Carolina Gonçalves Rodrigues

Relatórios de Estágio e Monografia intitulada "Impact of Surface Charged Nanostructured Carriers in Drug Delivery and Targeting" referentes à Unidade Curricular "Estágio", sob a orientação, respetivamente, do Dr. Filipe Silva, do Dr. Carlos Oliveira e da Professora Doutora Eliana Souto e apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas

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Universidade de Coimbra

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UNIVERSIDADE DE COIMBRA

I, Carolina Gonçalves Rodrigues, student of the Integrated Master's Degree in Pharmaceutical Sciences, with the number 2012146885, declare that I take full responsibility for the content of the Report of Internship presented to the Faculty of Pharmacy of the University of Coimbra, within the course unit Curricular Internship.

I further declare that this is an original work and that any statement or expression used by me is referenced in the References of this Internship Report, according to legally established bibliographic criteria, always safeguarding the Copyright, except for my personal opinions.

Coimbra, 17 of July of 2017

Carolina Gonçalves Rodrigues

(Carolina Gonçalves Rodrigues)

In spite of everything seems random, there is always a plan.

(my own saying)

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Part I – Report of Curricular Internship in Pharmaceutical Industry

Laboratórios Basi, Mortágua

Acknowledgements:

To Filipe Silva, PharmD, for providing me the chance of the internship as well as *Laboratórios Basi* for the opportunity,

To Rui, Goreti and Sofia for all the knowledge they have shared with me, all the availability, support, friendship and fellowship,

To Susana, Daniela and Joana Costa for all the kindness and for always being there,

To all the team of *Laboratórios Basi* for the welcoming and experience,

To the Faculty of Pharmacy of the University of Coimbra for the professional shaping these past five years,

To my family and friends for helping me giving more of myself,

To all, my sincere thank you.

ABBREVIATIONS

API	Active Pharmaceutical Ingredient
BASI	*Laboratórios Basi*
CEP/COS	Certificate of Suitability
CoA	Certificate of Analysis
CoC	Certificate of Conformity
EMA	European Medicines Agency
GMP	Good Manufacturing Practice
INFARMED, I.P.	*Autoridade Nacional do Medicamento e Produtos de Saúde, Instituto Público* (National Authority of Medicines and Health Products, Public Institute)
IPC	In Process Control
FFUC	*Faculdade de Farmácia da Universidade de Coimbra* (Faculty of Pharmacy of the University of Coimbra)
MA	Marketing Authorization
MPVP	Manufacturing Process Validation Protocol
MPVR	Manufacturing Process Validation Report
IMDPS	Integrated Master's Degree in Pharmaceutical Sciences
OOS	Out of specification result
PQR	Product Quality Review
QA	Quality Assurance
QAP	Quality Assurance Process
QC	Quality Control
QP	Qualified Person
SWOT	*Strengths, Weaknesses, Opportunities, Threats*

1. INTRODUCTION

As the end of course approaches, many choices have to be made in order to decide in which pharmaceutical areas we will do our final internships. Although I could have done Erasmus, hospital pharmacy, investigation and so many other good opportunities at the end of the course, I never had doubts that what I really wanted to experience would be pharmaceutical industry. Not only because my interest and curiosity for this area had increased over the course, while I had classes of Pharmaceutical Technology, Regulatory Affairs or Management and Quality Assurance, but also due to the fact that I wanted to learn more about this fascinating area in a real workplace and with professionals with a huge knowledge and experience.

The given chance of performing part of my final internship of the Integrated Master's Degree in Pharmaceutical Sciences course in an pharmaceutical industry is an enormous privilege for me, since this is an unique opportunity for the students of the Faculty of Pharmacy of the University of Coimbra in all country and also because it is an honor for me as a student to be received in a pharmaceutical industry as being part of the team.

From January to March of 2017, I had the perk of spending my days learning and training in *Laboratórios Basi*, in Mortágua, in the Quality Assurance Department and guided by Filipe Silva, PharmD.

In this report, an analysis will be done of my internship in the form of a SWOT analysis, which means that a sum up of the Strengths, Weaknesses, Opportunities, and Threats of this experience will be presented, while some examples of the activities which I have done are introduced.

2. *LABORATÓRIOS BASI*

Laboratórios Basi is a pharmaceutical industry, placed in Mortágua, which began its activity in 1956 and that since 2007 is integrated in a wider pharmaceutical group of companies, creating great conditions for the development of synergies[1].

BASI's missions concerned the worries of "provide treatments at the best possible price, with a guarantee of excellence based in several decades of activity and also to provide new medicinal products and therapeutic solutions with high standards of quality, innovation, competitiveness and safety, addressing the markets needs and demands"[1].

Nowadays, BASI is an industrial player of international reference with a high competitive level, currently present in 4 continents and more than 20 countries with a portfolio of above 200 medicinal products for human use in more than 50 different therapeutic areas and in all pharmaceutical forms[2].

Figures 1 – Pipeline and logo of BASI.

Source of the images: http://www.basi.pt/webbasi/portefolio/pipeline.aspx, accessed in 18-March-2017.

3. SWOT ANALYSIS

	POSITIVE ASPECTS	NEGATIVE ASPECTS
INNER ENVIRONMENT	**Strengths** • Welcoming in the first day • Time to input new knowledges • Working team • Continuous update • Responsibility and autonomy given • Frequency and lasting of the internship • Quality assurance area • English as main language at work • Diversity of situations and tasks • Making use of theoretical learning and of the simulated context into professional practice • Suitability of the course to future perspectives	**Weaknesses** • Lack of useful IT knowledge
OUTER ENVIRONMENT	**Opportunities** • Networking • Career Perspectives • Higher contact with English language along the course	**Threats** • Lack of formal recognition of the internship • Other well prepared professionals in places traditionally from pharmacists

3.1. STRENGTHS

3.1.1. WELCOMING IN THE FIRST DAY

The first day in a new place is an essential part of starting an experience as it is the final internship. Firstly, I was carefully received in the human resources that explained me everything important as schedule, internal rules and lunch periods (kindly offered by BASI). Then I was greeted in BASI by Filipe Silva, PharmD., who made me a global company presentation, introduced me to the team, presented me the Quality Assurance System and guided me to a tour through BASI's facilities.

3.1.2. TIME TO INPUT NEW KNOWLEDGES

In the initial days of my internship, there was given me the opportunity of study and learn accurately all the official documents related to Quality Assurance, namely international documentation as EudraLex volume 4, from the European Commission[3] or Quality Guidelines, from the ICH (International Conference on Harmonization)[4], as well as internal documents such as Quality General Procedures. This period was really important to a better preparation for the following days of the internship.

3.1.3. WORKING TEAM

The QA team as well as the elements of the QC and the Product Management were really friendly to me and have always been available to clarify my doubts and to teach me new subjects. This was an essential point in my internship since the team had a great background and experience and it was also dynamic and cheerful.

3.1.4. CONTINUOUS UPDATE

I had the chance to learn new different things while I was doing the tasks which were asked me to do and also in different moments of individual explanations on important information and databases in pharmaceutical industry, namely in European Directorate for the Quality of Medicines & Healthcare[5] and EudraGMDP[6] regarding essential information for example on Monographs in the European Pharmacopoeia, updated versions of the CEP of API or qualification of the manufacturers of raw materials.

3.1.5. RESPONSIBILITY AND AUTONOMY GIVEN

During all the internship, I felt that important and useful work have been asked me to do, with an important sense of responsibility and always with autonomy and confidence which allowed me to grow and to learn more as I felt part of the team.

3.1.6. FREQUENCY AND LASTING OF THE INTERNSHIP

From my point of view an internship in pharmaceutical industry of three months is an adequate duration once it has allowed me to experience the reality of the workplace and to have an idea of the tasks of each department and more deeply of QA Department. From 9th of January until 31th of March of 2017, I have made a total of 520hours of internship at QA Department at BASI.

3.1.7. QUALITY ASSURANCE AREA

When the opportunity of an internship in QA appeared, I had no doubt this was the area I wanted to experiment in my final internship once it was still an unknown area for me regarding the daily work. I have found a multidisciplinary area which allowed me to develop my knowledge about INFARMED, EMA and other similar entities around the world; to get to know the GMP guidelines better and how it works regarding its practical application in pharmaceutical industry; understand the differences between a CoA and a CoC, the reality of IPC and Hold-Times during manufacturing and of the followed stability studies; the stablished procedures for handling deviations, complaints or OOS, because the rule of a pharmacist in this area is to implement measures to avoid problems and when this is not possible, solve them as quickly and as better they can. I had also the chance to understand how an internal and external audit or inspection must be carried out and to develop and realize the need of documents as the PQR (opportunity to compile and analyze important information to draw conclusions about product quality), MPVP or MPVR. Moreover, QA also allowed me to improve my knowledge on Regulatory Affairs as far as MA dossier or other similar dossiers are concerned.

3.1.8. ENGLISH AS MAIN LANGUAGE AT WORK

All the work I have developed during the internship was in English due to the fact that Basi has multiple international customers and also because this is an increasingly international area where the languages background has a great positive impact.

3.1.9. DIVERSITY OF SITUATIONS AND TASKS

During the three months of internship there were days in which I have done always the same task, but this is the daily routine of work and what will be required always from pharmacists. In spite of that, I never had two similar days and many different situations were presented to me, which can be proved by the plan of activities carried out during the internship, presented in annex I. In annex II it is shown an example of a risk management tool of a hypothetical situation developed during the internship.

3.1.10. MAKING USE OF THE THEORETICAL LEARNING AND OF THE SIMULATED CONTEXT INTO PROFESSIONAL PRACTICE

The theoretical learning acquired along the course in curricular units as Pharmaceutical Technologies, Management and Quality Assurance, Regulatory Affairs and so many others, were really important to my preparation for this internship and despite the fact that I have learned everyday many new things, I felt I had a good background to the context into professional practice which was improved in my internship.

3.1.11. SUITABILITY OF THE COURSE TO FUTURE PERSPECTIVES

As already mentioned in the last point, from my point of view the course had prepared me well to this internship. Pharmaceutical industry, as many other pharmaceutical areas, requires a constant update and input of knowledge, however I believe I would be well prepared to future perspectives in pharmaceutical industry if I had that chance.

3.2. WEAKNESSES

3.2.1. LACK OF USEFUL IT KNOWLEDGE

The only weakness I can highlight in this internship was the lack of computer skills I have felt. The reality of nowadays work is really based on IT skills which is something not taught and explored during the course.

3.3. OPPORTUNITIES

3.3.1. NETWORKING

Like any professional experience, this internship was a great opportunity to meet new people, create new contacts, cross with new perspectives and other working methods.

Moreover, the professionals' experiences and backgrounds in different areas and pharmaceutical industries gave me a comprehensive view of the area in Portugal.

3.3.2. CAREER PERSPECTIVES

After this internship, in which I could really experience the daily routine of pharmaceutical industry, I am more confident and sure that this area is one of the most challenging for me and thus I will continue working to build career perspectives in this area. Who knows if the EMA have the chance to come to Portugal and open new doors for Portuguese pharmacists.

3.3.3. HIGHER CONTACT WITH ENGLISH LANGUAGE ALONG THE COURSE

Regarding the contact with English language along the course, I believe that it is a great opportunity to the Faculties have more curricular units in English and even works and exams in this language once it is not an optional skill anymore these days and it should be more present in our five years of course in order to prepare us to the reality we will face.

3.4. *THREATS*

3.4.1. *LACK OF FORMAL RECOGNITION OF THE INTERNSHIP*

The pharmaceutical degree within the European Union does not contemplate any other internship areas rather than community and hospital pharmacy[7]. This way, industry pharmacy is naturally left out in most faculties. Fortunately, in Coimbra it is given a chance to the students of performing extra hours so that they can have such experience. Despite recognizing this opportunity as an immense advantage, I believe that we should not be settled yet. I firmly believe that since these internship hours would not count for the curricular plan, some negative consequences arise, once we are constantly encouraged along the course to explore the several areas where the pharmacist can give a contribute and thus, if pharmacist want to conquer places in pharmaceutical industry this issue is extremely important.

3.4.2. *OTHER WELL PREPARED PROFESSIONALS IN PLACES TRADITIONALLY FROM PHARMACISTS*

Nowadays a huge variety of courses is focused on the different needed areas of a pharmaceutical industry. Biochemists, microbiologists, chemical and environmental engineers and as many others professionals that are essential for the proper functioning of a complex industry, as this one is. The pharmacists need to bear in mind that these professionals have an excellent background and they are well prepared to take the places which were

traditionally only occupied by pharmacists. From my point of view, it is really important that faculties of pharmacy all over the country take this in account and provide more visits and internships to pharmaceutical industries, in order to students to get to know their opportunities better.

4. FINAL CONSIDERATIONS

Right before starting this internship, I already knew this would be both an enriching and challenging experience. If on one hand I was confident that I would fit the theoretical and practical aspects of the areas within the pharmaceutical industry, on the other hand I feared if it would meet the expectations and if I could be up to the demands expected from such a multidisciplinary area as QA.

The three months that composed the duration of this practice ended seeming too short and I had completed the experience with much less uncertainties and much more confident about myself and about what I want to do in my professional future.

My curricular internship at BASI was essential to add and reinforce the apprenticeship along the IMDPS, allowing me to use the acquired knowledge in the real context of the professional activity, which permitted me to grow in a personal and professional way. Beyond that, the internship provided me with a more embracing vision of the management and daily situations that lie beyond the good functioning of a pharmaceutical industry and I bear in mind that all of that was only possible with a working team which always supported me and which was worried about teaching me the most.

Everything that I have learned and experienced these three months is impossible to write down, as well as the growth and autonomy that I achieved which makes me face the future with expectations and think that it would certainly be full of great challenges, but all of them will have a way to be overtaken and be full of new apprenticeship.

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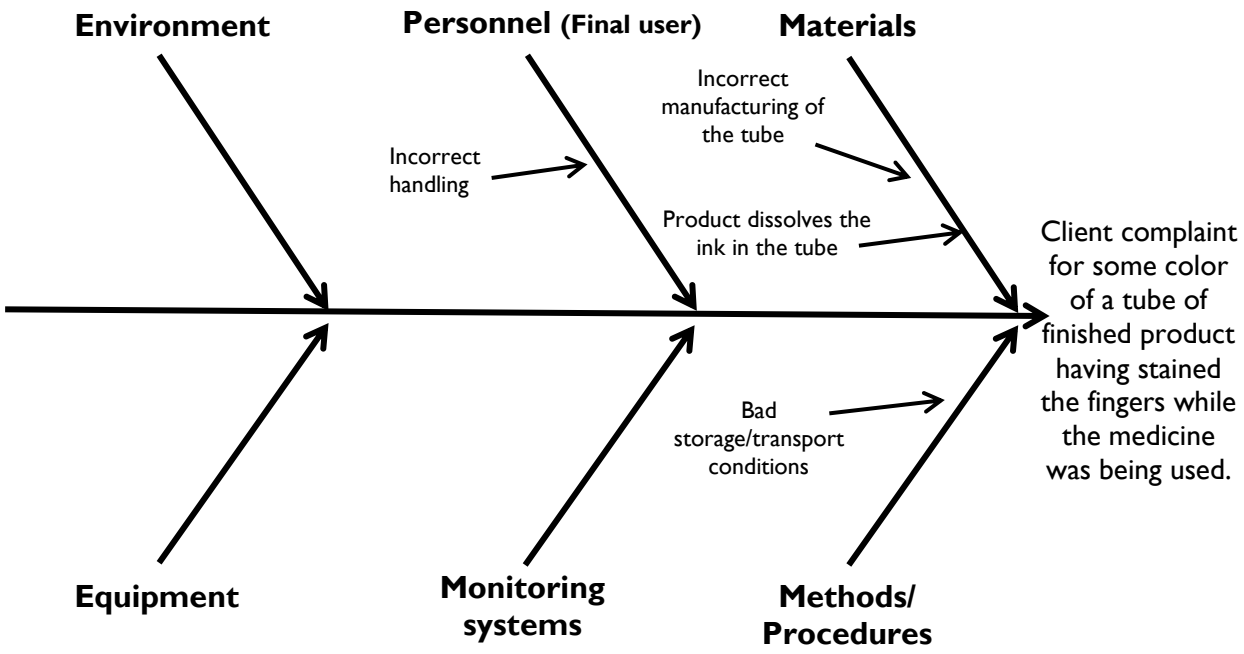
Annex I – Plan of activities carried out during the internship

Date	Activity	Deadline
09-01-2017	Training on "Quality Management Systems"	09-01-2017
09-01-2017	Guided tour throught BASI's facilities	09-01-2017
09-01-2017	Read/Analyze folder Site Master File (SMF)	09-01-2017
09-01-2017	Read/Analyze folder Validation Manufacturing Process	10-01-2017
09-01-2017	Read/Analyze QAP (most important: 1, 2, 3, 4, 5, 6, 7, 8, 10)	10-01-2017
09-01-2017	Read/Analyze Eudralex - volume 4, part I (chapters 1, 2, 3, 4, 5, 6, 7, 8, 9)	10-01-2017
09-01-2017	Globaly analyze Eudralex - volume 4, part II	11-01-2017
09-01-2017	Read/Analyze Eudralex - volume 4, part III (special attention on SMF, Q9, Q10)	11-01-2017
09-01-2017	Read/Analyze Eudralex - volume 4, annexes (most importants: 8, 9, 11, 15, 16, 19)	11-01-2017
12-01-2017	Checking the storage conditions of medicines, medical devices, suplements, cosmetics and biocides produced by BASI (several idioms)	13-01-2017
16-01-2017	Checking data on water analysis orders in the months of January to July 2016	17-01-2016
17-01-2017	Revision of PGQ (Quality General Procedure) on "Complaints follow up"	17-01-2016
18-01-2017	Follow up of complaints according to the Quality General Procedure - PGQ.0007- product A - use of risk management tools	19-01-2017
19-01-2017	Learn/Review terms on EDQM, CEP, ICH (namely ICH Q3C (R6))	19-01-2017
20-01-2017	Training on "PQRs and how to make them"	20-01-2017
20-01-2017	Elaboration of the PQR of Product B - 12 batches: gathering necessary data and completing the document	06-02-2017
25-01-2017	Reading the latest version of the PQRs model. Debating uncertainties.	26-01-2017
01-02-2017	Cleaning validation records of Products C and D	01-02-2017
01-02-2017	Elaboration of cleaning validation report for Product C (worst case product of medicinal drugs manufactured in pasty line) and Product D (worst case product manufactured in pasty line)	02-02-2017
06-02-2017	Elaboration of a PQR of Product E - 44 batches: data gathering and chart organization	08-02-2017
07-02-2017	Colection of data and creation of charts for a Validation Report of three Product F batches	07-02-2017
09-02-2017	Elaboration of the PQR of Product G - 14 batches: data gathering and making of the final document	16-02-2017
10-02-2017	Revision on APIs information - gathering storage conditions of APIs in the EP 9.0 and the USP	10-02-2017
17-02-2017	Elaboration of the PQR of PQR of Product H - 23 bacthes: data gathering and making of the final document	24-02-2017
27-02-2017	Elaboration of the PQR of Product I - 10 batches: data gathering and making of the final document	06-03-2017
06-03-2017	Elaboration of the PQR of Product J - 12 batches: data gathering and making of the final document	13-03-2017
07-03-2017	Explanation on EudraGMDP database	07-03-2017
10-03-2017	Colection of data and creation of charts for a Validation Report of three Product L batches	10-03-2017
10-03-2017	Sorting of old files for destruction - selection of one file per product per year to keep	10-03-2017
15-03-2017	Elaboration of the PQR of Product M - 12 batches: data gathering and making of the final document	29-03-2017
17-03-2017	Elaboration of the MPVR of Product E (bulk e HT) - 3 batches: data gathering and making of the final document	22-03-2017
20-03-2017	Visit to the storehouse of raw materials, finnished products, and batch samples (retention and reference samples)	20-03-2017
22-03-2017	Elaboration of the MPVR of Product N (bulk e HT) - 3 batches: data gathering and making of the final document	24-03-2017
23-03-2017	Elaboration of the MPVR of Product O (bulk e HT) - 3 batches: data gathering and making of the final document	24-03-2017
29-03-2017	Elaboration of the PQR of Product P - 8 batches: data gathering and making of the final document	31-03-2017
31-03-2017	Analyze the QAP related to Data Integrity as well as information from Medicines and Healthcare Products Regulatory (MHRA)	31-03-2017

Annex II – Example of risk management tool

FISHBONE DIAGRAM			
Process / Product / Equipment/ Computerized system	Product X - code Y	**Risk Analysis Report Nr.**	0001
Risk (s) / Hazard (s)	Inks may be toxic for the skin and loss of important information in the tube.	**Start Date**	18/01/2017
Team	Quality Assurance	**Conclusion Date**	18/01/2017
Example of a Fishbone Diagram of an hypothetic situation			



Overview	The fault probably originates from one of the causes presented in the diagram, being the incorrect use of the product the most likely once this was the only case verified in many batches.

Part II – Report of Curricular Internship in Community Pharmacy

Farmácia Barros, Pombal

Acknowledgements:

To Carlos Oliveira, PharmD. and Margarida Oliveira, PharmD., for providing me the chance of the internship in *Farmácia Barros* and for having shared with me his outstanding art of Pharmacy,

To Ana Paula, Aida, Andrea, Carlos, Fernanda and Regina for all the knowledge they have shared with me, all the availability, simplicity support, friendship and fellowship,

To all the clients of *Farmácia Barros* for patiently giving me the chance to learn and to grow in each different situation,

To the Faculty of Pharmacy of the University of Coimbra for the professional shaping these past five years,

To my family and friends for all the support and for helping me to give always more of myself,

To João who has always been there, no matter what, helping me to make it happen

To all, my sincere thank you.

ABBREVIATIONS

ANF	*Associação Nacional das Farmácias* (National Pharmacies' Association)
BPF	*Boas Práticas Farmacêuticas* (Good Pharmaceutical Practice)
CNP	*Código Nacional do Produto* (National Product Code)
FFUC	*Faculdade de Farmácia da Universidade de Coimbra* (Faculty of Pharmacy of the University of Coimbra)
IMDPS	Integrated Master's Degree in Pharmaceutical Sciences
INFARMED, I.P.	*Autoridade Nacional do Medicamento e Produtos de Saúde, Instituto Público* (National Authority of Medicines and Health Products, Public Institute)
MNSRM	*Medicamento Não Sujeito a Receita Médica* (Medicines which do not need a prescription to be dispensed)
MSRM	*Medicamento Sujeito a Receita Médica* (Medicines which need a prescription to be dispensed)
OTC	Over the counter: medicine a which does not require a medical prescription to be sold, nevertheless it should be out of hand for the costumers)
PVP	*Preço de venda ao público* (Retail selling prince)
SWOT	Strengths, Weaknesses, Opportunities, Threats

1. INTRODUCTION

To finish the course of Pharmaceutical Sciences, it is needed to make a total of 810 hours in a Community Pharmacy in order to be ready to the application of theoretical knowledge in daily practice, as well as to develop the needed skills of communication and pharmaceutical marketing and selling which only are possible to achieve in a real place of work.

During my five years of the IMDPS course at FFUC, the pharmacological knowledge was always in the main focus, once it is the basis of the role of the pharmacists and it is essential to a good pharmaceutical counselling in a community pharmacy and also to the health professional pharmacists are in all different areas of work. At the end of the course, it arrives the moment when the theoretical knowledge needs to be conjugated with practicable skills and the capacity to deal with a huge number of different situations, drugs and other health products which are available in a community pharmacy. Therefore, I want it to have a final internship in a community pharmacy which allowed me to learn all these new things, to grow and to experience varied situations which would prepare me to be more insightful and versatile.

Thus, from April 24th to July 13th of 2017, I had the outstanding opportunity of spending my days learning and training in *Farmácia Barros*, in Pombal, guided by Carlos Oliveira, PharmD and with an unconditional support from all the working team in this last moment of my course of IMDPS.

In this report, an analysis will be done of my internship in the form of a SWOT analysis, with a sum up of the Strengths, Weaknesses, Opportunities, and Threats of this experience, while some examples of the activities which I have done are introduced in order to better exemplified my experience during my internship in *Farmácia Barros* and how it was plenty advantageous for my learning and growth as a more complete and prepared pharmacist.

2. *FARMÁCIA BARROS*

Farmácia Barros is a community pharmacy placed in Pombal which was funded before 1935 and its current technical director, Carlos Oliveira, Pharm.D works there since 1982. Beside all its history, this community pharmacy is modern and it is located in the historic center of the city of Pombal.

Farmácia Barros is ruled by an outstanding sympathy, knowledge and experience of its workers and it presents a great heterogeneity of customers, since the elder ones, to young people or even migrants and tourists.

This pharmacy has got a huge variety of products and services being thus a space of health and well-being for the population.

One of the differential factors of this pharmacy and the main reason why I have chosen it to perform my final internship is the fact that *Farmácia Barros* is always in development process with much innovation and empowerment conjugated with a great proximity to their customers and a patient-centered care service.

Figure 1 – Main facade of *Farmácia Barros*, Pombal.

Source of the image: google maps of *Farmácia Barros*, in Pombal, accessed in 05-June-2017.

3. SWOT ANALYSIS

	POSITIVE ASPECTS	***NEGATIVE ASPECTS***
INNER ENVIRONMENT	***Strengths*** • Better understand the flow of work in Community Pharmacy • Time to input new knowledge • Working team • Continuous update/ formation • Variety of trainings performed • Range of pharmaceutical services • Responsibility and autonomy given • Frequency and lasting of the internship • Diversity of situations and tasks • Suitability of the course to future perspectives • Compounding drugs • Relationship with customers and their heterogeneity	***Weaknesses*** • Lack of useful IT knowledge – Sifarma 2000® course • Lack of knowledge concerning some pharmacotherapeutical groups • Counselling on OTC, herbal medicines and medical devices • Lack of commercial and sales preparation • Manual Prescription
OUTER ENVIRONMENT	***Opportunities*** • Dermopharmacy and cosmetics • Veterinary pharmacy • Clinical Pharmacy • Diversity of products in the pharmacy • Preliminary curricular internship • Emigrants and tourists • Counselling protocols • Drug interactions	***Threats*** • Selling of OTC and herbal products outside the pharmacies • Out-of-date information in the prescriptions • Untrustworthy information within the reach of everyone

3.1. STRENGTHS

3.1.1. BETTER UNDERSTAND THE FLOW OF WORK IN COMMUNITY PHARMACY

After three months in a pharmaceutical industry understanding the all process until the commercialization of a drug, it was very interesting to spend time realizing the flow of the drugs before, during and after their dispensing in the community pharmacy. The role of the pharmacists in all this process is very important and gives the change to improve the management, business and sales skills, beyond scientific capabilities. Below it is schematically represented the flow of work in a community pharmacy:

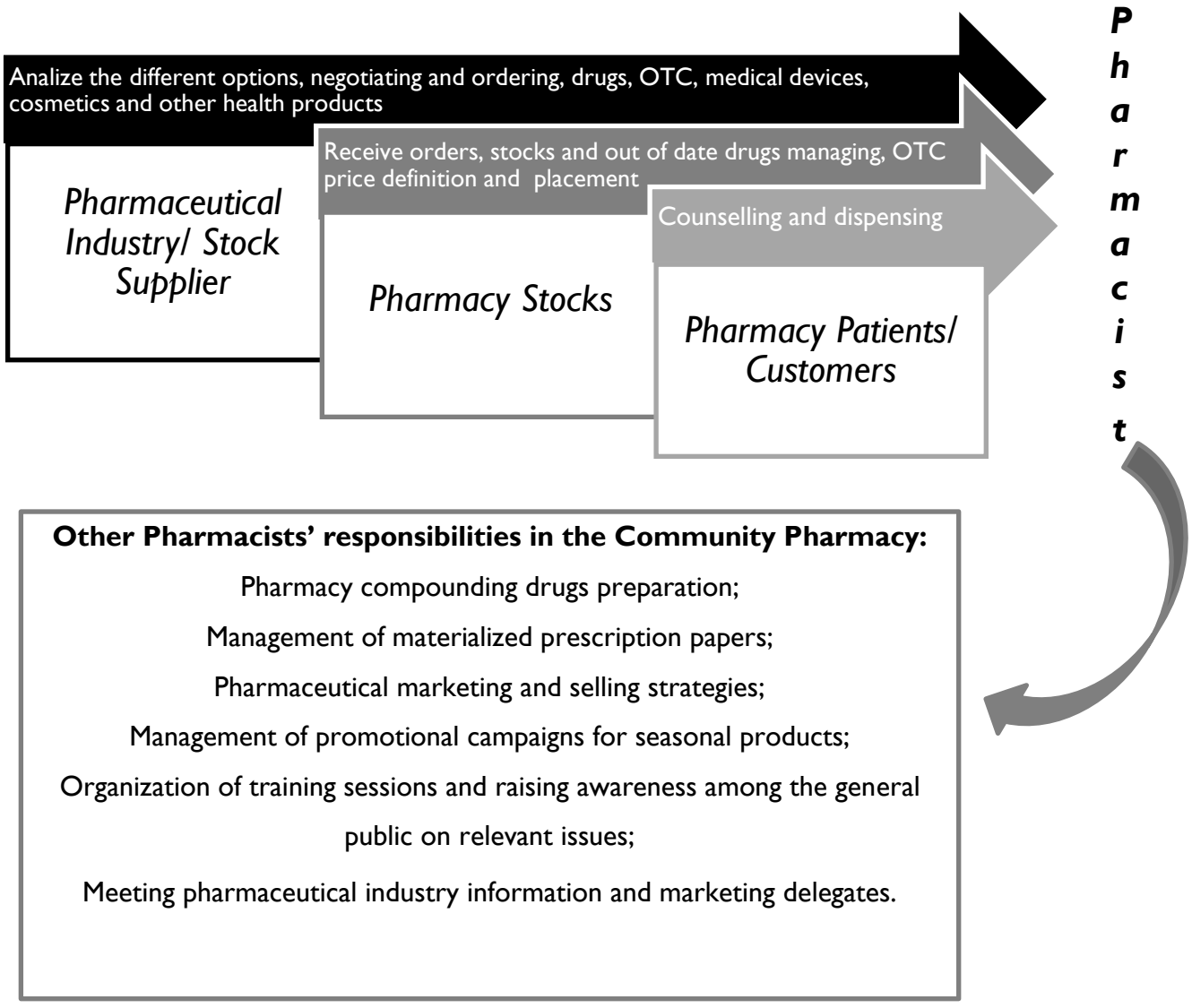


Figure 2 – Schematic flow of work of pharmacists in community pharmacy that I have experienced during my internship at *Farmácia Barros*.

3.1.2. TIME TO INPUT NEW KNOWLEDGE

After being through this community pharmacy in a summer internship before and returning three years later, there were quite a few important aspects that I could not recall. Luckily, both the duration of the internship and the approach my tutor had towards my internship helped me to catch up with most back office tasks performed at a pharmacy and to reinforce my knowledge. For instance, by taking my time in focusing in the reception of medicine's orders and storage of the same I could get acquainted with the determination of the PVP, the organization of the pharmacy, the *Kaizen* methodology, the "First expired, first out" method, the importance of the CNP, all the potential of the card *"Saúda"* from ANF[1], management of narcotic and psychotropic drugs or the checking and communication of prescriptions to the INFARMED.

All these issues are not realized by most of the costumers, however they underpin a good management of a pharmacy.

3.1.3. WORKING TEAM

I complete this experience with the idea that the personal growth I reckon I attained from the first day was not only due to my interest and effort but was augmented by the dynamic and proactive work team I proudly worked aside with. The constant accompaniment they provided allowed every doubt that I would have from time to time to become a chance of learning and their positive spirit, willingness to innovate and entrepreneurship mentality were a big part of my motivation and growth.

3.1.4. CONTINUOUS UPDATE/FORMATION

Pharmacology is a thriving branch of science, this is, unlike other knowledge areas, this is in constant evolution either with the development of new dosage forms, the discovery of new drugs or medical devices, among many other. This constant evolution requires a particular effort from the professional to keep up to date with the novels. I therefore acknowledge the importance that informative sessions by the industries' marketing representatives have. After witnessing a few from brands such as *Medela*, *Bioderma*, *ThermaPearl*, *Parondotax* I regard I got to know the products much better and consequently help me in achieving my ideals of a good pharmaceutical counseling.

3.1.5. VARIETY OF TRAININGS PERFORMED

Besides the informative sessions mentioned in the previous point, some companies would held trainings for the pharmacists. Despite the expected strong marketing influence during these events, these initiatives are, from my point of view, very fruitful for the ones who wish

to develop their skills. For instance, I was glad to be invited to a training session on Cross Selling by *Servier* as well as a free inscription in an online course related to sales techniques.

3.1.6. RANGE OF PHARMACEUTICAL SERVICES

The chance to provide some pharmaceutical services to the population such as measurement of biochemical parameters, preparation and simulation of administration of vaccines or nutrition and podiatry counselling sessions enriched my experience. Not only they proved the preparation I got from the course to face the challenges of practicing these services but also boosted my confidence in the role a pharmacist has and gave me a glimpse of the one we might have in the promotion of health in society.

3.1.7. RESPONSIBILITY AND AUTONOMY GIVEN

From the first day I was trusted with plenty autonomy. The idea of learning by doing, setting my own pace and always trust to have the support from the rest of the work team perfectly matched my ambitions with this experience and held, without any doubt, a major role in the progress I felt I made.

3.1.8. FREQUENCY AND LASTING OF THE INTERNSHIP

The duration of the internship (approximately 650 hours because I have also done another internship) is in my opinion well adjusted for a Pharmacist aspirant to develop any remaining skills and acquire enough knowledge for the beginning of his career.

3.1.9. DIVERSITY OF SITUATIONS AND TASKS

When working at the counter with different personalities at the other side, one might expect a wide variety of scenarios. Most pharmacies also provide services for retirement homes or other special institutions. For instance, *Farmácia Barros* served several retirement homes, a cooperative for handicapped and a kindergarten association. These relations raised even further the pool of differentiated care and tasks to be performed requiring constant adaptations of the knowledge a health sciences professional has according to the situation aspects (i.e. advising drugs to children). Bearing this in mind, I believe that the curricular path I was able to outline across my course helped to prepare better to relate subjects rather following an approach of direct application of knowledge to the situations in front of us. Therefore, I adamantly believe that the true value of a Pharmacist is when things drift from the ordinary and become complex. This is one of the skills I care for the most and expect to keep on improving throughout my professional career.

3.1.10. SUITABILITY OF THE COURSE TO FUTURE PERSPECTIVES

In general, when I was confronted with theorical doubts I was well prepared to search for a solution in a fast and reliable way. Also, the theorical background I built from the course made me comfortable with understanding from the advanced scientific bibliography in disposition as well as all the advices from the team of the pharmacy.

3.1.11. COMPOUNDING DRUGS

Farmácia Barros counts with a laboratory of excellence. Hence, since it was so well prepared for the production of compounds it is a reference in the area and receives some requests. With this chance I was able to perfect my practice in the laboratory with a vast set of different compounds. An example of a compounding sheet I did is attached in the annexes with the paste of menthol water and zinc oxide.

3.1.12. RELATIONSHIP WITH CUSTOMERS AND THEIR HETEROGENEITY

Despite the fact that many costumers had a close relation with the professionals working at the pharmacy, I took my time to shorten that bond by valuing a personal counseling and care rather than a fast sale. With these ideals I promptly developed was I think to be a good capacity of adaptation and resolution of the most varied situations.

3.2. WEAKNESSES

3.2.1. LACK OF USEFUL IT KNOWLEDGE – SIFARMA 2000® COURSE

Right before the students of the IMDPS started their curricular internship, training on the software Sifarma 2000® was held. I keep in mind that this event did not live up to its expectations, due to the fact that it skips the basics and it plunged right into some of the advanced tools the program displays. Apart from having to learn most when I reached the pharmacy I felt I were not able to make use of the maximum potential of the program, as for example, to the implementation of personalized campaigns.

3.2.2. LACK OF KNOWLEDGE CONCERNING SOME PHARMACOTHERAPEUTICAL GROUPS

Only now that I have the experience at the counter I could have a better insight of the most common conditions and medicines in the everyday life of a pharmacy. Therefore I am now able to conclude that the pharmacotherapeutical groups that we are taught in the course should be rethought. On one hand the attention given between different groups does not match with the frequency these drugs are dispensed and on the other hand more recent medicines (which are common because doctors tend to prefer them) are not as mentioned as older and less sold medicines.

3.2.3. COUNSELLING IN OTC, HERBAL MEDICINES AND MEDICAL DEVICES

From my point of view, the biggest immediate difference a pharmacist can make is when counseling medication. This first requires the health sciences professional to be comfortable with relating symptoms and signals with health conditions so he can advise a product or send the costumer to seek medical attention. Secondly, when he diagnoses a condition for which he can advise a product, he needs to have a great knowledge of these three classes (OTC, herbal medicines and medical devices) so that he can select the most appropriate. After completing the course I am lead to consider that none of these know-hows are given the right attention, furthermore, a class where one of the groups is discussed (medical devices) is not mandatory and many students end up not taking it.

3.2.4. LACK OF COMMERCIAL AND SALES PREPARATION

Some of the skills I felt I had to do the biggest effort to catch up were the ones related to the commercial management and purchases of the pharmacy. In fact, most aspects are not even mentioned throughout the course and they are essential.

3.2.5. MANUAL PRESCRIPTION

Portugal was a pioneer in implementing the fully electronic prescription. Despite the contrasting opinions, I believe this action has mostly got advantages, however, with the simultaneous existence of both the old and the two most recent versions I felt a certain difficulty in avoiding mistakes in the manual prescriptions.

3.3. OPPORTUNITIES

3.3.1. DERMOPHARMACY AND COSMETICS

Every time I was confronted with cosmetic sales it was not so difficult to explore the composition and doing a proper counseling afterwards. Nevertheless, I reckon that students could only benefit even further if during classes we focused in the complete product lines of several brands so that we can get better be aware of this market. I would also like to reaffirm the importance it would have to develop even further this class of products since it was in my opinion a good area to explore cross selling.

3.3.2. VETERINARY PHARMACY

With a slow yet steady increase in the quality of life I foresee that differentiated economic areas that did not have much space in the market, might arise its popularity. One of these examples is the veterinary care. The population is more interested in having pets, there is an increasing awareness in the welfare of the animals and therefore pharmacists might play an

important role in this revolution of mentality if we can prove to become specialists and good counselors in the area.

3.3.3. CLINICAL PHARMACY

Clinical pharmacy is a branch of pharmacy that makes use of several tools to provide a personalized care to the patient and which makes the pharmacists as part of a health chain which is nearer from the people. In line with the previously explored thoughts (3.3.2.), there is nowadays a higher interest in promoting welfare rather than healing sicknesses, not just towards animals but also and mainly towards humans. This is another concept that pharmacist must grab to define with it the future of its profession and become an even more important link in the society.

Along the internship, I could gladly apply some of the concepts underlining Clinical Pharmacy, one of these examples is the elaboration of pill boxes as the one in the picture attached in the annexes. Other examples are the informative sessions held at the pharmacy that I could take part into, as the ones for pregnant and the elaboration of one presentation entitled "Give years to life", also referred in the annexes.

3.3.4. DIVERSITY OF PRODUCTS IN THE PHARMACY

Pharmacies have the privilege to dispense the solution for an innumerous amount of needs. Not only the ones related with illnesses but also with cosmetics, chemical products for diverse purposes, baby and animal care, nutrition, and so on. With this in mind, I believe that if a pharmacy invested in the differentiated products and training of its collaborators in different areas, its services would be more requested.

3.3.5. PRELIMINARY CURRICULAR INTERNSHIP

From my perspectives, the concept of an early curricular internship in community pharmacy would help students to better determine the necessities and aspects to improve while they are still carving their academic path. Also, this would likely provide a better background to several of the subjects taught in the last years of the course, making them more relatable and hence more interesting.

3.3.6. EMIGRANTS AND TOURISTS

The city of Pombal is located in an area of a strong emigration tradition, also tourists are somewhat common and when they are here, they look for similar products to the ones they are used to. These situations are the link between the pharmacies and the foreign languages and ultimately lead to the disclosure of the Portuguese pharmacies beyond borders.

3.3.7. COUNSELLING PROTOCOLS

From the already made protocols that I contacted with I realized their potential especially when someone is giving its first footsteps in counselling since they give hints and tips that can be transversal to several other situations. From my standpoint I also think they help an intern to adapt quicker to the working reality.

3.3.8. DRUG INTERACTIONS

Being the pharmacist the health professional of the drugs he is expected to deal with the most complex aspects of the same to a degree that no one else can. An example of the most common aspects would most likely be interactions drugs have with each other and with other products. However, this area is far from being easily relatable by its whole due to its immensity but still it needs to be mastered and in useful time. Databases as the free one at the website *drugs.com* which was explored in Pharmacognosy classes might provide the solution needed to do a correct counsel even in more complex situations.[2]

3.4. *THREATS*

3.4.1. *SELLING OF OTC AND HERBAL PRODUCTS OUTSIDE THE PHARMACIES*

The authorized selling of MNSRM outside the pharmacies as well as a range of shops where is possible to buy herbal products where the questions related to drug interactions are not even considered are a complicated issue that I have understood at the counter of the pharmacy and where pharmacists have an important role explaining carefully to the customers.

3.4.2. *OUT-OF-DATE INFORMATION IN THE PRESCRIPTIONS*

One of biggest threats I have felt at the counter in my internship is the fact that prescriptions present commonly out-of-date information, namely the one related to the maximum prices for each reimbursed drug. This may happened due to the fact that the prescriptions may be from months ago but this leads to some difficult situations and nobody explains to the patients what does these prices mean and most of the times they say they want to take the brand or the pharmaceutical laboratory they are used to and then they think we are not being honest with them because the price is higher of the one in the prescription. In spite of the fact that we explain them the reason, I felt some people do not really understand it.

3.4.3. *UNTRUSTWORTHY INFORMATION WITHIN THE REACH OF EVERYONE*

Many times people come to the pharmacy with ideas and names of products which they have read about it on internet. This situation may be complicated once people not always have the right information and most of the times, the places where they get the information are not reliable and may induce them in mistakes.

4. FINAL CONSIDERATIONS

To sum up all the considerations of my SWOT analysis of my final curricular internship in *Farmácia Barros*, I believe this was an outstanding and enriching experience which clearly made me grow and learn a lot to complete my course as a better and more versatile professional. I have the feeling of accomplished mission and personal and professional fulfillment for all the evolution that I felt during the internship.

All the team of *Farmácia Barros* was absolutely crucial for my evolution and to all of them I will be forever grateful for the sharing, availability and kindness that they have placed in my training.

The community pharmacist is a professional with a huge knowledge and who is always learning scientifically and at the level of a range of needed skills. I believe in the essential role of pharmacists and in the value they have in getting better people's lives because I had the privilege of experience this in my internship, dispensing drugs and other health products within the pharmaceutical ethics or even some times only by counselling or pharmaceutical services. All the four real situation explained in the annexes are example of what I have referred and experiences to learn with and to keep in mind as memories of an amazing final internship.

5. REFERENCES

1. **Farmácias Portuguesas – Como funciona o Cartão Saúda?** Available in https://www.farmaciasportuguesas.pt/sauda/como-funciona, accessed in 01-july-2017.
2. **Drugs.com – Know more. Be sure.** Available in https://www.drugs.com/, accessed in 03-july-2017.
3. **Boas Práticas Farmacêuticas de Farmácia Comunitária.** Available in http://www.ordemfarmaceuticos.pt/xFiles/scContentDeployer_pt/docs/Doc3082.pdf, accessed in 02-july-2017.

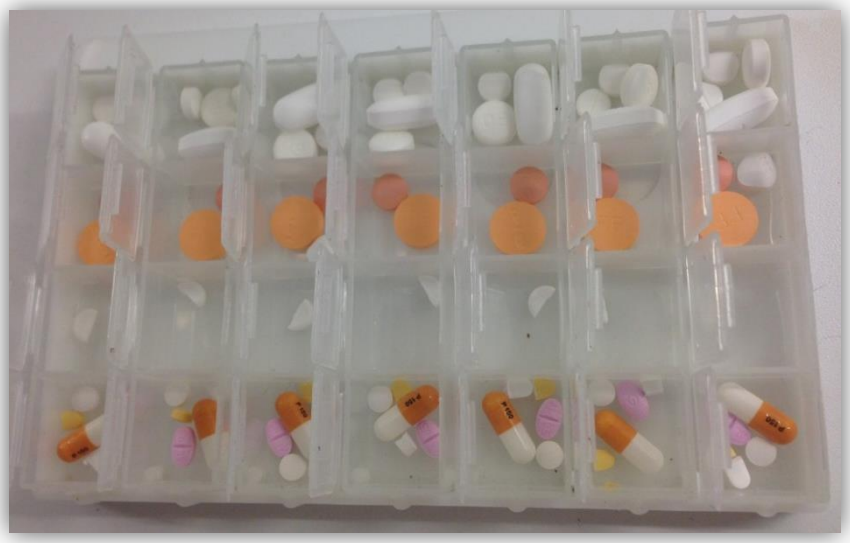
6. Annexes

REAL LIFE SITUATIONS

Situation 1

During the classes of Clinical Pharmacy in the 4th year of IMDPS, we have explored the role of pharmacists in the preparation of pill boxes for those patients who takes many different drugs and who are not able to take care of them by their own. In *Farmácia Barros* I had the chance to week after week follow the process of preparation of the pill box of a patient and to learn more about his therapeutic scheme:

Drug	Morning	Lunch	Afternoon break	Dinner
Valsartan 80mg	One			
Atorvastatin 10mg				One
Furosemide 40mg	One		Half	
Sertraline 50mg				Half
Alprazolam 0.5mg				One
Clopidogrel 75mg		One		
Etoricoxib 90mg	One – at lunch only at Wednesdays and Sundays			
Mirtazapine 15mg				Half
Pregabalin 150mg				One
Metformin 500mg	One			
Folic Acid + Iron, 1mg + 90mg		One		



Figures 3 – Photography of the patient pill box after being prepared for one week of medication.

Situation 2

In Pombal there are four pharmacies and the thus each one is on duty one week per month. In other words, in this specific week of the month, the pharmacy is open 24h per day, 7 days per week and it will be the pharmacy ready to all the needs of the population, namely prescriptions from hospital emergencies. In one of these weeks, at 22h it comes to *Farmácia Barros* a man with the follow prescription:

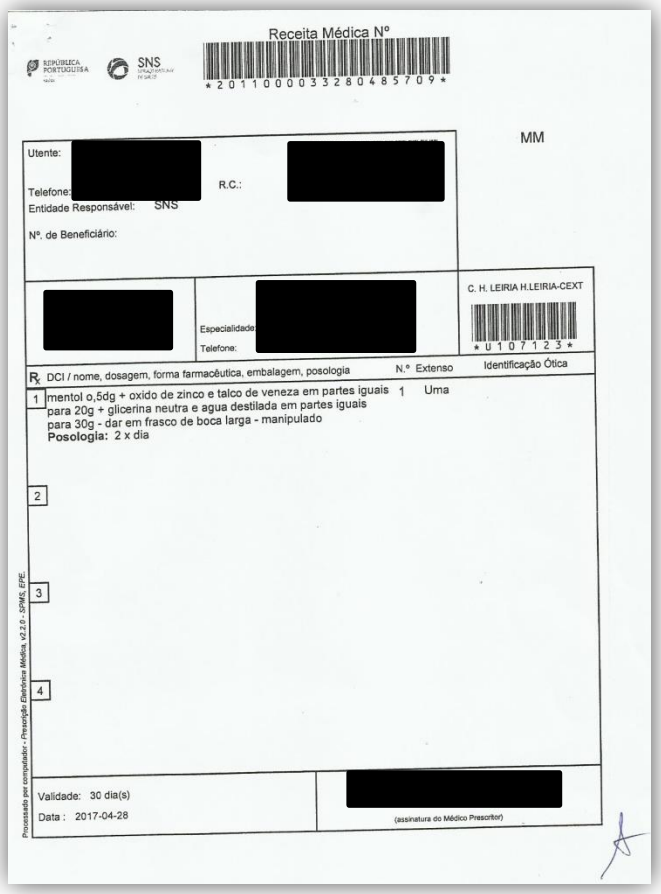
Prednisolone, 20mg, 20 tablets
Bilastine, 20mg, 20 tablets
Betamethasone + Fusidic acid, 1mg/g + 20mg, 15g of tube cream
Ofloxacin, 200mg, 16 coated tablets

I have realized that in the pharmacy there were all the drugs of the prescription with exception of the antibiotic in these specific dosage and presentation. There were no other near pharmacies to buy this antibiotic and sending from the supplier would take some hours, until the next delivery in the following morning. The solution founded for this situation was to call for the Emergency Service of the Hospital of Pombal and ask to talk with the doctor of the referred prescription in order to understand if the patient had already taken intravenous antibiotic in the hospital and he could wait for the ofloxacin next morning or if the doctor would prefer to prescribe another antibiotic instead. The doctor was very approachable and he showed up to be ready to help. He said the patient was already taken intravenous antibiotic in the hospital and once the patient get started to take the other three drugs, he could wait until the next morning to take the antibiotic. Thus, the ofloxacin was ordered from the supplier and in the next morning the patient started to take it.

Situation 3

The compound drugs made at the laboratory of the Pharmacy represent one wonderful solution for those situations in which there is no a drug in the market that fixes completely in the situation. Therefore, the doctor will prescribe an amount of components and the pharmacist must be able to prepare it according to the Good Pharmaceutical Practices for Community Pharmacy[3]. During my internship I had the opportunity to learn more about the preparation and also to prepare different pharmaceutical formulations in the Pharmacy's laboratory, which is very well equipped and thus gave me the chance to improve my knowledge in the production of compound drugs and the regulatory aspects related. One example of a compound drug which I learned how to prepare was the Paste of Menthol Water and Zinc Oxide which is used as adjuvant healing and restoring/soothing of the skin. The pharmacist need to be able to understand that the receipt is for a compounding drug, to write the compounding plan accordingly to the proper bibliography and to elaborate the label of the compounding drug with all relevant information. The prescription, the compounding plan used while it was prepared as well as the label are presented below:

Doctor's prescription to the Paste of Menthol Water and Zinc Oxide:


Receita Médica Nº

20110000332804857 09

MM

Utente: [redacted]

Telefone: [redacted] R.C.: [redacted]

Entidade Responsável: SNS

Nº. de Beneficiário:

[redacted] Especialidade: [redacted]

Telefone:

C. H. LEIRIA H.LEIRIA-CEXT

U107123

Rx DCI / nome, dosagem, forma farmacêutica, embalagem, posologia	N.º Extenso	Identificação Ótica
1 mentol o,5dg + oxido de zinco e talco de veneza em partes iguais para 20g + glicerina neutra e agua destilada em partes iguais para 30g - dar em frasco de boca larga - manipulado **Posologia:** 2 x dia	1 Uma	
2		
3		
4		

Validade: 30 dia(s)

Data : 2017-04-28

[redacted]

(assinatura do Médico Prescritor)

Processado por computador - Prescrição Eletrónica Médica, v2.2.0 - SPMS, EPE

Compounding plan of the Paste of Menthol Water and Zinc Oxide:

Farmácia Barros

Prop. e Dir. Técnica Dr. Carlos Manuel P. Oliveira
Tel.236 212 037
Largo das Almas 3100-446 Pombal

Ficha de Preparação

"Pasta de Mentol – 0.05g; Óxido de zinco e Talco de veneza em partes iguais – 20g; Glicerina e Água purificada em partes iguais para 30g"

Forma farmacêutica: pasta **Data de Preparação:** 03/05/2017

Número de Lote: 1417 **Quantidade a preparar:** 30g

Matérias-primas	Nº do lote	Origem	Farma-copeia	Quantidade para 100g (ou ml, ou unidades)	Quantidade calculada	Quantidade pesada	Rubrica do Operador e data	Rubrica do Supervisor e data
Mentol	151237-I-1	Acofarma		0.166g	0.05g	0.05g	03.05.17	03/05/17
Óxido de Zinco	42_16	José Manuel Gomes dos Santos, LDA.		33.33	10g	10g	03.05.17	03/05/2017
Talco de veneza	161589-P-2	Acofarma		33.33	10g	10g	03.05.17	03/05/2017
Glicerina neutra	15310	Laboratórios GSL		16.83	4.975	4.8	03.05.17	03/5/2017
Água purificada	0004.2016	José Manuel Gomes dos Santos, LDA.		16.83	4.975	4.8	03.05.17	03/05/2017

Preparação

Técnica	***Rubrica do operador***
1. Limpar a hélice do agitador mecânico com água destilada, secando-a, em seguida, com papel absorvente.	
2. Verificar o estado de limpeza de todo o material a utilizar.	
3. Pulverizar o mentol em almofariz de porcelana, pesar 0.5g e transferir para o almofariz de vidro.	
4. Pesar o óxido de zinco e o talco e adicionar ao almofariz de vidro e misturar.	
5. Transferir a mistura obtida em 4. para o recipiente unguator e dispersa-la na glicerina.	
6. Adicionar a água e misturar tudo no unguator.	
7. Fechar o recipiente e rotular.	
8. Lavar e secar o resto do material utilizado.	

Rubrica do Director Técnico:	Data:
	03.05.2017

Farmácia Barros

Prop. e Dir. Tecnica Dr. Carlos Manuel P. Oliveira
Tel.236 212 037
Largo das Almas 3100-446 Pombal

Embalagem

1. Embalar a suspensão em recipiente opaco.

Material de embalagem	Nº de lote	Origem
Embalagem unguator	7309/23 P25	Plural

Capacidade do recipiente: 50g Operador:

Rotulagem

1. Proceder à elaboração do rótulo de acordo com as normas de Boas Práticas de Farmácia
2. Anexar a esta ficha de preparação uma cópia, rubricada e datada, do rótulo da embalagem dispensada.

Operador:

Verificação

Ensaio	Especificações	Resultado	Rubrica do Operador
1. CARACTERÍSTICAS ORGANOLÉPTICAS			
1.1 Côr	côr branca	Conforme	
1.2 Odor	mentol	Conforme	
1.3 Aspecto	homogéneo	Conforme	
2. CONFORMIDADE COM A DEFINIÇÃO DA MONOGRAFIA "PREPARAÇÕES SEMI-SÓLIDAS PARA APLICAÇÃO LOCAL" DA FPVIII	**CONFORME A DEFINIÇÃO DA MONOGRAFIA "PREPARAÇÕES SEMI-SÓLIDAS PARA APLICAÇÃO LOCAL" DA FPVIII**	Conforme	
3. QUANTIDADE	30g (+/- 5%) (quantidade a preparar)	Conforme	

APROVADO ☑ ***REJEITADO*** ☐

Supervisor 03/05/17

Nome e morada do doente

[redacted]

Nome do prescritor

[redacted]

Rubrica do Director Técnico:	Data: 03-05-2017

Farmácia Barros

Prop. e Dir. Técnica Dr. Carlos Manuel P. Oliveira
Tel.236 212 037
Largo dos Almas 3100-446 Pombal

Cálculo do preço de venda

Matérias-primas:

Matérias-primas	Embalagem existente em armazém		Preço de aquisição de uma dada quantidade unitária (s/iva)		Quantidade a usar	Factor multiplicativo	Preço da m.p utilizada na preparação
	Quantidade adquirida	Preço de aquisição (s/IVA)	Quantidade unitária	Preço			
Mentol	5g	1.65€	1g	0.33€	**0.05g**	2.5	**0.041€**
Óxido de Zinco	100g	1.99€	1g	0.019€	**10g**	1.9	**0.361€**
Talco de veneza	100g	0.95€	1g	0.095€	**10g**	1.9	**1.805€**
Glicerina neutra	60ml	0.48€	1ml	0.008€	**4.98g**	2.5	**0.099€**
Água purificada	1L	0.75€	1ml	0.00075€	**4.98g**	2.5	**0.009€**
						Subtotal A	**2.32€**

Honorários de Manipulação:

	Forma farmacêutica	Quantidade	F(€)	Factor multiplicativo	Valor
Valor referente à quantidade base	Pasta	**30g**	**4,92€**	X **4.5**	= **22.14€**
Valor adicional					
				Subtotal B	**22.14€**

Material de embalagem:

Materiais de embalagem	Preço de aquisição (s/ IVA)	Quantidade	Factor multiplicativo	Valor
Embalagem Unguator	**1,85€**	X **1**	x 1,2	= **2.22€**
			Subtotal C	**2.22€**

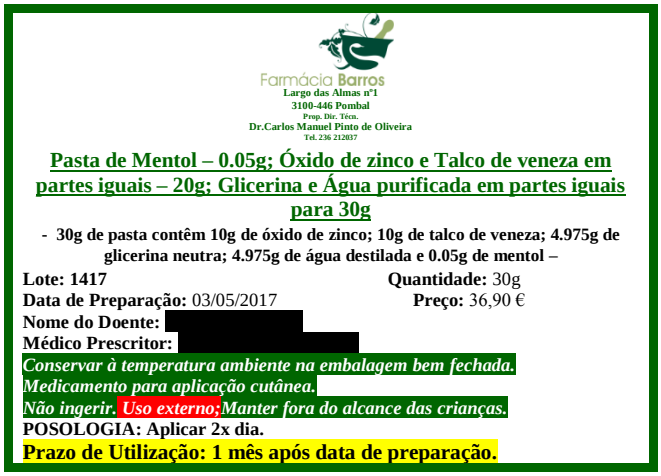
PREÇO DO MEDICAMENTO MANIPULADO: 1,3 x (A+B+C)	**34.68€**
+ IVA	6%
PREÇO FINAL =	**36.90€**

OPERADOR

SUPERVISOR

Rubrica do Director Técnico:	Data: 05-05-2017

Label of the Paste of Menthol Water and Zinc Oxide with all the relevant information to the patient:



Farmácia Barros
Largo das Almas nº1
3100-446 Pombal
Prop. Dir. Técn.
Dr.Carlos Manuel Pinto de Oliveira
Tel. 236 212027

Pasta de Mentol – 0.05g; Óxido de zinco e Talco de veneza em partes iguais – 20g; Glicerina e Água purificada em partes iguais para 30g

- 30g de pasta contêm 10g de óxido de zinco; 10g de talco de veneza; 4.975g de glicerina neutra; 4.975g de água destilada e 0.05g de mentol –

Lote: 1417 **Quantidade:** 30g
Data de Preparação: 03/05/2017 **Preço:** 36,90 €
Nome do Doente: [redacted]
Médico Prescritor: [redacted]

Conservar à temperatura ambiente na embalagem bem fechada.
Medicamento para aplicação cutânea.
Não ingerir. Uso externo; Manter fora do alcance das crianças.
POSOLOGIA: Aplicar 2x dia.
Prazo de Utilização: 1 mês após data de preparação.

Situation 4

The metabolic syndrome is one of the biggest issues on nowadays people's health. The combination of several diseases that lead to the correspondent parameters of metabolic syndrome is much more common in current times due to unbalanced diets (rich in salt and fat) and sedentary lifestyles. Within of the scope of the month of May of 2017 as the month of the heart it was asked me to prepare a presentation entitled "Give years to life" related to the theme of the Metabolic Syndrome and the non-pharmacologic ways of avoiding it, both in food and physical exercise even for the elderly and the people with reduced mobility. I believe the pharmacists as an important role in these actions as they are near the population and most of the times the pharmacy is the better place where they can receive information about these topics and not only about drugs, once that the chain of pharmacies allows an approximation to the population and a place of health within the reach of all people.

Part III – Final Thesis

"Impact of surface charged nanostructured carriers in drug delivery and targeting"

Acknowledgements:

To the Professor Eliana Souto, PharmD. for her mentoring and constant availability comprehension and priceless help.
To the Doctor Ana Rita Fernandes for explaining me everything she knew and to
To the Faculty of Pharmacy of the University of Coimbra for the professional shaping these past five years, all its professionals and important people in my path,
To my family and friends for all the support and for helping me to give always more of myself,

To all, my sincere thank you.

Abstract

One of the biggest challenges pharmaceutical technology is dealing nowadays is the targeted delivery of drugs directly to the site of action, with the aim to increase therapeutic effectiveness and decrease adverse side effects. Nanotechnology has a decisive role while meeting these demands, namely with the development of nanostructured carriers several advantages may arise from their use. Nanostructured carriers represent drug delivery systems with a promising approach to obtain a drug formulation with the intended pharmacokinetic and pharmacodynamic profiles. Nevertheless, nanostructured carriers' features as size, shape and surface charge will dictate the biodistribution of the drugs among the different organs. The study of these properties is therefore a priority, with the main goal of understanding their impact on the role of the nanostructured carriers and its consequences. This chapter focuses on the impact that the surface electrical charge of the nanostructured carriers has in drug delivery and targeting. As an example for understanding the importance of the design of nanoparticles for overcoming biological barriers to drug delivery, several studies have shown that different surface charge – positive, negative or neutral – may be the key element to understand why some drugs can cross the blood-brain barrier while others cannot. Or even how this property influences opsonisation, circulation life-time and interaction with endogenous molecules. Studying the impact of the surface charge on the profile and performance of nanoparticles, is therefore crucial to develop an effective drug formulation since this property has an obvious impact on the transport, delivery and release profile of the loaded drug.

Keywords: Nanostructured carriers; surface charge; opsonisation; biodistribution; drug delivery; drug targeting.

Resumo

Um dos maiores desafios que a tecnologia farmacêutica enfrenta nos dias de hoje diz respeito à cedência de fármacos directamente no local de acção, com o objectivo de aumentar eficácia terapêutica dos mesmos e diminuir os seus efeitos secundários. A nanotecnologia tem um papel decisivo na resposta a estas exigências, nomeadamente com o desenvolvimento de transportadores à escala nano, com as muitas vantagens e avanços que advém desta utilização. Transportadores nanoestruturados representam sistemas de cedência de fármacos com uma abordagem promissora para obter formulações de fármacos com o perfil farmacocinético e farmacodinâmico desejado. Contudo, as características dos transportadores nanoestruturados, como o tamanho, forma ou carga eléctrica de superfície irão definir a biodistribuição dos fármacos pelos diferentes órgãos. Desta forma, o estudo destas propriedades é uma prioridade, com o principal objectivo de entender o impacto destas no papel dos transportadores nanoestruturados e suas respectivas consequências. O presente trabalho disserte o impacto que a carga de superfície dos transportadores nanoestruturados tem ao nível dao direccionamento e cedência dos fármacos. Muitos estudos têm mostrado que a carga eléctrica de superfície – positiva, negativa ou neutra – pode ser o elemento chave para entender o porquê de alguns fármacos conseguirem ultrapassar a barreira hematoencefálica e outros não. Esta propriedade influencia a opsonização, tempo de circulação e interação dos fármacos com moléculas endógenas. Assim, o estudo do impacto da superfície de carga no perfil e desempenho das nanopartículas, respectivamente nos transportadores nanoestruturados e independentemente da natureza destes, é crucial no desenvolvimento de novas formulações de fármacos.

Palavras-chave:

Transportadores nanoestruturados; carga de superfície; opsonização; biodistribuição; entrega de fármacos; vetorização de fármacos.

Abbreviations

BBB	Blood-brain barrier
NP	Nanoparticles
SLN	Solid Lipid Nanoparticles
NLC	Nanostructured Lipid Carriers

1. Introduction

Pharmaceutical nanotechnology is an area of the science which is under continuous development, where technological advancements have to combine advantages of drug delivery and targeting, with the aim to improve current therapeutic approaches.

In the last decades, breakthrough in nanotechnology has created a range of new opportunities in the biomedical field, allowing the development of more effective tools for therapy and diagnostics (Cole and Holland 2015).

The recognised value and important outcomes of nano-sized strategies in pharmaceutical technology reasons the investment and efforts, attributed to variations in kinetics, modified drug distribution and release profile of drugs (Jaiswal, Gidwani et al. 2016). The role of nanotechnology in drug delivery and targeting is thereby a crucial element to understand how far the risk-benefit ratio of combining it with medicines is well balanced. Nanometrology, i.e. the measurements at nanoscale, as well as relevant legislation, also needs to be improved to offer consistency of the work being published worldwide, which ultimately ends in improved safety (Dini, Panzarini et al. 2015).

The need to develop new drugs, and the improvement of the existing ones, is related to the existence of a range of diseases which still do not have efficient treatments and ways of diagnosis. The scientific and technological advances have allowed to overcome this problem with the constant search for new solutions, among which a range of opportunities arise from the work at nanoscopic scale (Creech, Wang et al. 2017), including site-specific drug delivery (Hsieh, Wen et al. 2014). Indeed, a successful therapy is correlated with the design of drug delivery which must allow reaching an optimized concentration of the drug at the targeted site with the view of increasing effectiveness and decreasing adverse side effects (Souto 2010).

Biological systems are nevertheless very complex and there are many factors affecting the effectiveness of a therapy or even of a method of diagnosis. While nanoparticles have been exploited for personalized medicine (Cole and Holland 2015), several limitations have also been encountered not only because there are many types of non-toxic nanomaterials available (Palombo, Deshmukh et al. 2014), but also their distribution is very much dependent on the nanomaterials surface properties (Amin, Joo et al. 2015).

One of the challenges encountered in the development of new nanoformulations is to identify the nanoparticles properties that may influence the drugs' performance and thereby the therapeutic outcome.

Blanco et al. suggested that the main features which influence the rational design of nanoparticles to overcome biological barriers are the particle size and shape, surface charge, deformability and degradability (Blanco, Shen et al. 2015). There is a special emphasis on surface modifications since these will determine the interactions with the cells of the organism and consequently the effective delivery of the drugs at the targeted site (Amin, Joo et al. 2015). Surface charge represents the feature with higher influence on cellular uptake and cytotoxicity of nanoparticles used in medicine (Frohlich 2012).

This review will focus on the impact of the surface electrical charge of nanostructured carriers in drug delivery and targeting with an approach of global recent developments in the area. How this specific feature has an important role in the therapeutic efficiency of drug-loaded nanoparticles and in which way engineered nanoparticles can improve their performance will also be addressed.

2. Nanostructured carriers for drug delivery

Conventional drug formulations exhibit not only problems related to pharmacokinetic limitations, but also barriers to overcome the poor solubility, permeability and bioavailability of many usual and very useful drugs (Szabo and Zelko 2015). This has been one of the greatest challenges of pharmaceutical industry in the last years and the reason why nanoparticle based drug delivery platforms have strongly being investigated as suitable vehicles to overcome the identified limitations (Blanco, Shen et al. 2015). Nanostructured carriers have emerged in order to solve the mentioned problems and to present new possibilities for drugs that might have not been used (or at least without their maximum potentiality) and even to use them by different administration routes.

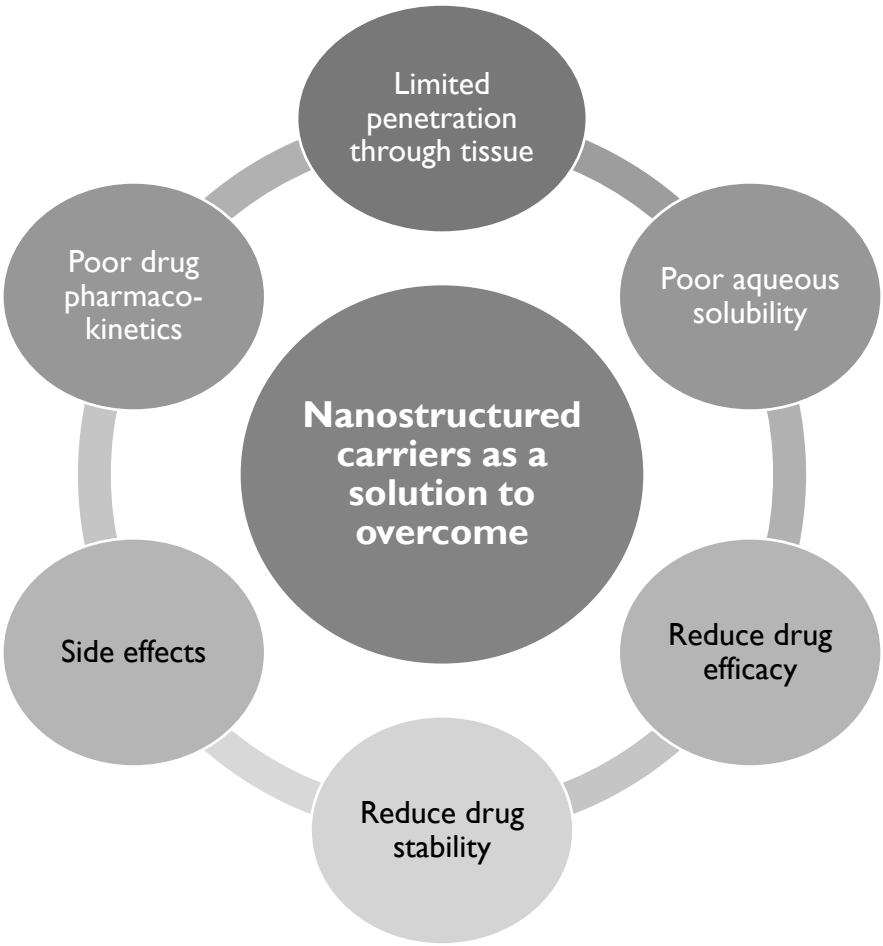


Figure 1: Identified limitations of classical drug therapy expected to be overcome by the use of nanostructured carriers. Adapted from (Soliman 2017).

Engineering nanostructured carriers with specific physicochemical properties will allow combining different options of nanomaterials and designs with high biodegradability and biocompatibility (Bilia, Guccione et al. 2014). Figure 2 summarizes the main properties nanostructured carriers should have to overcome biological barriers to achieve a therapeutic effect.

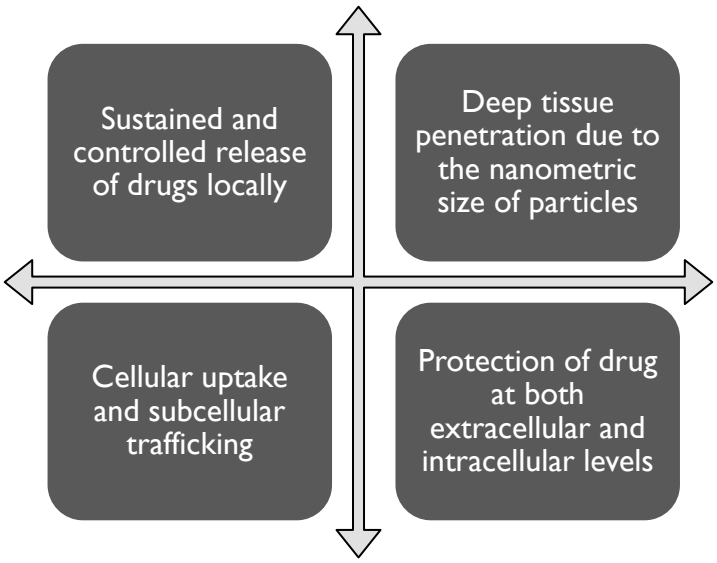


Figure 2: Main properties of nanostructured carriers to overcome biological barriers for targeted delivery of drugs. Adapted from (Bilia, Guccione et al. 2014).

Besides the targeted delivery of drugs, imaging and diagnosis are also tools influenced by the surface properties of the carriers and have been exploited for: (i) early cancer diagnosis (Liu, Miyoshi et al. 2007), (ii) assessment of real-time treatment (Rowland, Noe et al. 2012), (iii) site-specific drug delivery at high concentration (Lee and Wong 2011); (iv) mutations detection (Youns, Hoheisel et al. 2011), (v) identification of new targets for clinical research much faster and prediction of drug resistance (Heidel and Davis 2011).

Different types of nanostructured carriers have been developed depending on the type of nanomaterials, selected according to the properties of the drug to be loaded (Figure 3).

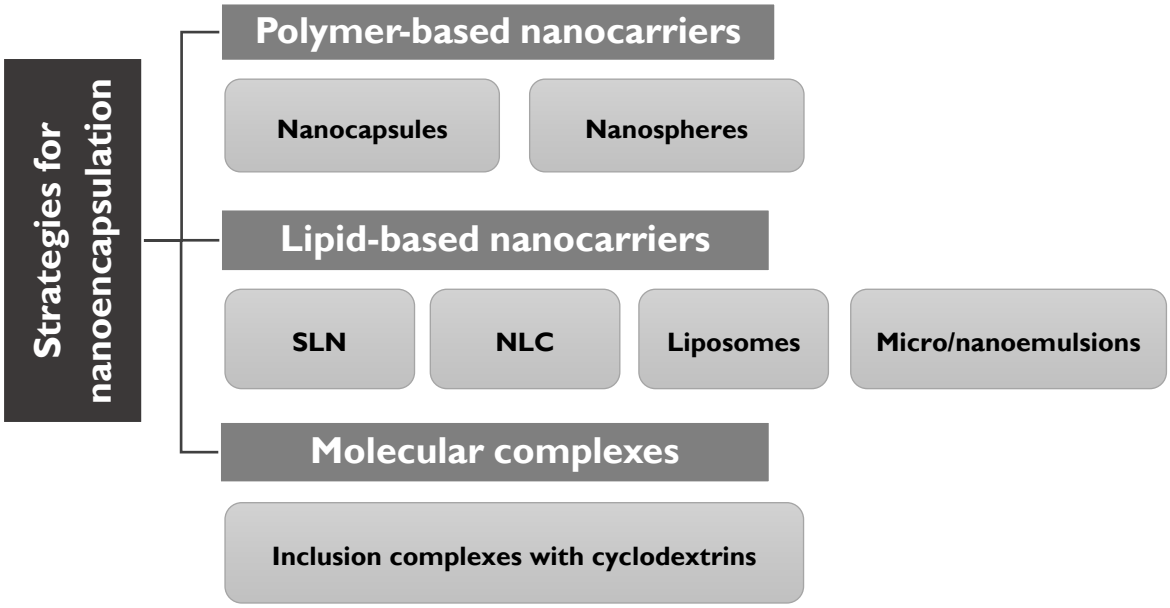


Figure 3: Examples of nanostructured carriers based on the type of nanomaterial. Adapted from (Bilia, Guccione et al. 2014).

The distinct nanosized drug delivery systems which present a functionality-related stability are considered according to the different chemical nature of the carrier of the drug molecules. As shown in Figure 3, polymeric nanoparticles include nanocapsules and nanospheres; the former are reservoir-based nanoparticles composed of an inner liquid phase surrounded by a polymeric layer controlling the release, whereas the latter are matrix-based nanoparticles from which the drug is released mainly by diffusion. Polymeric nanoparticles demonstrate feasibility of surface functionalization and a high level of control over characteristics (Szabo and Zelko 2015). Lipid nanoparticles have become interesting carriers for loading and delivery of lipophilic, poorly water soluble drugs (Teixeira, C. et al. 2017). These comprise solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), liposomes and nano and micro emulsions. SLN are characterized by the presence of a solid lipid with a melting point above 40°C, creating a lipid matrix similar as that of polymeric nanoparticles with the capacity to modify the release profile of loaded drugs and low acute/chronic toxicity (Doktorovova, Souto et al. 2014, Doktorovova, Kovacevic et al. 2016). NLC are composed of a mixture of solid lipid and liquid lipid (oil) that enhance the loading capacity and thermodynamic stability during storage (Souto, Mehnert et al. 2006, Souto and Doktorovova 2009). Microemulsions are composed of a mixture of oil, water and

surfactant in appropriate ratios that can be spontaneously formed without input of energy (Boonme, Junyaprasert et al. 2009). These systems have a high solubilizing power, offering the capacity to be loaded with lipophilic, hydrophilic, as well as amphiphilic drugs, and create high drug concentration gradient. Nanoemulsions are non-equilibrium systems, which properties depend not only on their composition but also on the preparation method. The droplet size of a nanoemulsion is typically within the range 20–200 nm.

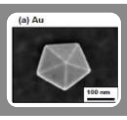
Inclusion complexes are based on the formation of host-guest complexes between the drug and the cyclodextrin, with the aim to depict the desired physicochemical properties and pharmacokinetic profile, without having a decrease of the safety of the drugs (Adeoye and Cabral-Marques 2017).

Nanostructured carriers are classified in a range of different types depending on specific criteria which mainly focus on the nature of the materials. Regardless the type of novel chemical, physical and biological properties in consequence of their reduced particle size once at least one dimension is about 1–100 nm (Safari and Zarnegar 2014).

Figure 4 shows an approach to classify different nanostructured carriers used for drug delivery and targeting. Depending on their nature and characteristics, nanostructured carriers may be more likely to load and delivery some kind of drugs and even to target those to some specific tissues and thus be used to the treatment of certain diseases. Considering the huge range of possibilities of nanostructured carriers available, the question of toxicity is a key aspect for their use in delivery and targeting. For example the carbon nanotubes, one of the recent developments in nanotechnology, besides being an attractive system with extraordinary mechanical, electrical and surface properties, they show cytotoxicity problems. To overcome this question, and the challenge of the insolubility of carbon nanotubes in most types of solvents, they usually need to be functionalized (Li, Song et al. 2014).

Inorganic Nanoparticles

Metal oxide particles or the particles which possess at least one metallic composition at nanoscale. Metallic and silica nanoparticles are examples of subtypes of inorganic nanoparticles.



Metallic Nanoparticles

Metal elements at nano range. Example of gold nanoparticles and magnetic nanoparticles. Their safety is still under discussion due to their toxicity.

Mesoporous silica systems

Show a great surface area and pore size with well-known surface properties, which make them desirable options for encapsulation of drugs.

Polymeric Nanoparticles - Nanospheres

Matrix-type solid nanoparticles. Drugs can be dissolved, dispersed, chemically bound or absorbed onto the polymeric matrix.

Polymeric Nanoparticles - Nanocapsules

Nano-vesicular nanoparticles in which drugs are enclosed within a cavity, surrounded by a polymeric membrane or coating. Drugs can be in solid or in liquid form.

Polymeric micelles

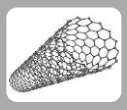
Unique amphiphilic properties with a core-shell structure.The inner core has hydrophobic properties which offers the opportunity to dissolve lipophilic drugs.

Liposomes

Spherical shaped artificial vesicles which are produced by natural non-toxic phospholipids and cholesterol. Their properties may be changed by their lipid composition, size, surface charge and preparation method.

Dendrimers

Monodisperse symmetric macromolecules with highly branched structures around an inner core. Due to their non-polar cavities, they can encapsulate hydrophobic drug molecules.



Carbon nanotubes (CNTs)

Attractive systems because of their excellent mechanical, electrical and surface properties. They need to be functionalised because of their insolubility in most types of solvents with risk of cytotoxicity effects.

Nanocrystals

Molecule aggregates which comprise the crystalline drug. They are especially used for poorly soluble drug molecules.

Hydrogels

Three-dimensional hydrophilic networks which are formed chemically or physically. They have some properties which must be optimised such as safety, biodegradability, drug loading capacity and drug-release kinetics.

Figure 4: Classification of nanostructured carriers used for drug delivery and targeting. Adapted from (S Caban 2014).

The advantages of the use of nanostructured carriers in drug delivery and targeting are manifold. As therapeutic materials they reveal great improvement in increasing the solubility, enhancing dissolution rate and increasing the bioavailability of drugs (Gaba, Fazil et al. 2015). These advantages have been already proven in distinct areas of therapeutics, namely some of those which do not have great solutions until now and which are critical in public health such as antibiotic resistance or chemotherapy (Jaiswal, Gidwani et al. 2016).

In specific diseases, as brain and retinal diseases, nanoparticles have concrete advantages as their ability to pass through biological barriers, namely the blood-brain barrier (BBB) or the blood-retinal barrier (BRB); these nanoparticles also help therapeutic drugs to stay more time in target organs and thus to reach better therapeutics' results, once the physical and chemical properties of the nanostructured carriers enhance the bioavailability of therapeutic agents both in local and systemic administration. Still increasing to this fact, it is the proved action of the nanoparticles as presenting therapeutic actions by themselves, as for example cerium oxide nanoparticles (nanoceria) which have shown to induce and anti-inflammatory effect only by themselves or some inorganic nanoparticles as gold, silica or silver nanospheres have also revealed "self-therapeutic" effect without having previously suffered any surface modification (Jo, Kim et al. 2015).

To sum up and bearing in mind the idea of the nanoscale level and the critical changes on the properties of the materials at this scale, as consequence of the increase of the surface-to-volume ratio and the quantum effects due to the electronic structure of nanoparticles being less visible and overlapping than in bulk materials (de Villiers P 2008),this way the pharmaceutical technology achieve the possibility to better control:

1. The delivery and targeting of therapeutic agent to the organs and tissues in a more specific and controlled way (Rowland, Noe et al. 2012);
2. An increase of the stability and a decrease of the toxicity of the drugs, driving to a biochemical protection of the these in the human body (Mishra, Hubenak et al. 2013);
3. The decrease of the adverse effects of the drugs once it is less probable the interactions of these with the biological surroundings (Rowland, Noe et al. 2012);
4. Long circulation times, as it will be better understanding in point 3.3, with an increase of the drug lifetime in the circulation (Mishra, Hubenak et al. 2013);
5. Biological degradation of the nanoparticles (Mishra, Hubenak et al. 2013);
6. More effective production of the drugs, with easier and not so expensive methods (Gaspar, Aksu et al. 2012);

7. An increase of the storage lifetime as consequence of the increase of the stability of the compounds (Duncan and Gaspar 2011).

All the advantages presented related to the production and development of nanostructured carriers for therapeutic agents make these technological formulations an outstanding solution to improve the efficacy and safety of drugs and a great technological advancement in the biomedical field (Demetzos and Pippa 2014).

3. Surface properties and drug delivery

A range of characteristics of the nanostructured carriers will influence their absorption and distribution of the loaded drugs. Some of these features are size, shape and the inevitable surface charge. This is why the principles of nanoparticle design are so important to overcome the limitations of low site-specific accumulation of drug, specifically at diseased sites due to the biological barriers (Blanco, Shen et al. 2015).

A large number of studies are focused on understanding the impact of the physicochemical properties in the therapeutic effects, also always bearing in mind tissue-specific microenvironments (Jo, Kim et al. 2015).

3.1. Physical properties

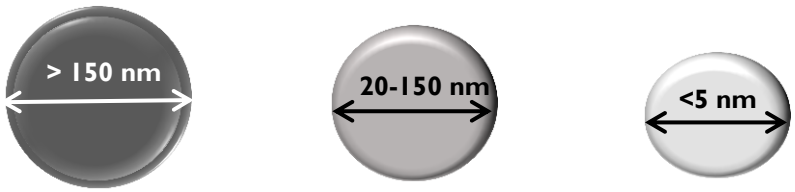
3.1.1. Size

The size of the nanostructured carriers is one of the features which make them completely distinct from a range of other pharmaceutical formulation studied before and this is why the impact of the changes in this characteristic is deeply analyzed. The compounds considered at a nano scale are those with features in the colloidal size range, in other words, with the size between 0.1 nm and 500 nm/ 1 μm (Eliana B. Souto 2007). However regarding systemic administration for therapeutic purposes, it is considered suitable the size of nanoparticles in the range between 2 and 200 nm (Jo, Kim et al. 2015).

The evaluation of the size is essential after the production of the carriers at nano-scale. This is commonly done using developed techniques as Dynamic Light Scattering (DLS) which allow to proceed to the detection of very low particle's size and even to analyze the polydispersity index (PDI). It is one essential feature of the stability and characterization of the particles and where the big goal is to achieve high monodispersed amounts of nanoparticles (Fangueiro, Gonzalez-Mira et al. 2013).

The size of the carriers which transport the therapeutic agent is related to the circulation half-lives of drugs (directly related to their effectiveness), the macrophages uptake of those and to the extravasation through leaky set of blood vessels. All this features will influence the capability of the carriers to deliver the drugs in the right place and during the enough time they are needed to have their action (Blanco, Shen et al. 2015).

Beside the importance of the size in the effectiveness of the nanostructured carriers, there is no a direct proportion between the size and the biodistribution of the drugs they transport once it depends on the therapeutic target (Blanco, Shen et al. 2015). Nevertheless, the size has direct consequences on the in vivo fate of drug-loaded nanoparticles (Figure 5).



> 150 nm	20-150 nm	< 5 nm
More nanoparticles are entrapped within the liver and spleen, as well as in the capillaries of the lungs.	**Nanoparticles extravasate through vascular fenestrations of tumors and escape filtration by liver and spleen.**	**Small-sized nanoparticles are readily filtered out by the kidneys.**

Figure 5: Influence of the size on the in vivo fate of drug-loaded nanoparticles.

A small particle size makes it able to escape of the removal procedure of lungs, liver, spleen and kidneys in order to can reach the target tissues. Nevertheless, if the nanocarriers are too small they will be vulnerable to suffer the action of the renal excretion or the procedure of clearance from the target tissues without having time to have their therapeutic action. Thus, it is essential to study the relation between the size of the nanocarriers and the impact of this in the effectiveness of the targeting and delivery of the drugs transported (Jo, Kim et al. 2015).

3.1.2. Shape

As happens with the size, also the shape has been found to be responsible for the interactions with the cells, the biodistribution and nanocarriers' fate, and the cellular uptake too (Champion, Katare et al. 2007). The existent range of achievable architectures and geometries of the nanoscale carriers turn possible to have distinct flow characteristics with obviously effects on pharmacokinetics and biodistribution of the drugs among the targeted organs (Blanco, Shen et al. 2015). The kinetics of internalization and efficacy of the nanostructured carriers used to deliver and target the therapeutic agents are also dependent of the local surface curvature of these (Amin, Joo et al. 2015).

The range of techniques to produce nanoscale particles, namely novel top-down and bottom-up techniques allow to structure the nanoparticles with the desirable shape and the one more appropriated to the target they are built to (Blanco, Shen et al. 2015). The referred techniques make it possible to produce nanoparticles with a range of shapes that comes from the sphere, the cube, rod or pyramid, until rectangular and triangular nanoplate, octahedron or icosahedron (Jo, Kim et al. 2015).

Blanco et al. in "The Principles of nanoparticle design for overcoming biological barriers to drug delivery" suggested that cylindrical and discoidal shapes also are achievable at nanoscale and it is the discoidal shape which allows to reach greater biodistribution in lungs, liver and spleen. At the level of the kidneys, being the shape of the nanoparticles spherical, cylindrical or discoidal, do not have any impact at the distribution of the drugs.

The zero-order release of pharmacokinetics of the nanoparticles was shown to be achieved when hemi-spherical particles are used (Hsieh, Rhine et al.).

Also interesting is the fact that hemodynamic forces, in other words, the forces in the blood circulation which nanoparticles found in the human body, are fluid resistant forces that act against the movement of the nanostructured particles in blood and this forces are proportional to the shape of nanoparticles if the Reynolds number of the fluid is moderate to high (Decuzzi, Pasqualini et al. 2008).

These findings were possible due to recent advances in computational modeling studies and also experimental studies using microfluidic devices which allow to understand the impact of the design of the drugs' carrier systems, specifically the shape and thus realize the benefits and challenges which arise from this information. Also some biological compounds from the cells help to improve the design of the nanocarriers giving some natural cues of the in vivo interactions which are more likely to happen (Sen Gupta 2016).

Deformability of the nanostructured carriers is defined as the ability of these to change their shape in a way to adapt and pass through narrow spaces. Thus, it is an essential feature to improve the mass penetration of the drugs, as an example the penetration through the skin without disrupting the lipid barrier. Studies have proven that nanoparticles with high deformability tend to have more effectiveness than the conventional liposomes in delivering and 6/targeting drugs in some diseases (Morilla and Romero 2016).

Some authors have taken their efforts to analyze the effect of the variation on the stiffness of the nanostructured carriers on the biodistribution and circulation times of the drugs. They have found that rigid nanoparticles are more likely to be easier cleared by the human body. Although it is possible to design for example nanogels with desirable rigidity taking advantage of zwitterionic monomers and cross-linkers, which have shown to turn the nanoparticles less rigid and more predisposed to deformability and consequently it has improved the circulation lifetimes and decreased the accumulation of the drugs in organs as the spleen (Cui, Bjornmalm et al. 2014).

Also the biodegradability of the systems to deliver drugs is a key to the development of these once that the stability of the particles has a critical role on the fate of the therapeutic agents. In spite of the fact that the older nanoparticles used for drug delivery and targeting were usually composed by lipids or polymers, the novel materials developed to build this nanostructured carriers are now more likely to create degradation components not harmful for the organism (Blanco, Shen et al. 2015).

Bearing in mind the logic idea of the clear connection between the kinetics degradation of the drugs' delivery systems and the release of the drugs themselves, it is understandable why biodegradation is also an important key to considerate in the design of nanoparticles. It is essential that the nanocarriers and the drugs they transport stay stable during its circulation trough the human body to avoid the accumulation of drugs in non-targeted and in healthy tissues and at the same time to increase their bioavailability in the targeted site, preventing this way also the adverse effects. Therefore, for example in the design of micelles, the critical micelle concentration (CMC) or in the nanoparticles the velocity of the dissociation into their component parts is all essential features to consider regarding the feasibility of the nanostructured carriers (Attia, Yang et al. 2013).

Despite being not a central key as the features described before, the surface texture of the structured nanocarriers can also affect significantly the interaction, uptake and cell attachment of these and consequently to change the drug delivery (Nel, Madler et al. 2009).

Nanoparticles used as drug delivery systems can be produce with different surface textures, namely ridges on surface, increasing this way the surface area, or even with surface roughness. These changes in the texture of the surface of the nanoparticles will directly affect other features as the drug release or even the circulation of the particles (Enlow, Luft et al. 2011).

The smoothness of the nanoparticles' surface represents a great alternative to control drug release, however roughness may also present some benefits once it improves cell binding by non-specific connection forces which lead to the increase of cellular uptake of the nanoparticles. Some combinations between different textures may also allow to direct penetration of nanoparticles into the cell and having hear an important role in the nanocarriers design. The roughness in the surface of the drug delivery systems is also associated with a decrease of the repulsive and opposing interactions as the electrostatic and hydrophilic interactions are, between the cells and the nanocarriers which transport the drugs, which lead to the improvement of the adhesion of the nanoparticles and a consequent easier action of these in the targeted cells (Nel, Madler et al. 2009).

One of the challenges of pharmaceutical technology in which the surface texture is an important development is the aerosols because of the fact that in this formulations the particles tend to become cohesive, amorphous and physically unstable, leading to an increase of the cohesion forces and consequently to powder deagglomeration and a harder delivery of the drugs to the lungs. Slightly changes in the surface texture, increasing the roughness is essential to improve the performance of the aerosols (Kwok, Tunsirikongkon et al. 2011). It is also described the advantages of the roughness in nanoparticles used for mucoadhesive deliver drug once it helps in mucoadhesion (Amin, Joo et al. 2015).

On the other hand, the smoothness of the nanoparticles' surface is also useful to decrease the variation in drug release and it has an important role in the biodistribution of the nanoparticles too (Niu, Yu et al. 2013).

To sum up, in spite of the fact that the proteins and the biomolecules are more likely to connect with nanoparticles with surfaces that present roughness than the ones which show smoother surfaces, the fact is that nanoparticles with smooth surfaces exhibit a higher in vivo stability when compared with the rough ones and this stability may be a critical factor to the effectiveness of these therapeutic systems (Sheng, Liu et al. 2009).

3.1.3. Surface charge

As far as the surface charge of the nanostructured carriers are concerned, this is one of the features of particles at nanoscale with more influence in the biodistribution on the drugs once they clearly affect the deliver and targeting of these therapeutic agents (Forest and Pourchez 2017), (Schipper, Iyer et al. 2009). Being the surface of the nanoparticles the first step of interaction between these carriers of drugs at nanoscale and the human cells, those where they should have their action and those where they preferably must not cause any change, it is easy to understand why the surface charge is one of the nanoparticles' physiochemical property suitable to determine the biodistribution of the drugs carried by the nanoparticles, the cellular uptake or yet the interaction with endogenous molecules (Jo, Kim et al. 2015).

Regarding nanoparticles, they may have different surface's charges, namely positive, negative or neutral charge and this will directly depend on the materials they are made of or even due to their own design and surface's changes purposely carried out in the nanoparticles' surface in order to adequate them to deliver or targeting in specific tissues (Blanco, Shen et al. 2015).

Taking the complexity of the human body into account and the importance of developing innovative pharmaceutical formulations capable of increasing the efficacy of drugs in target tissues and decreasing their adverse effects on adjacent tissues, it is essential the study of this feature not only by the analysis of the materials used to design the carriers of the drugs and their behavior at nanoscale, but also the deeply evaluation of the human body characteristic which may limit the results of the drugs in vivo (Forest and Pourchez 2017).

To better understand this issue, Figure 6 illustrates the comparative biodistribution of drug-loaded nanoparticles with positive, negative and neutral surface charges regarding four different target tissues of great therapeutic importance.

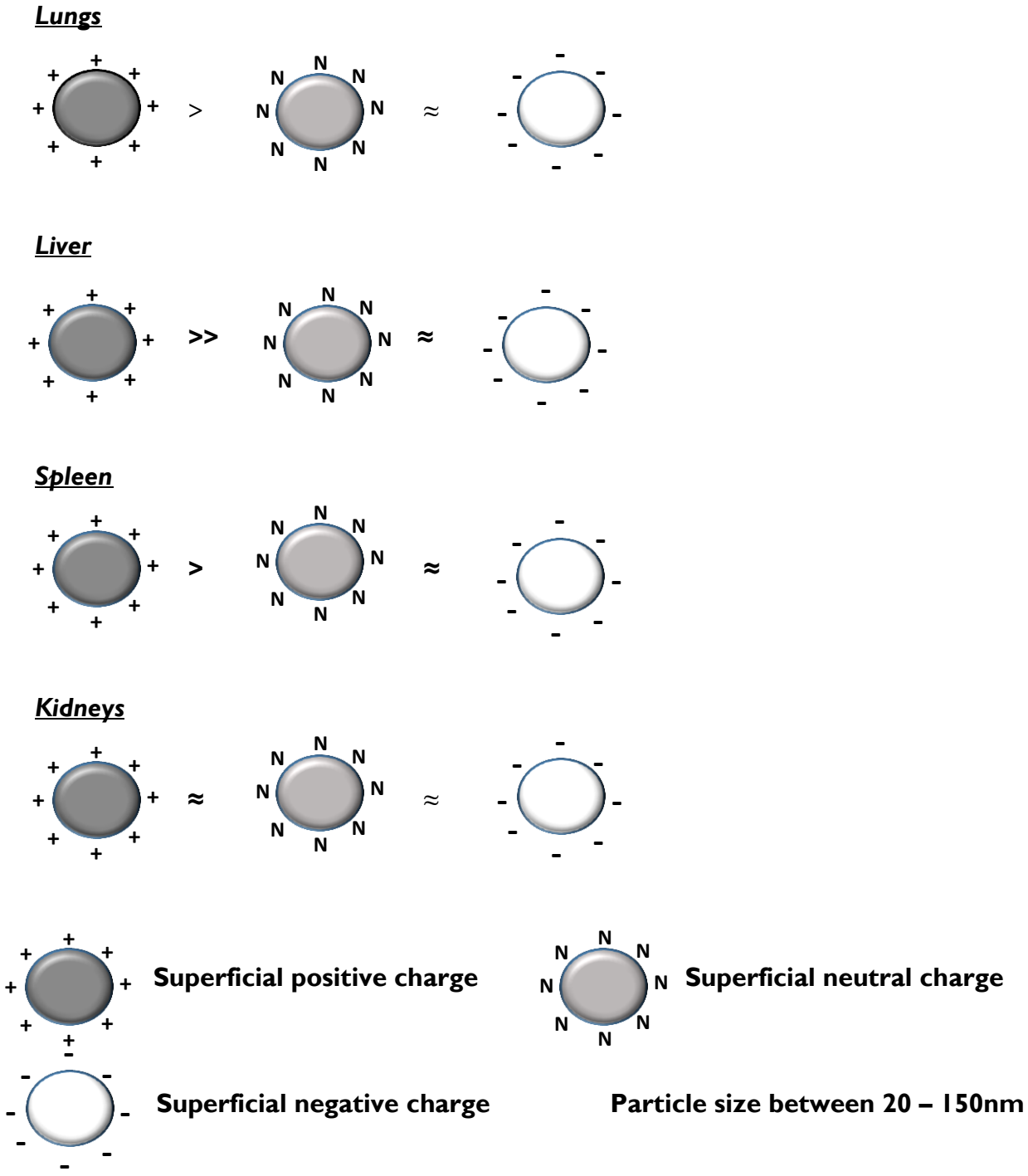


Figure 6: Comparative impact of the surface charge of drug-loaded nanoparticles on the biodistribution in different organs. Adapted from (Blanco, Shen et al. 2015).

Figure 6 gives a clear picture of how the surface charge is not a linear feature and that accordingly to the therapeutic target tissue of drugs, this feature can be tailored to increase the circulation lifetimes and to reach the accumulation of drugs in specific and selected sites of interest. Secondly it is easily understood that changes in the nanoparticles' surface charge will have impact on the opsonization, times of circulation and the interaction with

endogenous molecules, namely macrophages. Nanostructured carriers with positive surface charge are more likely to be sequestrated by endogenous macrophages in the lungs, liver and spleen, with an obvious accumulation of these nanoparticles in these organs, with special incidence and higher impact on this bioaccumulation in the liver, when compared with parallel delivery systems with negative or neutral surface charges. Regarding the nanoparticles with neutral and slightly negative surface charges, they have a lower tendency to enhance the accumulation of drugs in tissues once they are more prone to have longer circulation lifetimes. Finally, concerning the biodistribution of nanostructured carriers of drugs in the kidneys, they did not seem to lead to preferable accumulation of nanoparticles whether they were charged positively, negatively or with neutral load, in any of these cases they would show similar biodistribution in these critical organs (Blanco, Shen et al. 2015).

Alexis et al. suggested that the drugs delivery systems with negative and neutral surface charges have been proven to decrease the adsorption of serum proteins, with a consequence in the increasing of the circulation half-lives and a decrease of the biodistribution of these drugs (Alexis, Pridgen et al. 2008). Many authors have studied the advantages of drug delivery systems with surface charges of 1.3 mV (neutral) or -10.6 mV (anionic) to the increase of the time of circulation of drugs in the blood flow, being the negatively charged nanoparticles the ones which presented lower accumulation in the spleen and the liver (Blanco, Shen et al. 2015). Regarding now the nanoparticles with positive surface charges, they tend to be easily accumulated in nonspecific tissues, showing higher uptake in almost the entire cells of the organism. Moreover, the cationic liposomes are more likely to bind and to be internalized by endothelial cells of tumor-associated angiogenic or even to tissues with higher number of cellular components characteristics of chronic inflammation, when compared with the same process in normal blood vessels or healthy tissues (Hua 2013).

Some mechanisms, namely the "proton sponge effect" have been described as an increased likability for endosomal release by the nanoparticles with positive surface charge, which decrease the degradation's effect of the endosomal compartment on drugs' carriers. This way, some conclusions have been made regarding the potential of the use of nanoparticles to improve current diseases' therapies, namely the effectiveness of nanoparticle delivery systems to tumors, concluding they would preferably have a neutral or slightly negative surface charge to be administered by intravenous route, but it would be desirable a switch to a positively surface in the local of action of the drugs to take advantage of this charge at the tumor site (Nel, Madler et al. 2009). Aiming this, much work have been done to achieve

a rational design that combines the both charges in different times of the actions of the drugs' delivery systems in order to maximize their accumulation in the tumor and also a cellular uptake. These systems are known as zwitterionic nanoparticles and are capable to switch their surface charge depending on environmental stimulus (Yuan, Mao et al. 2012).

In spite of the already described and even of some studies in vitro with three dimensional models of fluorescein-carrying gold nanoparticles with positive surface charge have showed higher cellular uptake by proliferating cells than normal ones, there are also studies of tumors, such as tumor cylindroids, in which it was exhibit an increase of the velocity of diffusion in the tissues of nanoparticles with negative surface charge. Consequently, these different results in specific and similar situations shows the impact of the deeply study of all the features of nanostructured carriers' design (Kim, Han et al. 2010).

As far as the toxicity is concerned, the nanoparticles with positive surface charge have been reported as being more toxic to the cells since their capability to destabilize the negatively charged membrane of human body's cells through electrostatic interactions (Aillon, Xie et al. 2009). Moreover, the nanostructured carriers with positive surface charge may also damage the membrane of mitochondria, namely the outer one, once its electrochemical gradient, which is slightly negative, is very sensitive to changes. This way, the enhance accumulation of the positively charged nanocarriers on the outer side of the mitochondria will have influence on the balance of the potential of the mitochondrial membrane with a consequent damage of this and the release in the cytosol of pro-apoptotic proteins which will lead the cell to apoptosis and an increase of human toxicity (Schaeublin, Braydich-Stolle et al. 2011).

Regarding the nanoparticles with negative surface charge, functionalized with quaternary carboxylate groups (instead of the quaternary ammonium groups of the positively charged nanoparticles), they have proven not to be present toxicity to the cells, considering particles made from the same materials and only with changes at the level of the surface charge (Aillon, Xie et al. 2009).

Despite these conclusions, it is important to refer that the toxicity is also dependent on other surfaces' modifications such as the addition of specific groups (which also affect the surface charge) or also the materials which the nanoparticles are made of, whereby all these features need to be considered when the nanoparticles' design is studied. Nevertheless, on the opposite, the shape and the aspect ratio have shown not to have a strong impact on the toxicity of the nanostructured carriers (Yu, Malugin et al. 2011).

3.2. Surface properties

3.2.1. Steric stabilization

The stability of the nanostructured carriers is essential to lead the aim why they are used for, namely the achievement of drug delivery systems with a controlled drug release, an increase of the effectiveness of the drugs they transport and a decrease of their adverse effects. Thence, the stability of these nanoparticles has an essential role, namely the steric stabilization. In other words, the older concept of steric stabilization includes all the aspects related to the stabilization of the particles through the addition of nonionic macromolecules on the surface of these drug delivery devices, having this way an improvement of the stability by an increase of the thermodynamic balance of the strengths (Napper 1977).

One great advantage of the application of the drug delivery systems conjugated with nanotechnology is that it is proven the recognition of steric stabilization by these nanoparticles, which can lead to an increase of the stability of these in vivo and since this, use them as great alternatives to design drug delivery systems which allow the drug targeting and controlled release of drugs. To aim this, a desirable design study need to be focused on the surface ligands of the nanoparticles, the ones which can suffer surface's modifications to make them capable of specifically interact with the target molecules in the inner and in the outer of the cell and this way remain stable over all the process as therapeutic agents (Mahmoodi, Ghavidast et al. 2016).

A very well-studied way to reach the steric stabilization is using chains polyethylene glycol (PEG) and polyethylene oxide (PEO) to attach at the surface of the nanoparticles since this way it will prevent the adsorption of serum opsonins at the nanoparticles' surface (Sathyamoorthy and Dhanaraju 2016).

The best steric stabilizers, or by other words, the best ligands to attach to the surface nanoparticles to improve their in vivo stability, will depend on the target tissues of the drug delivery systems, however the polymers or the random, block or graft copolymers are described as being the great ligands in order to improve the stability of the nanoparticles (Shi 2002).

Some literature presents some advantages of steric stabilization instead of electrostatic stabilization (presented below), such as the relative lack of sensitivity to the presence of electrolytes; the equal efficacy in both aqueous and non-aqueous dispersion media; the equal efficacy at both high and low solids content and the reversibility of flocculation (Shi 2002).

Despite the great impact of the steric stabilization on the stability of the nanostructured carriers, some studies regarding liposomes have shown that the role of this parameter on the properties of these drug delivery devices is much smaller than the changes in the liposomal size, which have exhibit a stronger impact on the behavior of the nanoparticles in vivo (Biswajit, Laboni et al. 2015).

3.2.2. Electrostatic stabilization

Another way to improve the stability of the nanoparticles is through the electrostatic stabilization of these, which succinctly is the increase of the nanoparticles' stability by the addition of ionic ligands/materials (instead of the nonionic from the steric stabilization) at the surface of the nanoparticles in order to obtain electrostatic repulsion between the particles and therefore make them remain steady (Bohr, Water et al. 2015).

The addition of ionic ligands to the surface of nanoparticles will consequently change their surface's charge and thus will influence its stability and mainly its biodistribution and fate. Thereby, regarding nanoemulsions, the cationic oil/water nanoemulsions have shown to have advantages to be used in ocular therapies instead of the anionic oil/water nanoemulsions because of the electrostatic interactions between the charge of the nanoparticle and the negative ocular charge, which leads to an increase of the precorneal residence time and a greater ocular drug bioavailability (Royle, Matthews et al. 2008).

Moreover, also the positively charged oil nanodroplets have a preferable impact on improving the long-term stability of the nanoemulsions trough the repulsive electrostatic strengths generated between the negative ocular charge and the positive oil nanodroplets charge. This electrostatic stabilization is measured by the zeta potential of the nanoparticles and it is effective in the prevention of the merging of the nanodroplets and to avoid the coalescence process of the nanoemulsions as long as it is their shelf life (Daull, Lallemand et al. 2014).

The electrostatic stabilization of nanostructured carriers for drug delivery and targeting may be made by the deposition of natural polyelectrolytes, a process that is relatively simple and without a higher spending on energy but which can significantly affect nanoparticles' features, mainly their size, surface charge and dispersion stability as a consequence of the addition of these groups of polyelectrolytes at the surface of these nanodelivery systems (Kuroiwa, Kobayashi et al. 2015).

The polyelectrolytes referred below and used to electrostatic stabilization, have shown to strongly adsorb opposite surface charges which will have a consequently influence on the balance on the interaction forces (which may be measured by Atomic Force Microscopy, AFM). Electrostatic patch-charge interactions may yet generate attractive forces, very similar to the ones between proteins, hydrophobic and mineral surfaces and lead this way to an electrostatic stabilization of the nanoparticles (Popa, Gillies et al. 2009).

To sum up, successful stabilization of nanoparticles is achieved by a functionalization and dispersal stabilization resulting of a mixture of steric and electrostatic repulsion (Figure 7) (Unterweger, Subatzus et al. 2015):

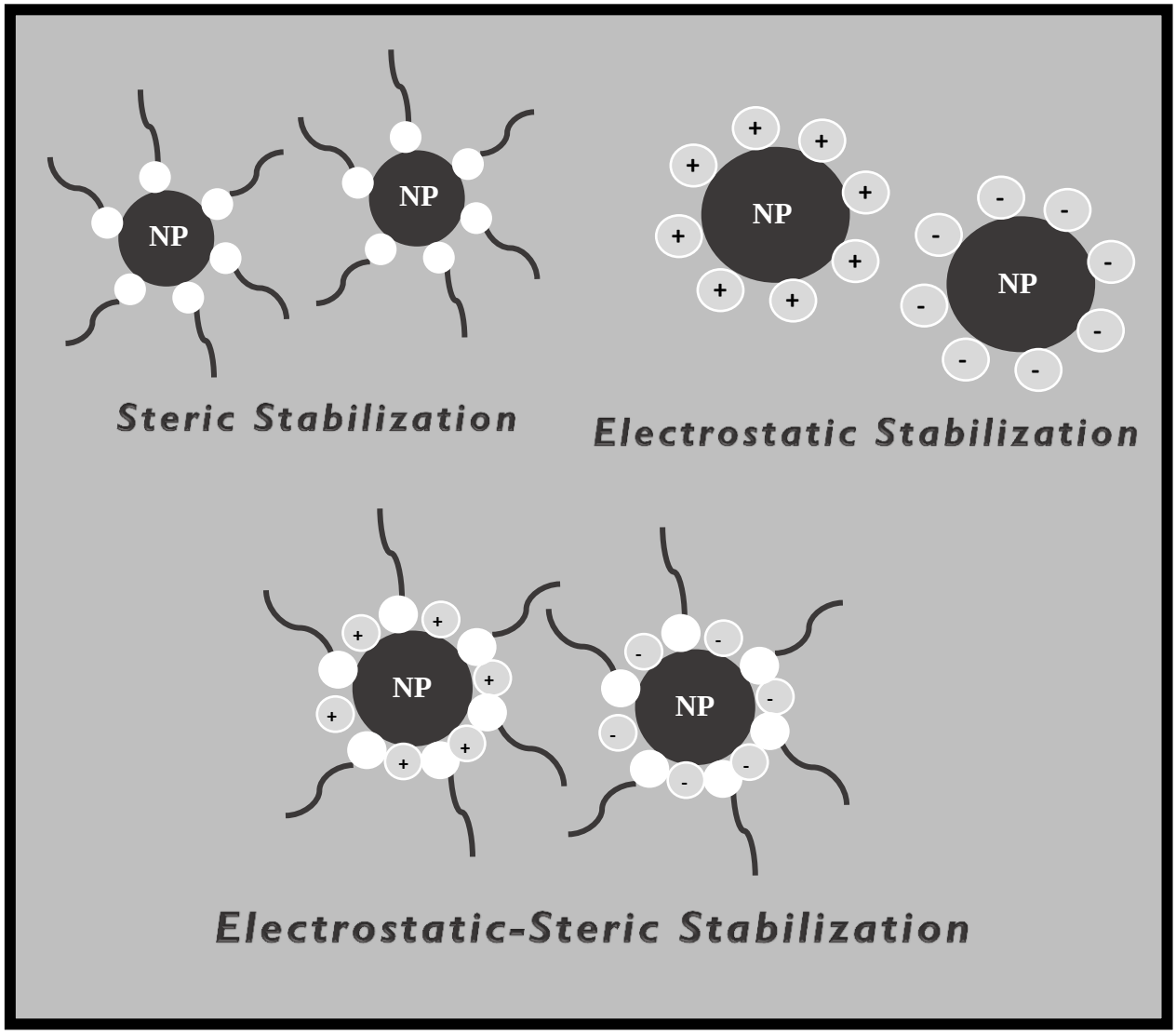


Figure 7: Steric and electrostatic stabilization of nanoparticles, and their respective steady-state combination.

3.3. Circulation time

The time of circulation of a drug in the blood flow is a determinant feature not only for the effectiveness of a drug but also to its biodistribution and fate. The time that a drug is in the circulation flow will be directly influenced by a range of factors, some of these have previously been described in this review and all of them play an important role in the design of the nanostructured carriers and in the improvement of the use of these in the current diseases' therapies. Thus, to meliorate the design of nanocarriers for drug delivery and targeting, many studies have been made in order to adjust the circulation times of the drugs to their therapeutic aims and this way there is being a focus on the physicomechanical aspects of carrier particles, such as their shape, size, and stiffness, as critical parameters with influence on the circulation times and therefore have special relevance in new design of the nanocarriers (Sen Gupta 2016).

The computational modeling studies and experimental studies which take advantage of microfluidic devices enable to better understand the impact of these factors in the behavior of the nanoparticles when they are in the hemodynamic circulation and even all the interactions during this circulation between the nanoparticles and the cells and which also influence the delivery and targeting of the drugs they transport. Moreover, the study of the components of the cells in circulation which will interact with the nanoparticles and the analysis of their behavior, is also giving information on how the design of drug delivery devices may be designed (Doshi, Swiston et al. 2011).

Regarding nanoparticles with a size below 10 nm, they are likely to spread over many organs, with low circulation times. Whether their size is higher than 10 nm, the degree of uptake of the nanoparticles from the reticuloendothelial system will directly affect the clearance of these from the blood circulation flow. Faster clearance rates are achieved when the nanoparticles have a size lower than the limit of renal barriers, which will have a critical influence on the effectiveness of a drug once it would not allow the drugs to have enough time to reach the target tissues. This is the reason why for tumor's therapies, in which the aim is to have a maximum of accumulation of drugs in the target tissues, the blood circulation time of nanostructured carriers of drugs have to be longer and large enough to avoid the fast penetration of the tissues. Thence, the surface design, mainly the surface charge of nanoparticles to drug delivery and targeting need to be deeply studied to obtain smart nanoparticles' surface coatings which can answer to all this challenges of current diseases (Nam, Won et al. 2013).

The conjugation of polyethylene glycol (PEG) in the nanoparticles' surface is one of the best strategies used in the design of nanostructured carriers to improve the circulation times of the nanoparticles in blood circulation flow and at the same time to decrease their renal clearance rates (Babu, Templeton et al. 2014).

Other nanoparticles surface's modifications may be done to improve the time of circulation of this drug delivery systems however the conjugation with PEG represent several advantages in the increase of the circulation times of nanoparticles once that it prevents the opsonization of the drugs' carriers and also decreases the uptake of these by the macrophages. Regarding the conjugation with chitosan, due to its positive charge, it can also be useful in drug delivery systems design because it has a strong interaction with cell membranes and mucosal surfaces, which may be an advantage in the design of some formulations. It is always important to study the impact of these surface's modifications in the behavior of the nanoparticles and sometimes to consider a mixture of modifications may be the best strategy (Kubiak 2014). Many studies have referred the conjugation of the nanoparticles' coating of PEG and chitosan has and outstanding step the nanodelivery systems' design to improve the circulation of times and consequently the effectiveness of drugs in tumor (Parveen and Sahoo 2011).

The polymeric nanoparticles have already proven their capability to long period circulation times, which make them very attractive to the development of drug delivery systems. Thus, the use of nanoparticles to carry drugs as paclitaxel with surface's modification to conjugate chitosan and polyethylene glycol coated PLGA (PLGA-CS-PEG) have been successfully developed to encapsulate and deliver such hydrophobic drugs which were a challenge until now to be able to do it. These surfaces' modifications avoid the phagocyte uptake of nanoparticles by decreasing the opsonization of these by the blood proteins which leads to an increase of the bioavailability of the drugs. Studies need to be done to improve the quantities of these compounds and the conditions of production of these nanoparticles in order to they do not affect other features of the carriers and consequently the effectiveness and *in vitro* cytotoxicity of drugs. Also some anti-proliferative effects and cell cycle inhibition were described as additional of the PLGA-CS-PEG nanoparticles by their own when compared with the paclitaxel only. This may be related to the easier cellular uptake of the drugs and thus a better anti-proliferative effect of the nanoparticles (Parveen and Sahoo 2011).

Still regarding the surface's modifications to improve the circulation times it is important to report the importance of the choice of the compounds to conjugate to the nanoparticles

once it have been described some problems related to the differences of action *in vitro* and in vivo of these compounds which may be solved by the choice of reversible PEGylation in which the portions of PEG are gradually released in blood circulation. Thence it is essential the development of pH-sensitive helper lipids, such as fatty acids and cholesteryl hemisuccinate (CHEMS) that together with the nanoparticles will be able to reach a balance between the need of stability of the drugs delivery systems, long systemic circulations times and the need of destabilization of nanoparticles inside the target cells to release the drugs and optimize their action (Cheng and Lee 2016).

3.4. Opsonization

The opsonization is one of the drawbacks of the use of nanostructured carriers for drug delivery and targeting once it makes harder to reach the therapeutic levels in target tissues. In a simple way, opsonization is a process of sequestration of the exogenous nanoparticles which starts by the opsonization of the nanoparticles in circulation flow by the plasma proteins, including serum albumin, apolipoproteins, complement components and immunoglobulins, with the adsorption of these molecules in the surface of the nanoparticles (Tenzer, Docter et al. 2013). Then, around the nanoparticles, it is generated the corona protein which is dependent on many features such as nanoparticles size, surface chemistry and charge or even their hydrophobicity (Nel, Madler et al. 2009). After this adsorption on the nanoparticles' surface, it occurs the attachment of these drug delivery devices to specific receptors presented in the surface of phagocytes which lead the nanoparticles to internalization and transportation to phagosomes where they are fused with lysosomes (Sahay, Alakhova et al. 2010).

The described process of opsonization is many times referred as directly associated to the mononuclear phagocyte system (MPS), which is composed by a system of phagocytic cells, mainly resident macrophages presented in the spleen, lymph nodes and in the liver. The MPS has showed the ability to sequester nanoparticles promptly soon after its intravenous administration (Blanco, Shen et al. 2015). Moreover, the opsonization not only increases the uptake of nanoparticles by the MPS, it also decreases the effectiveness of the strategies of targeting from the nanoparticles because of the deposition of the corona protein in their surface which masks the targeting ligands of these drug delivery devices and decreases their specificity and selectivity. It is still important to mention that the mechanism of corona protein deposition in the nanoparticles' surface, as well as the impact of other biological and dynamic components which influence the behavior of the nanostructured drug carriers will

have a strong impact on the stability, bioavailability, toxicity and fate of the drugs (Docter, Distler et al. 2014).

Now it is possible to realize that without a nanoparticles' surface modification, these drug delivery systems would be immediately marked by opsonin proteins and thus recognized by the phagocytes of MPS and therefore the opsonization need to be avoided. The opsonization of the nanoparticle may be influenced by a range of factors, namely the nanoparticles' features such as the surface charge, and it this characteristic in which drug delivery systems' design must be focused on (Mogosanu, Grumezescu et al. 2016).

The already referred surface modifications in the nanostructured drug carriers in order to improve their effectiveness may include coating the surface of nanoparticles with hydrophilic molecules such as the also already mentioned PEG which will help to restrict the uptake of nanoparticles by the reticuloendothelial system (S Caban 2014). In this way, a protective layer with a hydrophilic potential around the nanoparticles will be formed, which will reduce the absorption of opsonin proteins and thence avoid or at least delay the first step of opsonization (Owens and Peppas 2006).

Besides the PEGylation of nanoparticles, other strategies to functionalize these drug delivery systems have been studied, namely the attachment of the marker CD47 self-peptides to the surface of nanoparticles once they give information to the body that these molecules are not to be eaten and thus it is possible to reduce the phagocytic clearance (Rodriguez, Harada et al. 2013).

To summarize, opsonization is one biological mechanism affected by the nanoparticles' surface charge and which also influence this feature. Surfaces modification in the nanostructured drug delivery systems will give the nanoparticles the ability to delay phagocytic clearance by the liver and spleen and therefore allow higher concentrations of drugs in the target tissues. Computationally design bearing in mind all the parameters referred will be the key to find the balance between all the factors and features affecting the effectiveness of the delivery and targeting of these drug delivery systems at nanoscale (Ziemys, Klemm et al. 2016).

3.5. Interaction with endogenous molecules

The interaction of nanocarriers with endogenous molecules plays an important role on all the topics previously referred and it is critical by the inevitable interaction of the nanoparticles with endogenous molecules before arriving to the target tissues (Amin, Joo et al. 2015).

Since the biological cell membranes have charge, the nanoparticles' surface charge will have a core role in the interaction of the nanostructured drug delivery carriers with the endogenous molecules and thus, also a great impact in the cellular uptake of the carriers and consequently of the drugs. Nanoparticles with a surface charge negative or neutral have shown a reduced level of internalization when compared with the same nanoparticles with a positively surface charged and this is much probably due to the negative surface charge of the membrane cells of the human organism (Yuan, Mao et al. 2012).

The interaction with endogenous molecules as the enzymes also plays an important role in the drug delivery and targeting, once in some diseases the production of specific enzymes is increased and this way the surface of nanoparticles may also be designed with appropriate substrates in their surface in order to achieve drug targeting release (Mura, Nicolas et al. 2013).

Some cationic polymers may be attached to the nanoparticles' surface, namely as poly(L-lysine), poly(ethylenimine), chitosan, and diethylaminoethyl-dextran in order to improve the cellular uptake of these nanodelivery systems. The increase of density in the nanoparticles' surface due to the charged groups leads to a clear increase of the absorption of the drugs once it is reported as being correlated with higher membrane permeability (Huhn, Kantner et al. 2013).

As previously mentioned, the nanoparticles with positive surface charge tend to bind to negative surface charge compounds in the surface of the cells and it was studied that how much more the surface charge of nanoparticles is positive, more cellular internalization occurs by common uptake mechanisms in the human cells (Chung, Wu et al. 2007). Also the proteins from plasma tend to bind to nanoparticles with cationic surface charge and thus improving their cellular uptake. These specific nanoparticles are also related to the depolarization of the plasma membrane compounds and a consequently shaping of holes in the lipid bilayers as a result of a range of nanoparticles' features such as their surface charge and also their density, size, shape, chemical composition or deformability (Leroueil, Berry et al. 2008).

Still regarding cationic nanoparticles they have proven an effective drug delivery in mucosal tissues and an increase of the interactions with gastrointestinal tract mucosae, nasal mucosae, and the cornea, especially when presented in their surface molecules of chitosan or hyaluronic acid (Hillaireau and Couvreur 2009). At last, as far as the BBB is concerned, nanoparticles with positive surface charge have also shown to cross in a greater extent these

barriers, with the increase on brain penetration and absorption of drugs (Jallouli, Paillard et al. 2007).

In spite of all the advantages referred in the use of cationic nanoparticles concerning the interaction with the endogenous molecules, this is not mandatory characteristic to an efficient endocytosis of the nanoparticles once that neutral of negative surface charged nanoparticles have also proven their effectiveness in improving the cellular uptake of the drugs they carry, mainly when targeting ligands are attached to their surface, much probably by a process of internalization in the cationic places of plasma membrane and where it seems to occur non-specific binding and clustering of the anionic nanoparticles (Amin, Joo et al. 2015).

4. Conclusions and Perspectives

The present review reviews current achievements on the development of nanostructured carriers in drug delivery and targeting and the relevant characteristics of those, specifically the surface's charge, which may affect their utility and effectiveness in the improvement of current diseases' therapies.

The formulations of nanoparticles used as drug delivery systems have already proved their effectiveness in drug delivery and targeting drugs and they have been shown as a strong strategy to improve the current diseases' treatments. However, pharmaceutical industry is interested in the development of more effective and safety controlled production processes once that quality assurance and high cost of production are two great handicaps of the use of nanoparticles in the delivery and targeting of drugs. This is even more critical when nanoparticles' surface modifications are made and it is needed to attach multiple components coating the nanoparticles because this complex designs demand sophisticated methods of production and evaluation steps during this process. Thence this turns the scale up harder and more expensive, whereby there will be new challenges for pharmaceutical industry regarding these factors and the real and practicable improvement of nanostructured carriers for drugs delivery and targeting that should not forget any of the features referred in this review and their impact on the behavior of the nanoparticles because it is essential the involvement of pharmaceutical industry in these effective developments.

Much work is already done and it is clear the advantages of nanostructured drug carriers instead of other classical pharmaceutical formulations, as well as the features with impact on

the nanoparticles behavior and the interactions between them are even them well studied. To become this in an essential key of the personalized medicine and in a fundamental issue of nanotheranostic, it is essential to carefully and deeply evaluate all the conclusions regarding the nanoparticles' features and start to improve the scale up of methods of production as well as the methods of quality control and assurance.

The greatest challenge in the future is to achieve reproducible and effective surface modifications in the nanostructured drug delivery systems, taking advantage of all the knowledge regarding the features with higher impact, such as surface charge, and all the technological advance that allows an engineering design with computational programs and in a second phase with in vitro and in vivo devices of control and measure of effectiveness of this outstanding drug delivery devices.

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