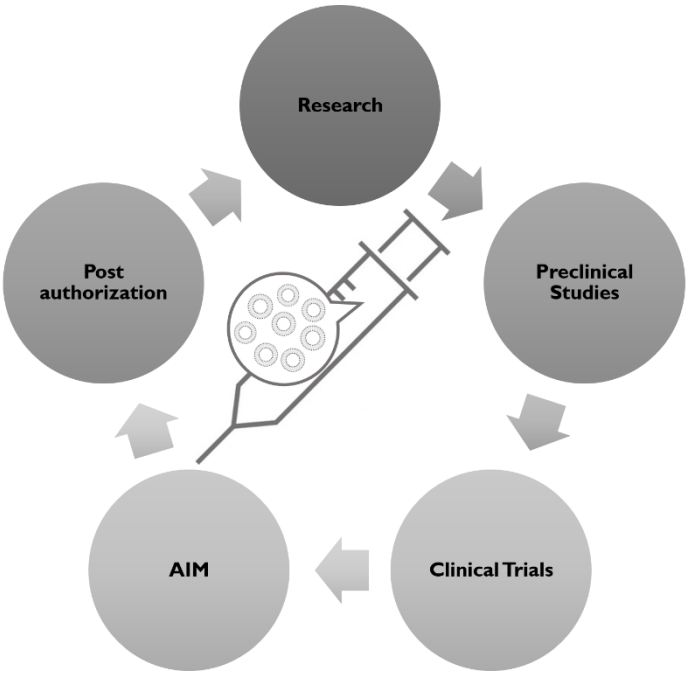

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Dissertação de Mestrado em Tecnologias do Medicamento, orientada pela Professora Doutora Eliana Maria Barbosa Souto e apresentada à Faculdade de Farmácia da Universidade de Coimbra.

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EPIGRAPH

"I am the best
specially when I'm
flying over the cuckoo's nest
but I would still rather
not to confess
all this pressure I'm holding
inside my chest
but I'm trying
hard enough to suppress
all these colliding particles of air
while digging my own lair
if only
I got over
all this resentment,
urge myself in being more independent
but I just can't deal with the commitment
of letting go of my sentiment."

Diana C Ferreira

AGRADECIMENTOS

Ao terminar esta etapa não posso deixar de destacar e agradecer a todos aqueles que me acompanharam e ajudaram durante a realização deste trabalho.

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ABSTRACT

The greatest challenges encountered in the development of nanopharmaceutics are related to their long-term stability, large-scale production of suitable formulations to be used in clinical trials, as well as the lack of harmonized regulation.

This review discusses the development of Good Manufacturing Practices (GMP) concerning nanopharmaceutics product lines from pilot to large scale development, in order to be capable of supplying clinical trials. The development of GMP for nanopharmaceutics could integrate a set of scientific principles and a strategy for regulatory implementation, since there is a huge gap in the regulation of these products.

To be clinically compliant, nanopharmaceutics must be fully characterized - physicochemically, biologically and toxicologically - since they can change the properties of a drug, such as its bioavailability and pharmacokinetics, due to the reduced particle size.

Also, the research and development plan for these products is extremely more complex than for other pharmaceutical products, from their basic research until there is a product with a marketing authorization.

Clinical trials are an essential part of the development plan for nanopharmaceutics. Quality, safety and efficacy are mostly established in these phases.

Nanopharmaceutics allow the improvement of drug bioavailability, reducing the dose frequency of the medicines and leading to a higher therapeutic compliance. So, creating a regulated system for the development of new and improved nanopharmaceutics following GMP, is an urgent measure.

Keywords: Nanopharmaceutics, Good Manufacturing Practices, Clinical Trials, Scale up, Regulation

RESUMO

Os maiores desafios encontrados durante o desenvolvimento de nanofarmacêuticos estão relacionados com a sua estabilidade a longo prazo e a produção em larga escala de formulações adequadas para o uso em ensaios clínicos. Assim como a falta de harmonização no que diz respeito à sua regulação.

Nesta revisão são abordadas as boas práticas de fabrico (BPF) que podem ser aplicadas à produção de nanofarmacêuticos, desde a escala piloto até à escala industrial, de modo a que possam ser usados em ensaios clínicos.

O desenvolvimento de BPF específicas para nanofarmacêuticos pode ser integrado num conjunto de princípios científicos e numa estratégia de implementação de regulação nesta área, uma vez que existe uma enorme lacuna no que concerne a regulação destes produtos farmacêuticos.

Para serem clinicamente compatíveis, os nanofarmacêuticos devem ser totalmente caracterizados – biológica, físico-química e toxicologicamente – uma vez que, devido ao reduzido tamanho das partículas, algumas propriedades do fármaco, tais como a sua biodisponibilidade e farmacocinética, podem ser alteradas.

A investigação e desenvolvimento destes produtos farmacêuticos é muito mais complexa do que qualquer outro produto, desde a sua investigação básica até à obtenção de uma autorização de introdução no mercado.

Ensaios clínicos são parte integral do plano de desenvolvimento de nanofarmacêuticos, sendo dados de qualidade, eficácia e segurança são obtidos maioritariamente nestes estudos.

O uso de nanofarmacêuticos permite melhorar a biodisponibilidade do fármaco, levando à redução da frequência das tomas de medicamentos, conduzindo a uma melhor adesão a terapêutica. Assim, torna-se fundamental e urgente, a criação de um sistema de regulação para novos nanofarmacêuticos seguindo BPF.

Palavras-Chave: Nanofarmacêuticos, Boas Práticas de Fabrico, Ensaios Clínicos, Transposição de escala, Regulação

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LIST OF ABBREVIATIONS

API	Active Pharmaceutical Ingredient
CEIC	Comissão de Ética para a Investigação Clínica
CMO	Contract Manufacturing Organization
CNPD	Comissão Nacional de Proteção de Dados
CRO	Contract Research Organization
CTD	Common Technical Document
CV	Curriculum Vitae
EMA	European Medicines Agency
ETPN	European Technology Platform Nanomedicine
EU	European Union
FDA	United States Food and Drug Administration
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
IB	Investigator's Brochure
ICH	International Council on Harmonization
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
Infarmed	Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.
IRB/IEC	Institutional Review Board/Independent Ethics Committee
LIC	Lei da Investigação Clínica
MedDRA	Medical Dictionary for Regulatory Activities
NIMP	Non-Investigational Medicinal Product
PoC	Proof of Concept
QbD	Quality by Design
R&D	Research and Development
RNCE	Rede Nacional de Comissões de Ética
RNEC	Registo Nacional de Estudos Clínicos
SCF	Supercritical Fluid

SLN	Solid Lipid Nanoparticles
SME	Subject-Matter Expert
SmPC	Summary of drug product
TRL	Technology Readiness Level

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1. Introduction

A large number of new technologies are being developed to respond to today's medical needs and so nanotechnology has the potential to offer solutions on imaging, diagnosis and therapy for some of these pharmaceutical gaps (1).

In the past two decades, several therapeutics based on nanotechnology have been successfully introduced for the treatment of cancer, neurodegenerative diseases, pain and infectious diseases.

Nanopharmaceutics has at least one dimension in the nanoscale (from 1 to 100 nanometers) and its properties depend on those dimensions (2).

Nanopharmaceutics also includes nano-enable devices. However, this review will be focusing only on nanopharmaceutics that refer to innovation regarding drug delivery and medicines based on the use of nanoparticles of the active ingredient (API) (3).

These nanopharmaceutics can target the delivery of drugs more precisely, improving their solubility and extending their half-life. They also improve the APIs therapeutic index, since they can cross many biological barriers (4).

Several types of nanopharmaceutics are already approved for human use (liposomes, polymeric micelles, PEGylated proteins, albumin-based formulations). These are the "first generation" nanopharmaceutics, where physico-chemical properties of the API are optimized and their pharmacokinetics and pharmacodynamics improved (5).

From the basic research of these nanopharmaceutics until their commercialization, an extremely detailed process of regulation and monitoring is mandatory. A full characterization must be provided to establish their quality, safety, efficacy, which also requires a complete series of clinical trials (6).

The drug development process is long and full of challenges, only a few percentage of new products achieve a marketing authorization. With nanopharmaceutics, besides the usual drug development process, new concerns, related to their scale up and possible nanotoxicity, create an even more complex development process (Figure 1). It is crucial to guarantee the pharmaceutical quality by adapting good manufacturing practices (GMPs) and overcome the regulation gaps regarding nanopharmaceutics (7).

The Competent Authorities need to be prepared to make regulations more robust and be ready for this new regulation paradigm, having in mind the benefit/risk of these new technologies.

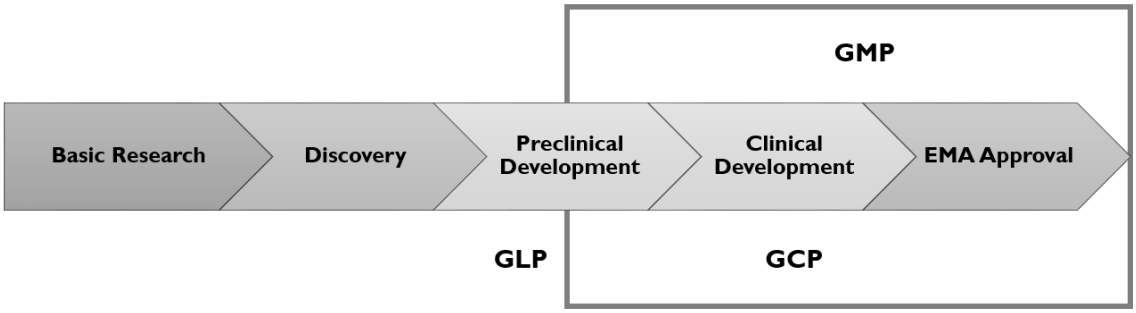

Figure 1 - The process of drug discovery and development.

The EU (European Union) has a very dynamic and multilevel regulation, since it includes both national and European legislation concerning medicines (8). However, it may not be enough to respond to the need of creating a system for nanopharmaceutics, due to the lack of harmonization of their large-scale production. This is still a project under construction, but when nanopharmaceutics are considered as advanced therapy medicinal products, the marketing authorization is only granted following a centralized procedure (3).

Many initiatives were created in order to advice and accelerate the process of research and development of nanomedicines, such as the ETPN (European Technology Platform Nanomedicine), a platform where industry, academia and the European Commission interact together to address the application of nanotechnology and its implications in healthcare (9).

For the Horizon 2020, ETPN proposed many actions, two of them are the following (10):

- "GMP manufacturing pilot lines for clinical batches, which will both assist academic groups and especially SMEs (Subject-Matter Expert) to develop their nanomedical materials for validation in clinical trials, before transfer to CMOs (Contract Manufacturing Organizations);
- A strong link with existing European clinical networks or organizations to help transfer and provide efficient early clinical trials in nanomedicine."

Through this investment, the R&D process of nanopharmaceutics will became much easier and harmonized and their quality/safety well characterized (11).

Another alternative, is the implementation of the TRL (Technology Readiness Level) to the R&D process. According to Nasa (12), TRL can be described as a system that can be used to

measure the level of development/maturity of a specific technology, providing information regarding. There are nine different levels for this evaluation, TRL 1 is the lowest and TRL 9 is the highest (Table 1).

Table 1 Technology Readiness Levels Scale.

TRL 1	System ready for full scale deployment
TRL 2	System incorporated in commercial design
TRL 3	Integrated pilot system demonstrated
TRL 4	Prototype system verified
TRL 5	Laboratory testing of integrated system
TRL 6	Laboratory testing of prototype component or process
TRL 7	Critical function: proof of concept established
TRL 8	Technology concept and/or application formulated
TRL 9	Basic principles observed and reported

This system can be used in technology before the beginning of the development of the full system and further adapted to the specific needs of an organization (Table 2), for example, it can be applied to the development of nanopharmaceutics, from the basic research until it's ready for marketing authorization.

Table 2 Adapted definition of the TRL scale used by the US department of Health and Humans Services (13).

TRL 1	**Review Scientific knowledge base**
TRL 2	Development of hypotheses and experimental designs
TRL 3	Target Identification and characterization of preliminary candidate(s)
TRL 4	Candidate optimization and non-GLP in vivo demonstration of activity and efficacy
TRL 5	Advanced characterization of candidate and initiation of GMP process development
TRL 6	GMP Pilot Lot Production, IND Submission and Phase 1 Clinical Trials
TRL 7	Scale-up, Initiation of GMP Process Validation and Phase 2 Clinical Trials
TRL 8	GMP Validation Consistency Lot Manufacturing, Efficacy Studies, Phase 3 Clinical Trials and FDA Approval
TRL 9	Post-Licensure and Post-Approval Activities

TRL 1 and 2 correspond to the basic research, and there is very little experimental work. TRL 3 is achieve when some strategic measures are defined and analytical and laboratory studies are necessary, a proof-of-concept model is also constructed (Figure 2).

Following these steps, the technology under evaluation in this case, goes to TRL4. Some in-vivo demonstration of efficacy is reproduced, to optimize the process.

From TRL4 to TRL5, the nanopharmaceutics pilot production requires GMP, because there's a strong possibility to be developed for clinical trials. Testing must be more precise and rigorous, under an environment close to the reality and where it's going to be used.

In the TRL6 should be a batch GMP-compliant able to be provided for First in Human/Phase 1 Clinical Trials. Pharmacokinetics and pharmacodynamics are evaluated, which means, this is a functional prototype and there is a second proof-of-concept. Once a drug enters a clinical trial phase, it cannot be improved, it is practically definitive.

For TRL7, the batch size requires a GMP scale-up, in order to supply a Phase 2 Clinical Trial and safety is also evaluated.

Finally, TRL8 and TRL9 are the last phases of this evaluation. TRL8 corresponds to Phase 3 Clinical Trials and market authorization, TRL9 corresponds to post approval activities. There has been an attempt to accelerate the development of nanopharmaceutics.

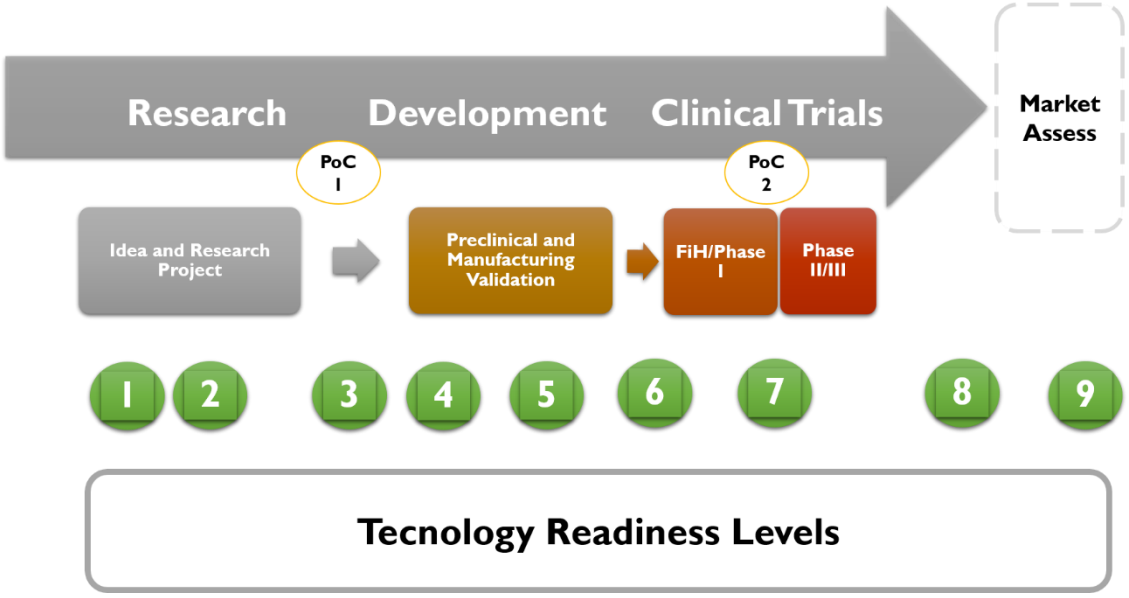

Figure 2 - Adapted from Chang et al. (2015): Technology readiness levels applied to R&D of a nanomedicine (6).

Nevertheless, in vitro and in vivo characterization must be completed and clinical trials are mandatory. Different stakeholders are working on a translational way to make the R&D of nanopharmaceutics more efficient and fast (14).

2. Clinical Trials

The development of a new drug follows a very complex investigation process and it can be divided in research and development. This process can take at least 5 to 10 years with a major financial investment.

The research comprehends the study of a disease, identification of new therapeutic targets and the choice of some suitable APIs capable of acting in those targets to change the progression of a disease.

In the pre-clinical phase, the new API is tested on animals to extrapolate data for the next phase and it can last from 3 to 6 years.

About 5 0000 to 10 000 molecules enter the first phase of research, however only 250 get to the pre-clinical phase and around 5 can get to the clinical development.

The clinical phase can be extended for 6-7 years being the most crucial part of the drug development.

A clinical trial can be defined as a prospective study comparing the effects and value of intervention against a control in human beings. Note that a clinical trial is prospective, rather than retrospective. Study participants must be followed forward in time (15).

It has the purpose of evaluate the most appropriate treatment for patients with a specific condition. These trials are designed to evaluate the efficacy and safety of some treatments or to investigate some adverse reactions to the medicines under development and/or to study its absorption, distribution, metabolism and excretion. Also, it can be determined the efficacy and safety of the product and compared to the standard medication (16).

Usually, a clinical trial with medicines can be classified in four phases: I, II, III, IV, depending on, for example, the stage of the drug development and post-marketing authorization. A phase of a clinical trial cannot be striated defined (17). However, the following definitions are based on their objectives and clinical development:

Phase I (Clinical Pharmacology and Toxicity): first evaluation of the drug safety, pharmacokinetics and pharmacodynamics on human volunteers, usually healthy. To determine the drug dosage the accepted drug dosage, without any major side effect. This information can be obtained by dose escalation. In a phase I clinical trial, the number of subjects varies from 10 to 100 for about 1,5 years.

Phase II (Initial Clinical Investigation for Clinical Effect): studies with a main objective of testing the safety and efficacy of a treatment, the subjects in these studies have a pathology and the treatment is specific for that pathology. The number of patients varies from 100 to 200 for 2

years, the dose/response can also be adjusted. It has a comparative design (placebo-controlled) (18).

Phase III (Full-Scale Evaluation of Treatment): the treatment is usually given to larger groups of patients and it has the purpose of comparing the drug under investigation to the standard treatment for a longer period (about 3 years). These trials are more extensive and more rigorous, since they should confirm the effectiveness of the treatment, monitoring side effects and drug interactions is also required. In these trials, the more common study design is randomized, double-blind. The conditions of these studies must be the closest the real use.

Phase IV (Postmarketing Surveillance): Usually are studies developed after the marketing approval. The SmPC (summary of drug product) is the base of the investigation and the purpose is to keep monitoring side effects.

Figure 3 shows a summary of the phases of clinical trials and their objectives. It also shows that it is very difficult to defined only one objective and can "overlap" with more than a phase (15). For example, a pharmacology study is generally done in a phase I clinical trial, but it can also be analyzed in other phases. Or the confirmation of the effectiveness of a treatment, is usually done in a phase II clinical trial, but in phase II or IV can also be demonstrate.

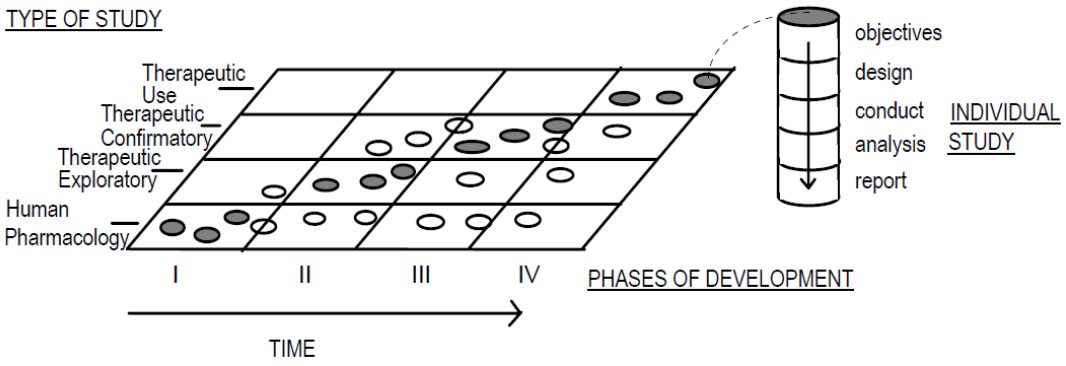

Figure 3 Correlation Between Development Phases and Types of Study (19).

2.1. European and Portuguese Legislation on Clinical Trials

2.1.1. European Legislation

The European legislation concerning clinical trials is extremely rigorous and has evolved, especially during the last decade. Directive 2001/20/EC of the European Parliament was one of the first attempts to work on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use (20).

The International Council on Harmonization guidelines concerning Good Clinical Practices (ICH E6) also include the European legislation through the directives 2001/20/EC and 2005/28/EC.

Directive 2003/94/EC refers to the good manufacturing practices of the investigational medicinal product (IMP) and the annex 13 only describes IMPs.

EudraLex is a collection of the rules and regulations governing medicinal products in the European Union. The body of European Union legislation in the pharmaceutical sector is compiled in Volume 1 and 5 of the publication "The rules governing medicinal products in the European Union" and the basic legislation is supported by a series of guidelines that are also published in the following volumes of "The rules governing medicinal products in the European Union". The legislation concerning clinical trials is all part of the Volume 10 (21).

2.1.1.1. Directive 2001/20/EC Of the European Parliament And Of The Council of 4 April 2001

Directive 2001/20/EC on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use had, as main objectives, the application of the GCP to all clinical trials, the protection of the subjects in a clinical trial.

It also translated a much more harmonized system in the European territory, deadlines were implemented and a data base was developed (EudraCT). This new paradigm was able to instigate some competition related to the pharmaceutic industry, because there's more transparency in the R&D (20).

2.1.1.2. Commission Directive 2003/94/EC of 8 October 2003

This directive includes the principles and guidelines of good manufacturing practice in respect of medicinal products for human use and IMPs for human use. The production of the IMP must follow the GMP of the Volume IV from Eudralex.

A conformity with GMP is obligated, the manufacturers outside the European Community must have equivalent certified good manufacturing standards that can guarantee the quality of the product, as well as keeping up with the scientific progress so it can review the manufacturing process.

Since these medicinal products are used in clinical trials, there's an amplified risk for the subjects when compared to other commercialized products, being a reason to have more rigorous rules concerning the development of IMPs. The GMP can assure the quality of the IMP and guarantee an adequate efficacy (22).

2.1.1.3. EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use - Annex 13

Annex 13 from the Volume IV of the Eudralex is only related to IMP, that can be defined, according to the annex 13 as "a pharmaceutical form of an active substance or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the authorized form, or when used for an unauthorized indication, or when used to gain further information about the authorized form".

The production of IMPs demands a stricter regulation, since the risk for the subjects is elevated when comparing to others marketed products due to the variety of clinical trials, demanding an adapted product that can supply all the needs of the study (packaging designs, labeling, etc).

In fact, some clinical trial designs can also influence the production of an IMP. The quality system adopted must take into consideration the GMP guidelines. The premises and equipment must be prepared for the specifications of the IMP, since, for example, its toxicity may not be fully described.

Regarding the documentation needed, specification and instructions of the whole process should be very clear and updated when necessary.

The quality system implemented must guarantee that each batch achieved its specification.

During the production, critical parameters should be well identified and justified. Keeping records is also important, in order to prepare the documents for the submission for a marketing authorization.

The packaging of IMPs is a crucial step, because depending on the clinical trial design NIMPs (Non-Investigational Medicinal Product), other medication that can be used in a clinical trial, like rescue medication. Also, is there is a placebo, it has the same aspect as the IMP, but are different products so, the packaging must be performed by trained personnel to prevent any mix up.

Just like the packing, the labelling requires a special care and should be compliant with the conditions of Directive 2003/94/EC. Specific information is required on labels, such as:

- name, address and telephone number of the sponsor, contract research organization (CRO) or investigator;
- pharmaceutical dosage form, route of administration, quantity of dosage units, and in the case of open trials, the name/identifier and strength/potency;
- the batch and/or code number to identify the contents and packaging operation;
- a trial reference code;
- the trial subject identification number/treatment number and where relevant, the visit number;
- the name of the investigator;
- "For clinical trial use only";
- period of use;
- "keep out of reach of children".

Release of the IMPs is another critical step and it cannot happen before the Qualified Person certified all the requirements and the assessment of each batch is based on some key elements, as the batch records, the production conditions and, of course, GMP compliance.

For the development of any batch with the purpose of be used in a clinical trial needs to meet all the specifications mentioned on Annex 13, its absence has to be completed justified (23).

2.1.1.3.1. IMPD

The Investigational Medicinal Product Dossier (IMPD) is a mandatory document for a submission of a clinical trial. Its format should be similar to CTD (Common Technical Document)

(Module 3 – Quality), referring the drug substance and the drug product specifications (Annex 1) (24).

The IMPD can be simplified in some circumstances (25), for example, the preclinical and clinical parts can be referenced to their location on the Investigator's Brochure (IB). Making a reference to a SmPC if the IMP as marketing authorization.

2.1.1.4. Commission Directive 2005/28/EC of 8 April 2005

This Directive refers to the principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorization of the manufacturing or importation of such products. Also refers to the documentation related to clinical trials and its inspection.

The member-states are advised to establish Ethics Committees, in order to assure the protection and safety of the subjects on the clinical trials.

Some other specifications are given for the content of the IB, manufacturing or import authorization, the trial master file, archiving and inspections procedures (26).

2.1.1.5. International Conference on Harmonization - Good Clinical Practice E6

The International Council for Harmonization was established to eliminated the major differences concerning drug development in the USA, European Union and Japan. Since the very beginning, the process as evolved. The ICH englobes guidelines for Safety, Quality and Efficacy topics and also other multidisciplinary topics, including MedDRA (Medical Dictionary for Regulatory Activities) and the CTD (Common Technical Document) (27).

In 1996, the ICH institute the guideline E6 on Good Clinical Practices (GCP), that became the standard for the conduct of clinical trials and they must be conducted in accordance with the ethical principles from the Declaration of Helsinki.

The document lists all the entities involved in a clinical trial, from the sponsor to the ethics committee, and their responsibilities in this process. It also describes the format of the documents needed for a clinical trial (IB, study protocol). The GCP didn't have a legal status, until the directive 2001/20/EC stated the GCP must be part of the national legislation for the conduct of a clinical trial.

The Institutional Review Board/Independent Ethics Committee (IRB/IEC) is responsible for the protection of the wellbeing of the trials' participants, the informed consent is written and revised by the IRB/IEC. The subjects in the study have to sign an informed consent with the clarification of the purpose of the trial, the procedures involved in the process, all of the subjects' responsibilities and, of course, their rights. The risks or possible risks involved in their participation must be fully explained, as well as the benefit from their participation in the study.

Documents like the study protocol and its amendments, IB, are also reviewed by the IRC/IEC, so they can be approved/refused or can require modifications.

The investigator is a qualified professional, trained in GCP and responsible for the conduct of the trial. Their CV (curriculum vitae) should be available for the sponsor, IRB/IEC and regulatory authority. The investigator has to know how to use the IMP and must follow the IB.

The Sponsor main focus is to ensure the quality of the results obtain in the clinical trial and the subjects protection. In order to achieve that purpose, a quality management system should be implemented based on the risk (identification, evaluation, control, communication, review and reporting). A sponsor may transfer any or all the sponsor's duties and/or functions to a CRO (Contract Research Organization), however the responsibility to ensure the quality of the data is always a sponsor's responsibility.

Regarding a clinical trial protocol, some general information as the protocol title, identification number, date, the investigators name is included. This document describes the whole purpose of the trial. Having information about the trial objectives and purpose, design, inclusion and exclusion criteria, treatment of subjects, assessment of efficacy/safety and also statistics.

IB englobes the clinical and nonclinical data related to the IMP. This document helps the investigators understand some specifications of the protocol, as the dose frequency/interval and methods of administration.

GCP has also other essential documentation for all the phases of a study (before, during, after) that complements the information provided by the study protocol and IB, especially in what concerns the administrative situation (28).

2.1.1.6. Regulation (EU) No 536/2014 OF THE European Parliament And Of the Council of 16 April 2014

Regulation (EU) No 536/2014 on clinical trials on medicinal products for human use repeals the Directive 2001/20/EC.

According to the European Commission, the number of clinical trials dropped after the directive 2001/20/EC, time and human resources increased the investment made by sponsors for clinical trials.

The legal form of a Regulation has more advantages than a directive, creating a more stable environment for sponsors/investigators, since the divergences of approach between different Member States will be minimum, there are no exceptions or national particularities, which make its application equal.

Citing the European Commission website: "The main characteristics of the new Regulation are:

- A streamlined application procedure via a single-entry point, the EU portal;
- A single set of documents to be prepared and submitted for the application defined in Annex I of the Regulation;
- A harmonized procedure for the assessment of applications for clinical trials, which is divided in two parts. Part I is jointly assessed by all Member States concerned. Part II is assessed by each Member State concerned separately;
- Strictly defined deadlines for the assessment of clinical trial application;
- The involvement of the ethics committees in the assessment procedure in accordance with the national law of the Member state concerned but within the overall timelines defined by the Regulation;
- Extension of the tacit agreement principle to the whole authorization process which, without compromising safety, will give sponsors, in particular SMEs and academics, increased legal certainty;
- Simplified reporting procedures which will spare sponsors from submitting broadly identical information separately to various bodies and different Member States.
- Increased transparency regarding clinical trials and their outcomes.
- Union controls in Member states and third countries to ensure that clinical trials rules are being properly supervised and enforced" (29).

Clinical trials conducted outside the EU, but referred to in a clinical trial application within the EU, will have to comply with regulatory requirements that are at least equivalent to those applicable in the EU.

Regulation (EU) No 536/2014 was approved on 16 April 2016, but it will only be fully applied when the data base and EU Portal is completed (30).

2.1.2. National Legislation

2.1.2.1. Law No 21/2014

Law No 21/2014 (LIC – Lei da Investigação Clínica) regulates clinical investigation in Portugal, including not only clinical trials with medicinal products for human use but also medical devices and cosmetics.

With this law, the development of a National portal for the submission and management of clinical trials is mandatory (RNEC: Registo Nacional de Estudos Clínicos). Following Law No 21/2014, RNEC allows an electronic submission of all documents necessary for an approval/amendment of a clinical trial, but also works as a data base, enabling the research anonymous data from studies.

It was also created a National networking of ethics committee (RNCE: Rede Nacional das Comissões de Ética).

Aspects like these, facilitate the whole process of submission and management of a clinical trial. Deadlines are smaller, allowing a quick approval from the competent authority (Infarmed, I.P.) and also from the Ethics Committee (CEIC) (31).

2.1.2.2. First Amendment to LIC: Law No 73/2015

First amendment to LIC clarifies the aspects related to the information available to auditors/inspectors about the subjects in a study.

During a clinical trial, quality control has to be assured, in order to protect the well-being of the subjects and following a study protocol is necessary to achieve solid conclusions. The mechanisms used to perform an inspection from the competent authorities verify all the activities

and documents related to the study under evaluation and the compliance to the established legislation (32).

During a conduct of a clinical trial, the need for audits is clear and is part of GCP applied to all clinical trials in the European Union. Since an audit assumes an access to patients' clinical data, there should an official informed consent signed by the subject. After LIC came into force, (CNPD), Comissão Nacional de Proteção de Dados, issued numerous reports to stop auditors from having access to patients' clinical information, based on their interpretation of LIC, because even transposing GCP that mentioned the role of auditions in a clinical trial and the access to the information, LIC was not so clear. In order to end this discussion, otherwise auditing clinical trials would been impossible, this amendment emerge and clarifies the role of an auditor/auditions during the course of a clinical trial.

2.1.2.3. Other Applicable Law Concerning Clinical Trials in Portugal

In Portugal, the conduct of clinical trials with medicines for human use is legislated by Law No 21/2014,16 April (LIC) amended by law No 73/2015.

All further national and European regulation, included in Volume X from Eudralex is applicable (33).

In the interest of creating a more competitive and beneficial environment for clinical investigation, the Regulation (EU) No 536/2014 was approved on 16 April 2014 for clinical trial using medicines for human use. European laws became more harmonized in what concerns clinical trials, since the Regulation is applied to all member states and becoming a more transparent process, from the authorization to the results, through their publication on the data base.

2.2. Clinical Trials with Nanomedicines

A variety of nanoparticle-based drug delivery systems have been developed for cancer treatment, cardiovascular diseases and other pathologies. These have different features and functionalities and even some differences in sizes (in the nanometer-scale) (34).

During the last decades, nanomedicines earned their place in the pharmaceutical market, the number of nanoparticle-based therapeutic products have increased (35). Which means, the development of nanomedicines is part now a reality.

There are several projects financed be the European Commission concerning nanotechnology applied to the health sector. According to Bremer-Hoffman, S. et al. (2015),

after analyzing several platforms, concluded that between 1996 and 2015 in the European Union there were (1):

- 131 Drug development projects related to nanomedicines;
- 69 Clinical trials with nanomedicines;
- 30 nanomedicines authorized.

From 2015 to May 2017, the results from EU Clinical Trials Register (36), presented 18 Clinical trials with nanopharmaceutics (nanoemulsions, nanosuspensions, nanobodies and liposomes) (Table 3).

Through some research of clinical trials data bases and literature, it is evident that are nanomedicines in all phases of the R&D, even in clinical trials or already holding a marketing authorization (37). Their therapeutic use is wide, from cancer treatment to autoimmune diseases and their formulation is also varied, using nanoparticles, liposomes polymeric micelles.

Since nanomedicines began to be widely use and their advantages evident, a need for a more accurate regulation is eminent. Sharing information and creating a harmonized approach for nanopharmaceutics is the first step towards the development of good manufacturing practices, without creating barriers to this kind of innovation.

Table 3 - EU clinical Trials Register results.

Nanopharmaceuticals	Number of Clinical Trials (from 01/01/2015 to 03/05/2017)
Nanoelmulsion	1
Nanosuspension	1
Nanobody	5
Nanoparticle	5
Liposome	6

3. Good Manufacturing Practices (GMPs) applied to nanomedicines

Nanomaterials have different and unique properties related to their size and so their regulation must be adapted, because it cannot follow the same rules as other medicines or health products. Those characteristics require specific legal standards and they must be flexible enough to include all those specifications that come with different nanomaterials.

Appropriate GMP concepts should be applied in the production of medicines for use in clinical trials with a suitable mechanism of approval of each batch (38).

For example, FDA (Food and Drug Administration) wants to enable the responsible development of products with new and beneficial properties, such as nanopharmaceutics. To achieve that, it will provide the necessary tools, to facilitate a pathway for the development of products like nanomedicines (39).

In 2013, FDA started the development of a plan concerning nanotechnology: Nanotechnology Regulatory Science Research Plan, to implement and coordinate regulatory issues on nanomaterials or any nanotechnology product. The main goal of the plan is to compensate the gap, in the US related to nanomedicines.

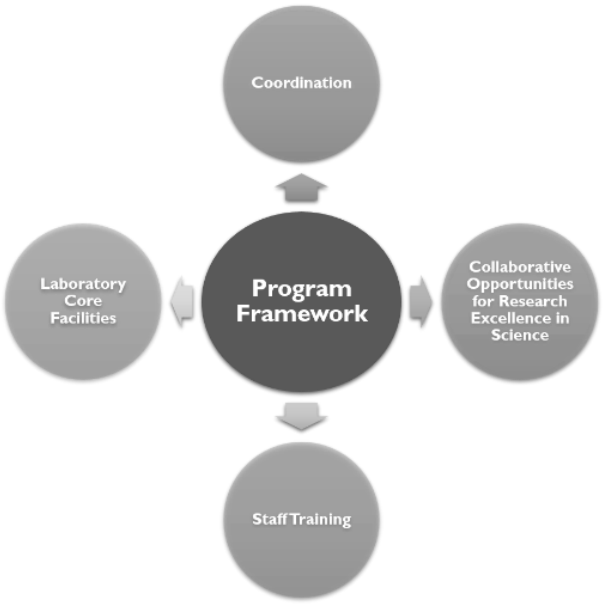

Figure 4 – Adapted from FDA Nanotechnology Regulatory Science Program (39).

The plan has to provide different approaches to assure quality, safety and efficacy of the nanomaterials. The Program comprehends four areas (Figure 4):

1. Staff Training and Professional Development;
2. Laboratory Core Facilities;
3. Collaborative Opportunities for Research Excellence in Science (CORES) Program;
4. FDA Coordination.

Regarding nanomedicines marketing authorization, EMA (European Medicines Agency) has reflection papers to help developers prepare the documentation for submission and can also ask for scientific advice (40). These reflection papers are very specific and should be followed using, simultaneously with other guidelines mentioned in the documents.

3.1. Data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product

This guideline helps to create valid quality, non-clinical and pharmacokinetic clinical comparative data, in order to support a marketing authorization document for an intravenous iron-based nano-colloidal product that claims to be similar to the one innovative product already on the market.

To treat iron deficiency, Iron-based products are used and they consist of a polynuclear iron core, generally present in the iron (III)-oxyhydroxide form, stabilized by a complex carbohydrate coating which leads to nano-sized colloidal aggregates (41).

Through the analysis of the current experience, the characterization of such nano-sized colloidal preparations based only on quality methods isn't enough, to assume that the products are equivalents. Since the pharmacokinetic studies are not sufficient to assure the toxicological and pharmacological effects of the products. This guideline reflects the need for non-clinical data besides human clinical pharmacokinetics data (42).

Guideline's index:

- Quality:
    - Quality characterization of the test product;
    - Establishing pharmaceutical comparability between test and reference product;
- Non-Clinical:
    - Methods of analysis;
    - Bio-distribution studies;
- Clinical:
    - Pharmacokinetics studies;
    - Efficacy and Safety studies;
    - Pharmacovigilance / Risk Management Plan.

3.2. Data requirements for intravenous liposomal products developed with reference to an innovator liposomal product

This document can help to create relevant quality, non-clinical and clinical data to support a marketing authorization for biosimilar intravenous liposomal products. The encapsulation or binding of an API to liposomes allows a better control of the drug delivery/targeting. However, some products using liposomes had pharmacokinetics problems, such as premature drug release.

The complete characterization of the stability, pharmacokinetics of a new liposomal product is critical to establish safe and effective use. Differences between the applicant's product and innovator product with regard to manufacturing process steps and formulation may substantially modify efficacy/safety due to changes in specific liposome-cell interactions and liposome distribution characteristics which are not detectable by conventional bioequivalence testing alone.

To demonstrate its safety and efficacy, the new liposomal product, must be tested in different ways, not only through bioequivalence studies. A small change to a step of the manufacturing process can be extremely relevant.

This document is simply a guide for the complete development of a liposomal product with reference to an innovator discusses the principles for assessing liposomal products developed with reference to an innovator one (43).

Guideline's index:

- Pharmaceutical Quality:
    - Quality characterization;
    - Establishing pharmaceutical comparability;
    - Pharmaceutical development of the applicant's product;
- Non-Clinical and Clinical Requirements:
    - General Aspects;
    - Methods of Analysis;
    - Non-Clinical Studies;
    - Clinical Studies.

3.3. Development of block-copolymer-micelle medicinal products

This guideline helps the pharmaceutical development of block-copolymer-micelle medicinal products and also some guidance for non-clinical studies and first stages of clinical trials.

Block copolymer micelles are formed by the self-assembly of either amphiphilic or oppositely charged copolymers in aqueous medium (44) and the API is incorporated into the inner core of the block copolymer micelle product (45).

Guideline's index:

- Chemistry, manufacturing and controls:
    - Pharmaceutical Quality;
    - Description and composition;
    - Quality characterization;
    - Manufacturing process and process control;
    - Product specification;
    - Stability;
    - Changes in manufacturing during development;
- Non-clinical studies:
    - General considerations;
    - Non-clinical pharmacokinetics;
    - Non-clinical pharmacodynamics;
    - Safety pharmacology;
    - Toxicology;
- Considerations for first-in-human studies.

3.4. Surface coatings: general issues for consideration regarding parenteral administration of coated nanomedicine products

This document emphasizes the important requirements for the development of coated nanomedicines products for parenteral administration.

During the development of nanomedicines, the coating is part of the product, due to its advantages, such as stability improvement and prolonging plasma circulation. So, it is clear that that presence of a coating influences the properties of a nanomedicine concerning its efficacy and safety. Because of this, the product characterization must be very explicit and complete (46).

Guideline's index:

- General considerations;
- Product characterization.

4. Production Scales

The scale-up of nanomedicines from pre-clinical laboratory scale to the quantity and quality needed for clinical trials has been a challenge, in a way that only a few nanomedicines achieve the market access, such as, Abraxane (paclitaxel/albumin nanoparticles, Abraxis Bioscience) and Doxil (doxorubicin pegylated liposomes, Janssen) (47).

From lab to large-scale development, many steps must be taken, because there is no direct translation from a laboratory scale to a large scale. Some check points must be done, and are intermediate steps through the hole process.

The first step is the production of a laboratory scale batch (5-40 ml). It is smaller than pilot scale batch and is manufactured for development purposes.

The pilot scale batch (500-1000 ml) is manufactured by a procedure representative and simulative of the one to be applied to a full production scale batch.

A large-scale production batch (> 1000ml) is manufactured at a production scale, using production equipment in a production facility as specified.

Scale-up has mainly been observed to affect the characteristics of nanopharmaceutics, like particle size, drug encapsulation, process residual materials, colloidal stability and surface morphology (48). These effects can be major challenges during large-scale production and they depend on the method of production used.

However, scale-up is a good reference to guarantee the quality of batches GMP compliant, some limitations only appear during this process on transferring a technology for a larger scale (49).

Depending on the nanomaterial, the production method used for the scale-up needs to be validated by the regulatory authority and should also be cost effective (50).

For a successful scale-up, some adaptations to the production method must be done to make sure the effectiveness and stability of the final product is kept and the toxicological parameters remain the same, even if are some differences from batch to batch (51). Since the batches produced are meant to be used in clinical trials, closer monitoring is required and GMP have to be followed.

5. Nanomaterials

Nanomaterials can be defined as one of the main products of nanotechnologies and contain nanoparticles, smaller than 100 nanometres in at least one dimension (2).

They represent one of the most complex and interesting systems for researchers due to their properties. In the past few decades, many studies using nanomaterials have been developed for health (diagnosis and treatment of diseases), cosmetics, electronics (52).

Even with some nanomedicines in the market, there are aspects to explore and some applications under investigation (53).

Lipids and polymers represent two organic nanomaterials widely used for nanoscale systems.

Lipids are a large group of molecules with biological properties as being components of cell membranes and energy storage (54) and amphiphilic lipids have the ability to self-assemble, creating structures like micelles and vesicles (55).

Polymers are a well-studied class of large molecules, natural and synthetic, to be used in the production of nanomaterials. They provide wide opportunity to tailor their length and hydrophilic or hydrophobic nature, and can incorporate functional groups (56).

Inorganic nanomaterials also have been used for imaging and as therapeutic agents, due to some of their specific properties. Gold, silver and iron are usually chosen as metal nanomaterials (57).

Their application is more relevant for the treatment of cancer, allowing a better tissue penetration and for cardiovascular medicine, since their delivery become more effective (58).

5.1. Production Methods

In order to produce nanomaterials, it is essential to defined some conditions, depending on the type of nanomaterial and its application.

Properties such as size, shape and crystallinity will be influenced by the conditions previously chosen (temperature, pH, concentration) (59).

There are two main principals of production: top-down and bottom-up. The first one consists on creating nanoscale structures from bigger materials to smaller ones using, for example, a milling process (60).

On the other hand, a bottom-up strategy, begins with smaller materials and make them into more complex nanomaterials.

Considering the kind of nanomaterial, lipid, polymer or metal, the production methods vary a lot, which is translated in different final products (Figure 5).

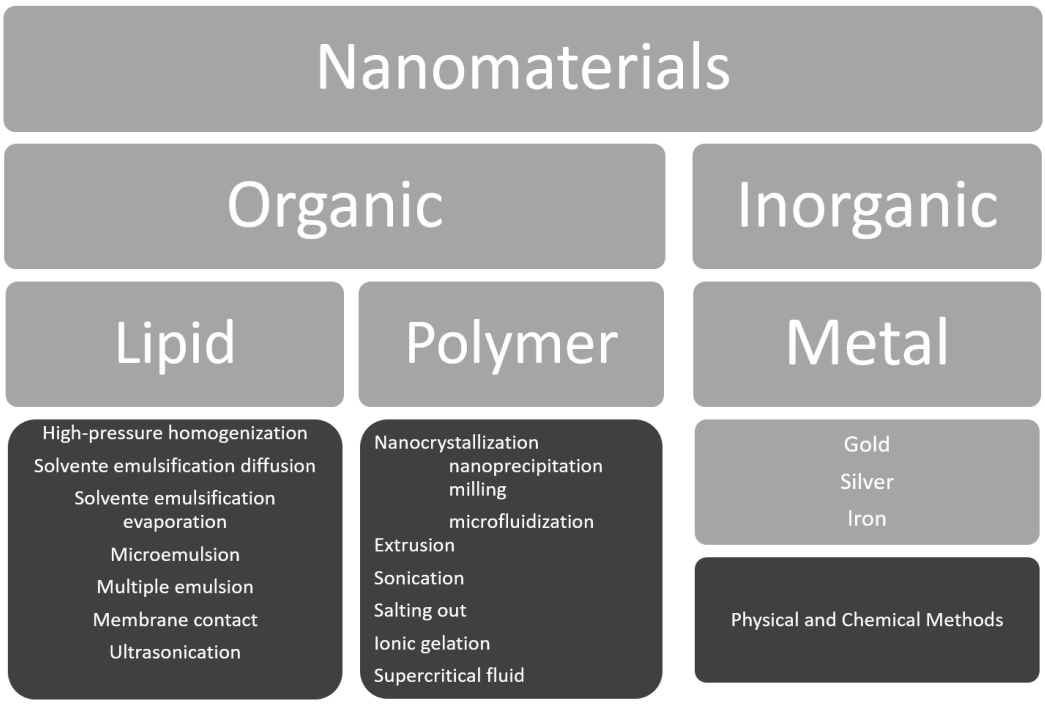

Figure 5 Production methods for different nanomaterials, adapted from (61).

5.1.1. Lipid Nanomaterials

5.1.1.1. High pressure homogenization

Lipid nanoparticles compared to polymeric nanoparticles can be produced by high pressure homogenization. This is a technique well established on the large scale and it is used in the pharmaceutical industry.

The two basic production methods for lipid nanoparticles are the hot homogenization technique and the cold homogenization technique (62).

The hot and cold homogenization techniques are described on Müller's "Solid lipid nanoparticles (SLN) for controlled drug delivery" article.

For the hot homogenization technique, the drug-containing melt is dispersed under stirring in a hot aqueous surfactant solution with identical temperature. The obtained pre-emulsion is homogenized using a piston-gap homogenizer, the produced hot oil/water nanoemulsion is cooled down to room temperature, and then the lipid recrystallizes leading to solid lipid nanoparticles.

In case of highly temperature-sensitive compounds, the cold homogenization method can be applied. This technique can also be used when formulating hydrophilic drugs because they would partition between the melted lipid and the water phase during the hot homogenization process.

For the cold homogenization technique, the drug containing lipid melt is cooled, the solid lipid ground to lipid microparticles and these lipid microparticles are dispersed in a cold surfactant solution yielding a pre-suspension. This pre-suspension is homogenized at room temperature; the cavitation forces are strong enough to break the lipid microparticles directly to solid lipid nanoparticles.

This process avoids the melting of the lipid and therefore minimizing loss of hydrophilic drugs to the water phase. Of course, the difference between the melting point of the lipid and the homogenization temperature needs to be large enough to avoid melting of the lipid in the homogenizer (63).

5.1.1.2. Solvent emulsification diffusion

Using the solvent emulsification diffusion method, nanoparticles are produced from an emulsion with a partially water-miscible solvent and the solvents used have low toxicity.

In order to produce the precipitation of the drug dissolved in the organic solvent, it is necessary to promote the diffusion of the organic solvent (64). The solvent is eliminated by evaporation or filtration, in accordance to its boiling point.

5.1.1.3. Solvent emulsification evaporation

This method consists on the evaporation of the internal phase of an emulsion by agitation. The API is dispersed or dissolved in an organic solvent. Then the organic phase is emulsified under agitation. Once the emulsion is stabilized, agitation is maintained and the solvent evaporates after diffusing through the continuous phase (65).

The solvent emulsification evaporation method has been used because of the scale-up and easy process and has low residual solvent (66).

5.1.1.4. Microemulsion

Microemulsion consists in a system of lipids, surfactant, co-surfactant and water. The drug is dissolved in an oil phase of the microemulsion and in the water phase is added the surfactant. Then the cosurfactant is added slowly with gradual stirring.

The microemulsion system can be affect by interfacial tension, viscosity, pH, refractive index, diffusion, and bioavailability (67).

Using microemulsions as a production method as some advantages like the fact that is a simple process to repeat and to scale-up and the drug is entrapped and remains stable (68).

5.1.1.5. Multiple emulsion

Multiple emulsions are a new method to incorporate hydrophilic compounds with high entrapment and more controlled diameters.

According to Kuroiwa et al. (2016): "multiple emulsions are prepared by multistep emulsification applying shear force to break down multiple droplets, consequently, some of the inner components leak, and droplet size varies during emulsification" (69).

Which means, this method can be unstable and difficult to reproduce in a large-scale environment.

5.1.1.6. Membrane Contact

According to Charcosset, C., El-Harati, A., Fessi, H. (2005), the membrane contact method uses melted lipids and presses then through the membrane pores, in order to form droplets with very small sizes. Then, inside the membrane, an aqueous phase circulates to remove the droplets away from the pore outlets.

This method as some advantages related to the control of particle size, its ability to be scaled up and it is not a difficult process to execute (70).

5.1.1.7. Ultrasonication

The use of ultrasounds can be very helpful, for example, to produce liposomes to entrap active ingredients (71).

This method can transform bigger particles into nanoparticles, through the variation of the intensity and/or frequency. The main problems concerning this technique are the possible contamination by metal (72).

5.1.2. Polymeric Nanomaterials

5.1.2.1. Nanocrystallization

Nanocrystallization is used specially for poorly water soluble APIs. The fact that crystals have an higher stability is translated into a more stable dissolution than the amorphous state (73). Besides, the dissolution rate increase by decreasing the particle size.

The small size of these crystals also enables them to circulate more freely, favoring their cellular uptake (74).

Nanocrystals can be produced using nanoprecipitation, milling and microfluidization.

5.1.2.2. Nanoprecipitation

Nanoprecipitation is a bottom-up technique where the nanoparticle is formed instantaneously in a single step using two miscible solvents, which is an advantage compared to other methods. The API and the polymer should be dissolve in a single solvent. Then, nanoprecipitation begins when the solution containing the polymer is added to the one with the other solution (non-solvent) (75).

5.1.2.3. Milling

A common top-down method to produce nanocrystals is milling, it is cost-efficient and can be used for scale-up (76).

In this process, the energy from the impact of the milling with the API results in the disintegration of particles to nanoparticles. It is used water, a stabilizer and a buffer. When the parameters of the milling process are optimized, nanoparticle size from batch to batch is practically the same conferring quality to the final product (77).

5.1.2.4. Microfluidization

Microfluidization is a high-pressure homogenization method (78) and is also called as air-jet milling or jet stream homogenization. Using a high-pressure air jet induced by the collision of two fluid streams, the particles are fragment until obtain nanoparticles (79).

5.1.2.5. Extrusion

Extrusion is one of the most common methods to produce nanoparticles. Hydrophobic drugs are transformed into nanoparticles using polycarbonate membranes (80). This method is reproducible from batch to batch.

5.1.2.6. Sonication

Sonication an important step, in order to prepare drug loaded nanoemulsion, since this procedure can increase the temperature resulting in the inactivation of APIs.

The increase of sonication time leads to the increase of the applied energy and the size of the nanoparticles obtained is smaller (81).

5.1.2.7. Salting Out

The salting out method is, sometimes, a modification of the emulsification-solvent diffusion. The drug plus the polymer used are dissolved in a solvent like acetone and later is emulsified into an aqueous gel containing the salting-out agent (electrolytes) and a colloidal stabilizer.

The obtained emulsion is diluted with an aqueous solution and enhance the diffusion of the solvent into the aqueous phase resulting in the formation of the particles. A salting out agent can influence the encapsulation efficiency of the drug, so it is a crucial step (82).

5.1.2.8. Ionic gelation

In order to prepare polymeric nanoparticles, ionic hydrophilic polymers such as chitosan and gelatin can be used. The ionic gelation method involves a mixture of two aqueous phases and the transition of material from liquid to gel due to ionic interactions at room temperature (83).

The nanoparticles formed have less than 500 nm, based on the properties of chitosan (84).

5.1.2.9. Supercritical Fluid

Supercritical fluid (SCF) can be considered a useful tool for material production in the last two decades (85).

SCF techniques have advantages related to the control of particles size and it is a clean process, since organic solvents are not used. When particles have small sizes and distribution and also smooth surface, they can be processed through determined SCF methods (86).

5.1.3. Metal Nanomaterials

5.1.3.1. Physical and Chemical Methods

To produce metallic nanoparticles, bottom-up approaches are commonly chosen, from chemical to physical methods. The general chemical method is the reduction of metal complexes in dilute solutions.

A variety of physical methods to produce metallic nanoparticles synthesis has been explored, such as supercritical fluids, pulsed laser, microwave irradiation, gamma radiations and chemical vapour deposition vapour phase synthesis of metal salts **(87).**

6. Requirements to Produce Clinical Trials Batches

The production of nanopharmaceutics batches for clinical trials requires special attention and specifications. A full description of the parameters to consider or a guidance to produce nanopharmaceutics is not a reality.

Nanopharmaceutics create some difficulty, because it is hard to have clear definitions and, at the same time, a very effective regulation (36).

However, many actions have been taken, in order to assist during the R&D process of nanopharmaceutics. For now, using the current legislation and guidelines to produce GMP compliant batches is the first step.

Using the Volume X from Eudralex and following the guideline *"guideline on the requirements to the chemical and pharmaceutical quality documentation concerning investigational medicinal products in clinical trials"*, can be helpful, the specifications for the development of an IMP are all described.

Since the production of nanopharmaceutics require different parameters, because corresponds to the production of a new medicinal product and it doesn't follow a conventional regulatory process, scientific advice should be sought to assure quality, safety and efficacy of the new product to be used in clinical trials. The Member states are responsible for monitoring the safety of medicines and creates measures to assure the benefit/risk of the new product is kept positive (88).

Usually, nanopharmaceutics use a known API, with a known safety profile. However, the final product needs to be fully described, since the new formulation can have a different toxicity due to the improvement of its solubility, for example.

For the use of these batches in clinical trials, labels also need to be adapt to the clinical trial in question (full description on Annex 13 concerning IMP), in order to be easily identified.

The batches also need to supply the population of the study. For a phase I clinical trial, where the maximum tolerated dose is probably determined be escalation of the medicinal product and the population varies from 10 to 100.

When it comes to phase II or phase III clinical trials, the number of subjects is much higher, the safety and control of the batches and IMPD is very restrict, since the pharmacokinetics, pharmacodynamics, safety and efficacy is almost defined. Other concern for these kind of batches is the stability and the storage conditions.

Using a quality-be-design approach for the production of the nanopharmaceutics adds an extra control over the quality, efficacy, and safety of the formulation development, and these

processes become more clear since it englobes critical process parameters, critical product quality attributes, and clinical properties as safety and efficacy (89).

The scale-up method, should guarantee the quality of each batch and keep minimum differences between them. Otherwise, they cannot be used for clinical trials. Also, the results from all the clinical trials phases will be integrated in the IMPD serving as the base for the documentation needs for a marketing authorization. The CTD as a similar format to the IMPD, which means, efforts must be made in these phases, otherwise the market access will be hard to achieve.

This is one of the greatest challenges for nanopharmaceutics, all the information available (guidelines, scientific advice given by a regulatory authority) must be followed to gain clinical acceptance. The investment made during the past decade, to study size, shape, and surface properties of nanopharmaceutics, can facilitate the marketing authorization (90).

7. Conclusions

Nanomedicine had big investments in the past two decades. It represents the new era of medicinal products and multiples stakeholders are developing new and improved nanopharmaceutics to respond to today's medical needs.

This new paradigm also brings new concerns for all industry and regulatory authorities, being in such an early development of new legislation for nanopharmaceutics.

During the R&D process of nanopharmaceutics products, many steps must be taken, to create a safe and, above all, effective product to be used in human subjects.

Clinical trials are mandatory for the development of medicinal products, but since there are subjects participating in these studies, regulation is very strict to ensure the safety of the volunteers. National and European legislation have all the rules to make clinical trials safe and to guarantee that the ratio benefit/risk is always positive.

Also, guidelines for clinical trials and the construction of an IMPD are incredible useful for the submission to test the new product, but to facilitate the next step – marketing authorization.

In what concerns nanopharmaceutics, there has been a big effort from all authorities and industry to develop GMP compliant nanopharmaceutics. However, there are no specific guidelines or regulation for them. To overcome this problem, scientific advice must be asked and projects to develop guidelines should continue.

Since there are many production methods of these products, their advantages and ease scale up should be taken under consideration. Because, if the method is optimized, the final product will have better pharmaceutical quality and stability.

The key for the advantages of nanopharmaceutics are their basic properties such as morphology, size and shape. These can only be effective if, from batch to batch, the quality is the unaltered, so a reproducible production method has to be chosen and validated.

At the present, there is no approved method that includes all parameters needed to guarantee quality control of the scale -up.

From basic research to clinical trials, some projects are evolving and so is the regulatory system, creating a good environment for the development of new and improved nanopharmaceutics, following GMP and harmonized guidelines.

To conclude, nanomedicine is an emergent area and some nanopharmaceutics already entered the clinical market. Which means, even if this is a longer process than other medicinal products, the investment made from every stakeholder will have a positive impact on the development of nanopharmaceutics.

REFERENCES

(1) BREMER-HOFFMANN, S., AMENTA, V., & ROSSI, F. - **Nanomedicines in the European translational process.** European Journal of Nanomedicine (2015), 191–202.

(2) Official Journal of the European Union - **COMMISSION RECOMMENDATION of 18 October 2011 on the definition of nanomaterial**. [Accessed on 28 Feb. 2017]. Available on Internet:
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0038:0040:EN:PDF

(3) DORBECK-JUNG, B. R. and CHOWDHURY, N. - **Is the European Medical Products Authorisation Regulation Equipped to Cope with the Challenges of Nanomedicines?** Law and Policy, The University of Denver (2011), 276–303.

(4) PETROS, R. A., DESIMONE, J.M. - **Strategies in the design of nanoparticles for therapeutic Applications**. Nature Reviews (2010), 615-627.

(5) ELIASOF, S. - **Correlating preclinical animal studies and human clinical trials of a multifunctional, polymeric nanoparticles.** Proceedings of the National Academy of Sciences (2013), 15127-15132.

(6) CHANG, E. H. et al. – **Nanomedicine: Past, present and future – A global perspective.** Biochemical and Biophysical Research Communications 468 (2015), 511-517.

(7) TINKLE, S. et al. – **Nanomedicines: Addressing the scientific and regulatory gap**. Annals of the New York Academy of Sciences (2014), 35–56.

(8) DORBECK-JUNG, B. R. - **What can Prudent Public Regulators Learn from the United Kingdom Government's Nanotechnological Regulatory Activities?** NanoEthics, (2007), 257–270.

(9) ETPN (European Technology Platform on Nanomedicine) – **About ETPN** [Accessed on 1 Mar. 2017]. Available on Internet: http://www.etp-nanomedicine.eu/public/about/

(10) ETPN (European Technology Platform on Nanomedicine) - **NANOMEDICINE 2020 Contribution of Nanomedicine to Horizon 2020**, (2013). [Accessed on 1 Mar. 2017]. Available on Internet: http://www.etp-nanomedicine.eu/public/press-documents/publications/etpn-publications/etpn-white-paper-H2020

(11) ETPN (European Technology Platform on Nanomedicine) **- Nanomedicine Strategic Research & Innovation Agenda,** (2016).

(12) NASA – **Technology Readiness Level**. USA: Thuy Mai, Oct. 28, 2012, Last Updated: Jul. 31, 2015. [Accessed on 2 Mar. 2017]. Available on Internet: https://www.nasa.gov/directorates/heo/scan/engineering/technology/txt_accordion1.html

(13) EARTO (European Association of Research and Technology Organizations) - **The TRL Scale as a Research & Innovation Policy Tool, EARTO Recommendations**, (2014), 1–17. [Accessed on 2 Mar. 2017]. Available on Internet: http://www.earto.eu/fileadmin/content/03_Publications/The_TRL_Scale_as_a_R_I_Policy_Tool_-_EARTO_Recommendations_-_Final.pdf

(14) BAWARSKI, W. E., et al. - **Emerging nanopharmaceuticals.** Nanomedicine: Nanotechnology, Biology, and Medicine, (2008), 273–282.

(15) FRIEDMAN, L. M., et al. - **Fundamentals of Clinical Trials**. 5th Ed. New York: Springer, 2015. ISBN 978-3-319-18538-5.

(16) POCOCK, S. J. – **Clinical Trials, A Practical Approach.** 1st Ed. New York: Wiley, 1983. ISBN 0- 047-190155-5

(17) MACHIN, D., DAY, S., GREEN, S. - **Textbook of Clinical Trials**. 2nd Ed. England: John Wiley & Sons Ltd, 2004. ISBN N 0-471-98787-5.

(18) F. Hoffmann-La Roche Ltd – **What is a clinical trial and how does a trial work?** [Accessed on 5 Mar. 2017]. Available on Internet: http://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/what_is_a_clinical_trial.htm?tab_id=tab1

(19) ICH Expert Working Group - **ICH Harmonised Tripartite Guideline - General Considerations for Clinical Trials E8** (1997). [Accessed on 5 Mar. 2017]. Available on Internet: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E8/Step4/E8_Guideline.pdf

(20) Official Journal of the European Communities - **DIRECTIVE 2001/20/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 4 April 2001.** (2001). [Accessed on 5 Mar. 2017]. Available on Internet: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32001L0020&from=EN

(21) European Commission – **EudraLex – EU Legislation**. [Accessed on 7 Mar. 2017]. Available on Internet: https://ec.europa.eu/health/documents/eudralex_en

(22) Official Journal of the European Communities - **COMMISSION DIRECTIVE 2003/94/EC of 8 October 2003** (2003). [Accessed on 7 Mar. 2017]. Available on Internet: http://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/dir_2003_94/dir_2003_94_en.pdf

(23) EudraLex The Rules Governing Medicinal Products in the European Union Volume 4 - **Good Manufacturing Practice Medicinal Products for Human and Veterinary Use Annex 13** (2010). [Accessed on 10 Mar. 2017]. Available on Internet: http://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2009_06_annex13.pdf

(24) The Rules Governing Medicinal Products in the European Union Volume 10 - **GUIDANCE DOCUMENTS APPLYING TO CLINICAL TRIALS GUIDANCE ON INVESTIGATIONAL MEDICINAL PRODUCTS (IMPS) AND 'NON INVESTIGATIONAL MEDICINAL PRODUCTS' (NIMPS).** (REV. 1, 2011). [Accessed on 10 Mar. 2017]. Available on Internet: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/imp_03-2011.pdf

(25) Committee for Medicinal Products for Human Use (CHMP) - **GUIDELINE ON THE REQUIREMENTS TO THE CHEMICAL AND PHARMACEUTICAL QUALITY DOCUMENTATION CONCERNING INVESTIGATIONAL MEDICINAL PRODUCTS IN CLINICAL TRIALS (2006).** [Accessed on 10 Mar. 2017]. Available on Internet: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/18540104en_en.pdf

(26) Official Journal of the European Communities - **COMMISSION DIRECTIVE 2005/28/EC of 8 April 2005** (2005). [Accessed on 14 Mar. 2017]. Available on Internet: http://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/dir_2005_28/dir_2005_28_en.pdf

(27) The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) – **Mission.** [Accessed on 14 Mar. 2017]. Available on Internet: http://www.ich.org/about/mission.html

(28) The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) **- INTEGRATED ADDENDUM TO ICH E6(R1): GUIDELINE FOR GOOD CLINICAL PRACTICE E6(R2) (Current Step 4 version dated 9 November 2016).** [Accessed on 14 Mar. 2017]. Available on Internet: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6/E6_R2__Step_4.pdf

(29) European Commission **– Clinical Trials - Regulation (EU) No 536/2014.** [Accessed on 14 Mar. 2017]. Available on Internet: http://ec.europa.eu/health/human-use/clinical-trials/regulation_en

(30) Official Journal of the European Union - **REGULATION (EU) No 536/2014 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 April 2014** (2014). [Accessed on 14 Mar. 2017]. Available on Internet: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/reg_2014_536/reg_2014_536_en.pdf

(31) Assembleia da República - **Lei n.º 21/2014, de 16 de abril** (2014). [Accessed on 18 Mar. 2017]. Available on Internet: http://www.infarmed.pt/documents/15786/1068535/036-B1_Lei_21_2014_1alt.pdf

(32) Assembleia da República - **Lei n.º 73/2015 de 27 de julho**. Diário da República, 2015. [Accessed on 18 Mar. 2017]. Available on Internet: http://www.ceic.pt/documents/20727/38721/Lei+n.%C2%BA+73-2015+de+27+de+julho/a04332bb-581e-46a2-b0de-ef714aeb1112

(33) INFARMED - Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.- **Medicamentos de Uso Humano – Ensaios Clínicos.** [Accessed on 18 Mar. 2017]. Available on Internet: http://www.infarmed.pt/web/infarmed/entidades/medicamentos-uso-humano/ensaios-clinicos

(34) GODIN, B., et al. - **Emerging applications of nanomedicine for therapy and diagnosis of cardiovascular diseases.** Trends in Pharmacological Sciences (2010), 199–205.

(35) ZHANG, L. et al. – Nanoparticles in Medicine: Therapeutic applications and Developments. CLINICAL PHARMACOLOGY & THERAPEUTICS (2008), 761-769.

(36) European Union – **Clinical Trials Register website**. [Accessed on 25 Mar. 2017]. Available on Internet: https://www.clinicaltrialsregister.eu/

(37) ETHERIDGE, M. L., et al. - **The big picture on nanomedicine: The state of investigational and approved nanomedicine products.** Nanomedicine: Nanotechnology, Biology, and Medicine, (2013) 1–14.

(38) ICH Expert Working Group - **ICH Harmonised Tripartite Guideline – Good Manufacturing Practice Guide For Active Pharmaceutical Ingredients Q7** (2000). [Accessed on 25 Mar. 2017]. Available on Internet: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q7/Step4/Q7_Guideline.pdf

(39) FDA – Food and Drug Administration - **FDA's Approach to Regulation of Nanotechnology Products.** USA: Last Updated May 8, 2015. [Accessed on 25 Mar. 2017]. Available on Internet: https://www.fda.gov/scienceresearch/specialtopics/nanotechnology/ucm301114.htm

(40) EMA -European Medicines Agency - **The European Medicines Agency's scientific guidelines on nanomedicines help medicine developers prepare marketing authorisation applications for human medicines.** [Accessed on 25 Mar. 2017]. Available on Internet: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000564.jsp&mid=WC0b01ac05806403e0

(41) GaBI – Generics and Biosimilars Initiative Journal – **EMA issues draft reflection paper for iron-based nano-colloidal products.** Belgium: Feb. 11, 2014 [Accessed on 10 Apr. 2017]. Available on Internet: http://gabi-journal.net/news/ema-issues-draft-reflection-paper-for-iron-based-nano-colloidal-products

(42) Committee for Medicinal Products for Human Use (CHMP) - **Reflection paper on the data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product.** EMA: 2015. [Accessed on 10 Apr. 2017]. Available on Internet: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/03/WC500184922.pdf

(43) Committee for Medicinal Products for Human Use (CHMP) - **Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product.** EMA: 2013. [Accessed on 10 Apr. 2017]. Available on Internet: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/03/WC500140351.pdf

(44) GAUCHER, G. et al. - **Block copolymer micelles: Preparation, characterization and application in drug delivery.** Journal of Controlled Release (2005), 169–188.

(45) Committee for Medicinal Products for Human Use (CHMP) - **Joint MHLW/EMA reflection paper on the development of block copolymer micelle medicinal products.** EMA: 2013. [Accessed on 10 Apr. 2017]. Available on Internet: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2014/01/WC500159411.pdf

(46) Committee for Medicinal Products for Human Use (CHMP) **- Reflection paper on surface coatings: general issues for consideration regarding parenteral administration of coated nanomedicine products.** EMA: 2013. [Accessed on 10 Apr. 2017]. Available on Internet: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/08/WC500147874.pdf

(47) THOMAS, O., LAGARCE, F. - **Lipid nanocapsules: A nanocarrier suitable for scale-up process.** Journal of Drug Delivery Science and Technology (2013), 555–559.

(48) MUTHU, M. S., WILSON, B. - **Challenges posed by the scale-up of nanomedicines.** Nanomedicine (2012), 307–309.

(49) GALINDO-RODRÍGUEZ, S. A. et al. - **Comparative scale-up of three methods for producing ibuprofen-loaded nanoparticles.** European Journal of Pharmaceutical Sciences (2005), 357–367.

(50) SHEGOKAR, R., SINGH, K. K., MÜLLER, R. H. - **Production & stability of stavudine solid lipid nanoparticles - From lab to industrial scale.** International Journal of Pharmaceutics (2011), 461–470.

(51) LANDESMAN-MILO, D., PEER, D. - **Transforming Nanomedicines from Lab Scale Production to Novel Clinical Modality.** Bioconjugate Chemistry (2016), 855–862.

(52) FAROKHZAD, O. C., LANGER, R. - **Nanomedicine: Developing smarter therapeutic and diagnostic modalities.** Advanced Drug Delivery Reviews (2006).

(53) DAVIS, M. E., CHEN, Z. G., SHIN, D. M. - **Nanoparticle therapeutics: an emerging treatment modality for cancer.** Nature Reviews - Drug Discovery (2008), 771–782.

(54) FAHY, E., et al. - **A comprehensive classification system for lipids.** Journal of Lipid Research (2005), 839–861.

(55) CLAWSON, C., ESENER, S., ZHANG, S. – **Lipid-Polymer Nanomaterials.** Nanomaterials for the Life Sciences (2011), 259-284.

(56) BENNET, D., KIM, S. - **Polymer Nanoparticles for Smart Drug Delivery.** InTech (2014), 257-309.

(57) MODY, V. V et al. - **Introduction to metallic nanoparticles.** Journal of Pharmacy and Bioallied Sciences (2010), 282–289.

(58) CAI, W. et al. - **Applications of gold nanoparticles in cancer nanotechnology.** Nanotechnology, Science and Applications (2008), 17–32.

(59) RAAB, C. et al. - **Production of nanoparticles and nanomaterials**. NanoTrust Dossiers, Institute of Technology Assessment of the Austrian Academy of Sciences (2011).

(60) BISWAS, A. et al.- **Advances in top-down and bottom-up surface nanofabrication: Techniques, applications & future prospects.** Advances in Colloid and Interface Science (2012), 2–27.

(61) PALIWAL, R., BABU, R. J., PALAKURTHI, S. - **Nanomedicine Scale-up Technologies: Feasibilities and Challenges.** AAPS PharmSciTech (2014), 1527–1534.

(62) SILVA, A. C. et al. - **Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): High pressure homogenization versus ultrasound.** Colloids and Surfaces B: Biointerfaces (2011), 158–165.

(63) MÜLLER, R. - **Solid lipid nanoparticles (SLN) for controlled drug delivery: a review of the state of the art.** European Journal of Pharmaceutics and Biopharmaceutics (2000), 161–177.

(64) TROTTA, M., DEBERNARDI, F., & CAPUTO, O. - **Preparation of solid lipid nanoparticles by a solvent emulsification-diffusion technique.** International Journal of Pharmaceutics (2003), 153–160.

(65) XIE, S. et al. - **Effect of PLGA as a polymeric emulsifier on preparation of hydrophilic protein-loaded solid lipid nanoparticles.** Colloids and Surfaces B: Biointerfaces (2008), 199–204.

(66) POOJA, D. et al. **- Optimization of solid lipid nanoparticles prepared by a single emulsification-solvent evaporation method.** Data in Brief (2016), 15–19.

(67) THAKKAR, H. et al. - **Formulation and characterization of lipid-based drug delivery system of raloxifene-microemulsion and self-microemulsifying drug delivery system.** Journal of Pharmacy and Bioallied Sciences (2011), 442-448.

(68) GHOSH, P. K. et al. - **Design and development of microemulsion drug delivery system of acyclovir for improvement of oral bioavailability.** AAPS PharmSciTech (2006).

(69) KUROIWA, T. - **Efficient Encapsulation of a Water-Soluble Molecule into Lipid Vesicles Using W/O/W Multiple Emulsions via Solvent Evaporation.** JAOCS, Journal of the American Oil Chemists' Society (2016), 421-430.

(70) CHARCOSSET, C., EL-HARATI, A., FESSI, H. - **Preparation of solid lipid nanoparticles using a membrane contactor.** Journal of Controlled Release (2005), 112–120.

(71) Hielscher – Ultrasound Technology – **Ultrasonic Liposome preparation.** [Accessed on 26 Apr. 2017]. Available on Internet:
https://www.hielscher.com/ultrasonic-liposome-preparation.htm

(72) BOSE, S., MICHNIAK-KOHN, B. - **Preparation and characterization of lipid based nanosystems for topical delivery of quercetin.** European Journal of Pharmaceutical Sciences (2013), 442–452.

(73) ZAKIR, F. et al. - **Nanocrystallization of poorly water soluble drugs for parenteral administration.** Journal of Biomedical Nanotechnology (2011), 127–129.

(74) FATEMINIA, S. M. A., WANG, Z., LIU, B. - **Nanocrystallization: An Effective Approach to Enhance the Performance of Organic Molecules.** Small Methods (2017).

(75) DE WAARD, H., FRIJLINK, H. W., HINRICHS, W. L. J. - **Bottom-up preparation techniques for nanocrystals of lipophilic drugs.** Pharmaceutical Research (2011), 1220–1223.

(76) PELTONEN, L., HIRVONEN, J. - **Pharmaceutical nanocrystals by nanomilling: Critical process parameters, particle fracturing and stabilization methods.** Journal of Pharmacy and Pharmacology. (2010), 1569–1579.

(77) VAN EERDENBRUGH, B., VAN DEN MOOTER, G., AUGUSTIJNS, P. - **Top-down production of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation into solid products.** International Journal of Pharmaceutics (2008), 64-75.

(78) KHAN, A. et al. - **Optimization of microfluidization for the homogeneous distribution of cellulose nanocrystals (CNCs) in biopolymeric matrix.** Cellulose (2014), 3457–3468.

(79) KALE, M. R. S., MISHRA, B. - **Drug nanocrystals: A way toward scale-up.** Saudi Pharmaceutical Journal (2016), 386-404.

(80) GUO, P. et al. - **Preparing amorphous hydrophobic drug nanoparticles by nanoporous membrane extrusion.** Nanomedicine (2013), 333–341.

(81) BOHREY, S., CHOURASIYA, V., PANDEY, A. - **Polymeric nanoparticles containing diazepam: preparation, optimization, characterization, in-vitro drug release and release kinetic study.** Nano Convergence (2016).

(82) PINTO REIS, C. et al. - **Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles.** Nanomedicine: Nanotechnology, Biology, and Medicine (2006), 8–21.

(83) DUTTA, P. K., RAVIKUMAR, M. N. V., DUTTA, J. - **Chitin and Chitosan for Versatile Applications.** Journal of Macromolecular Science, Part C: Polymer Reviews (2002).

(84) PANTA, P. et al. - **Protein Drug-Loaded Polymeric Nanoparticles.** J. Biomedical Science and Engineering (2014), 825–832.

(85) BYRAPPA, K., OHARA, S., ADSCHIRI, T. **- Nanoparticles synthesis using supercritical fluid technology - towards biomedical applications.** Advanced Drug Delivery Reviews (2008), 299–327.

(86) SHETH, P. et al. - **Nanoparticles in the pharmaceutical industry and the use of supercritical fluid technologies for nanoparticle production.** Current Drug Delivery (2012), 269–84.

(87) CHARITIDIS, C. et al. **- Manufacturing nanomaterials: from research to industry.** Manufacturing Review (2014).

(88) HAFNER, A. et al. - **Nanotherapeutics in the EU: an overview on current state and future directions.** International Journal of Nanomedicine (2014), 1005-1023.

(89) DUNCAN, R., GASPAR, R. - **Nanomedicine(s) under the microscope.** Molecular Pharmaceutics (2011), 2101–2141.

(90) WANG, R., BILLONE, P. S., MULLETT, W. M. - **Nanomedicine in Action: An Overview of Cancer Nanomedicine on the Market and in Clinical Trials.** Journal of Nanomaterials (2013).

ANNEXES

Example of The Format of an IMPD

Investigational medicinal product dossier
<PRODUCT>, VERSION <xx.yy>
<date>

AUTHORS

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