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Chapter

Nanocosmetics: Production, Characterization, and Performance Improvement

Júlia Scherer Santos

Abstract

Nanocosmetics are personal care products containing nanocarriers or nanoparticles. Nanocarriers have been used in sunscreens, moisturizers, perfumes, and anti-aging and hair products. These carriers increase formulation efficacy and promote controlled release of active ingredients. Polymeric nanocarriers or lipid nanocarriers containing sunscreens have enhanced ultraviolet protection. In addition, these nanocarriers protect unstable ingredients from degrading ultraviolet radiations. Furthermore, nanocarriers also impart moisturizing effect due to their reduced particle size. This chapter describes issues related to nanocarrier-based cosmetics production, characterization, and biological evaluation.

Keywords: nanocarriers, polymeric nanocarriers, lipid nanocarriers, nanocosmetics, biological assays

1. Introduction

Nanocosmetics are skin formulations containing nanocarriers or nanoparticles. They have several advantages over conventional cosmetics (ie, cosmetics without nanotechnology). Nanocarriers are nanometric carriers having a substance entrapped inside. They are classified into lipid nanocarriers and polymeric nanocarriers. The first ones include solid lipid nanoparticles, nanostructured lipid carriers, and nanoemulsions. Polymeric nanocarriers include nanocapsules, nanospheres, and micelles. In order to demonstrate the importance of these nanocarriers in cosmetics, an initial examination was performed in Scopus databases and Web of Science database until 2019. The terms “nanocarriers” and “cosmetics” were crossed searching for abstract, article title, and keywords, and the results are shown in Figure 1 and Table 1. The number of publications retrieved from the databases was similar although the Web of Science database recovered a smaller number of publications.

Figure 1 shows the number of publications from search 01 over time. An exponential increase in publications is observed in both databases. It is also noteworthy that the first publications using nanocarriers applied to cosmetics date from the mid-2000s, showing, therefore, that this term is of recent use.

In order to detect the number of publications regarding lipid nanocarriers and polymeric nanocarriers, two new searches were performed. From the search 01, the total number of publications was further crossed with the term “lipid” and “polymer” in search 02 and search 03, respectively (Table 1). In this context, the
The vast majority of publications refer to lipid due to its ingredients’ biocompatibility. Although polymeric nanocarriers had a smaller number of publications, they are still important, mainly in relation to the use of natural polymers.

Nanoparticles applied to skin formulations comprise inorganic nanoparticles and organic nanoparticles. Inorganic nanoparticles such as titanium oxide nanoparticles and oxide zinc nanoparticles are common in sunscreen formulations. On the other hand, organic nanoparticles have polymers or lipids in their composition. The approach to cosmetics containing inorganic nanoparticles is beyond the scope of this chapter. The main aspects of production and characterization of lipid nanocarriers and polymeric nanocarriers will be addressed in this chapter. Further, in vitro and/or in vivo evaluation of nanocarriers and nanocosmetics will also be described.

2. Nanocosmetics production and characterization

Nanocosmetics are also called nanocosmeceuticals: cosmeceuticals containing nanotechnology [1]. Cosmeceuticals are cosmetics with benefits over traditional cosmetics. They are defined as quasi pharmaceuticals as they come from the
combination of cosmetics and pharmaceuticals. Cosmeceuticals must have an established mechanism of action in skin cells or tissues. These cosmetics are often tested regarding efficacy studies, and in this sense, they resemble pharmaceutical products [1]. Nanocarrier production methods are classified into bottom-up or bottom-down [2] and vary accordingly to each nanoparticle type (ie., matrix types and depot-types) [2, 3]. After nanocarrier production, characterization studies should be conducted. They include the following assays: determination of particle size, zeta potential, encapsulation efficiency, and in vitro release study [2]. Also, characterization studies should be performed on nanocosmetics.

2.1 Nanocarrier production

Nanocarriers are nanometric carriers entrapping the desired substance in its structure. As this substance is enclosed in the nanocarrier, a performance improved could be achieved. There are several methodologies applied to nanocarriers and the present work does not intend to exhaust all existing ones. Table 2 below shows some of the methods applied to the following nanocarriers: nanoemulsions, solid lipid nanoparticles, nanostructured lipid carriers, nanocapsules, and nanospheres [2, 4].

Polymeric nanocarriers (nanocapsules or nanospheres) are composed of biodegradable and/or biocompatible polymers such as poly (D,L-lactide-co-glycolide) [26] and chitosan [23, 25] and they have a size ranging from 100 to 1000 nm. The substance is encapsulated, dispersed, or adsorbed on the surface of these particles. Nanocapsules are depot-like systems formed by a polymeric wall and an oily core. On the other hand, nanospheres do not contain oil in their composition. Therefore, nanospheres are formed by a polymeric matrix where the substance is dispersed or dissolved [2, 4]. To obtain these particles, methods such as interfacial deposition of pre-formed polymer, emulsification/solvent diffusion, and interfacial polymerization can be employed. Recently, the focus has been on the use of natural polymers [23, 25, 28].

| Nanocarrier       | Method                                           | References                  |
|-------------------|--------------------------------------------------|-----------------------------|
| Nanoemulsions     | Ultrasonic emulsification-solvent evaporation    | [5, 6]                      |
|                   | Ultrasonication                                  | [7]                         |
|                   | Emulsification/evaporation                       | [8]                         |
| Lipid nanocarriers| High pressure homogenization                     | [9–14]                      |
|                   | High shear homogenization/high pressure homogenization | [15]                     |
|                   | Melt emulsification coupled with high shear homogenization | [16, 17]              |
|                   | Double emulsion                                  | [18]                        |
|                   | Hot melt microemulsion                           | [19]                        |
|                   | Microemulsion coupled with probe sonication      | [20]                        |
|                   | Ultrasonication                                  | [7, 21, 22]                 |
| Polymeric nanocarriers | Ionic crosslinking                               | [23]                        |
|                   | Interfacial deposition of pre-formed polymer     | [24, 25]                    |
|                   | Polymerization of monomers                       | [8]                         |
|                   | Emulsification/solvent evaporation               | [26, 27]                    |
|                   | Two-step desolvation                             | [28]                        |

Table 2. Nanocarrier preparation methods.
Lipid nanocarriers differ from polymeric nanocarriers since they have solid/liquid lipid in their composition. These lipids account for their high biocompatibility. Solid lipid nanoparticles (SLN) are formed by a solid lipid matrix and were developed in the 90s. However, SLN have low long-term stability [4]. Nanostructured lipid carriers (NLC) were developed in order to increase the long-term stability of solid lipid nanoparticles.

Although nanoemulsions are lipid nanocarriers, just as SLN and NLC, they have an internal phase with a nanometric droplet size and different production methods. Therefore, the method to be selected will depend on the type of nanocarrier and also on the equipment and raw materials available.

2.2 Nanocarrier characterization

Nanocarrier characterization is performed to provide information such as particle size, zeta potential, entrapment efficiency [13, 18, 29], morphological aspect [27, 30], and pH [25]. Other analyses also include nanocarrier physical stability [11] and compatibility between ingredients of nanocarriers [31].

Particle size distribution and zeta potential analyses are essential, as they are directly related to nanocarriers' biological behavior. Nanocarriers have different particle size distributions varying according to carrier type [12–14, 23, 25, 26, 30] and also according to their composition [13, 14, 25, 31, 32]. In this sense, lipid nanocarriers' particle size distribution range from 100 nm to 200 nm [13, 14, 21, 29], or they may have particle size distribution ranging from 60 nm to more than 200 nm [32]. For nanoemulsions, droplet size may be smaller than 100 nm [30] or greater than 200 nm [6]. Regarding polymeric nanocarriers, sizes smaller than 100 nm [27], in the range between 100 and 200 nm [23], or even greater than 200 nm [26] are described.

Zeta potential reflects the particle surface charge and is related to its composition. Negative zeta values are the most reported [18, 24, 25]. Nevertheless, positive zeta potential is also reported for nanocarriers since there is an increase in its biological efficacy compared to negatively charged nanocarriers [29]. Entrapment efficiency, on the other hand, is related to the preparation method and to the physicochemical properties of the encapsulated substance [23]. This methodology sets the percentage encapsulated in nanocarriers [6, 16, 26–28, 32]. High encapsulation efficiency can increase biological efficacy [16, 26].

In addition, methodologies such as atomic force microscopy [19, 27], transmission electron microscopy [12, 22, 27, 30], scanning electron microscopy [8, 20, 23], and optical microscopy [9, 20] have been used as way to complement size determination and to evaluate particle morphology. Moreover, pH measurement of nanocarriers [25, 30, 33, 34] allows assessing the compatibility of the skin to the nanocarrier.

Physical stability is another approach used to monitor the formulation behavior by measuring particle size distribution, zeta potential, and entrapment efficiency over a predetermined period of time [9, 11, 13, 23, 34]. Physical stability has also been showed by equipment that detects instability phenomena, such as sedimentation and cremation [25].

Furthermore, ingredient compatibility [15, 26–28, 35] must be also be performed prior to the development/preparation of any nanocarrier using methodologies such as thermal analysis. Ingredient interaction is not desired in many cases. Nevertheless, in some situations, ingredient interaction can be beneficial since it causes increase in cosmetic efficacy [28]. Thermal analyses are also used for other purposes: to evaluate lipid crystallinity, to measure the physical stability of lipid nanoparticle, and to show that substance is encapsulated within the nanoparticle [17, 29]. Finally, in the case of nanoemulsions, other analyses can also be performed: electrical conductivity [30, 33, 34], phase diagram [30], and interfacial tension [33].
2.3 Nanocosmetics production and characterization

Once properly characterized, nanocarrier can be added in an appropriate semi-solid form since nanocarriers are usually aqueous liquid forms that have low viscosity [4]. Nevertheless, it is also possible to thicken the nanocarrier solution/dispersion [34]. Cosmetics forms employed in nanocosmetics (Table 3) include mainly creams [13, 20, 24, 26], hydrogels [15, 18, 21, 24, 25], or gel-creams [19].

From the moment that nanocosmetics are obtained, they must be characterized by tests such as pH, organoleptic characteristics, and rheology [20, 24–26]. As previously mentioned, pH determination is a prime analysis to ensure formulation compatibility with the skin. In contrast, organoleptic characteristics such as appearance and color [25] can be visually determined. In the case of color determination, it is also possible to monitor it by equipment [21]. Rheological analysis defines the product ease/difficulty of flow. Consequently, rheological behavior is crucial because it is directly related to spreadability of cosmetics in the skin [24].

Stability determination of a nanocosmetic is a common assay comprising analyses such as organoleptic characteristics, pH, rheology, and entrapped substance content [19, 20, 24, 25]. Taking into consideration that stability test submits the product to temperature variations, changes in pH, rheology, and substance content are expected. In that respect, nanoencapsulation has shown the ability to increase the physical stability of cosmetics [24]. Besides, there may be incompatibility of the nanocarrier with the semi-solid vehicle. Hence, compatibility of ingredients from nanocosmetics can also be performed [20].

Further, other tests can also be applied to nanocosmetics such as extrudability and nanocosmetic particle size determination. The first one determines the ease of formulation be removed from its packaging [20]. The second one detects the presence of nanometric particles in the semi-solid form [25].

3. Biological evaluation of nanocosmetics

Biological evaluation of nanocosmetics comprises efficacy and toxicity tests [13, 18, 20, 35] aiming to demonstrate its performance and safety profile [26]. Firstly, the type of nanocarrier to be prepared must be established. This choice should take into account the properties of each nanocarrier. In such a way, if the nanocosmetic must have a moisturizing effect, lipid nanocarriers may be an option.

Table 3.

| Cosmetic form | Main component | References |
|---------------|----------------|------------|
| Cream         | Stearic acid/triethanolamine | [20, 27] |
|               | Sodium polyacrylate/dimethicone/cyclopentasiloxane/trideceth-6/PEG/PPG-18/18 dimethicone | [24] |
|               | Glyceril monostearate | [26] |
| Hydrogel      | Hydroxyethyl acrylate/sodium acryloyldimethyl taurate | [28] |
|               | Ammonium acryloyldimethyl taurate/VP copolymer | [24] |
|               | Carbomer | [15, 18, 21] |
|               | Chitosan | [25] |
| Gel-cream     | Cetearyl alcohol/dicetyl phosphate/Ceteth-30 phosphate/Acrylates/C10-30 alkyl acrylate crosspolymer | [19] |
since they have moisturizing activity [11, 21]. If a sunscreen formulation is intended, lipid nanocarriers can also be prepared [17] due to their light scattering properties. Regardless of the carrier type, biological assessment can be performed directly on the nanocarriers and also on the nanocosmetics [27]. Sometimes, researchers choose at first to perform tests only with nanocarriers [6, 12, 31, 36] in order to establish their properties and potential applications before adding them into a semi-solid form. In other cases, efficacy tests can be done directly on nanocosmetics [18–21, 24].

Performance assays are selected according to the benefit promoted by nanoencapsulation. Thereby, an appropriate efficacy test for a photounstable substance loaded in the nanocarrier is the photostability test [36]. On the other hand, for a substance with a limited skin distribution profile or with undesirable skin permeation, skin permeation/penetration study may be performed [6, 35, 37]. Furthermore, safety assays aim to demonstrate nanocarrier safety profile when applied to a culture of cells [29, 38].

3.1 Biological evaluation of nanocarriers

Nanocarriers can be submitted to efficacy and safety tests [26] after a proper characterization. Efficacy tests assess its performance. Safety tests such as cell viability sets the nanocarrier cellular toxicity [26, 29]. Table 4 below summarizes some of the most used methodologies/tests. In vitro release and skin permeation/penetration tests are widely used [10, 12, 14, 31] as efficacy tests. Additionally, assays such as cell uptake [6, 38], antioxidant activity [13], moisturizing effect [14], and content of nanoencapsulated substance [25] are also employed as efficacy tests.

In vitro release simulates the substance release, and for this reason, it is widely used. Nanocarriers usually have a better performance than conventional formulations (without nanocarriers), favoring the release of the nanoencapsulated substance [23]. Besides, nanocarriers show an initial burst release followed by a prolonged release [12, 14, 22, 31, 35]. This prolonged action is a desirable effect as it reduces the number of product reapplications. In addition, there are also some differences regarding release profile of each nanocarrier [12, 22] (for example, see [22]). Hence, depending on the objective, it is possible to prepare NLC instead of SNL in order to favor the substance release. Furthermore, variations in nanocarriers components are also alternatives to modulate biological effect. In this sense, quantitative variations in nanocarriers composition affect in vitro release and moisturizing effect [14].

Skin penetration/permeation studies are conducted to detect the location of a substance in skin layers (penetration) and/or its presence in the receptor medium (indicative of skin permeation) [6, 36]. Skins from different sources are used (See Table 3). In the same way as for in vitro release tests, nanocarrier type also impacts the skin distribution profile. NLC promote greater skin retention than nanoemulsions. [7, 12], and nanoemulsions may have a small degree of permeation. However, regardless of nanocarrier type, they promote a greater skin penetration than conventional formulations [9]. In the case of cosmetic products, penetration/retention in the epidermis or dermis is desired, and its permeation (detection in the blood circulation) is unwanted [9]. It is yet noteworthy that nanoencapsulation of vegetable ingredients promotes its better retention in the epidermis and dermis. [6, 10, 35]. As there is a trend of the use of natural ingredients in cosmetics, this result is relevant because it assures an increased efficacy of vegetable ingredients loaded in nanocarriers [6].

Cell uptake is a complementary study to skin permeation allowing to confirm the skin location/deposition of nanoencapsulated substances. In this way,
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nanoencapsulation of vegetable ingredients modulates its skin distribution and allows its skin retention as evidenced by confocal microscopy [38]. Besides, a strategy to increase cellular uptake is the use of nanocarriers with positive electrical charge (CSLN) [29].

In contrast, as previously mentioned, cell viability is a safety assay. As nanosystems may cause cell/tissue toxicity, assessing their safety is important. Nanocarriers with low cytotoxicity are considered safe [12, 26, 27]. Regarding other efficacy assays, nanocarriers also have a better performance. Nanoencapsulation of vegetable

| Biological studies                  | Methodology             | Nanocarrier | References |
|-------------------------------------|-------------------------|-------------|------------|
| Penetration/permeation study        | Permeation in mice skin | PNC         | [38]       |
|                                     |                         | NLC         | [10]       |
|                                     | Penetration/permeation in porcine ear skin | NE         | [6]       |
|                                     | Penetration study in human skin | NLC         | [12]       |
|                                     | Permeation/permeation in albino rats | NLC         | [14]       |
|                                     | Permeation in dermis pig ear | SLN, NLC   | [9]       |
|                                     | Penetration in stratum corneum/ epidermis from human skin | NLC, NE   | [36, 7] |
| **In vitro release**                | Membrane dialysis       | NLC         | [31]       |
|                                     |                         | NLC, NE     | [12]       |
|                                     |                         | NLC         | [14]       |
|                                     |                         | PNC         | [23]       |
|                                     |                         | NLC         | [22]       |
|                                     | Franz diffusion cell    | CSLN        | [29]       |
|                                     |                         | NLC         | [35]       |
| **Cellular uptake**                 | CLSM                    | PNC         | [38]       |
|                                     | CLSM                    | NE          | [6]        |
|                                     | Flow cytometer          | CSLN        | [29]       |
| **Cell viability**                  | Sulfobrodhamine assay   | PNC         | [38]       |
|                                     | MTT                     | CSLN        | [29]       |
|                                     |                         | PNC         | [26]       |
| **Antioxidant activity**            | Chemiluminescence       | NLC         | [31]       |
|                                     |                         | NLC         | [13]       |
|                                     | FRAP                    | NLC, NE     | [12]       |
|                                     | DPPH                    | NLC         | [14]       |
| **Moisturization effect**           | Occlusion factor        | NLC         | [14]       |
|                                     | TEWL and skin hydration | NLC         | [11]       |
| **Photostability**                  | Content of tocopherol   | NLC, NE     | [12]       |
|                                     | Content of rose hip oil | PNC         | [25]       |

Table 4. Biological evaluation of nanocarriers.

PNC: polymeric nanocarrier, NLC: nanostructured lipid carriers, SLN: solid lipid nanoparticles, NE: nanoemulsion, CLSM: Confocal laser scanning microscopy, MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, CSLN: cationic solid lipid nanoparticles, FRAP: ferric reducing antioxidant potential, DPPH: (1,1-diphenylpicryl-hydrazyl), TEWL: transepidermic water loss.
ingredients increase its antioxidant activity [13, 27]. Nanocarriers also promote skin moisturization [11, 34] where the moisturizing effect depends on the carrier composition [11]. Nanocarriers increase photostability of entrapped substances [12, 25], which affects its antioxidant activity. Since there is a less amount of substance degraded by light, a greater proportion of it will be available to act as an antioxidant.

### 3.2 Biological evaluation of nanocosmetics

Biological assessment of nanocosmetics comprises the same assays employed to nanocarriers such as penetration/skin permeation, skin moisturization, or antioxidant activity. However, other tests are also used for semi-solid forms such as the sun protection factor (SPF) and ultraviolet A protection factor (UVA-PF) [31]. Table 5 below exhibits the biological tests applied to nanocosmetics, whether they are efficacy or safety tests.

As described previously, skin penetration/permeation determines substance accumulation in skin layers and its permeation, if any. Penetration and/or permeation will be desirable depending on the type of product. For antioxidants, penetration in the epidermis and dermis is desirable [6]. Besides, ingredients of nanocosmetics also affect skin permeation/permeation, whether they are ingredients of the nanocarrier or the ingredients of semi-solid form [26]. As reported, nanocosmetics have a better skin retention/permeation than conventional cosmetics [27].

SPF and UVA-PF are efficacy tests used to evaluate UVB and UVA protection, respectively [15]. Nanocosmetics have shown superior performances regarding these assays. Cosmetics containing nanocarriers with different quantitative compositions also have different SPF and PF-UVA values [16]. In addition, nanoencapsulation also improves ultraviolet protection (for example, see [17]). Nanocosmetics containing association of vegetable oils have also been used to promote ultraviolet protection [13].

Moisturizing effect is commonly performed in vitro [20, 27] or in humans [19, 21] and is usually an important measure for moisturizing and anti-aging cosmetics [21]. Cosmetics containing lipid nanocarriers have moisturizing properties due to their adhesive characteristics, to their composition [19, 21], and to the composition of semi-solid vehicle [18]. Nanocosmetics also preserve antioxidant activity even after irradiation exposure. Once there is depletion of endogenous antioxidants with ultraviolet radiation exposure, these nanocosmetics can be used as anti-aging cosmetics [27]. Another important test is skin toxicity assessment whose goal is to detect any irritation caused by nanocosmetics [20]. Additionally, nanocosmetics have a photostabilizing effect. Nanocosmetics have the ability to reduce the degradation of nanoencapsulated ingredients [24].

| Biological assay                        | References                        |
|-----------------------------------------|-----------------------------------|
| Skin penetration/permeation             | [20, 26, 27]                      |
| Sunscreen protection factor (SPF)       | [13, 16, 17, 20, 26, 27, 31]      |
| Ultraviolet A protection factor (UVA-PF)| [13, 17, 31]                     |
| Skin moisturization                     | [18–21, 27]                      |
| Antioxidant activity                    | [27]                              |
| Toxicity                                | [20, 26, 27]                      |

Table 5. Biological assays applied to nanocosmetics.
4. Conclusion

Nanotechnology-based cosmetics are increasingly common due to their many benefits. Understanding nanocarrier properties is a complex and expensive task that requires professionals with expertise in nanotechnology as well as requiring a high investment in the development of nanotechnology-based products. After deciding the suitable nanocarrier, its composition, and preparation method, characterization must be accomplished. Subsequently, nanocarrier and/or cosmetics containing nanocarriers must be evaluated regarding biological tests to assure an effective and safe product.

Ultimately, there is a trend to use biocompatible ingredients as well as the use of natural ingredients in nanocarriers. An association/co-encapsulation of multifunctional ingredients is also reported. Therefore, multifunctional nanocosmetics represent a great alternative to improve the performance of cosmetics.
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