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Review

# On the use of nanotechnology-based strategies for association of complex matrices from plant extracts

Giovanni Konat Zorzi[a], Edison Luis Santana Carvalho[b], Gilsane Lino von Poser[a], Helder Ferreira Teixeira[a,*]

[a] Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[b] Programa de Pós-graduação em Produtos Bioativos e Biociências, Universidade Federal do Rio de Janeiro, Campus Macaé, Rio de Janeiro, RJ, Brazil

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ABSTRACT

Depending on the method of extraction, plant extracts can contain an enormous variety of active molecules, such as phenolic compounds, essential oils, alkaloids, among others. In many cases, from a pharmacological point of view, it is interesting to work with crude extract or fractions instead of a single isolated compound. This could be due to multi-targeting effect of the extract, lack of knowledge of the active compounds, synergistic effect of the extract compounds, among others. In any case, in order to achieve a final product some issues must be overcome, including poor stability, solvent toxicity, and low solubility of the bioactive compound. Recently many nanotechnology-based strategies have been proposed as an alternative to solve these problems, especially liposomes, nanoemulsions and nanoparticles. In this sense, the present work aims to review the main nanotechnological approaches used for association of different plant extracts and the main achievements from using these technologies.



## Introduction

Mankind always had a close relationship with plants, using them for thousands of years as source of potential medicines to relieve both physical and spiritual pain. Throughout the centuries, the therapies have evolved and the use of complexes mixtures as plant extracts has been systematically replaced by therapies using a single isolated compound. Nevertheless, the plants are still a major source of bioactive compounds in modern medicine (Rates, 2001).

Ideally a single, pure and isolated compound should be used in the development of a formulation. Nevertheless, this is not always the most viable or successful approach. Among the reasons of using complex mixtures, emphasis could be given to the synergism with different compounds, the loss of activity after isolation, chemical instability, difficulty in the purification and the possibility to act in multiple targets at once (Gertsch, 2011; Kim et al., 2008; Rando et al., 2009). However, seldom a plant extract itself can be used as a final product. As for pharmaceutical dosage forms, some technological process must be performed in order to obtain a final medicine that can assure quality, safety and efficacy (Prista et al., 2011).

In the past years, the number of publications relating to the use of nanotechnology-based systems association plant extract has been growing. There is already a well-documented literature about the benefits of associating isolated compounds to nanotechnological drug delivery systems and extending these studies to more complexes matrices, such as plant extracts, was just a matter of time. There is a clear division among the works relating to plant extract and nanotechnology and two well defined groups can be distinguished. The first considers the use of plant extract for the formation of metallic nanoparticles (Mittal et al., 2013) and the second, the use of nanotechnology-based systems to improve biopharmaceutical and technological properties of plant extracts (Ajazuddin and Saraf, 2010; Bonifacio et al., 2014). The later will be the focus of this review.

Many nanostructures have been proposed for drug delivery, each one having their own advantages and drawbacks. This manuscript revises the use of lipid- and polymer-based nanostructures in the association of different extract, establishing a relation between the type of nanosystems and its preparation method to the different plant extracts and most abundant compounds.

## Nanosystems: characterization and preparation techniques

So far, the association of plant extracts has been described for liposomes, nanoemulsions and nanoparticles (either lipid or

* Corresponding author.
*E-mail:* helder.teixeira@ufrgs.br (H.F. Teixeira).

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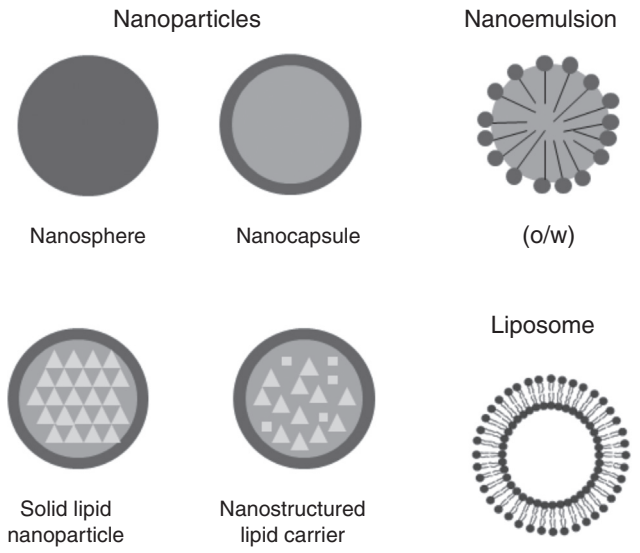


**Fig. 1.** Different architectures of nanosystems used to improve biopharmaceutical properties of plant extracts.

polymer-based nanoparticles) (Fig. 1). Factors such as polarity of active compound, solubility, presence of organic solvent and volatility must be taken into consideration when selecting the nanosystem and its preparation technique. For example, essential oils are rich in lipophilic compounds and extremely volatile molecules. In this sense, lipid-based systems prepared at low temperatures and without solvent evaporation after the addition of the oils, such as liposome or nanoemulsion are more likely to be successful.

Regardless the type of nanosystem or preparation technique, there are some basic characterizations of the system. The basic physicochemical parameters are average size, surface charge and association efficiency.

Determination of the size of colloidal structures can be done by several techniques such as transmission electron microscopy, laser diffraction, and small angle X-ray. Among them, dynamic light scattering offers several advantages such as wide range of measurement, simplicity and reliability (Berne and Pecora, 2003). It is a non-destructive technique that requires a small amount of sample (normally diluted). Nevertheless, for polydispersed samples the fluctuations of angle-dependency of scattered light may lead to unrealistic results. As alternative, the use of CONTIN algorithm for dynamic light scattering or nanoparticle tracking analysis is recommended, being the later also used to determine the concentration of nanoparticles (Filipe et al., 2010). Reduction of size results in the increase of surface free energy and, in this way, the preparation method must be chosen wisely (Shaw, 1992). The reduction of interfacial tension also decreases the amount of energy required for lowering the average size, as describe by the Laplace equation (Butt et al., 2003). In the nanometric world, some forces that play a major role in the macroscopic world are minimized. The Brownian motion, for example, is more significant than the gravity. In a similar way, the reduction of size can alter some physicochemical properties of bulk material (Hiemenz, 1997). Perhaps the most emblematic is the increase in the solubility. Due to the high surface area of nanostructured colloids, the solubility of lipophilic compound is increased, as dictated by the Kelvin equation (Butt et al., 2003; Cosgrove, 2010). Ultimately, this can result in loss of the active compound associated and increase in size due to the Ostwald ripening (Butt et al., 2003; Myers, 1999).

Size, however, seldom comes as a single value but rather a representative value of a distribution that can be narrow, moderate or broad. In this sense, the polydispersity index is a mathematical approach to measure the width of the particle size distribution, being the square of the division of standard deviation by mean diameter for dynamic light scattering. This property varies from 0 to 1, where lower values indicate narrower distribution (Berne and Pecora, 2003). Many factors affect the polydispesity of a system. For freshly prepared formulations, the manufacturing technique as well as type and amount of constituents plays a major role. Change of polydispersity through time is often associated to stability issues (Washington, 1992).

**Table 1**
Amount of extract (dry weight) usually associated to each nanotechnology-based system.

| System | Amount of extract (%) |
|---|---|
| Nanoemulsion | 0.5–20 |
| Nanoparticle | 0.5–2.0 |
| Liposome | 0.1–2.0 |

Estimation of the surface charge is important to predict colloidal stability and adequability of route of administration. According to the DLVO theory, colloidal stability is basically dependent on steric hindrance and the balance of attractive/repulsive forces (Hunter, 1989, 2001). In this way, keeping the surface charge far from neutrality is a way to favor the system stability. In the same way, the module and intensity of surface charge may result in undesired interactions with body fluids and tissues (Peeters et al., 2005; Rabinovich-Guilatt et al., 2004). For example, depending on the charge, molecules associated to nanosystem may permeate more or less in the skin layers (Gillet et al., 2011; Ogiso et al., 2001; Venuganti and Perumal, 2009). So, a given charge may be required depending on route of administration. The surface charge is easily estimated based on the quantification of the zeta potential, the difference of potential on the slipping plane of the colloid (Birdi, 2009; Butt et al., 2003). This determination of zeta potential is done by electrophoretic and electroacoustic methods, specially evaluating the electrophoretic mobility of the particles (Hunter, 1989).

Thus, zeta potential can be modulated by a judicious selection of materials that would be on the interface of nanostructure-continuous phase, such as surfactants, and compounds that would be adsorbed on the surface of the structures (e.g. polymers or compounds found in the plant extract) (Zorzi et al., 2011, 2015).

Another important factor is the determination of the association efficiency, *i.e.* the amount of active compound associated with the colloidal structure. This is a tricky task, however, because most extracts are not fully characterized and/or a marker is used to represent the total content. The best way to proceed is determining the dry residue and quantify the amount of marker (s) in respect to the dry residue, even if the extract is not used in its dried from. A judicious selection of the marker must be done and the method should be previously validated. Recently, Nemitz et al. presented a validated method to quantify isoflavone aglicone fraction from *Glycine max* ethanolic extract that was incorporated into nanoemulsion (Nemitz et al., 2015). Three relevant isoflavones, daidzein, glycitein, and genistein, could be simultaneously quantified and the method was validated to different matrices (nanoparticles, porcine skin, and esophagic mucosa). Similarly, Dias et al. have validated a head-space GC–MS method for the quantification of *Copaifera multijuga* essential oil in nanoemulsions (Dias et al., 2012). HPLC-UV validation of *Achyrocline satureioides* hydroethanolic extracts association to nanoemulsions was also recently reported. Three flavonoids with reported biological activity could be simultaneously determined (quercetin, 3-O-methylquercetin, and luteolin) (Bidone et al., 2014a).

Overall, the amount of extract that can be incorporated into nanosystem varies with its type (Table 1). Nanoemulsions are