Sustainable Agriculture Reviews 44

Ankit Saneja
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Eric Lichtfouse *Editors*

# Sustainable Agriculture Reviews 44

## Pharmaceutical Technology for Natural Products Delivery Vol. 2 Impact of Nanotechnology

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# Sustainable Agriculture Reviews

Volume 44

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Sustainable agriculture is a rapidly growing field aiming at producing food and energy in a sustainable way for humans and their children. Sustainable agriculture is a discipline that addresses current issues such as climate change, increasing food and fuel prices, poor-nation starvation, rich-nation obesity, water pollution, soil erosion, fertility loss, pest control, and biodiversity depletion.

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## Pharmaceutical Technology for Natural Products Delivery Vol. 2 Impact of Nanotechnology

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# Preface

The science of today is the technology for tomorrow (Edward Teller).

Natural products are a golden source for drug discovery due to their high chemical diversity that has been designed by living organisms. However, classical drug administration is often poorly efficient. Here, the recent development of nanotechnologies has open new routes because nanomaterials have unique properties that improve pharmacodynamic and pharmacokinetic properties of active chemicals. This book presents recent advances in nanoscience for improving the therapeutic efficacy of natural products and discusses lipid nanoarchitectonics, inorganic particles, and nanoemulsions for delivering natural products.

In Chap. 1, Bilia et al. reviews nanotechnology applications for natural products delivery. They also present nanocarriers made of polymers or lipid constituents for the delivery of artemisinin, curcumin, andrographolide, resveratrol, honokiol, salvianolic acid B, green tea catechins, and silymarin. In Chap. 2, Gonçalves et al. highlight the use of biosynthesized nanomaterials as a viable alternative to conventional techniques. They also discuss plant extracts as a source of new nanomedicines, acting as an ally or alternative to existing therapies (Fig. 1). In Chap. 3, Saka and Chella explore the various delivery options of bioactive molecules and extracts using nanocarriers made of polymers, lipids, and inorganic materials. The role of nanocarriers for improving therapeutic efficacy is highlighted by case studies.

In Chap. 4, Barradas et al. describe the main aspects of nanoemulsion formulations, type of surfactants and oil phases, and techniques for characterizing nanoemulsions. They also discuss applications of nanoemulsions for improvement of bioactive oil bioavailability and solubilization, masking unpleasant aspects of oils, and enhancement of essential oils pharmacological activity. In Chap. 5, Chaudhari et al. discuss lipid nanoarchitectonics in natural product delivery for the treatment of cancer therapy. In Chap. 6, Meena et al. describe the properties, synthesis, advantages, and toxicities of inorganic particles made of silver, gold, iron oxide, and silica and their application for the delivery of natural products. Finally, in Chap. 7, Padhi and Behera discuss the delivery of camptothecin for increasing stability and solubility using novel drug delivery platforms.

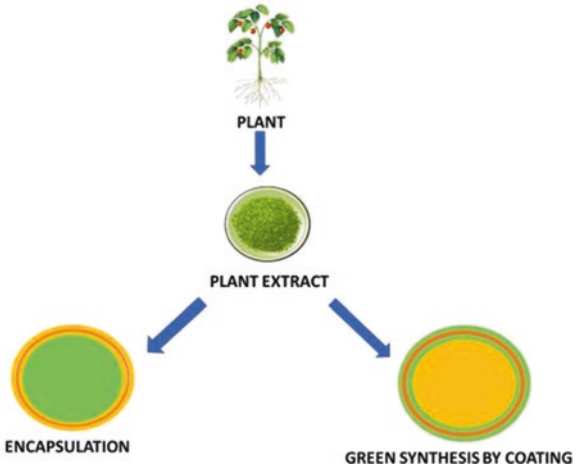


**Fig. 1** Plant extracts in nanotechnology. Left: encapsulation of plant extracts to enhance therapeutic efficiency. Right: green synthesis of nanoparticles, during which plant compounds nucleate and stabilize nanoparticles. From Chap. 2 by Gonçalves et al.

We extend our sincere gratitude to all contributors who had made significant contribution to prepare high-quality chapters. We also extend our thanks to Melanie van Overbeek, Assistant Editor at Springer Nature, for her support during this whole process.

New Delhi, India — Ankit Saneja
New Delhi, India — Amulya K. Panda
Aix-en-Provence, France — Eric Lichtfouse

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# About the Editors

**Ankit Saneja** currently works as Research Associate in the Product Development Cell, National Institute of Immunology, New Delhi, India. He has received his PhD from the Academy of Scientific and Innovative Research (AcSIR) at the CSIR-Indian Institute of Integrative Medicine, Jammu. He has been recipient of several international awards such as the CEFIPRA-ESONN Fellowship, Bioencapsulation Research Grant, and PSE Travel Bursary. He has to his credit several international publications. His research interest includes exploring different types of formulations for various biomedical applications.

**Amulya K. Panda** has been a Scientist at the National Institute of Immunology (NII), New Delhi, for the last 29 years. Currently, he is the Director of NII. He completed his master's degree in Chemical Engineering from IIT Madras and his PhD in Biochemical Engineering and Biotechnology from IIT Delhi. He has been a Visiting Scientist with Prof. Harvey Blanch at the Chemical Engineering Department, University of California, Berkeley, USA, and in the Department of Pharmaceutical Science at the University of Nebraska Medical Center, Omaha, USA. His research interest includes bioprocess engineering, recombinant fermentation process development, high-throughput protein refolding from inclusion bodies, and vaccine delivery using biodegradable polymer particles. He has published more than 130 research papers and 10 book chapters and is an inventor of more than 30 issued or pending patents. His honors include Samanta Chandra

Sekhara Award from Odisha Bigyan Academy for the year 2000; Biotech Product and Process Development and Commercialization Award 2001 from the Department of Biotechnology, Government of India, on May 11, 2001; Young Asian Biotechnologist Prize from The Society for Biotechnology, Japan, for the year 2004; Tata Innovation Fellowship 2010 from the Department of Biotechnology, Government of India; and GB Manjrekar Award of AMI at AMI 2010 Conference at BIT Mesra in December 2010. His research group was also awarded Second Prize at Intel-UC Berkeley Technology Entrepreneurship Challenge 2008 and Third Prize at World Innovation Summit, Barcelona, on June 16, 2009. He was the President of the Association of Microbiologist of India (AMI) for the year 2017.

**Eric Lichtfouse, PhD** is Geochemist and Professor of Scientific Writing at Aix-Marseille University, France, and Visiting Professor at Xi'an Jiaotong University, China. He has discovered temporal pools of molecular substances in soils, invented carbon-13 dating, and published the book *Scientific Writing for Impact Factor Journals*. He is Chief Editor and Founder of the journal *Environmental Chemistry Letters* and the book series Sustainable Agriculture Reviews and Environmental Chemistry for a Sustainable World. He has awards in analytical chemistry and scientific editing. He is World XTerra Vice-Champion.

# Chapter 1
# Nanotechnology Applications for Natural Products Delivery


**Anna Rita Bilia, Vieri Piazzini, and Maria Camilla Bergonzi**

**Abstract** Natural products are fascinating molecules in drug discovery for their exciting structure variability and for their interaction with various biological targets, which represent the best approach to develop successful medications for many diseases. The scarce water solubility, low lipophilicity and inappropriate molecular size of many natural compounds, which undergo structural instability in biological milieu, rapid clearance and high metabolic rate, have severely limited their use in clinic. Nanomedicine represents an excellent tool to increase bioavailability and activities of natural products. Generally, nanosized delivery systems provide large surface area increasing dissolution properties and can overcome anatomic barriers. In addition, passive and active targeting can optimize the performance of the nanocarriers. Passive targeting takes advantage of the unique pathophysiological characteristics of inflamed and tumor vessels, enabling nanodrugs to accumulate in the tissues. The effect is called enhanced permeation and retention, generally obtained by the decoration with polyethylene glycol the vector surface. An intriguing strategy is to decorate the nanocarriers with special ligands in order to recognize and bind to target cells through ligand–receptor interactions. Although the active targeting strategy looks intriguing, nanodrugs currently approved for clinical use are relatively simple and generally lack active targeting or triggered drug release components.

In this review different nanocarriers made of polymers or lipid constituents mainly based on artemisinin, curcumin, andrographolide, resveratrol, honokiol, salvianolic acid B, green tea catechins, silymarin and other extracts are reported. Each nanosystem has its own advantages, disadvantages, and characteristics. Polymeric nanoparticles are solid in nature and include nanosphere and nanocapsule. They are ideal candidates to enhance the bioavailability of the natural products after various routes of administration, principally oral and parenteral, but also nasal and intraocular, as well as to cross physiological barriers including blood brain barrier. Polymeric micelles have high safety, worthy stability and low cost. Polymeric micelles are very stable in physiological media with a consequent controlled drug

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release of drugs, while the hydrophilic shell protects the encapsulated drug from the external medium and prevents the interaction with plasma components, resulting in in vivo long circulating properties. Dendrimers are characterized by low polydispersity, good biocompatibility, able to cross cell barriers via both paracellular and transcellular pathways, very versatile able to carry both lipophilic and hydrophilic drugs. Lipid nanocarriers include vesicles, nanocochleates, micelles, solid lipid nanoparticles and nanostructured lipid particles, emulsions with nano scale, including nanoemulsions and microemulsions. Vesicles include liposomes and niosomes are the first nano drug delivery systems that have been successfully translated into real-time clinical applications. They are extremely versatile in terms of route of administration and characteristics of the loaded drug. Due to their similarity to biological membranes provides unique opportunities to deliver drug molecules into cells or subcellular compartments. Liposomes can be converted to nanocochleates, which are unique nanovectors, after treatment with divalent ions, which are very useful for both oral and parenteral administration. Solid lipid nanoparticles and nanostructured lipid particles are easy to scale-up, low cost of production, relative nontoxic nature, biodegradable composition, and stability against aggregation or coalescence. Mostly lipid drugs can be loaded in these nanoparticles to avoid extrusion. Nanoemulsions and microemulsions are the most interesting nanostructures to essentially increase drug loading and enhance bioavailability. They both give reproducible plasma drug profile and can also be used for sustained and targeted drug delivery.

**Keywords** Nanosized delivery systems · Natural products · Polymeric and lipid nanosystems · Bioavailability · Efficacy

## 1.1 Introduction

Natural products from plants, animals, and minerals have represented for millennia the only resource to maintain health, for prophylaxis or to cure human and animal diseases. Still currently, between 65 and 80% of populations in developing countries use medicinal plants as therapeutic remedies for their primary healthcare (Cameron et al. 2011).

The tangible importance of natural products to the drug discovery process and their possible role in therapy is unquestionable and numerous strong scientific evidences have confirmed the reputation of traditional knowledge suggesting that the worldwide natural products' sources represent a varied pool of key drugs (Bilia et al. 2014a, b, 2017; 2018a, b). Interestingly, the impact of natural products in the clinic is quite marked, about 69% of anti-infective (ca. 195 molecules) are naturally derived or inspired. Among the antitumor drugs (ca. 172 molecules), 75% are represented by natural products or inspired to them (Newman and Cragg 2012). Natural products still

represent a main source of drugs thanks to their enormous structural and chemical diversity, which is incomparable by any synthetic libraries (Bilia et al. 2017). In the Dictionary of Natural Products, over 300,000 natural products describing the available chemical, physical and biological properties are reported (Dictionary of Natural Products. http://dnp.chemnetbase.com). Natural products are also very attractive molecules because they can modulate multiple targets activating various signalling or functional pathways increasing their therapeutic value against multifactorial and complex diseases, especially cancer and diabetes. Conversely, the *in vivo* performance could results limited because of low hydrophilicity and poor intrinsic dissolution rate, physical or chemical instability. In addition, they may present low absorption, scarce biodistribution, first-pass metabolism, poor penetration and accumulation in the organs of the body, or trivial targeting efficacy (Bilia et al. 2017, 2018a, b).

### *1.1.1 Nanotechnologies Strategies to Optimize the Clinical Use of Natural Products*

Diverse strategies are used to optimise the bioavailability of natural products, including the development of semisynthetic compounds or synthetic analogues, the production of prodrugs, and the technological approach for the production of appropriate formulations (Bilia et al. 2017; 2018a). The latter strategy to optimize the biopharmaceutical performance of natural products is the development of suitable drug delivery systems, in particular those nano-sized, generally between 50 and 300 nm up to 1 μ, which correspond from the size of one-half of DNA diameter and one eighth of the red blood cell diameter (Fig. 1.1). Some nanosized delivery

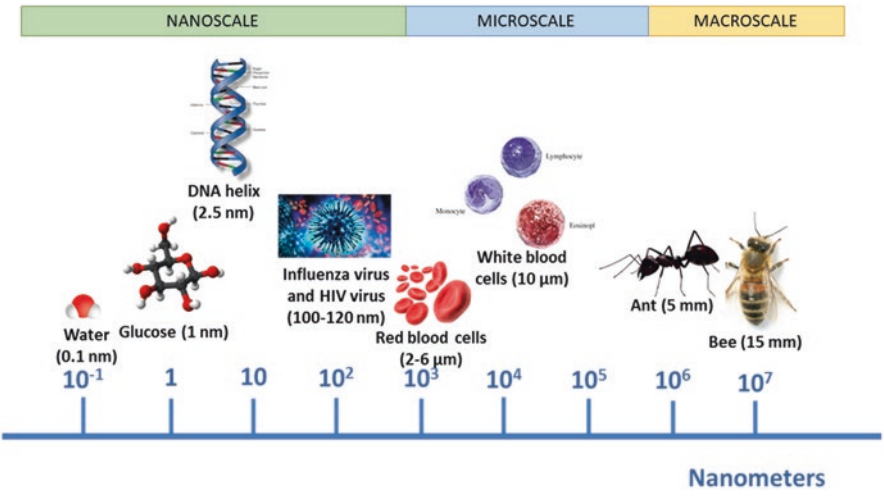


**Fig. 1.1** Scale of nature. (Reproduced with permission from Bilia et al. 2017 from Thieme)

systems have already entered in the clinic because they can offer an advanced approach to optimized the therapeutic efficacy targeting definite tissues or organs or capable to cross biological barriers with the aim to increase efficacy, safety profile and compliance of drugs. An efficacious drug delivery system should possess optimal drug loading, ideal release characteristics, extended shelf-life, with a consequent considerable greater clinic effectiveness and lesser side effects (Bilia et al. 2017, 2018a).

Different types of nanomaterials can be used to prepare nanocarriers which are capable of being loaded with hydrophobic and hydrophilic drugs and generally are classified as polymer-based systems and lipid-based systems. Roles of size, shape, charge, hydrophobic/hydrophilic character, and surface chemistry of nanocarriers to optimize *in vivo* behaviour including active intracellular delivery and improved pharmacokinetics and pharmacodynamics of drugs, modifying some physiological parameters, including hepatic filtration, tissue extravasation, tissue diffusion, and kidney excretion (Fig. 1.2).

Passive and active targeting is generally obtained modifying the surface of nanosystems (Fig. 1.3). Nanocarriers are generally recognized as extraneous structures, and consequently are opsonized by the reticuloendothelial system, thus decreasing the availability of the drug.

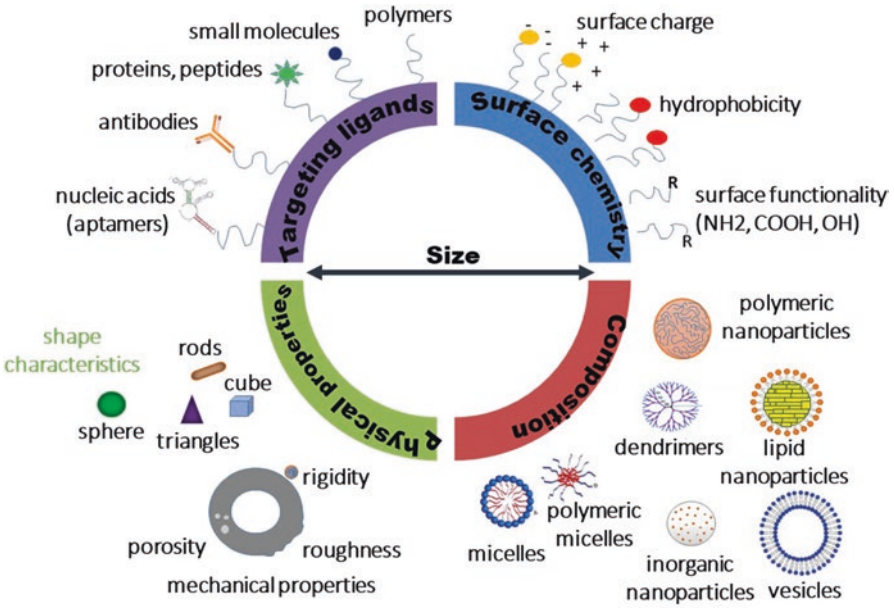


**Fig. 1.2** Roles of size, shape, charge, hydrophobic/hydrophilic character, and surface chemistry of nanocarriers to optimize *in vivo* behavior including active intracellular delivery and improved pharmacokinetics and pharmacodynamics of drugs, modifying some physiological parameters, including hepatic filtration, tissue extravasation, tissue diffusion, and kidney excretion. (Reproduced with permission from Bilia et al. 2017 from Thieme)

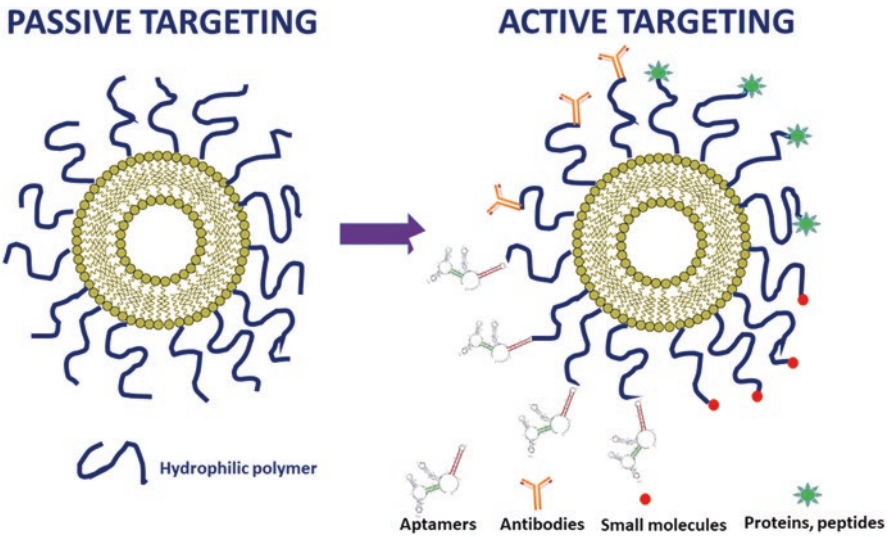


**Fig. 1.3** Passive and active targeting. (Reproduced with permission from Bilia et al. 2018a from Wiley)

It has been observed that nanocarriers decorated in the superficial area with hydrophilic polymers such as polyethylene glycol are not recognised by the reticuloendothelial system obtaining a "stealth effect", resulting in an enhancement of the pharmacokinetic properties of the drug, increasing drug solubility and drug stability. In addition, these long circulating nanocarriers can better penetrate that inflamed tissues, tumours other pathological conditions in the body, because of the more permeable blood vessel walls with respect to the normal tissues and a consequent enhanced permeation and retention effect, reaching up to 50-fold accumulation in tumours compared to physiological tissues (Fig. 1.4, Bilia et al. 2017, 2018a, b). A further approach is to decorate the surface of the nanovectors with diverse elements to obtain the active drug targeting, also called ligand-mediated targeting (Fig. 1.3). Ligands are selected to target surface molecules or receptors overexpressed in diseased organs, tissues, cells or subcellular domains. Active targeting increases internalization of drugs without altering the overall biodistribution, because of the recognition of the ligand by its target substrate. Various ligands can be used, namely small molecules, antibodies, nucleic acids, proteins and peptides. Peptide-targeting fragments are ranging from 2 to 50 amino acids. Numerous peptide receptors are overexpressed in tumour cells and include luteinizing hormone-releasing hormone, bombesin and somatostatin receptor. Peptides can also target integrins, which are transmembrane receptors, whose role is vital in the adhesion between cells and surrounding tissues and are generally overexpressed in tumour neovasculature. Small molecules such as folic acid and sugars are often used as active targeting, because of the small production costs, lack of degradation, low immunogenic properties and ease of conjugation to nanocarriers (Bilia et al. 2018a).

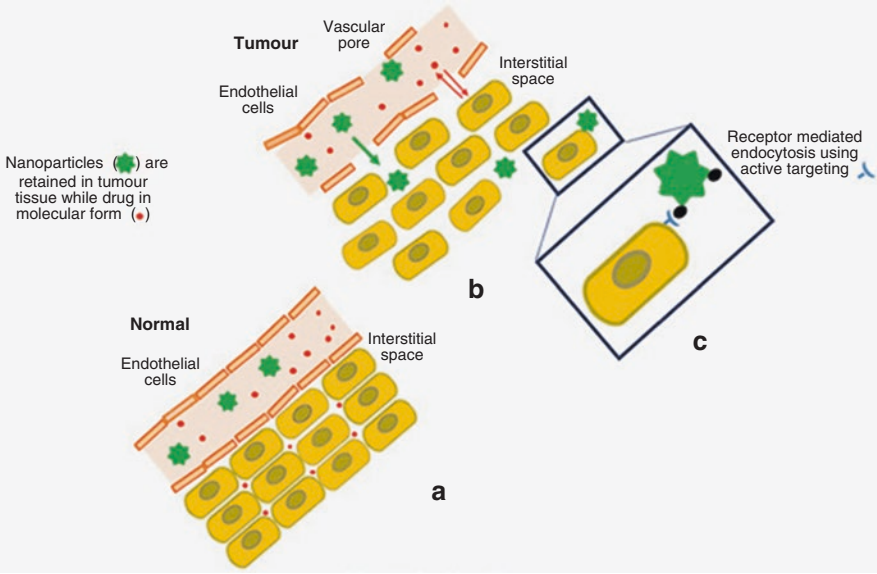


**Fig. 1.4** The role of enhanced permeation and retention effects for nanocarrier behavior in healthy tissues (**a**), their accumulation in inflamed or tumor tissues (**b**), and active drug targeting of nanocarriers (**c**). (Reproduced with permission from Bilia et al. 2017 from Thieme)

## 1.2 Polymeric Nanocarriers

Nicolas et al. (2013) Polymeric nanocarriers are composed of natural, semisynthetic or synthetic polymers, and their selection is mainly based on their biodegradation properties, biocompatibility, surface characteristics, in order to develop of new and safe carriers with controlled and targeted drug delivery concept.

Natural polymers, also called biopolymers, are obtained from bacteria, fungi, animals, and plants, and are mainly represented by polysaccharides and proteins.

Polysaccharides derived from plant kingdom are cellulose, pectin, starch, alginic acid, carrageenan, Arabic gum. Those from animal or bacterial origin are chitosan, and gums including xanthan, gellan. Nanocarriers based on polysaccharides are very suitable for water-soluble or hydrophilic drugs. They are characterised by high stability, extraordinary safety, low cost, great biodegradability and no toxicity. Several natural proteins are also used to produce nanocarriers and include gelatin, casein, albumin and soy proteins hydrolysate. Among the synthetic polymers, polyvinyl alcohol, poly-cyanoacrylate alkyl esters, polyglycolic acid, polylactic acid, polylactic glycolic acid, and poly(N-vinyl pyrrolidone), are among the most used (Bilia et al. 2017, 2018a, b).

Polymeric nanocarriers include nanospheres and nanocapsules, polymeric micelles and dendrimers (Fig. 1.5).

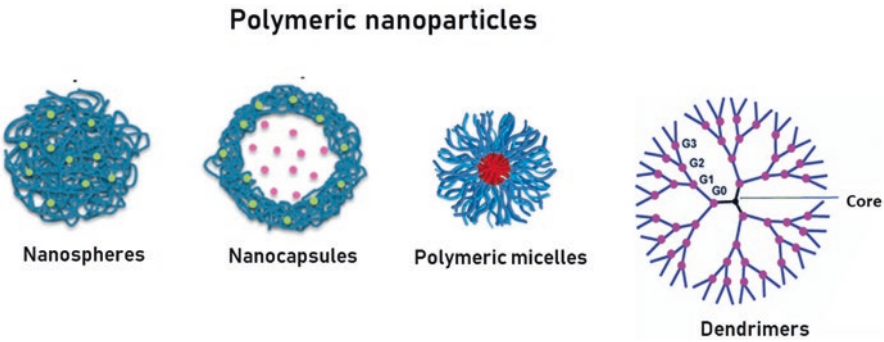


**Fig. 1.5** Polymeric nanoparticles. Reproduced with permission from Bilia et al. 2018a from Wiley

### *1.2.1 Polymeric Nanoparticles*

Nanoparticle production is based on chemical reactions of polymerization, or can be produced directly from a macromolecule or a preformed polymer. Nanoparticles include nanospheres and nanocapsules (Fig. 1.5). Nanospheres are matrix systems in which the drug is physically and uniformly dispersed, while in nanocapsules the drug is confined to a cavity surrounded by a polymeric membrane. Diverse nanocarriers have been formulated in the form of polymeric nanoparticles to increase membrane permeability, bioavailability, to obtain sustained release, or to selectively cross barriers, in particular the blood brain barrier.

Injectable nanoparticles formulated monomethoxy polyethylene glycol and poly lactide-co-glycolide and co-encapsulated with ginkgolides A, B, C and bilobalide, the active components of *Ginkgo biloba* extract were developed. A mean diameter of 123.3 ± 44.0 nm and zeta potential of −30.86 ± 2.49 mV characterised the developed nanoparticles. The encapsulation efficiency of the total ginkgo terpenes in the optimal formulation was 78.84 ± 2.06%, with a loading dose of 11.90 ± 0.31 mg of terpenes in 150 mg of polymer. The particles exhibited a sustained and synchronized release of the four components, both *in vitro* and *in vivo*. In addition, the half-life time of the four terpenoid compounds was also significantly improved after their loading into the nanoparticles (Han et al. 2012).

A further example is represented by quercetin encapsulated in polylactic acid nanoparticles whose release patterns was studied over a period of 96 h. Within the first half hour, 40–45% of the quercetin was released. This quick burst was attributed to the quercetin at the surface of the particle diffusing into the surroundings. Over the next 96 h, the release was slower and reached a maximum of 87.6%. This slower release was attributed to the diffusion of the quercetin from deeper within the nanoparticle (Kumari et al. 2010).

In a pharmacokinetic study, curcumin loaded in polylactic glycolic acid nanoparticles improved the relative oral bioavailability by 563%, compared to free curcumin. The increased bioavailability of curcumin was due to the inhibition of

P-gp-mediated efflux. The authors tested this hypothesis by adding verapamil, a P-glycoprotein inhibitor, to curcumin or curcumin loaded in polymeric nanoparticles. After 120 min of treatment, the remaining curcumin in the jejunum was evaluated. There was no significant difference in the residual curcumin content in the presence of verapamil and the values obtained with curcumin nanoparticles without the addition of verapamil. Accordingly, it was found that nanoparticles inhibit P-glycoprotein, which allows increased drug permeability and bioavailability (Xie et al. 2011).

Bioavailability of curcumin loaded in PEGylated polyester nanoparticles was in investigated in a Caco-2 cell model. PEG polymers (from 2000 to 5000 Da) and polylactic or polylactic polyglycolic acid scarcely modify the transcellular transport of nanoparticles. This was optimised using PEG with a molecular weight of 5000 and polylactic acid with a molecular weight of 40,000, which demonstrated a greater drug loading and slower release properties (Song et al. 2013).

Diverse studies suggest that polymeric nanoparticles represent very useful drug delivery systems to improve solubility and transport across physiological barriers, in particular polymeric nanoparticles are useful drug-delivery systems to cross the blood-brain barrier. The blood-brain barrier hinders the passage of systemically delivered therapeutics and the brain extracellular matrix limits the distribution and durability of locally delivered agents.

Poly alchylcyanoacrylate represent an interesting class of polymers to successfully cross blood-brain barrier.

In a recent study, nanoparticles made of poly ethylcyanoacrylate coated with polysorbate 80 were developed, and characterised in terms of dimensional analysis, polydispersity and zeta potential, morphology, encapsulation efficacy, and loading capacity (Grossi et al. 2017). Nanoparticles' distribution and fate were evaluated after intracerebral injection in healthy rats. Furthermore, their ability to reach the brain tissues and the lack of their toxicity was demonstrated after systemic administration in rats. After intracerebral injection in healthy rats, nanoparticles were distributed within the injected hemisphere and mainly interacted with microglial cells, presumably involved in their clearance by phagocytosis. Furthermore, nanoparticles were able to pass the blood-brain barrier after systemic administration in rats, and the lack of toxicity in C57/B6 mice chronically administered was highlighted (Fig. 1.6). Nanoparticles were loaded with salvianolic acid B, the bioactive constituent from *Salvia miltiorrhiza* which represents a molecule to prevent degeneration in several animal models by various biological mechanisms (Bonaccini et al. 2015), with a poor chemical stability and low bioavailability which limits its clinical application for central nervous system neuronal injury and degeneration. Encapsulation efficacy and loading capacities were 98.70% ± 0.45 and 53.3% ± 0.24, respectively. In addition, the nanoparticles were suitable for parental administration because their mean diameter smaller than 300 nm, with a polydispersity of 0.04 ± 0.03, and a ζ-potential of −8.38 mV ± 3.87. The *in vitro* release of salvianolic acid B from the nanoparticles was sustained and prolonged during 8 h, suitable for a promising clinical application (Grossi et al. 2017).

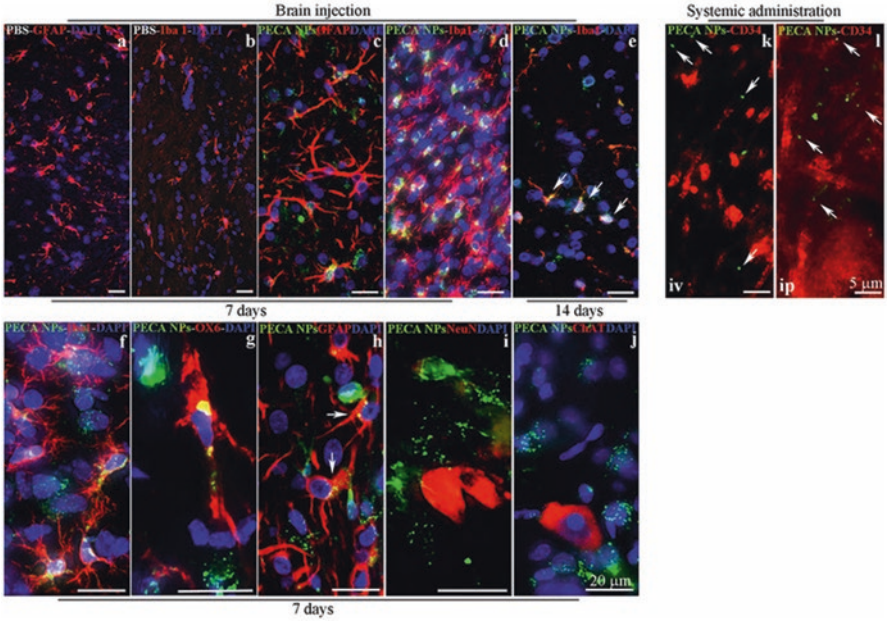


**Fig. 1.6** Intracerebral injection of poly ethylcyanoacrylate nanoparticles loaded with loaded with the florescent probe and coated with polysorbate 80 and their blood brain barrier crossing abilities after systemic administration. (**c**–**j**) Double labelling experiments between poly ethylcyanoacrylate nanoparticles loaded with loaded with the florescent probe and coated with polysorbate 80 (green) and glial markers GFAP/Iba-1/OX6 (red, arrows) or neuronal NeuN/ChAT antibodies (red) plus 4′,6-Diamidine-2′-phenylindole dihydrochloride (DAPI, blue) in injected rats, 7 (**c**, **d**, **f**–**j**) and 14 (**e**) days after surgery. GFAP- and Iba 1-immunopositive cells (red) with resting morphology and no poly ethylcyanoacrylate nanoparticles loaded with loaded with the florescent probe and coated with polysorbate 80 (green) signal were detected in PBS-injected rats (**a**, **b**, **k**, **l**). The lack of colocalization between poly ethylcyanoacrylate nanoparticles loaded with loaded with the florescent probe and coated with polysorbate 80 (green, indicated by the arrows) and the brain vascular endothelium marker CD34 (red) demonstrated their ability to cross the blood brain barrier after an acute i.v. (**k**) and i.p. (**l**) administration. (Reproduced with permission from Grossi et al. 2017 from Thieme)

A further approach to penetrate blood-brain barrier is the use of albumin nanoparticles. In particular albumin when polymerised results a versatile nontoxic, non-immunogenic, biocompatible and biodegradable protein having high binding capacity of various natural products and being well tolerated without any serious side effects (Bilia et al. 2018a). A recent publication reported the production of albumin nanoparticles without the use of organic solvents to obtain useful drug-delivery systems to cross the blood-brain barrier. Human serum albumin nanoparticles have gained considerable attention owing to their high loading capacity for various drugs and the fact that they are well tolerated. Two different cross-linking methods, the chemical and the thermal ones, were investigated to produce the nanoparticles (Bergonzi et al. 2016). Nanoparticles were chemically and physically

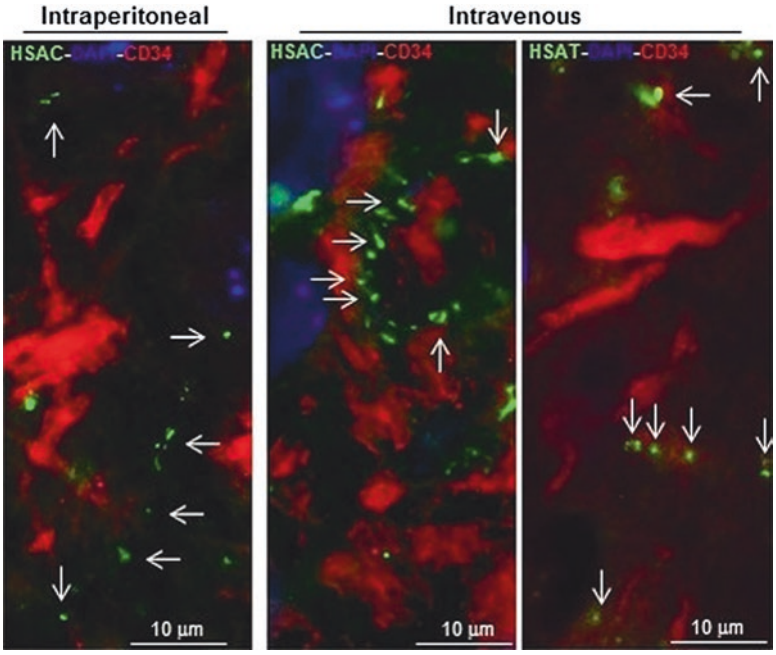


**Fig. 1.7** Crossing blood brain barrier abilities of the albumin nanoparticles prepared with heat (HSAT) or chemically synthetized (HSAC) after injection in rats. The lack of co-localization of the nanoparticle loaded with a florescent probe (green, arrows) with the brain vascular endothelium marker CD34 (red), the brain vascular endothelium marker, indicates the ability of the two nanoformulations to cross the blood brain barrier after an acute systemic administration. (Reproduced with permission from Bergonzi et al. 2016 from Wiley)

characterized by dynamic light scattering, transmission electron microscopy, and high-performance liquid chromatography coupled with diode array and fluorimetric detectors. A first set of studies evaluated the *in vivo* distribution and the fate of albumin nanoparticles in healthy rats, after intracerebral injection. Then the ability of formulations to cross the blood-brain barrier and reach the brain tissues was demonstrated by intravenous and intraperitoneal administration in healthy rats (Fig. 1.7). In addition, the toxicity of nanoparticles was excluded by behavioural tests.

The toxicity of the developed carriers was estimated by behavioral tests. Nanoparticles were observed to be located in different brain tissues depending on the mode of injection, and did not induce an inflammatory response. Behavioral tests demonstrated no locomotor, explorative, or cognitive function impairment induced by the nanoparticles. Despite the similar results obtained by *in vivo* tests, thermal cross-linking method was consider superior to chemical cross-linking in terms of formulation parameters such as a decrease in costs and the time necessary for the preparation (Bergonzi et al. 2016).

In a further study, andrographolide the major diterpenoid of the Asian medicinal plant *Andrographis paniculata* having exciting pharmacological potential for

treatment of neurodegenerative disorders (Casamonti et al. 2019a) was loaded into human serum albumin based nanoparticles and poly ethylcyanoacrylate nanoparticles and characterized in terms of size, zeta potential, polydispersity, and release studies (Guccione et al. 2017). The ability of free andrographolide and andrographolide loaded in both types of polymeric nanoparticles for their crossing BBB properties were investigated using an *in vitro* BBB model based on human cerebral microvascular endothelial cell line (hCMEC/D3). Free andrographolide did not permeate the blood-brain barrier model, as also predicted by in silico studies. By contrast, albumin nanoparticles improved by two-fold the permeation of andrographolide while maintaining the integrity of the cell layer, while poly ethylcyanoacrylate nanoparticles temporarily disrupted blood-brain barrier integrity (Guccione et al. 2017).

In a successive study andrographolide encapsulated in human albumin nanoparticles was investigated for the distribution in brain and activity effects in TgCRND8 mice, an Alzheimer's disease mouse model. Developed nanoparticles had proper size (mean size: 159.2 ± 4.5 nm), size distribution (nearby 0.12 ± 0.01) and ζ-potential (−24.8 ± 1.2 mV) with a remarkable encapsulation efficiency (more than 99%). In the step down inhibitory avoidance test, nanoparticles administered to TgCRND8 mice significantly improved their performance ($p < 0.0001$) reaching levels comparable to those displayed by wild type mice. In the object recognition test, treated and untreated animals showed no deficiencies in exploratory activity, directional movement towards the objects and locomotor activity. No cognitive impairments (discrimination score) were detected in TgCRND8 mice ($p < 0.0001$) treated with nanoparticles. After acutely i.v. administration or chronical i.p. administration, nanoparticles were detected in the brain parenchyma of TgCRND8 mice. The immunofluorescent analyses evidenced the presence of nanoparticles both in the pE3-Aβ plaque surroundings, and inside the pE3-Aβ plaque, indicative of the ability of these nanoparticles to cross the BBB and to penetrate in both undamaged and damaged brain tissues (Bilia et al. 2019).

Finally, human serum albumin nanoparticles coated with chitosan were developed as drug delivery systems to be used as nose-to-brain carrier. The mean particle sizes was 241 ± 18 nm and 261 ± 8 nm and the zeta potential was −47 ± 3 mV and + 45 ± 1 mV for albumin nanoparticles and albumin nanoparticles coated with chitosan, respectively. The optimized formulations showed excellent stability upon storage both as suspension and as freeze-dried product after 3 months. The mucoadhesion properties were assessed by turbidimetric and indirect method. Nanoparticles were loaded with sulforhodamine B sodium salt as model drug and the effect of chitosan coating properties was investigated by *in vitro* release studies, permeation and uptake experiments using Caco-2 and hCMEC/D3 cells as model of the nasal epithelium and blood-brain barrier, respectively. Furthermore, *ex vivo* diffusion experiments were tested using rabbit nasal mucosa and the ability of the formulations to reversibly open tight and gap junctions was explored by western blotting and RT-PCR analysing in both Caco-2 and hCMEC/D3 cell (Piazzini et al. 2019a).

### *1.2.2 Polymeric Micelles*

Polymeric micelles are nanostructures (20–200 nm) made up of amphiphilic polymers consisting of block copolymers of hydrophobic and hydrophilic monomer units, that spontaneously self-associate in aqueous solution, forming a micelle at concentrations higher than the critical micellar concentration (Fig. 1.5). Generally, the sequence of the hydrophilic polymer (A) represents the shell, while the core is formed by an hydrophobic polymer (B) arranged to obtain a di-block or a multiblock polymer. The most common used hydrophilic portion is represented by polyethylene glycol, while poly(D, L)-lactic acid, polypropyleneoxide, poly-caprolactone, and poly(L)-aspartic acid. A very common polymers used to prepare micelles are Pluronics (polyoxyethylene polyoxypropylene block copolymers) (Bilia et al. 2017, 2018a).

Principal advantages of polymeric micelles are their high safety, worthy stability and low cost. Polymeric micelles are very stable in physiological media with a consequent controlled drug release of drugs, while the hydrophilic shell protects the encapsulated drug from the external medium and prevents the interaction with plasma components, resulting in *in vivo* long circulating properties. Moreover, the small particle size prolongs the residence time in blood circulation, bypassing the liver and spleen filtration and the glomerular elimination, and enhances cellular uptake and the ability to cross epithelial barriers. All these aspects result in increased drug bioavailability (Bilia et al. 2017, 2018a).

A study reported the formulation of a polymeric micelle of about 142 nm obtained by esterification of oleoyl chloride and polyethylene glycol 400 and loaded with curcumin was developed and tested for the inhibition of human brain(glioblastoma astrocytoma) cells proliferation, a model of glioblastoma. In addition, the sensitivity of adult human bone marrow stromal cells and regular human fibroblastic (HFSF-PI3) cell lines to formulations were also investigated. The micelle significantly suppressed the proliferation of human brain(glioblastoma astrocytoma) cells in a time- and dose-dependent manner, with the half maximal inhibitory concentration of 20 μM after 24 h and 48 h, which declined to 10 μM at 72 h ($p < 0.001$). Moreover, the viability of human brain(glioblastoma astrocytoma) was not affected by free curcumin. Cell viability of adult human bone marrow stromal cells cell line after 24 h of micelle exposure declined to 67% after treatment with 25 μM micelle ($p < 0.01$) and to 35 and 31% after treatment with 30 μM and 35 μM micelle, respectively ($p < 0.001$). The viability of regular human fibroblastic (HFSF-PI3) cell line was not significantly affected by the micelle treatment and no inhibitory effect was detected on these cells ($p > 0.05$)at the dose of 20 μM; but, at 25 μM concentration, the viability of the cells decreased to 50% ($p < 0.01$). Therefore, in concentrations suppressive for cancer cells, no harmful effects connected to micelles were observed in stem cells and normal fibroblast cells, showing the safety of this formulation as an anticancer treatment agent on normal cells (Tahmasebi Mirgani et al. 2014).

In a further study, Soluplus a polymer constituted of polyvinyl caprolactam (57%), polyvinyl acetate (30%) and polyethylene glycol (13%), and soluplus plus

containing also d-α-tocopherol polyethylene glycol 1000 succinate, also known as vitamin E TPGS (ratio Soluplus/TPGS was 20:1) were employed as amphiphilic polymers for the development of micelles able to improve the oral bioavailability of poorly water-soluble drugs. Micelles were loaded with increasing amounts of silymarin, from 0.5 mg/mL to 4 mg/mL. Micelles loaded up to 3 mg/mL of silymarin (encapsulation efficiency >92%) had similar average diameter (ca. 60 nm) and homogeneity (polydispersity index ≤0.1) to the empty micelles. Solubility studies demonstrated that the solubility of silymarin increased by ca. six-fold when loaded into micelles. Micelles avoided silymarin degradation in the gastrointestinal tract and showed a slow release of silymarin observed for both types of micelles. The potential increase of the intestinal absorption of silymarin was investigated by the parallel artificial membrane permeability assay, which confirmed a significant or borderline improvement the passive diffusion of silymarin when formulated into micelles. Transport studies employing Caco-2 cell line demonstrated that mixed micelles statistically enhanced the permeability of silymarin compared to polymeric micelles and unformulated silymarin. The study eveidenced that micelles entered into Caco-2 cells via energy-dependent mechanisms (Piazzini et al. 2019b).

### *1.2.3 Dendrimers*

Dendrimers are well-defined hyperbranched polymers which form semiglobular to globular structures (Fig. 1.5), mostly with a high density of functionalities on the surface together with a limited "volume." Dendrimer originate from the Greek word δένδρον (dendron), translated as tree. Diameters of these nanoparticles are generally less than 10 nm, but can be modulated by varying dendrimer generations (G0, G1, G2, G3 etc.).

The dendrimer is characterised by a central core surrounded by branches of repeating units. The surface can be characterised by the presence of functional groups, which have a crucial role in dendrimer characteristics. Drug molecules can be linked to superficial moieties or loaded in the interior cavities. Diverse polymers including polyamidoamine (PAMAM), polyethylenglycol, poly(L-glutamic acid), polyethyleneimine, polypropyleneimine, are used to synthetize dendrimers. They are nanocarriers with vast benefits including the possibility to select the nanometric size range, simplicity of variation by modifying their ends, easy to prepare (Bilia et al. 2017; 2018a). In order to avoid bioavailability limits of curcumin, nanoparticles made by a new dendrimer G0.5 based on polyamidoamine was developed. The developed dendrimer G0.5 had no cytotoxic effects in breast cancer MCF-7 cells and produced spherical NPs of ca. 150 nm. After the loading of curcumin [molar ratio G0.5/curcumin 1:1 (formulation 1) and 1:0.5 (formulation 2)], both dendrimers were very homogenous. Formulation 1 was further tested for the drug release properties because the highest encapsulation efficiency and loading capacity. Curcumin solubility for both formulations was strongly enhanced (ca. 415 and 150 times more soluble than aqueous solubility for formulation 1 and 2, respectively (Falconieri et al. 2017).

## 1.3 Lipid Nanocarriers

Lipid-based nanocarriers are based on lipids, natural glycerides, waxes or long chain fat acids or alcohols or diverse synthetic molecules represented by glycerides, sterols, aliphatic molecules, long chain acids or alcohols or their esters. Surfactants from natural (mainly lecitins) or synthetic origins are also included in the formulations. Generally, excipients are carefully chosen from food excipients, which are marketed under the denomination "Generally Recognized As Safe" (GRAS).

Lipid nanocarriers include vesicles, nanocochleates, micelles, solid lipid nanoparticles (SLN) and nanostructured lipid particles (NLC), emulsions with nano scale, including nanoemulsions and microemulsions (Fig. 1.8). Vesicles include liposomes and niosomes and are obtained using amphiphilic lipids, phosphatidylcholine derivatives and non-ionic surfactants. Liposomes can be converted to nanocochleates, which are unique nanovectors, after treatment with divalent ions (Bilia et al. 2018a).

### *1.3.1 Micro and Nanoemulsions*

Nanoemulsions and microemulsions are formulated with an oil phase, an aqueous phase, a surface active agent and probably a co-surfactant (McClements 2012). Microemulsions are defined as homogeneous thermodynamically stable transparent dispersions of two immiscible liquids stabilized by an interfacial film of surfactants. They have droplet size above 100–500 nm and require very low energy to formulate

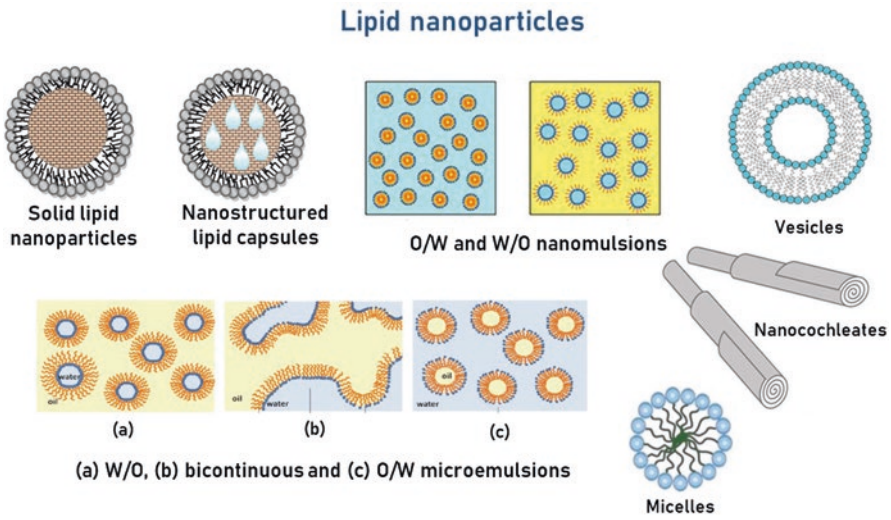


**Fig. 1.8** Lipid nanoparticles. (Reproduced with permission from Bilia et al. 2018a from Wiley)

emulsion, since they form spontaneously when aqueous, oil, and amphiphilic components are brought into contact, besides having a lower production cost compared to nanoemulsions. They are characterised by three systems, thus in addition to oil in water and water in oil emulsions, a unique biphasic system called "bicontinuos systems" can be also formulated (Fig. 1.8). Nanoemulsions are non-equilibrium systems with a spontaneous tendency to separate into the constituent phases, prepared using lower surfactant concentrations than microemulsions. They have droplet covering the size range of 20–500 nm and referred to as miniemulsions, ultrafine emulsions, and submicrometer emulsions (Bilia et al. 2017; 2018a). Although both nanoemulsions and microemulsions show long-term stability, microemulsions are much more sensitive to environmental changes, such as temperature, ionic strength, composition (adding/removing molecules to/from the aqueous continuous phase). A drawback when comparing to nanoemulsions, is that microemulsion formation requires the use of relatively large amounts of surfactant, *i.e.*, their loading capacity is significantly lower than this of comparable nanoemulsion delivery systems, especially when using triglycerides as the dispersed oil phase (McClements 2012). A further and very successful approach to improve the solubility, chemical stability, and oral bioavailability of poorly water soluble molecules is self-microemulsifying drug delivery systems. They are defined as isotropic mixtures of oil, surfactant, cosurfactant and drug, which form oil-in water microemulsion with droplet size less than 100 nm when exposed to aqueous media with gentle agitation or motility of gastrointestinal tract (Kang et al. 2004). Different studies are present in the literature about the application of micro/nanoemulsion to deliver the natural compounds and recently some researches are concerning the formulation also of the plant extracts due to the high versatility of the formulations.

Astaxanthin, as other carotenoids, has a very low absorption by the human body due to its low water solubility, and it is quite unstable to high temperature, pH changes, oxygen and light, limiting its use. The formulation of astaxanthin in oil-in-water nanoemulsions using gypenosides (a natural mix of triterpenoid saponins from *Gynostemma pentaphyllum*) as natural emulsifiers was investigated and compared with a synthetic emulsifier, Tween 20 (Chen et al. 2018). Gypenosides produced nanoemulsions with a volume mean diameter (125 ± 2 nm), which was similar to those prepared using Tween 20 (145 ± 6 nm). In addition, gypenosides was able to protect the oil droplets from coalescence during thermal processing at elevated temperatures for 30 min, even if the rate of astaxanthin degradation increased with increasing temperature. All nanoemulsions showed excellent stability when stored at 5 °C and 25 °C for at least 30 days, regardless of emulsifier type. Gypenosides provided better protection against astaxanthin degradation than did Tween 20, probably due to the free radical scavenging ability of gypenosides (Chen et al. 2018).

Oil-in-water astaxanthin-loaded nanoemulsions were also prepared using ginseng saponins as natural emulsifiers (Shu et al. 2018). An oil phase (5% w/w) was obtained by dispersing 2% w/w of astaxanthin in soybean oil. The aqueous phase (95%) was prepared by dissolving saponins (0.08–1.2%, (w/w) in water. Saponins were capable of producing nanoscaled droplets (mean values were ca. 125 nm). The

droplet size of the nanoemulsions decreased with increasing emulsifier concentration and homogenization pressure. The nanoemulsions were stable without droplet coalescence against thermal treatment, and over a narrow range of pH values (ranging from 7 to 9) but not in acidic conditions (pH range from 3 to 6) and in the presence of salt (Shu et al. 2018).

β-Carotene is as another important carotenoid with high instability and low oral-bioavailability. A formulation based on tea polyphenols and β-carotene was developed. It was an oil-in-water nanoemulsion with the core oil phase containing the carotenoid and the water phase containing tea polyphenols (Meng et al. 2019). The polyphenol extracts from green tea contained 20.04% of (−)-epigallocatechin, 11.17% of (−)-epicatechin gallate, 3.37% of (−)-epicatechin, 2.13% of (−)-gallocatechin gallate, 1.98% of (−)-gallocatechin and 0.51% of (+)-catechin. Corn oil was used as oily phase. During storage at three different temperatures (4, 25 and 35 °C), the nanoemulsion had a better stability and higher retention rate of β-carotene than the nanoemulsion containing only β-carotene. An *in vitro* simulated digestion assay indicated that the β-carotene recovery rates of the nanoemulsion containing also the tea polyphenols at digestion phases I and II were significantly increased compared to the nanoemulsion containing only the carotenoid. An *in vivo* absorption study showed that the nanoemulsion contain the tea polyphenols showed a higher conversion from β-carotene to vitamin A compared to the nanoemulsion containing only the carotenoid (Meng et al. 2019).

An oil-in-water nanoemulsion containing capsaicin used for management of pain and inflammatory disorders, was prepared using Tween 80 and Span 80 as non-ionic surfactants, ethanol as co-surfactant, olive oil as oil phase and water as external phase (Ghiasia et al. 2019).

The formulation had a droplet diameter of 13–14 nm and was stable for more than 8 months at both 4 °C and 45 °C. The nanoemulsion was then formulated into topical cream and gel to compare its efficacy and safety profiles with conventional cream of capsaicin. Carbopol® was selected as gel matrix base, while the cream base consisted of cetyl alcohol, cholesterol, liquid paraffin, soft paraffin and beeswax. Both formulations contained 0.075 w/w of capsaicin.

Skin irritation study showed that topical application of capsaicin nanoemulsion was safe and no sign of oedema or erythema was observed. The preparation significantly decreased inflammation of rats paw oedema compared to the conventional formulations and control group. Capsaicin nanoemulsion loaded in gel with showed very good resistance to the pain caused by heat stimulus in rats (Ghiasia et al. 2019).

Curcumin as other lipophilic small molecules, has a low hydrophilicity and intrinsic dissolution rate(s), low physical and chemical stability, rapid metabolization with low nanomolar levels of the parent compound and its glucuronide and sulphate conjugates found in the peripheral or portal circulation, low absorption, poor pharmacokinetics and bioavailability, and low penetration and targeting efficacy (Bilia et al. 2017). Three microemulsions were developed and characterized, stabilized by non-ionic surfactants macrogolglycerol ricinoleate (Cremophor EL), polysorbate 20 (Tween 20), polysorbate 80 (Tween 80), or lecithin and contained a variety of oils, namely, olive oil, wheat germoil, and vitamin E. The oral absorption

of curcumin microemulsions was investigated in vitro using parallel artificial membrane permeability assay. The optimal formulation consisted of vitamin E (3.3 g/100 g), Tween 20 (53.8 g/100 g), ethanol (6.6 g/100 g), and water (36.3 g/100 g), obtaining a percentage of permeation through the artificial membrane of about 70% (Bergonzi et al. 2014).

In a further study, nanoemulsions containing 0.5% w/w corn oil containing 0.4% w/w curcumin, sodium-alginate (1.0% w/w) and 0.5, 1.0 or 2.0% w/w of surfactant (Tween 20, lecithin or sucrose monopalmitate), were developed (Artiga-Artigas et al. 2018). Nanoemulsions showed particle sizes $\leq$400 ± 3 nm and effectively reduced droplets interfacial tension with negative ζ-potential values ($\leq -37$ mV), regardless the concentration of surfactant. Nanoemulsions with 2.0% w/w lecithin were stable during 86 days of experiment, whereas those containing Tween 20 or sucrose monopalmitate at the same concentrations were not stable after 5 days or along 24 h, respectively (Artiga-Artigas et al. 2018).

A study by Machado et al. (2019) developed a curcumin-nanoemulsion to be used as a photosensitizing agent in photodynamic therapy in an *in vitro* breast cancer model, breast cancer MCF-7 cells. The nanoemulsion containing 0.1 mg/mL showed an efficient internalization in the fibroblast and adenocarcinoma mammary line, as well as absence of cytotoxicity. The oil phase was prepared containing medium-chain-triglycerides and natural soy phospholipids. Poloxamer 188 was used as an anionic surfactant. The physical parameters of NE were: size 199 ± 0.2 nm; Zeta potential −46.3 (mV) polydispersity index 0.179. Curcumin had phototoxic effects, significantly decreased the proliferation of breast cancer MCF-7 cells and stimulating the reactive oxygen species production in combination with photodynamic therapy (Machado et al. 2019).

Green tea catechins (Polyphenon 60) and caffeine were encapsulated in a microemulsion to obtain synergistic antibacterial activity against selected pathogens (Gupta et al. 2014). Combination of two natural compounds would advocate two different mechanisms on the bacterial growth thereby providing for better antibacterial activity. Thermodynamically stable microemulsion was developed by using Labrasol as an oil phase, Cremophor EL as surfactant, and glycerol as cosurfactant. The combination of the above two natural compounds was proficient in lowering the MICs of individual agents. Higher antimicrobial effect of microemulsions can be attributed to the formation of nanodrops that increase the surface tension and thereby force themselves to merge with the lipids present in the bacterial cell membrane. Results of 2,2-diphenyl-1-picrylhydrazyl assay indicated that microemulsion system preserved the long term antioxidative potential of P60 and caffeine (Fig. 1.9). The cytotoxicity of the optimized formulation on Vero cell line by MTT assay was found to be nontoxic to mammalian cells (Fig. 1.10).

I a further study, mangiferin nanoemulsions were developed using hyaluronic acid of different molecular weights, in absence or presence of Transcutol-P (Pleguezuelos-Villa et al. 2019). Mangiferin (1%) and glycerine (3%) were added to distilled water in a glass tube in absence or presence of hyaluronic acid (1%) to obtain aqueous phases. Lipoid® S75 (5%), polysorbate 80 (1%), tocopherol (0.1%) and almond oil (10%) were also mixed with or without Transcutol-P (4%) to obtain

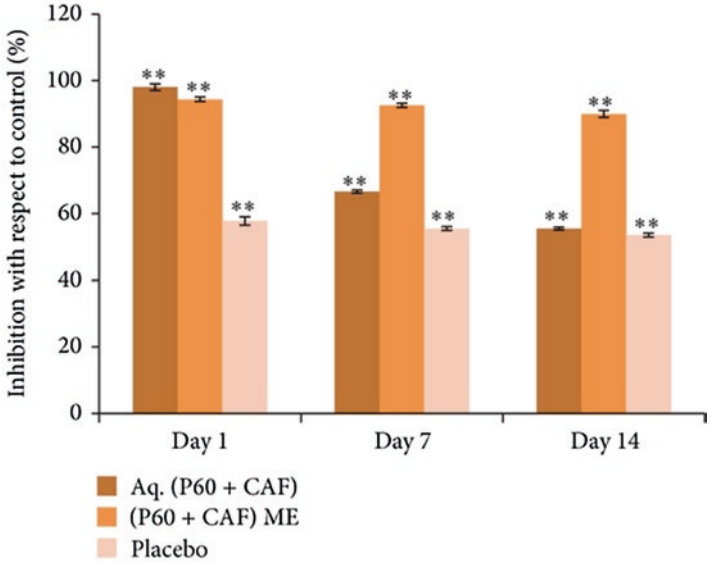


**Fig. 1.9** Antioxidative effect of the aqueous green tea extract plus caffein, loaded in the microemulsion, and placebo via 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Data are represented as percentage of inhibition with respect to control. Mean values of three independent experiments and S.E. are shown. **Significant at $P < 0.005$. (Reproduced with permission from Gupta et al. 2014 from Hindawi)

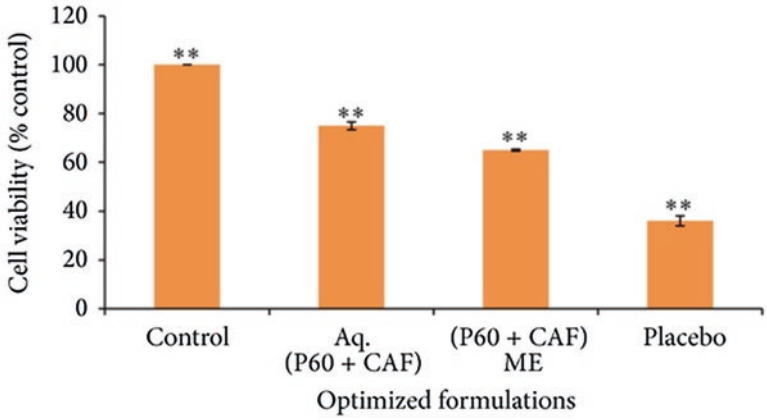


**Fig. 1.10** Cytotoxicity analysis of the aqueous green tea extract plus caffein, loaded in the microemulsion, and placebo on Vero cell lines after 24 hrs via MTT assay. Data are represented as percentage of Vero cell viability. Mean values of three independent experiments and S.E. are shown. (Reproduced with permission from Gupta et al. 2014 from Hindawi)