticles (NPs) that mimic biological actions, "nanomachines", nanofiber and polymeric nanoconstructors of potential use as biomaterials and nanoscale devices (*e.g.*, silicon microchips for drug delivery), sensors, and laboratory diagnostics [2,4]. In this context, several innovative techniques exploit the unique characteristics of “nano” by applying them to obtain new pharmaceutical forms of controlled release and drug delivery systems that may increase the action of the active agents [3,5].

The concept of drug delivery systems was introduced in the early twentieth century by Paul Ehrlich with the "magic pill", in which the drug is released precisely in its exact site of action, thereby increasing its effectiveness. In this regard, the application of nanotechnology tends to make this more feasible due to the ideal small size of the particles, releasing the substance in the correct location and time [2,6]. Among the different systems used as drug carriers (nanocarriers) are the submicron emulsions (nanoemulsions, microemulsions, liquid crystals), polymeric micelles, cyclodextrins, liposomes, and NPs (polymeric, lipid, and metallic) [4].

These nanosystems are able to offer a therapeutic protection system to the drug against possible instability in the body, promoting maintenance of plasma levels at constant concentration; increased therapeutic efficacy; gradual and controlled release of the drug by conditioning the environmental stimulation

(sensitive to changes in pH or temperature); significant decrease in toxicity by reducing plasma levels of maximum concentration; reduced instability and decomposition of sensitive drugs; possibility of directing to specific targets; incorporation of both hydrophilic and lipophilic substances in the same formulation; and reduction of the therapeutic dose and number of administrations, leading to a consequent improvement in patient compliance and convenience [5]. Depending on the particle charge, surface properties, and relative hydrophobicity of the nanosystems, they can be designed to be adsorbed in organs or tissues preferentially, releasing the drug in high concentration in the desired location. The main disadvantages of nanoscaled particles are difficulties in production, storage, and administration due to physical instability phenomena such as aggregation. The choice of wall material and production method is therefore of paramount importance and combined with the ability to modify drug release makes such nanosystems ideal candidates for therapeutic purposes presenting a wide range of applications, such as cancer therapy, administration of vaccines, contraceptives, delivery of anti-microbial and anti-viral agents, *etc*. [6].

23.1.1. Essential oils (EOs)

Essential oils (EOs) can be defined as a "product obtained from a natural raw material of plant origin, by steam distillation, hydrodistillation or by mechanical processes from the epicarp of citrus fruits" [7]. They are complex mixtures of volatile substances, usually aromatic. Therefore, they are also known as volatile oils, ethereal oils, or essences. The term oil is related to the oily liquid appearance at room temperature. EOs differ from fixed oils due to their main characteristic of high volatility. Another important point is the pleasant and intense aroma, hence their being called essences [8].

Spices have been used since ancient times for a large number of purposes, such as medicine, perfume, preservatives, and to add aroma and flavour to food. The first distillation of EOs appeared in the Orient (Egypt, India, and Persia) more than 2000 years ago and was improved in the ninth century by the Arabs. However, only in the thirteenth century were EOs made by pharmacies and their pharmacological effects described in pharmacopoeias. Paracelsus von Hohenheim used the term "essential oil" for the first time in the sixteenth century when naming the effective component of a drug as "Quinta essential" [9]. Nowadays, EOs are widely used in perfumes and make-up products, as food preservers and additives, in sanitary products, in agriculture, and as natural products. Moreover, EOs are used in massages or baths, most frequently in aromatherapy. They still represent an important part of the traditional medicine in pharmaceutical preparations, and several monographs are reported in the official pharmacopoeias. In fact, more than three thousand EOs are known, and about 10 % of them have commercial importance for industries [10,11].

EOs are synthesised by all plant organs and stored in secretory cell cavities, canals, epidermic cells, or glandular trichomes. It seems that they play important roles in self-defense against bacterial and fungal infections and in pollination, as well as in intraspecific communication [12]. They are generally complex mixtures of volatile organic substances produced as plants' secondary metabolites composed by hydrocarbons (terpenes, sesquiterpenes, and phenylpropanoids) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, and phenol ethers), characterised by low molecular weight. Despite the complex content of EOs, compounds are present at quite different concentrations. Generally, two or three are major components at fairly high concentrations compared to others present in trace amounts. These major components are usually used as a quality parameter of the EO and are frequently related to its biological properties. However, the assumption that only active phytochemicals are involved in the biological 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 or play a synegistic role [13]. For example, the geometric isomers neral (39.50 %) and geranial (51.44 %) (known together as citral) are the major components of the *Cymbopogon citratus* EO, popularly known as lemongrass [14]. The quality parameter of lemongrass EO is related to citral content over 75 % [15].

Different factors such as climate, soil composition, plant organ, age, and vegetative cycle stage can interfere in the chemical profile of the EO, as observed with any other vegetal material. Furthermore, the extraction method is closely related to the quality of the EO. An inappropriate extraction procedure can damage or alter the action of the chemical signature of the EO, which can result in change in bioactivity and natural characteristics. EOs can be obtained by different methods, such as distillation (steam distillation, hydrodistillation, and hydrodiffusion), solvent extraction, supercritic carbon dioxide, pressurised hot water, expression (cold pressing), or microwaves. Most of the commercialised EOs are chemotyped by gas chromatography and mass spectrometry analysis. Analytical monographs have been published to ensure the good quality of EOs [10,11,16].

Besides their high volatility, EOs can easily decompose. Such a characteristic is especially related to heat, humidity, light, or oxygen exposure. Degradation of EO constituents is due to oxidation, isomerisation, cyclisation, or dehydrogenation reactions, activated either enzymatically or chemically, and normally influenced by the conditions during the processing and storage of the plant material, upon distillation, and in the course of subsequent handling of the oil itself. The degraded product may result in a loss of quality and pharmacological properties [17]. In addition, some aged EOs as well as oxidised terpenoids are known for their skin-sensitising capacities [18].

At present, promising approaches have been reported using EOs or their isolated compounds in medicinal products for human or veterinary [17] use as

well as in medical devices [12]. They have been widely used for pharmaceutical, sanitary, cosmetic, agricultural, and food applications [10]. Traditionally, EOs have been used for many biological properties which have been extensively described in literature, as shown in Table 1.

Table 1. Some biological properties of EOs

Biological property	Essential oil
anti-microbial	lemongrass [14], peppermint [19], clove, tea tree, thyme, geranium, marjoram, palmarosa, rosewood, sage, mint [20], salvia [21], peppermint [22], patchouli, vanilla, ylang-ylang [23], melissa [24]
antioxidant	clove, cinnamon, nutmeg, basil, oregano, thyme [10], rosemary [25]
anti-diabetic	rosemary [25]
anti-viral	star-anise [25], peppermint [19]
hypotensive	lemongrass [14]
insecticidal	star-anise [25]
sedative	lemongrass [14], peppermint [19]

In order to overcome the intrinsic limitations of the EOs described above and explore their biological properties, a significantly large part of the current scientific work in this area applies nanotechnology on the encapsulation of EOs. Nanosystems are often designed to protect the active compounds against environmental factors (*e.g.*, oxygen, light, moisture, and pH), decrease oil volatility, transform the oil into a powder, and control the release of active compounds. Additionally, due to the subcellular size, nanocarriers may increase the cellular absorption mechanisms, reducing toxicity and increasing bioefficacy.

The aim of this chapter is to offer an overview on nanoencapsulation of EOs that covers the present status of the field and development perspectives. Different nanosystems were developed intending to be EOs delivery systems. This review focuses on inclusion complex with cyclodextrins, submicron emulsions, NPs (polymeric, lipid, and magnetic) and liposomes.

The nanotechnology combined to EOs could bring an important contribution to the development of new delivery systems. These new delivery systems carring EOs are promising candidates as future and new products for health improvement.

23.2. EOs NANODELIVERY SYSTEMS

23.2.1. Challenges in development

At this point, it is well established that the encapsulation of EOs in nanocarriers has many advantages, especially in providing stabilisation against volatilisation and degradation as well as controlling release. However, many aspects must be observed when designing nanodelivery systems for such a complex mixture with delicate characteristics as EOs. As previously mentioned, nanocarriers can be structured by a great variety of material and processes. The choice of the type of nanocarrier (*e.g.*, NP, nanoemulsion) to be developed and the wall material to be employed must be strongly related to its target. The choice of the method is equally important, since the difficulty of scaling up for production and particle aggregation which can occur due to physical instability during storage and administration. In the particular case of encapsulating EOs, the selected method should avoid any physical treatment that could increase the loss of its compounds by volatilisation or degradation, such as heat, humidity, light, or oxygen exposure and high pressure. When designing these nanocarriers it is important to keep in mind that the primary goal is to obtain spherical particles with a smooth surface, low mean diameter, homogeneous distribution (polidispersity index), high encapsulation efficiency (EE), and low aggregation potential, which is directly related to the zeta potential (ZP).

Particle size, shape, and surface properties of the NPs play a crucial role in the uptake of nanosized delivery systems across the mucosal membrane. NPs between 50–300 nm, positive ZP, and hydrophobic surface were found to have a preferential uptake as compared to their counterparts [11].

In a previous work, Falcão *et al.* had compared the encapsulation of *Cymbopogon citratus* EO (lemongrass) in *β*-cyclodextrins by precipitation method and poly(caprolactone) (PCL) polymeric nanoparticles (PNPs) by oil in water (o/w) emulsion method using ultrasonic emulsification. The EO : *β*-cyclodextrin inclusion complex obtained was irregular in shape and had a larger mean diameter (441.2 nm) and lower inclusion efficiency (9.46 %), and the NPs (240.0 nm) were spherical in shape and demonstrated a higher EE (36.51 %). Moreover, according to the chemical comparison of the oil composition before and after the encapsulation processes, it was demonstrated that the NPs incorporated more compounds than the cyclodextrin complex. In the PCL NPs, the chemical profile indicated the loss of most volatile compounds, probably during the emulsification step due to slight heating resulting from the ultrasound treatment. The major compounds (neral and geranial) were almost completely incorporated, showing that these substances have an affinity for the polymer used as the wall material. On the other hand, the inclusion profile of EO compounds in the inclusion complex was different. With the exception of *trans*-caryophyllene, the sesquiterpenes present in the original oil were not identified in the complex. It is likely that

this type of molecule was not included in the central cavity of the host molecule due to steric effects. The authors concluded that, in the employed methods, encapsulating with PCL is a better choice to develop *Cymbopogon citratus* EO delivery systems than the inclusion complex using *β*-cyclodextrin as a host molecule [14].

23.2.2. Inclusion complex of cyclodextrins with EO

A molecular complex refers to the physical association between a host and a guest (active ingredient) molecule, and in the case of EOs the complexes are reported as cyclodextrins. Cyclodextrins in hydrophobic molecules such as EOs can form inclusion complexes that can enhance their aqueous solubility. This is possible due to their hydrophobic internal cavity and hydrophilic exterior, which compose cyclodextrins' structure.

Ciobanu *et al.* studied the complexation and retention capacities of *α*-cyclodextrin, *β*-cyclodextrin, *γ*-cyclodextrin, hydroxypropyl-*β*-cyclodextrin, randomly methylated-*β*-cyclodextrin, a low methylated-*β*-cyclodextrin, and cross-linked *β*-cyclodextrin polymers for the four major components of peppermint (*Mentha piperita)* EO (menthol, menthone, pulegone, and eucalyptol). The controlled release of aroma compounds from cyclodextrins was evaluated by multiple headspace extraction experiments. The obtained results indicated the formation of a 1 : 1 inclusion complex for all the studied compounds. *β*-cyclodextrins presented the greater aqueous phase formation constant and retention ability. Furthermore, it was observed that *β*-cyclodextrin confers protection against evaporation of the aroma, so it is possible to have a better release efficiency by using cyclodextrin polymers [19].

Haloci *et al.* produced inclusion complexes between the *Satureja montana* EO and *β*-cyclodextrin by co-precipitation method with different oil : *β*-cyclodextrin ratios. The chemical composition and biological properties of the EO before and after inclusion in *β*-cyclodextrin were studied. The best inclusion efficiency was achieved at the ratio of 20 : 80, showing 94.07 % of powder recovery. The qualitative and quantitative chemical composition of the EO showed no significant change after the inclusion process, which is related to the maintenance of the biological properties of the EOs. However, the complex obtained with an oil : *β*-cyclodextrin ratio of 10 : 90 showed an increase in anti-fungal and antioxidant activities when compared to the free oil [26].

EOs from species like *Eugenia caryophyllata* and *Piper nigrum* usually present in their composition a major compound known as *β*-Caryophyllene, which has interesting biological properties. Employing the co-precipitation method, Liu *et al.* developed an inclusion complex of *β*-caryophyllene and *β*-cyclodextrin with inclusion efficiency of 62.04 %. The study compared the pharmacokinetic characteristics after oral administration between the free

β-caryophyllene and the complex in rat plasma. An increase in oral bioavailability for *β*-caryophyllene was observed in the inclusion complex. It was demonstrated after 12 h of administration of the *β*-caryophyllene/*β*-cyclodextrin complex compared to the free β-caryophyllene. Furthermore, other pharmacokinetic parameters corroborate this result [27].

Lippia sidoides EO was encapsulated using *β*-cyclodextrin by suspension method, followed by the removal of water by employing a spray drying technique. Particles of 10–12 nm were obtained and an inclusion efficiency of 70 % in the EO : *β*-cyclodextrin ratio of 1 : 10 water in water (w/w). The mentioned oil is a potent anti-microbial of local use, and by the described process an important reduction in volatilisation was observed that enhanced thermal stability [28].

Carvacrol, a phenolic monoterpene usually present in the EO of oregano (*Origanum* spp.), was encapsulated with *β*-cyclodextrin to increase its stability and solubility by slurry complex method and compared with a physical mixture (PM). Particle size (PS) of the developed inclusion complex (7.5 μm) were much smaller than those of PM (80.2 μm) and *β*-cyclodextrin alone (14.8 μm). The complex showed longer hyperalgesic effects (about 24 h) than the free substance that possesses durability of approximately 9 h. Furthermore, without complex formation, significant changes in nociceptive responses were not observed. Another factor that should be mentioned is the use of minor amounts of active compound to achieve the desired effect, which also is advantageous [29].

23.2.3. Submicron emulsions containing EO as delivery systems

Emulsions can be classified by the particle size as macroemulsion, nanoemulsion, and microemulsion. When the droplet size is in the nanometer range, they are also called submicron emulsions [30].

The encapsulation by emulsification promotes protection from volatilisation of EOs [31-33]. Due to their intrinsic properties, nanoemulsions may present several advantages for encapsulating functional lipophilic compounds over macro and microemulsions [34]. Salvia-Trujillo *et al.* studied the characteristics of microfluidised EO nanoemulsions. Microfluidisation involves the application of high pressure on a coarse emulsion for the production of nanoemulsions. The macroemulsions containing EOs (lemongrass, clove, tea tree, thyme, geranium, marjoram, palmarosa, rosewood, sage, or mint) were prepared by high shear homogenisation and passed through a microfluidisation system, obtaining nanoemulsions in the range 2.22–20.88 nm of the average droplet size and low polydispersity index. Moreover, the results showed that nanoemulsions containing lemongrass, clove, thyme, or palmarosa EOs demonstrated the strongest anti-microbial activity. Only in the case of nanoemulsions with lemongrass or clove EOs could an enhanced activity against *Escherichia coli* (*E. coli*) be observed [20].

Dias *et al.* compared nanoemulsions containing Copaiba oil prepared with high pressure homogenisation and spontaneous emulsification. The results indicated that the advantages are greater when using high pressure homogenisation because of the higher *β*-caryophyllene contents and physical stability of the formulation. These nanoemulsions showed reduced loss of volatile fraction within 90 days of storage at 4 °C [35].

Severino *et al.* evaluated the anti-bacterial activity of modified chitosan-based coatings containing nanoemulsion of EOs against *E. coli* O157 : H7 and *Salmonella Typhimurium*. The nanoemulsions were obtained by high pressure homogenisation. The droplet size of the four different nanoemulsions, made of carvacrol, mandarin, bergamot, and lemon EOs, respectively, were 133 nm, 161 nm, 164 nm, and 177 nm. Also, the nanoemulsions were compared in terms of minimum inhibitory concentration (MIC) against the two bacteria evaluated *in vitro* using the micro-broth dilution assay. Carvacrol nanoemulsion proved to be the most effective anti-bacterial agent and was therefore selected to be incorporated into modified chitosan to form a bioactive coating [36].

A study conducted by Kim *et al*. developed nanoemulsions of lemongrass oil by homogenisation using a vortex mixer, high shear probe mixer, or dynamic hot pressure (DHP) treatment. The nanoemulsions formed by DHP processing presented mean droplet sizes of 56.4 and 87.9 nm following the oil concentration of 0.5 and 4.0 g / 100 g, respectively. Also, the stability of the emulsions was improved when employing DHP, making this method a better choice than the others used in the study. In addition, the nanoemulsion containing lemongrass oil showed anti-microbial affects against *Salmonella typhimurium* and *E. coli* [37].

EOs formulated in submicronic emulsions are a great tool for the development of products that can be applied in the pharmaceutical, cosmetic, and food industries. The encapsulation in nanoemulsions increases the effectiveness of EOs' action because of the extremely small particle size, improving the stability of formulations and interaction with microorganisms, cells, and receptors and protecting the EOs from volatilisation. Also, the surfactant and method employed influence the physical stability and biological activity through the particle size, interaction between surfactant and EOs, and bioavailability.

23.2.4. EO loaded in polymeric nanoparticles

PNPs are classified as nanocapsules and nanospheres. Nanospheres are matrix systems. Nanocapsules have two compartments: a polymeric wall and a core, which is typically oily. The EO may be conjugated with the polymer (matrix or wall) or solubilised in the oily core. The polymers, whether of natural or synthetic origin, must be biocompatible and biodegradable.

PCL NPs were prepared by a solvent emulsification-diffusion technique for encapsulating eugenol, a volatile constituent of many EOs, showing the

effectiveness of the polymeric shell on improving its stability during storage and protecting against oxidative reactions caused by light [38]. Gomes *et al.* encapsulated eugenol also in NPs, but by using poly(D,L-lactide-*co*-glycolide) (PLGA) as a wall material by emulsion evaporation method. An EE of 98.27 % and PS of 179.3 nm were observed. Eugenol-loaded NPs showed anti-microbial properties inhibiting the growth of *Salmonella* spp. and *Listeria* spp. The authors related improvement on efficacy on the increased hydrophilicity of the active compound, sustained release, and small particle size [39].

To increase the stability of the oregano EO (*Origanum vulgare*), Hosseini *et al.* encapsulated in biodegradable NPs of chitosan by a two-step process: o/w emulsification and gelation of chitosan with sodium tripolyphosphate (TPP). Chitosan NPs showed PS between 40–80 nm and EE and loading capacity of about 21–47 % and 3–8 %, respectively, when the initial oil content was 0.1–0.8 g g^{-1} chitosan. Furthermore, the nanoencapsulation of oregano oil into chitosan NPs improved its thermal stability and provided release control. It is important to emphasise that the increase of the initial content of oregano EO resulted in an increase in PS and loading capacity as well as reduction in the EE [40]. These results were consistent with those reported by Keawchaoon and Yoksan when carrying out the loading of carvacrol into chitosan-TPP NPs by the same method. Carvacrol-loaded NPs showed anti-microbial activity against *Staphylococcus aureus*, *Bacillus cereus*, and *E. coli* and a sustained release that reached a plateau level after 30 days [41].

Another approach to encapsulate an EO was studied by Lertsutthiwong *et al*. In order to reverse problems regarding volatility and low water solubility, they incorporated turmeric oil into alginate nanocapsules by a three-step procedure using o/w emulsification, gelification with calcium chloride, and solvent removal. The results showed that particle size can be affected by the solvent employed in oil solubilisation, and the presence of a surfactant and the sonication step is crucial to the size homogeneity. However, during the process of preparing alginate nanocapsules, about 42 % of the turmeric oil was lost and the good physical stability in long-term storage was observed only at 4 °C. Better physical stability at a higher temperature (25 °C) and less oil loss was obtained by adding chitosan during preparation of the nanocapsules [42,43].

Decreased volatilisation and stronger aroma of tea tree oil (*Melaleuca alternifolia* EO) were achieved by Flores *et al.* through its incorporation into PCL nanocapsules. Employing the interfacial polymeric deposition method, it was possible to obtain PS of 212 nm, negative ZP (–13.5 mV), and oil content of 95.7 %. Tea-tree-loaded nanocapsule suspension showed high anti-fungal activity against *Trichophyton rubrum* in different *in vitro* models of dermatophyte nail infection. It was considered by the authors as a promising topical therapy of onychomycosis [44,45].

Lippia sidoides oil, rich in thymol, was encapsulated in PNPs formed by a matrix of chitosan and angico gum and evaluated with respect to the *in vitro*

release profile and activity against *Stegomyia aegypti* larvae [46]. The polymeric nanocarriers were obtained by spray drying an emulsified coacervate using the same proportions of chitosan : angico gum (1 : 10) but varying the proportion of EO : angico gum-chitosan (1 : 2, 1 : 4, 1 : 10, and 1 : 20). Among the tested ratios, higher loading (6.7 %), EE (77 %), and an EO sustained release were obtained for the EO : angico gum-chitosan (1 : 10) sample. Moreover, this release profile provided increased bioavailability, enhancing its larvicidal effect with 85 and 92 % mortality after 24 and 48 h, respectively. Subsequent studies published regarding *L. sidoides* oil encapsulation in NPs presented complex biopolymers as wall materials such as chitosan/cashew gum [47,48] and alginate/cashew gum [49], thus showing the potential use of these nanosystems as a larvicide for fighting dengue.

23.2.5. Solid lipid nanoparticles and nanostructured lipid carriers for delivery of EO

A good way to improve the use of EOs is by encapsulating them in lipid NPs – more specifically, the solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) – due to the many advantages that utilising this kind of system can provide to volatile oils. Firstly, the EOs are mostly lipophilic, presenting a good miscibility with the lipid matrix, improving the entrapment efficiency [50]. Lipids in a solid form protect the EOs, decreasing the sensitivity to air, light, and temperature and reducing the volatilisation [51,52] and degradation of the active components.

During the first decade of the current century, studies involving the encapsulation of EOs in SLN and NLC began to emerge. The most widely used method is high-pressure homogenisation by the hot homogenisation technique [51-53], where the aqueous phase (around 5 to 10 °C above the lipid melting point) is added into the oil phase, under stirring. The reduction of droplet size is obtained employing a high pressure homogeniser. Another tool also quite explored to reduce the size of the pre-formed emulsion is the ultrasound probe [54,55]. However, in some cases, it is possible to produce particles in nanometric range even with vigorous stirring [56], but the right choice of surfactants is imperative, otherwise, only micrometer particles are obtained [57,58].

SLN were produced by a high-pressure homogenisation method containing frankincense and myrrh oil (FMO) in a ratio of 1 : 1, and the *in vivo* anti-tumour activity was evaluated. The average diameter was 113.3 nm and the EE was 80.60 %. The stability of the entrapped oil in SLN was higher when compared to the free FMO during the 6 days of storage. The anti-tumour efficacy of the free FMO was compared with SLN-FMO and an inclusion complex with *β*-cyclodextrin (FMO-*β*-CD). SLN-FMO showed higher activity, 43.66 % of the inhibition rate, compared to the other formulations FMO suspension (31.23 %) and FMO-*β*-CD (34.81 %) [52].

Zedoary turmeric oil (ZTO) (*Curcuma zedoaria*) was encapsulated in NLC by melt-emulsification technique. NPs were formulated in pure Crodamol® SS or with Miglyol® 812N using soybean phosphatidylcholine as a surfactant. When comparing pure Crodamol® SLN (PS: 399 nm; EE: 81.1 %) and Crodamol® with Miglyol® NLC, a decrease was observed in the average diameter and an increase in EE as the proportion of Miglyol®/Crodamol® increased (*e.g.*, with 30 % [wt] of Miglyol®, PS: 128.2 nm; EE: 94.2 %). The greater complexity of the matrix decreases the surface tension, leading to a reduction of particle size and larger space for insertion of the drug molecules in the lipid matrix, increasing the EO entrapment. *In vitro* drug release experiments indicated that NLC could enhance the drug release rate over the SLN, and the drug release rate could be adjusted by the liquid lipid content in lipid NPs. NLC showed a burst release for the first 8 h, explained by the oil release from the outer surface, followed by a sustained release until 90 h due to the matrix erosion. In SLN, the EO was released more slowly because oil diffusion through the solid lipid is more difficult than through the liquid lipid in NLC. When the percentage of the liquid lipid increases, the release of the oil is accelerated. These results were corroborated by *in vivo* study. ZTO-NLC also showed a prolonged acting time when compared to free ZTO after intravenous administration [54].

β-elemene, an isolated compound from the EO from the same species (*C. zedoaria*), was formulated into SLN by the method combining probe sonication and membrane extrusion. Glycerol palmitostearate, monostearin, or a combination of 70 and 30 % of both, respectively, were employed as a lipid. The surfactant (poloxamer 188) was added in the oil or aqueous phase. A smaller mean diameter (26.5 nm) and a bigger entrapment (99.9 %) were obtained using only lipid monostearin with the addition of the surfactant in the oil phase. This could be explained by the presence of more hydrophilic groups from the monostearin, which facilitates the emulsification process. A premix of surfactant with lipids may also facilitate the breakdown of the lipid phase into smaller droplets. The most stable formulation concerning size and *β*-elemene content parameters, after 8 months of storage, was obtained employing a combination of 70 % glycerol palmitostearate and 30 % monostearin with the surfactant added in the lipid phase. The *in vitro* release profile showed a steady release with the SLN depletion after 80 h, which may suggest a model of solid solution [50,55]. Although a significant difference was not observed in the pharmacokinetic parameters between SLN-*β*-elemene and commercial emulsion after intravenous administration *in vivo*, the tissue distribution in the liver, spleen, and kidney was higher using SLN-*β*-elemene, whereas the *β*-elemene concentration was lower in the heart and lung [55], corroborating the already reported opsonisation and uptake from NPs by the reticuloendothelial system [59].

The *Artemisia arborescens* EO was encapsulated in SLN by the hot pressure homogenisation technique utilising Compritol® 888 ATO as a lipid matrix and Poloxamer 188 (SLN-1) or sodium cocoamphoacetate (SLN-2) as a surfactant.

SLN-2 showed no difference in particle size (207 nm) between SLN-oil and SLN-placebo as well as a greater EO entrapment (92 %). Since the formulations have been developed for use as a pesticide, they were sprayed. There was no variation in particle size in SLN-2 before or after spraying, whereas for SLN-1 there was a small increase; when stored at 40 °C, the SLN-1 gelled, preventing spraying. However, SLN-1 showed a better ability to avoid oil volatilisation. After 48 h at 35 °C, SLN-1 lost 37.07 % of the oil, whereas SLN-2 evaporated 45.51 % [51]. The same NPs were also evaluated for skin permeation in Franz diffusion cells. With SLN the oil accumulated in the outer layers of skin, whereas with the pure oil the permeation was larger and with a low accumulation [60]. These results suggest that SLN can reduce pesticide toxicity.

23.2.6. Functionalised magnetic nanoparticles loading EO

Magnetic nanoparticles (MNPs) are widely studied for their potential biomedical applications, such as diagnostic imaging, drug targeting, drug delivery, stabilisation of EOs and inhibition of microbial biofilm development, improved surfaces with anti-adherent properties, hyperthermia, and cancer treatment. Fluorescent magnetic nanoparticles (FMNPs) are being used in an increasing number of medical applications, offering chemical groups designed to permit the specific attachment of drugs and to improve their biocompatibility. The magnetic component of the MNPs in general is magnetite, Fe_3O_4, a proven biocompatible iron oxide [12,21,61].

Due to the significant increase of actual anti-microbial drug resistance, new alternative strategies for combating microbial infections have been studied to explore the widely known properties of EOs. The combination of the stabilising carrier properties of MNPs with EOs (or isolated compounds from EOs) could improve stability and represent a successful approach for the development of novel materials and surfaces, refractory to microbial biofilms formation [12,21,61].

Anghel *et al.* developed and characterised a novel nanostructured phytoactive coated rayon/polyester wound dressing surface, refractory to *Candida albicans* adhesion, colonisation, and biofilm formation, based on FMNPs and *Anethum graveolens* and *Salvia officinalis* EOs with potential application for wound care. The FMNPs were prepared by wet chemical precipitation from aqueous iron salt solutions by means of alkaline media and showed a spherical shape with an average diameter of 15 nm, estimated by transmission electron microscope (TEM). The amount of EOs entrapped in the FMNPs evaluated by thermogravimetry (TG) analysis was 1.22 % for *S. officinalis* and 6.65 % for *A. graveolens*. The NPs were able to stabilise and control the release of EOs, significantly enhancing the anti-biofilm effect for at least 72 h. The nanobiocoatings preferentially inhibit the early stages of biofilm formation (after 24 h), but also reduce the formation and development of mature

biofilms. While the inhibitory effect of *A. graveolens* reached the maximum intensity on 48 h biofilms, the effect of *S. officinalis* gradually increased with the biofilm age, exhibiting a maximum efficiency at 72 h. According to the authors, the difference on activity from both systems is related to the different release rate of the two phytocompound from the NP carrier [21]. In a similar study, *Satureja hortensis* EO inhibited *C. albicans* adhesion and biofilm formation for up to 72 h of incubation. FMNPs were developed with an average size of 10 nm and 14.37 % of EO content [62].

In other studies, a core/shell/EO nanofluid was used to create a coated shell on a prosthetic medical device. The catheter pieces were coated with suspended core/shell by applying a magnetic field on nanofluid, and the EO was applied by adsorption in a secondary covering treatment. FMNPs were synthesised by precipitation method. The microbial adherence ability and biofilm development were investigated up to 72 h in catheter pieces with and without a hybrid NPs/EO nanosystem [22,61,63].

R. officinalis EO coated NPs of up to 20 nm in size were fabricated by Chifiriuc *et al.* by employing oleic acid as a surfactant. The nanobiosystem strongly inhibited the adherence ability and biofilm development of the *Candida albicans* and *Candida tropicalis* tested strains to the device surface [63].

Core/shell/*Eugenia carryophyllata* EO nanostructures of up to 20 nm in size were obtained, and the activity was evaluated against different strains of *Candida* spp. The nanosystem drastically decreased the number of biofilm embedded cells, especially after 24 and 48 h of incubation. For some of the tested strains, the fungal adherence and biofilm development was totally inhibited at 24 h [61].

Anghel and Grumezescu reported the development of a 5 nm core/shell magnetic nanostructure combined with *Mentha piperita* EO. Magnetite coated with lauric acid was prepared and characterised. The *M. piperita* EO content was estimated by TG analysis as approximately 17.3 %. An improvement was observed on resistance to *S. aureus* adherence and development of biofilm for up to 72 h [22].

More recently, iron oxide NPs functionalised with myristic acid exhibiting a size below 20 nm were fabricated with the EOs of ylang-ylang (*Cananga odorata* subsp. *genuina*), patchouli (*Pogostemon cablinsyn*. *P. patchouli*, *P. heineanus*), and vanilla (*Vanilla planifolia*) to be further used as coating agents for medical device surfaces. NPs were prepared by co-precipitation of Fe^{+2} and Fe^{+3} in basic aqueous dispersion of myristic acid and then characterised. The amount of patchouli, vanilla, and ylang-ylang was estimated by TG analysis as 7.74 %, 9.94 %, and 15.58 %, respectively. According to Bilcu *et al*., the content of EOs adsorbed on the surface of NPs seems to be related to the polarity of EOs. Vanilla EO loaded NPs strongly inhibited both the initial adherence of *S. aureus* cells after 24 h and the development of the mature biofilm at 48 h. Patchouli and ylang-ylang EOs mostly inhibited the initial

adherence phase of *S. aureus* biofilm development. In the case of *K. pneumoniae*, all tested nanosystems exhibited similar efficiency, being active mostly against the adherence to the tested surface. The tested oils did not exhibit any significant influence on the biofilms after 72 h, indicating that the bactericidal effect is related to the release rate from the iron based nanocarrier attached to the catheter surface [23].

Magnetic microspheres consisting of magnetite NPs functionalised with poly(lactic acid)—chitosan polymers and *Melissa officinalis* EO were described by Grumezescu *et al*. MNPs/EO not exceeding 20 nm were prepared by co-precipitation of Fe^{+2} and Fe^{+3} in a basic aqueous solution. Microspheres with diameters ranging between 350 and 530 nm were pelliculised by Matrix--Assisted Pulsed Laser Evaporation (MAPLE) and characterised. The obtained MAPLE-deposited thin-films showed excellent anti-adherence and anti--*S. aureus* biofilm formation. The microspheres exhibited a great impact on microbial colonisation, imparing the normal formation and maturation of the biofilms. This effect was probably mediated by the release profile of the EO from the polymeric nanosystem, which remained significant up to 72 h of incubation [24].

23.2.7. Liposomes as EO nanocarriers

Liposomes or (phospho)lipid vesicles are spherical self-assembled colloidal particles formed spontaneously by a curved lipid bilayer which entraps part of the solvent [64]. Liposomes are interesting carriers of drug systems, especially due to their biocompatibility, intracellular delivery, targeting and entrapment of hidrophylic molecules in an aqueous core and lipophylic molecules into a lipid bilayer [65].

Considering EOs' delivery, the main reasons to use a liposome as a nanocarrier for EOs are for the improvement of solubility and absorption of lipophylic drugs and to enhance cellular membrane interactions, thereby increasing therapeutic effectiveness [66-70].

Depending on the size and number of bilayers, liposomes can be classified as multilamellar vesicles (MLVs), large unilamellar vesicles (LUVs), and small unilamellar vesicles (SUVs), which present specific characteristics regarding loading capacity and different preparation methods [71]. The PS, bilayer fluidity, and stability as well as the EE are important parameters for therapeutic efficiency and could be affected by physical chemical properties of EO or isolated compounds and by the composition of liposomes [64].

It was observed that EE is slightly lower when comparing SUVs to MLVs for *Zanthoxylum tinogassuiba* [72] and *Artemisia arboresens* [73] EOs, which can be attributed to a smaller bilayer area for SUV. These results were not observed with *Santolina insularis* EO; the entrapment efficiency was not considered different for SUVs and MLVs, but it was related that MLVs had a more irregular size distribution than SUVs [74].

Regarding the liposome characterisation, it was observed that the loading of EO could reduce [69,73-75] or slightly increase the PS [68,72]. This indicates that the effect of oil incorporated into the packaging and accommodated in the lipid bilayer depends on the type of substance and the employed process [72].

Considering the liposome composition, the inclusion of a non-ionic surfactant in the phospholipid bilayer reduced the EE of *Artemisia arborescens* EO [73]. Yoshida *et al*. evaluated the effects of different cryprotectors on *Eugenia uniflora* EO liposomes. They observed that threalose is a more effective agent cryoprotector, although other agents, such as sucrose, can be used in specific situations [75].

Liposomes have been studied for many diseases, including cancer. It can be due the passive targeting to the tumoural tissues and other possibilities as increase the circulation time by using hidrophylic polymers or active targeting by coating liposome with antibodies [76].

Celia *et al.* studied anti-cancer activity in an *in vitro* cell model of free bergamote EO and the same EO encapsulated in liposomes. The liposomes were prepared by hydratation of lipid thin-film composed by dipalmitoyl--*sn*-glycero-3-phosphocholine monohydrate (DPPC), cholesterol, and 1,2-Distearoyl-phosphatidylethanolamine-methyl-poly(ethylene glycol) conjugate-2000 (DSPE-mPEG2000). Then the MLVs were extruded through a stainless-steel extrusion device. It was observed that the liposomal forms presented a greater reduction in cell viability and a greater increase in cell mortality than free EO, indicating an improvement in activity, which can be attributed to the increase of cellular uptake of the liposomes [68].

A similar study was carried out with free *Zanthoxylum tingoassuiba* EO entrapped in liposomes using human glioblastoma G-15 cells. The MLVs were prepared by thin-film hydration method and the SUVs were prepared by sonication of MLVs. The free EO was separated from liposomal EO by centrifugation. The liposomes, especially SUVs, are more efficient in reducing the cell viability than free EO. In addition, an increase was observed in thermal--oxidative stability for the liposomal form [72].

The capability of liposomes to enhance intracellular drug delivery is useful to intracellular therapy such as viral infection. The anti-viral effect against HSV-1 was improved when EO from *Artemisia arborescens* was encapsulated in liposomes. As in other studies, the MLVs were prepared by thin-film hydration following sonication to form SUV. The entrapped oil was separated from free oil by untracentrifugation. Three formulations were tested considering different surfactants. The effect was dependent on the composition and liposome size, with MLVs showing better results than SUVs due to the higher drug leakage from SUVs [73].

It is common for EOs have anti-microbial activity. This can be explained by their lipophylic characteristic that perturbs and interacts with membrane

cells [77]. Thus, the use of liposomes could improve anti-microbial activity by furthering the membrane interaction. This was observed with tea tree oil loaded in liposomes prepared by reversed phase evaporation, with or without silver ions, in comparison with free EO against *Candida albicans, Staphylococcus aureus*, and *Pseudomonas aeruginosa* [78].

A similar effect was expected with *Zanthoxylum tingoassuiba* EO entrapped on MLVs prepared by thin-film hydration. Despite the vesicle size (greater than 1 μm), an incomplete release was observed after 15 h, suggesting a possible increase in EO penetration through the cytoplasmatic membrane, being enhanced by reduced particle size [69].

It is evident that, due to their hydrophobic compartment, liposomes are useful nanocarriers to EO delivery, especially for intracellular therapy and any kind of treatment that depends on the interaction between biomolecules and membrane cells.

23.3. OUTLOOK

Nanotechnology is an innovative, multidisciplinary approach that has potential applications in medicinal and health research. Nanocarriers can display specific and controlled chemical and physical properties, interacting with cells and tissues with a high degree of functional specificity. When applied to natural products, more specifically EOs, nanotechnology can be an important tool in the development of new formulations.

EOs are widely known for their biological properties and are extensively used in folk medicine for promoting health, as well as preventing and treating different diseases. However, their physicochemical characteristics, such as high volatility, low water solubility and stability, together with the side effects associated with their use have limited their application in medicine. They are more commonly used for external routes, such as topicals, gargles, and mouthwashes, or for inhalation. Oral administration is rarer and requires previous dilution. Processing such oils in delivery systems is an alternative for overcoming these intrinsic issues and increasing their medical application, since it can also expand the range of administration routes to be explored, such as oral and parenteral.

Several studies have reported the development of nanocarriers for EO delivery, and an improvement in stability and controlled release are normally described. However, the challenge in designing this kind of system is even greater when encapsulating such a complex mixture. The EE, particle size distribution, surface characteristics, and release profile must be carefully evaluated. A high content of EO seems to be the most challenging of all, since many fabrication processes employ high-energy methods to achieve the nanometer range that can accelerate the volatilisation and degradation of the oils, such as ultrasound

and high pressure homogenisers. The affinity between oil and encapsulating material seems to play an important role in optimising the entrapment and can modulate the release profile, acting as a useful tool according to the intended purpose.

Despite great challenges, a well-designed nanosystem can make an important contribution to the development of promising candidates for new products for the improvement of human or veterinary health.

REFERENCES

1. G.A. Silva. *Surg. Neurol.* **61** (2004) 216–220.
2. S. Shrivastava, D. Dash. *J. Nanotech.* **2009** (2009) 1–14.
3. S.K. Sahoo, S. Parveen, J.J. Panda. *Nanomedicine* **3** (2007) 20–31.
4. S.M. Moghimi, A.C. Hunter, J.C. Murray. *FASEB J.* **19** (2005) 311–330.
5. L.F. Pimentel, A.T. Jácome-Júnior, V.C.F.Mosqueira, N.S. Santos-Magalhães. *Braz. J. Pharm. Sci.* **43** (2007) 503–514.
6. O. Kayser, A. Lemke, N. Hernández-Trejo. *Curr. Pharm. Biotechnol.* **6** (2005) 3–5.
7. EN ISO 9235: Aromatic natural raw materials (2013).
8. C.M.O. Simões, V. Spitzer, in *Farmacognosia: da planta ao medicamento*, C.M.O. Simões, E.P. Schenkel, G. Gosmann, J.C.P. Mello, L.A. Mentz, P.R. Petrovick, Eds., 6th Ed., Editora da UFRGS:Editora da UFSC, Porto Alegre:Florianópolis, Brazil, 2007, p. 467–495.
9. E. Guenther, *The Essential Oils*, Krieger Publishing Company, Malabar, USA, 1972, p. 3–4.
10. A. Properzi, P. Angelini, G. Bertuzzi, R. Venanzoni. *Med. Aromat. Plants* **2** (2013) 1–4.
11. A.R. Bilia, C. Guccione, B. Isacchi, C. Righeschi, F. Firenzuoli, C. Bergonzi. *Evid. Based Complement Alternat. Med.* (2014) 1–14.
12. A.M. Grumezescu. *Curr. Org. Chem.* **17** (2013) 90–96.
13. A. São Pedro, I. Espirito Santo, C.V. Silva, C. Detoni, E. Albuquerque, in *Microbial pathogens and strategies for combating them: science, technology and education*, A. Méndez-Vilas, Ed., Formatex Research Center, Badajoz, Spain, 2013, p. 1364–1374.
14. D.Q. Falcão, A.R. Santos, B. Ortiz-Silva, R.P. Louro, R. Seiceira, P.V. Finotelli, J.L.P. Ferreira, S.G. De Simone, A.C.F. Amaral. *Lat. Am. .J Pharm.* **30** (2011) 765–772.
15. B.T. Schaneberg, I.A. Khan. *J. Agric. Food Chem.* **50** (2002) 1345–1349.
16. P. Tongnuanchan, S. Benjakul. *J. Food. Sci.* **79** (2014) R1231–R1249.
17. C. Turek, F.C. Stintzing. *Compr. Rev. Food Sci. F* **12** (2013) 40–53.
18. J.B. Christensson, P. Forsström, A.-M. Wennberg, A.-T. Karlberg, M. Matura. *Contact Dermatitis* **60** (2009) 32–40.
19. A. Ciobanu, I. Mallard, D. Landy, G. Brabie, D. Nistor, S. Fourmentin, *Food Chem.* **138** (2013) 291–297.
20. L. Salvia-Trujillo, A. Rojas-Graü, R. Soliva-Fortuny, O. Martín-Belloso. *Food Hydrocolloids* **43** (2015) 547–556.

21. I. Anghel, A.M. Holban, E. Andronescu, A.M. Grumezescu, M.C. Chifiriuc. *Biointerphases* **8** (2013) 1–12.
22. I. Anghel, A.M. Grumezescu. *Nanoscale Res. Lett.* **8** (2013) 1–6.
23. M. Bilcu, A.M. Grumezescu, A.E. Oprea, R.C. Popescu, G.D. Mogoşanu, R. Hristu, G.A. Stanciu, D.F. Mihailescu, V. Lazar, E. Bezirtzoglou, M.C. Chifiriuc. *Molecules* **19** (2014) 17943–17956.
24. A.M. Grumezescu, E. Andronescu, A.E. Oprea, A.M. Holban, G. Socol, V. Grumezescu, M.C. Chifiriuc, F. Iordache, H. Maniu. *J. Sol-Gel Sci. Technol.* **11** (2014) 1–8.
25. C.P. Fernandes, M.P. Mascarenhas, F.M. Zibetti, B.G. Lima, R.P.R.F. Oliveira, L. Rocha, D.Q. Falcão. *Braz. J. Pharmacog.* **23** (2013) 108–114.
26. E. Haloci, V. Toska, R. Shkreli, E. Goci, S. Vertuani, S. Manfredini. *J. Incl. Phenom. Macrocycl. Chem.* **80** (2014) 147–153.
27. H. Liu, G. Yang, Y. Tang, D. Cao, T. Qi, Y. Qi, G. Fan. *Int. J. Pharm.* **450** (2013) 304–310.
28. L.P. Fernandes, W.P. Oliveira, J. Sztatisz, I.M. Szilágyi, C. Novák. *J. Therm. Anal. Calorim.* **95** (2009) 855–863.
29. A.G. Guimarães, M.A. Oliveira, R.S. Alves, P.P. Menezes, M.R. Serafini, A.A.S. Araújo, D.P. Bezerra, L.J.Q. Júnior. *Chem. Biol. Interact.* **227** (2015) 69–76.
30. J.L. Salager, in *Pharmaceutical emulsions and suspensions: drugs and the pharmaceutical sciences*, F. Nielloud, G. Marti-Mestres, Eds., Marcel Dekker, New York, USA, 2000, p. 19–72.
31. N. Uson, M.J. Garcia, C. Solans. *Colloids Surf. A* **250** (2004) 415–421.
32. D.S. Bernardi, T.A. Pereira, N.R. Maciel, J. Bortoloto, G.S. Vieira, G.C. Oliveira, P.A. Rocha-Filho. *J. Nanobiotechnology* **44** (2011).
33. W. He, Y. Tan, Z. Tian, L. Chen, F. Hu, W. Wu. *Int. J. Nanomed.* **6** (2011) 521–533.
34. X. Li, N. Anton,T.M.C. Ta, M. Zhao, N. Messaddeq, T.F. Vandamme. *Int. J. Nanomed.* **6** (2011) 1313–1325.
35. D.O. Dias, M. Colomboa, R.G. Kelmanna, S. Kaiser, L.G. Luccaa, H.F. Teixeira, R.P. Limberger, V.F. Veiga-Jr., L.S. Koeste. *Ind. Crop Prod.* **59** (2014) 154–162.
36. R. Severino, G. Ferrari, K.D. Vu, F. Donsi, S. Salmieri, M. Lacroix. *Food Control* **50** (2015) 215–222.
37. I. Kim, Y.A. Oh, H. Lee, K.B. Song, S.C. Min. *Food Sci. Technol. Int.* **58** (2014) 1–10.
38. M.J. Choi, A. Soottitantawat, O. Nuchuchua, S.G. Min, U. Ruktanonchai. *Food Res. Int.* **42** (2009) 148–156.
39. C. Gomes, R.G. Moreira, E. Castell-Perez. *J. Food Sci.* **76** (2011) 16–24.
40. S.F. Hosseini, M. Zandi, M. Rezaei, F. Farahmandghavi. *Carbohydr. Polym.* **95** (2013) 50–56.
41. L. Keawchaoon, R. Yoksan. *Colloids Surf. B* **84** (2011) 163–171.
42. P. Lertsutthiwong, K. Noomun, N. Jongaroonngamsang, P. Rojsitthisak, U. Nimmannit. *Carbohydr. Polym.* **74** (2008) 209–214.
43. P. Lertsutthiwong, P. Rojsitthisak, U. Nimmannit. *Mater. Sci. Eng. C* **29** (2009) 856–860.
44. F.C. Flores, R.F. Ribeiro, A.F. Ourique, C.M.B. Rolim, C.B. Silva. *Quim. Nova* **34** (2011) 968–972.
45. F.C. Flores, J.A. Lima, R.F. Ribeiro, S.H. Alves, C.M.B. Rolim, R.C.R. Beck, C.B. Silva. *Mycopathologia* **175** (2013) 281–286.

46. H.C.B. Paula, F.M. Sombra, F.O.M.S. Abreu, R.C.M. de Paula. *J. Braz. Chem. Soc.* **21** (2010) 2359–2366.
47. H.C.B. Paula, F.M. Sombra, R.F. Cavalcante, F.O.M.S. Abreu, R.C.M. de Paula. *Mater. Sci. Eng. C* **31** (2011) 173–178.
48. F.O.M.S. Abreu, E.F. Oliveira, H.C.B. Paula, R.C.M. de Paula. *Carbohydr. Polym.* **89** (2012) 1277–1282.
49. E.F. Oliveira, H.C.B. Paula, R.C.M. Paula. *Colloids Surf. B* **113** (2014) 146–151.
50. R.H. Müller, K. Mäder, S. Gohla. *Eur. J. Pharm. Biopharm.* **50** (2000) 161–177.
51. F. Lai, S.A. Wissing, R.H. Muller ,A.M. Fadda. *AAPS PharmSciTech* **7** (2006) E1–E9.
52. F. Shi, J.-H. Zhao, Y. Liu, Z. Wang, Y.-T. Zhang, N.-P. Feng. *Int. J. Nanomed.* **7** (2012) 2033–2043.
53. N.A. Alhaj, M.N. Shamsudin, N.M. Alipiah, H.F. Zamri, A. Bustamam, S. Ibrahim, R. Abdullah. *Am. J. Pharmacol. Toxicol.* **5** (2010) 52–57.
54. X.-L. Zhao, C.-R. Yang, K.-L. Yang, K.-X. Li, H.-Y. Hu, D.-W. Chen. *Drug Dev. Ind. Pharm.* **36** (2010) 773–780.
55. Y. Wang, Y. Deng, S. Mao, S. Jin, J. Wang, D. Bi. *Drug Dev. Ind. Pharm.* **31** (2005) 769–778.
56. E. Moghimipour, Z. Ramezani, S. Handali. *Curr. Drug Deliv.* **10** (2013) 151–157.
57. P. Lertsatitthanakorn, S. Taweechaisupapong, C. Aromdee , W. Khunkitti. *Int. J. Essen Oil Ther.* **2** (2008) 167–171.
58. E. Gavini, V. Sanna, R. Sharma, C. Juliano, M. Usai, M. Marchetti, J. Karlsen, P. Giunchedi. *Pharm. Dev. Technol.* **10** (2005) 479–487.
59. G.M. Barratt. *Pharm. Sci. Technol.* **3** (2000) 163–171.
60. F. Lai, C. Sinico, A. De Logu, M. Zaru, R.H. Muller, A.M. Fadda. *Int. J. Nanomed.* **2** (2007) 419–425.
61. A.M. Grumezescu, M.C. Chifiriuc, C. Saviuc, V. Grumezescu, R. Hristu, D.E. Mihaiescu, G.A. Stanciu, E. Andronescu. *IEEE T. NanoBioSci.* **11** (2012) 360–365.
62. I. Anghel, A.M. Grumezescu, A.M. Holban, A. Ficai, A.G. Anghel, M.C. Chifiriuc. *Int. J. Mol. Sci.* **14** (2013) 18110–18123.
63. C. Chifiriuc, V. Grumezescu, A.M. Grumezescu, C. Saviuc, V. Lazăr, E. Andronescu. *Nanoscale Res. Lett.* **7** (2012) 209–216.
64. D.D. Lasic, *Liposomes from Physicas to Application*, Elsevier, Amsterdam, Netherlands, 1993, p. 573.
65. M. Ranson, A. Howell, S. Cheeseman, J. Margison. *Cancer Treat. Rev.* **22** (1996) 365–379.
66. S.C. Mourão, P.I. Costa, H.R.N. Salgado, M.P.D. Gremião. *Int. .J Pharm.* **295** (2005) 157–162.
67. A. Fahr, P.V. Hoogevest, S. May, N. Bergstrand, M.L.S. Leigh. *Eur. J. Pharm. Sci.* **26** (2005) 251–265.
68. C. Celia, E. Trapasso, M. Locatelli, M. Navarra, C.A. Ventura, J. Wolfram, M. Carafa, V.M. Morittu, D. Britti, L.D. Marzio, D. Paolino. *Coll. Surf. B Biointerf.* **112** (2013) 548–553.
69. C.B. Detoni, E.C.M. Cabral-Albuquerque, S.V.A. Hohlemweger, C. Sampaio, T.F. Barros, E.S. Velozo. *J. Microencapsulation* **26** (2009) 684–691.
70. E. Moghimipour, N. Aghel, A.Z. Mahmoudabadi, Z. Ramezani, S. Handali. *J. Nat. Pharm. Prod.* **7** (2012) 117–122.
71. A. Sharma, U.S. Sharma. *Int. J. Pharm.* **154** (1997) 123–140.

72. C.B. Detoni, D.M. Oliveira, I.E. Santo, A.S. Pedro, R. El-Bacha, E.S. Velozo, D. Ferreira, B. Sarmento, E.C.M. Cabral-Albuquerque. *J. Lip. Res.* **22** (2012) 1–7.
73. C. Sinico, A.D. Logu, F. Lai, D. Valenti, M. Manconi, G. Loy, L. Bonsignore, A.M. Fadda. *Eur. J. Pharm. Biopharm.* **59** (2005) 161–168.
74. D. Valenti, A. Logu, G. Loy, C. Sinico, L. Bonsignore, F. Cottiglia, D. Garay, A.M. Fadda. *J. Lip. Res.* **11** (2001) 73–90.
75. P.A. Yoshida, D. Yokota, M.A. Foglio, R.A.F. Rodrigues, S.C. Pinho. *J. Microencapsulation* **27** (2010) 416–425.
76. R.D. Hofheinza, S.U. Gnad-Vogy, U. Beyer, A. Hochhaus. *Anti-Cancer Drugs* **16** (2005) 691–707.
77. M. Cristani, M. D`Arrigo, G. Mandalari, F. Castelli, M.G. Sarpietro, D. Micieli, V. Venuti, G. Bisignano, A. Saija, D. Trombetta. *J. Agric. Food Chem.* **55** (2007) 6300–6308.
78. W.L. Low, C. Martin, D.J. Hill, M.A. Kenward. *Lett. Appl. Microbiol.* **57** (2013) 33–39.