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Introductory Chapter: Reduce the Gap from Bench to Bedside for Nanomedicines Increasing the Stability to Long-Term Storage

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1. The issue of physical stability in nanosized drug delivery systems

Stability to storage of a new pharmaceutical product is a key step for medical and commercial success. As with other pharmaceutical products, nanomaterials and nanotechnological drugs (nanomedicines) must be rigorously designed and tested to demonstrate the favorable benefit/risk ratio needed by health authorities to issue marketing authorizations.

Innovative pharmaceutical products, such as micro and nanocarriers, have to face two important aspects for regulatory approval: firstly, the demonstration of efficacy and safety profile is necessary, and secondly suitable storage conditions must be identified for the final product. In the case of colloidal (nanosized) carriers, it is important to obtain nearly unchanged physico-chemical properties during storage [1, 2].

The issue of stability to long-term storage of nanosized drug carriers will be briefly commented in this chapter, since this aspect can become, and often actually is, crucial for the translation of basic researches to clinically valid products. Liposomes, that would often be promising colloidal systems for drug delivery and targeting, are a sound example of how the question of stability strongly influences the translation from lab scale to therapeutics. Apart some few products which were able to reach a clinical significance, a huge number of projects, although promising at the preliminary phases, are unable to undergo industrial and commercial scaling-up, in most cases because of their very limited physico-chemical stability and difficulty to preserve the chemical integrity of liposomal formulations.

Another important aspect is related to the requirement of sterilization, mandatory for products that must come in contact with blood, eye or other damaged or sensitive tissues. Gamma

or UV radiation and autoclaving or chemical (ethylene oxide) sterilization techniques are usually applied to sterilize polymer-based products or devices. These processes expose the polymeric materials to a certain degree of physico-chemical stress and thus fundamental to ensure that the sterilized biomaterial retains its characteristics and features.

Of course, for some applications of biomaterials, a robust chemical stability is paradoxically unwanted. For resorbable materials, for instance, such as materials for sutures or fixing screws for bone fractures, a relatively rapid degradation in the body after the healing process is required.

Considering colloidal systems, we should pay attention to three principal properties, such as size and size distribution (homogeneity), surface charge, and shape (the rule of 4S), that should not significantly change during storage and that influence physico-chemical stability (Figure 1) [3].

Looking into this matter, we can highlight some important properties that separately influence physico-chemical stability. It is widely known that a mean particle size below 100 nm, correlated with a narrow size distribution ($PdI < 0.2$), allows increasing the stability of a nanosized system. These experimental evidences are necessary but not sufficient conditions, for instance also zeta potential values (i.e., a surface charge not less than ± 35 mV) stabilize the nanoformulation. When this situation is not verified, several instability conditions have been reported.

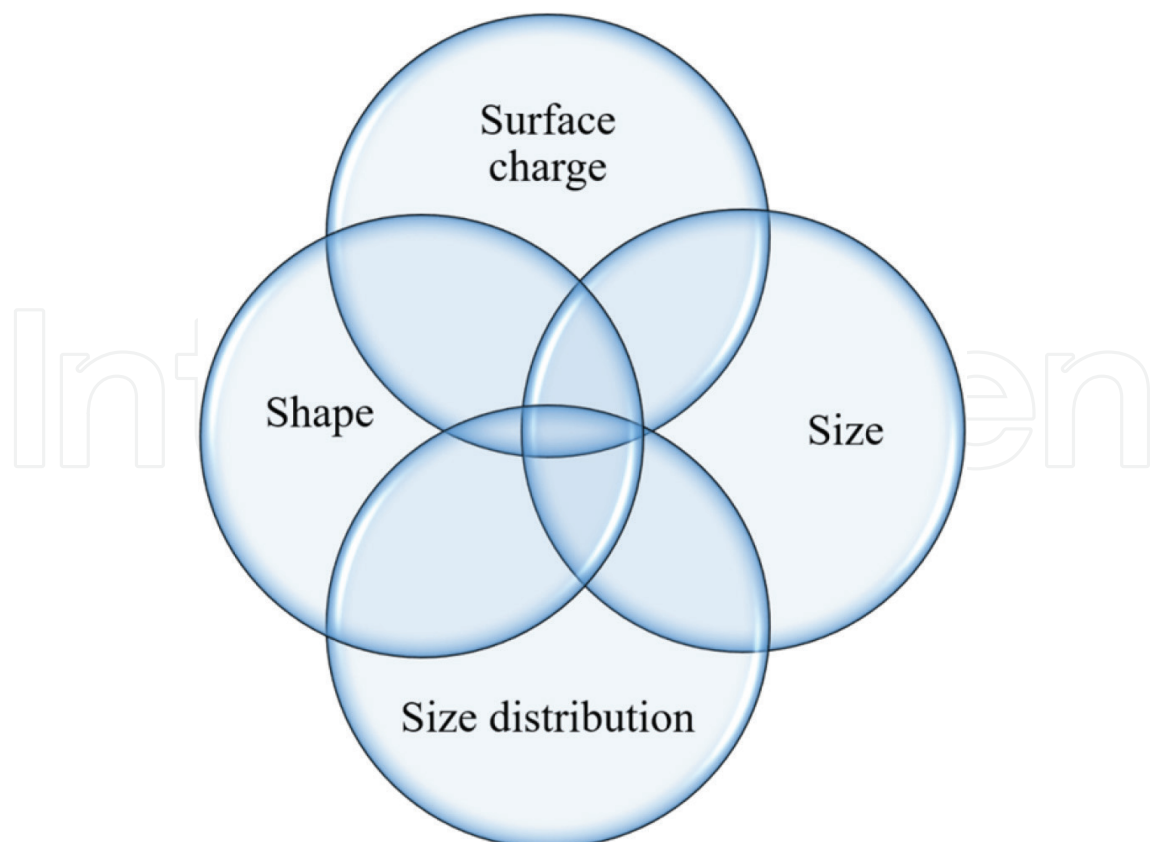


Figure 1. Venn diagram on properties that influence the physico-chemical stability of colloidal suspensions.

2. Freeze-drying (lyophilization) as a strategy to increase nanocarrier stability

To improve the stability of colloidal delivery systems upon storage, the removal of solvents, if required during the preparation method, is necessary, to obtain a final aqueous suspension. Storage of aqueous suspensions could determine some changes in the product such as increase of size, due to coalescence or aggregation phenomena, or even a reduction of it, due to degradation of the polymeric matrix, undesirable drug leakage, changes in the surface charge, and so on.

To remove water, freeze-drying (lyophilization) is one of the most efficient techniques used in the pharmaceutical industry and a strategy to reduce and control the instability phenomena [4, 5]. However, several physico-chemical phenomena, such as air adsorption and modification of nanoparticle surface during the various steps of the process may lead difficulty in redispersion of colloidal carriers in aqueous media for the subsequent administration [6, 7]. The addition of inert additives, defined as lyoprotectants or cryoprotectants) at relatively high concentrations (10–30%, but in some instances also up to 50% by weight), could protect the colloidal suspension against stress induced by the freeze-drying process [8–10].

During the last three decades, different studies were performed to select the additives to use during the freeze-dried process to prevent lyophilization stress. Several authors described the protective effect due to the formation of an amorphous vitreous layer on the surface of particles. Carbohydrates and polyalcohols are the most investigated ones [11].

Figure 2 shows how this field is a current topic of interest for scientists, as highlighted by the increased number of papers published in the last two decades (Pubmed source).

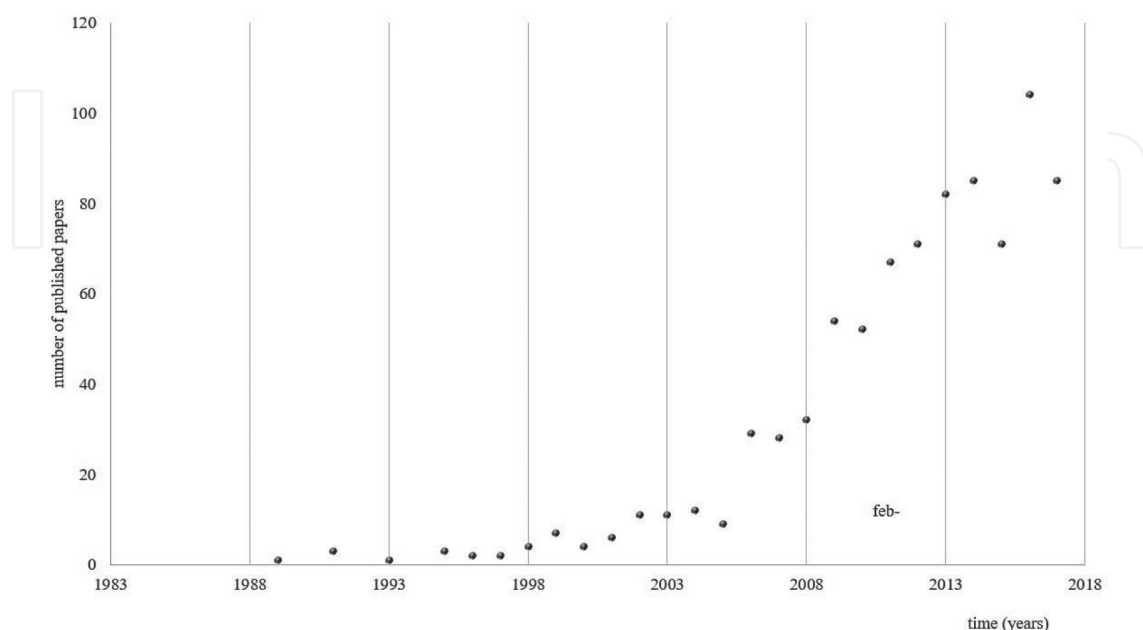


Figure 2. Trend of article publication in the field 'freeze-dried and nanoparticles' (Pubmed database; update: February 2018).

The protective action of cryoprotectants is principally a surface phenomenon, for which the proper amount of this component should also be evaluated. The concentration should be established as a function of total superficial area correlated to the size and the presence or not of the adsorbed drug onto the surface that could change the disposition of cryoprotectant at a molecular level on the surface. In the case of sugars, it was showed a reduction in the suitable amount to be used going from monosaccharides and disaccharides to oligosaccharides, therefore as a function of the type [9].

To better understand what happens during the freeze-drying process, the involved steps are recalled below. Scientists do not often distinguish between cooling and freezing steps and they consider as a single process. Conversely, cooling is related to the decrease of temperature of the chamber and the product inside it, while freezing corresponds to the modification of the physical state of the formulation from liquid (water) to solid (ice). During this step, water starts to nucleate and ice crystals are formed. Supercooling occurs if the formulation is cooled below its freezing point.

Lyophilization of nanosystems would however deserve a peculiar discussion. The first question is whether it is better a slow or fast freezing speed to obtain redispersible nanoformulations? The answer should consider some aspects: first of all, the state of excipient (e.g., the cryoprotectant) during freezing. For instance, mannitol crystallizes during slow freezing, while it is made more amorphous upon a fast freezing that makes mannitol to be able to protect nanoparticles. Another aspect is the concentration; several authors reported an enhanced protective action with increasing the amount of cryoprotectant. In summary, fast freezing rate and high concentration of cryoprotectant should produce a dry nanoformulation that preserve its better initial physico-chemical properties upon redispersion with an aqueous medium. However, a critical concentration for each cryoprotector seems to exist, above which an additional amount is prejudicial in many cases [12].

During primary drying, it is appropriate to obtain a non-collapsed cake, whose consequence would be a long time necessary for the redispersion of the freeze-dried nanoparticles. If primary drying occurs below the T_c of the nanoformulation, it is possible to avoid this phenomenon and the product will preserve the initial macroscopic structure.

Also the secondary drying is very important to be considered. Several authors sometimes underestimate the evaluation and standardization of operative conditions during this step. Currently, the drying time and shelf temperature can be optimized using simulation softwares, thus reducing experimental errors [13].

Raw materials used for the preparation of the colloidal carriers should be considered in the design and in the selection of the cryoprotectant. Liposomes are more sensitive to the freezing step, while polymers used to realize micro and nanocarriers are resistant to the low temperatures, but could suffer in the dehydration process, with a difficulty to restore the initial particle morphology and size (e.g., because of aggregation).

3. Regulatory aspects

Polymer- and lipid-based nanomedicines are checked during stability assays for physico-chemical, chemical, and microbiological aspects, such as macroscopic and microscopic physical

appearance, particle mean size and size homogeneity, zeta potential (surface charge), morphology and surface chemistry, drug loading/content and release profile, and *in vitro* drug stability/degradation. ICH guidelines (e.g., ICH Q1A and Q1C [14, 15]) can be helpful to project a suitable and efficacious stability plan, in terms of temperature/humidity conditions and duration.

For formulations that contain or encapsulate biotechnological products, like monoclonal antibodies, peptides, proteins, and gene material, the procedures described in ICH guideline Q5C for stability testing of these compounds must be considered [16].

Moreover, for medical devices and especially for sterile devices, apart an extrapolation from the above ICH guidelines, the ISO 13485:2016 rules are useful to manage the quality and stability requirements of these products, with the aim of demonstrating their ability to meet customer needs and regulatory requisites.

In April 2014 in London, during the “SME workshop for micro-, small- and medium-sized enterprises: focus on quality for medicines containing chemical entities”, organized by EMA, Dolores Hernán Pérez de la Ossa presented a report entitled “Quality aspects of nano-based medicines” [17].

The report identifies the challenges that a nanomedicine product should overcome to pass the regulatory process and safely reach the market. Such document fixes not only the physico-chemical aspects of the proposed nanomedicine, but also any complex supramolecular structure, for example the presence of ligands on the surface. These molecules determine the interactions with biological substrates and influence the physical stability of the so-called third generation nanoformulations. The presence of these substances often limits the effects of others additives added to stabilize the colloidal carriers. Cryoprotectant agents could not cover the surface and not preserve from aggregation during a freeze-dried process. The ligands could significantly modify the zeta potential value, a predictable parameter of colloidal carrier stability.

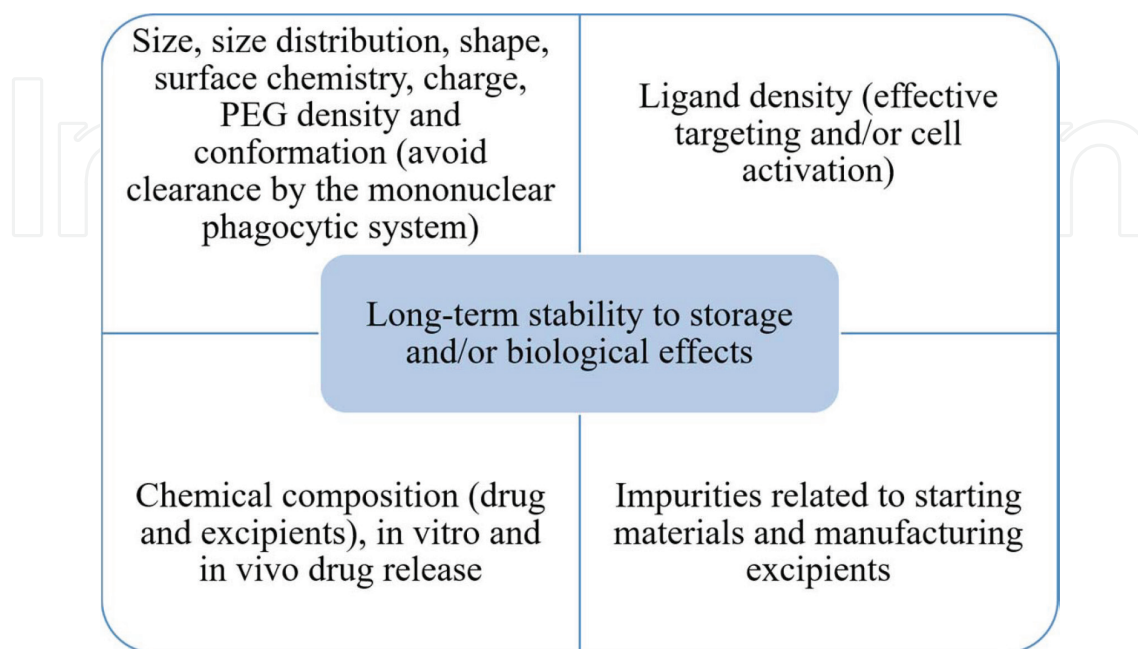


Figure 3. The main properties of nanomedicines that influence both long-term storage stability and biological interactions.

Another important aspect worthy of note is that only a small number of scientists, during the planning of an experimental work, taken into account the regulatory aspects, which instead must be considered before the development of a project. The biggest threshold is the scalable, controlled, and reproducible manufacturing of nanomedicines under good manufacturing practice (GMP) and, in many cases, under sterile conditions.

Why it is important to control physico-chemical properties of nanocarriers? Safety and efficacy of nanomedicines upon biological substrates are strictly correlated with these properties (**Figure 3**).

For this reason, there is a growing interest in quality-by-design approach (QbD): it is in fact very important to establish which critical quality attributes (CQA) govern the quality, safety, and efficacy of nanomedicines, also considering their specific route of administration or application. Applying this or a similar approach in preclinical studies helps to obtain reproducible drug batches [18–20].

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