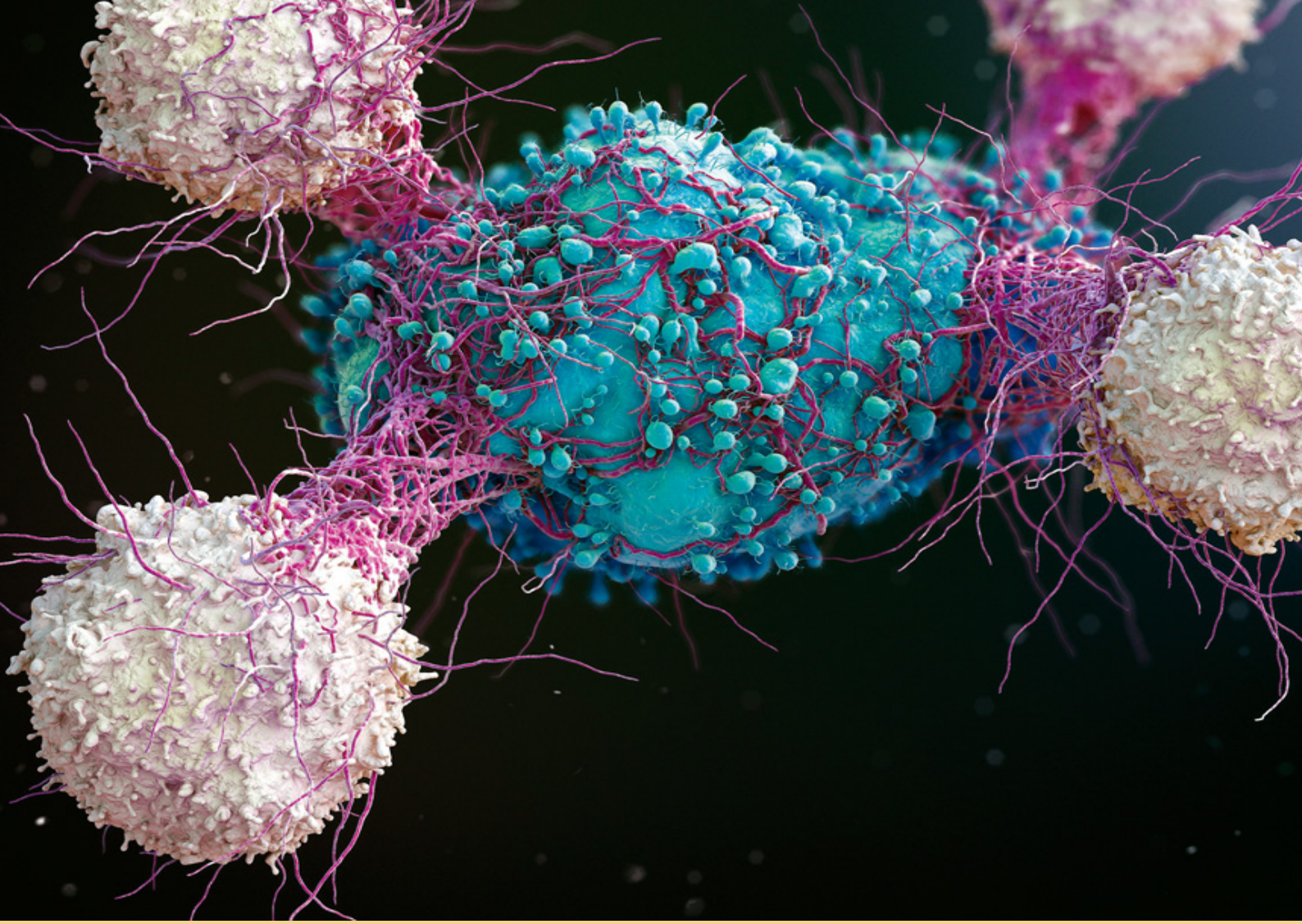

Mariana Guerra da Silva

Relatórios de Estágio e Monografia intitulada "Current Applications of Nanoemulsions in Targeted Cancer Therapie" referentes à Unidade Curricular "Estágio", sob orientação do Dr. João Oliveira, da Dra. Maria Ângela Lima 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

Setembro 2018

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Universidade de Coimbra

Mariana Guerra da Silva

Internship Reports and Monograph titled "Current Application of Nanoemulsions in Targeted Cancer Therapy" referent to the subject *Curricular Internship* under the orientation of João Oliveira, *Pharm. D.,* Maria Ângela Lima, *Pharm. D*., and Professor Eliana Souto, *Pharm. D.,* respectively, presented to the Faculty of Pharmacy, University of Coimbra (FFUC) for the appretiation of the work developed on the Integrated Master in Pharmaceutical Sciences (MICF).

September 2018

UNIVERSIDADE DE COIMBRA

Eu, Mariana Guerra da Silva, estudante do Mestrado Integrado em Ciências Farnacêuticas, com o nº 2012168404, declaro assumir toda a responsabilidade pelo conteúdo do Documento Relatórios de Estágio e Monografia intitulada "Current Applications of Nanoemulsions in Targeted Cancer Therapy" apresentados à Faculdade de Farmácia da Universidade de Coimbra, no âmbito da unidade Estágio Curricular.

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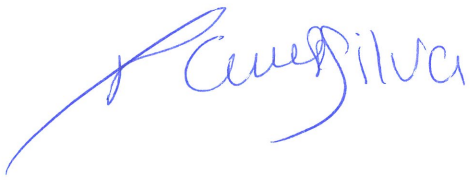

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Publications

The issue of this monograph is currently in aprovation for two publications in Springer Verlag (2019) with the respective names: “Current Applications of Nanoemulsions in Cancer Therapeutics” and “Nanoemulsions: Methods of Production”.

Index

Abbreviations

ABC | ATP-Binding Cassette

AN | Gold Nanoparticles

ANF | National Pharmacies Association

API | Active Pharmaceutical Ingredient

ATP | Adenosine Tryphosphate

AV | Anisidine Value

BBB | Brain-Blood Barrier

Bcl-2 | B-cell lymphoma 2

BLI | Bioluminescence Imaging

BSE | Bovine Spongiform Encephalopathy

CC | Change Control

CEP | Certificate of Suitability

CEU | Contrast Enhanced Ultra-Sound

CD | Cluster of Differentiation

CI | Combination Index

CoA | Certificate of Analysis

CSC | Cancer Stem Cell

CT | Computed Tomography

DHA | Docosahexanoic Acid

DLS | Dynamic Light Scattering

DMF | Drug Master File

DOX | Doxorubicin

ECM | Extracellular Matrix

EM | Electron Microscopy

EFGR | Epidermal Growth Factor Receptor

EMT | Epithelial Mesenchymal Transition

FDA | Food and Drugs Administration

FFUC | Faculty of Pharmacy of University of Coimbra

FP | Finished Product GAS | Safe Gradient Excipient

GMP | Good Manufacturing Practice

HA | Hyaluronic Acid

HER3 | Human Epidermal Growth Factor Receptor-3

HPLC | High Performance Liquid Chromatography

HPN | HA-complexed PTX Nanoemulsion

IC_{50} | 50% of Inhibitory Concentration

IPC-MS | Inductively Coupled Plasma-Mass Spectometry

LAL | Limulus Amebocyte Lysate

LCT | Long Chain Triglycerides

LP | Licopene

MA | Manufacture Authorization

MICF | Integrated Master in Pharmaceutical Sciences

MMP | Matrix Metalloproteinase

MPS | Mononuclear Phagocytic System

MRI | Magnetic Ressonance Imaging

MSDS | Material Safety Data Sheet MDR | Multidrug Resistance

NF-kB | Nuclear Factor-kappa B

PARP | Poly (ADP-ribosomo) Polymerase

PCS | Photon Correlation Spectroscopy

PCX | Paclitaxel

PDGF | Platelet-Derived Growth Factor

PDI | Polydispersity Index

PEC | Micelle Polyelectrolyte Complex

PEG | Polyethylene Glycol

PET | Positron Emission Tomography

P-gp | P-glycoprotein

PN | Nanoemulsion with PTX

PrC | Prostate Cancer

PSMA | Prostate-Specific Membrane Antigen

PV | Peroxide Value

Pt | Platinum

PUFA | Polyunsaturated Fatty Acid

QMP | Quality Management Procedure

QMS | Quality Management System

QP | Qualified PersonSCT | Short Chain Triglycerides

SEM | Scanning Electron Microscopy

siRNA | Small Interfering RNA

SMF | Site Master File

SPECT | Single-Photon Emission Tomography

SPR | Surface Plasmon Ressonance

SWOT | Strengths, Weaknesses, Opportunities and Threats

TEM | Transmission Electron Microscopy

TIC | Tumor Initiating Cell

TME | Tumor Microenvironment

TOP | Technic Operational Procedure

TSE | Transmissible Spongiform Encephalopathy

VEGF | Vascular Endothelial Growth Factor

SECTION I

MONOGRAPH

Abstract

Pharmaceutical formulations containing nanometer particles are called nanoemulsions. This type of formulation is known for being able to encapsulate drugs which are poorly or even not soluble at all in water as their cores are hydrophobic. Not only this, but they are also composed of Safe Gradient Excipients, contributing for a safer and stable deliver of the drug to carry. For decades, multiple researches have tried to find a reliable therapy for cancer with little success as the substances developed failures, mainly due to low solubility, multidrug resistance and increased toxicity to healthy cells.

Safe and efficient cancer therapy may be achieved with the help of nanoemulsions. The dosage form is capable of mitigating water-solubility problems, targeting cancer cells and be designed to survive multi-drug resistance mechanisms. Nanoemulsions possess the ability of being modified using ligands to target molecules at the tumor cell surface or even to overcame multi-drug resistance. This way, multifunctional nanoemulsions have become a main topic of researches in distinct areas.

A wide variety of researches were developed to confirm that nanoemulsions may be of skillful use in cancer therapy - specifically the case of targeted nanoemulsions to treat colon, ovarian, prostate and lung cancer. The referred studies proved the efficiency of this dosage form in the targeting of tumor cells, reducing of tumor growth, decrease of toxicity to healthy tissues and low rate of cancer cells migration to different organs.

Keywords: multifunctional nanoemulsions, targeted delivery, passive targeting, active targeting, multidrug resistance, cancer.

1. Introduction

1.1. Nanoemulsions in oncologic context

1.1.1. Description

Nanoemulsions are dosage forms which serve the purpose of being drug vehicles (specially for drugs with low water solubility), nucleic acids and imaging agents (1). They are, fundamentally, emulsions on submicron range, composed of Safe Gradient Excipients (SGE) (2). These pharmaceutical dosage forms are heterogeneous dispersions of nanometer droplets in another liquid, possessing a high capacity to solubilize hydrophobic drugs, an easier production and enhanced stability (3). Nanoemulsions are able not only to solubilize vast amounts of hydrophobic drugs but, as well, to protect them from hydrolysis, enzymatic degradation and even from recognition by the mononuclear phagocytic system (MPS), with additional capacity of increase the half-life of molecules (4). Overall, all these points turn this formulation into a suitable option for parenteral administration.

Usually, the oil phase of a nanoemulsion is dispersed as droplets in an aqueous phase, using for that purpose reliable emulsifying agents to stabilize the system (Figure 1). These emulsifying agents are, indeed, amphiphylic compounds (or surfactants) which are used to reduce interfacial tension between two immiscible liquids, since they are preferably adsorbed on their surface - as in the case of the compound Tween 80®. Their chemical profile presents hydrophobic hydrocarbonated tails that tend to place in non-polar liquids, such as oils, and a polar head that usually places itself in polar liquids, like water. The droplet size must be comprised between 50 nm to 200 nm, depending on its composition and production. A nanoemulsion has a translucid and transparent profile with great surface area due to the small particle size (5).

Nanoemulsions can also reduce the side effects of the drug they bear. Cremophor EL® is associated with nefrotoxicity, hypotension and bronchospasm, leading to anaphylactic reactions. As a preeminent solution for such side effects, a propofol emulsion with SGE excipient, Diprivan®, is used. Soybean oil and safflower oil-based emulsions are often chosen for parenteral nutrition, being a source of essential fatty acids as Ω-3 and Ω-6, polyunsaturated fatty acid (PUFA), non-glucidic calories and vitamins E and K (6).

Some drugs formulated in nanoemulsions are already available commercially (Table 1) (1). The big advantage in this nanoformulation is the fact that hydrophobic drugs are encapsulated in the hydrophobic core of the nanoemulsion, increasing the drug loading capacity and the delivery efficiency (6).

1.1.2. Composition

The strategy of engineering a nanoemulsion is the key factor for its physical and chemical properties and it can be designed aiming a certain profile. Choosing the materials wisely has a profound influence on the way the nanoemulsion to reacts to stimuli or binds to cell surface receptors (1).

When the particles of an emulsion reach sub-micron sizes, the formulation is named nanoemulsion (7). The usual non-lipophilic core can be composed by a monoglycerol, a diglycerol or a triglycerol. The oil must be carefully selected since it has significant impact on the therapeutic payload, physical-chemical properties, particle size and stability (8). It is possible to prepare nanoemulsions using long chain triglycerides (LCT) which originate larger size particles, with a diameter of 120 nm or short chain triglycerides (SCT) resulting in smaller particles, with a diameter of 40 nm. The smaller the particle, the higher the stability of the formulation which is useful against floculation, gravitational force and Brownian motion. It is, however, important to highlight that SCT oils are quite soluble in water, favouring Ostwald's ripening. The prior phenomenon occurs when larger particles are generated from smaller ones with consequent increase in the solubility of the material (Figure 2). Long chain oils have higher molecular weight and, therefore, low water solubility, resulting in a small chance of going through Ostwald's ripening. The nanoemulsion size also impacts its optical properties: the lower the size of the particle, the higher the transparency (9).

The choice of emulsifying agents also plays a determinant role in the stability of the formulation and in the design of its size and features. The ideal emulsifying agent should be able to reduce interfacial tension, rapidly adsorb at interface and stabilize the surface by electrostatic or steric interactions. It might be any amphiphilic molecule: surfactants (Tween 80®), amphiphilical proteins (caseinate), phospholipids (soy lecithin), polysaccharides (modified starch) ou polymers (PEG) (10). PEG-modified nanoemulsions provide specific targeting and longer circulation time (11).

Texture modifiers, weighting agents or ripening retarders are also of great utility. Sugars, polyols, polysaccharides and proteins can be texture modifiers since they increase the aqueous phase viscosity which contributes to lower the possibility of gravitational separation. A weighting agent must be lipophilic, be able to mix with the oil and also able to prevent gravitational separation (12). A ripening retarder is also a lipophilic compound added to the the oil regarding the hindering of ripening of Ostwald (13). Other materials responsive to

stimuli, displaying changes in their properties, can be useful in the process of enhancing the drug loading capacity using lipidic nanocarriers.

Thermo-sensible nanoemulsions can be developed from lipids that change their behaviour, because of phase transition, a result of an external stimuli, inducing leaks in the formulation which releases its payload (14).

There is also the possibility of creating delivery systems responsive to pH oscillations that are stable at pH=7.4, but change their properties when exposed to acidic environments, leading to drug release. Liposomes are often used as pH-responsive nanocarriers: dioleoyl phosphatidylethanolamine (DOPE) is one example of these (15).

1.1.3. Physical and chemical characterization

Several physical and chemical properties can influence the behaviour of nanoemulsions as following described.

Size Distribuition

The polydispersion index (PDI) and particle size are characterized by dynamic light scattering (DLS) - photon correlation spectroscopy - that has on basis the principle that colloidal particles are scattered by light, acquiring Brown motion, that is detected by a photomultiplier tube. The intensity of dispersed light floculation is critical to find the diffusion coefficient of the particle, allowing Stoke radius (mean hydrodynamic radius) to be calculated (based on intensity and weight) through Stokes equation. It is critical to be aware of the parameters to be kept in mind in order to attain the PDI: high particle concentration, size range, changes in sample during analysis and dust presence. Photon-correlation spectroscopy (PCS) is still the most used method to study the size of nanoemulsions due to its simplicity, easiness to prepare the sample and fast output. Nevertheless, since the size to measure is extremely small, PCS is incapable of detecting small populations of big droplets, contributing for errors in the process (1).

Zeta Potential

The surface charge of the system is also a main subject as it impacts stability and electrostatic interactions. It is measured through electrophoretic movement using a magnetic field. The electrophoretic movement of the particules adopts Henry's equation:

$$\mu = \frac{2\varepsilon\zeta f\,(ka)}{3\eta} \quad (1)$$

μ = electrophoretic mobility

ε = dielectric constant

ζ = zeta potencial

f(Ka) = Henry's function

η = viscosity

Oil Droplet Morfology

To evaluate the morphology and size of the oil droplet, the electron microscopy (EM) is, usually, the best-known method. EM allow not only the direct visualization of morphology and contaminants, but also proportionates correct measurements. It reveals small populations of immense size droplets, which are not available under PC (16). Transmission electron microscopy (TEM) is the largest used technique for imaging nanoemulsions. A staining method is used, since nanoemulsions cannot produce enough contrast (17). Cryo-TEM is a more suitable technique for imaging and consists on frozing the samples before analysis. Scanning electron microscopy (SEM) and environmental SEM (e-SEM) are also used to obtain nanoemulsion visualization (18).

Drug encapsulation

The lipidic solubility and aqueous partition behaviour of the nanoemulsion have significant impact on the drug loading, directly influencing the success of drug encapsulation. The efficiency of this process is studied using centrifugal filter devices. A sample is placed in the upper donor chamber and centrifuged at high speed to obtain the aqueous phase alone in a sample recovery chamber, standing the oil and the drug, in the donor chamber. The aqueous phase is evaluated by analysis of partition and encapsulation efficiency determined by mass balance. The usual choice methods are UV-Vis spectroscopy, fluorescent spectroscopy, high-pressure liquid chromatography (HPLC) and mass spectroscopy (MS) (19).

Stability

Stability studies are performed to evaluate the integrity of excipients and drug in the nanoemulsion. Having this purpose in mind, nanoemulsions are centrifuged on a range of 3,000 rpm to 3,500 rpm for a period of 30 minutes in order to speed creaming process. Stability measurements are based on: the height of the oil layer on top, the emulsion phase in the middle and aqueous phase at the bottom. The study involves the incubation of the formulations at 4.0 °C and 25.0 °C for a period of 3 to 6 months and periodic remotion of samples to evaluate size, charge and efficiency of encapsulation. If there is no change the nanoemulsion is stable at the storing conditions mentioned. Since FDA states that the stability of a formulation must be evaluated in long term, intermediate and accelerated time, processes of heating, cooling and freeze-thaw are highly used (20). Oil stability largely contributes to increase the stability of the formulation. Oils tend to be oxidated, reaction which culimantes with rancidity and side products useful to access the stability. The peroxide value (PV) estimates the quantity of the primary side product of oil, hydroperoxides. PV may not give a completely right value of the stability of the oil since its value gets lower as hydroperoxides form other products like carbonyls. At this point, anisidine value (AV) is more accurate and it takes the dissolution of the oil in iso-octane reacting with anisidine in acid and evaluated spectrocopically. TOTOX (Total Oxidation Value) gives a value for all the oxidation processes. The oil oxidation process is accelerated by factors as temperature, presence of oxygen, light and presence of transition metals (1).

pH

The value of pH can impact all the components present in nanoemulsions. Some substances are sensitive to factors like humidity and pH and their nanoemulsions are prepared at a specific pH to assure that the encapsulated drug is in its active form. Oil oxidation processes changes pH and, therefore, the conditions of the system. Modifications in pH can alter the color of the nanoemulsion and even its size (21).

Drug release

This aspect affects drug delivery, as it is responsible for bioavailability, absorption and kinetics. To evaluate this parameter, the dialysis bag method is the reference. The sample in the bag is dialyzed using a buffer in the sink receiver compartment, under stirring conditions at 37.0 °C. A portion is isolated periodically and replaced by fresh buffer. The drug is separated from the withdrawn volume to be quantified (1). Kinetical models can mimic the drug release and all of them can be used in nanoemulsions (22). However, recent studies

show that the dialysis method is not suitable for nanoparticules, since they must diffuse through the dialysis chamber to reach the membrane and then the receiver – a process similar to a double-membrane transport (23).

Sterility

The treatment can only be considered safe if the formulation is sterile. Autoclavation is not suited for nanoemulsions due to the elevated temperatures and pressure that would turn the method used much more expensive. Nanoemulsions are usually filtered with 220 nm filters, being the process validated with spectroscopy and plating (24).

1.1.4. Production of nanoemulsions

The dissolution of substances in nanoemulsions is achieved depending on their solubility in oil or water, although both phases can disperse emulsifiers. A general representation is pictured in Figure 3.

The two phases are heated and mixed (with adequate temperature and agitation) in order to acquire a dispersion as homogeneous as possible. After this, the emulsion faces sheer force homogenization to attain minimal particle size. When this process ends, particles will have a layer of emulsifying agents separating the lipophilic inside from the aqueous phase. This barrier bares repulsive forces (electrostatic, steric or electrosteric, depending on the emulsifier) that stabilize the nanoemulsion (1).

High-sheer methods resort to high pressure homogenizers, microfluidizers and ultrasonificators (25). Instruments, energy, time, temperature and formulation composition have impact on the size of the particle. High-pressure homogenization originates particles with small size. During a high-pressure homogenization process the contribute of multiple forces - hydraulic, turbulence and cavitation - results in the small size of particle. In microfluidization, a high-pressure pump impulses the drug into the interaction chamber which has microchannels. From the microchannels, the substance goes to an impingement area where particles reach nano range. Ultrasonication is also an option, as it produces cavitation forces, leading to disruption and downsizing of particules. High-pressure homogenization and microfluidization are more used since they can be developed at laboratory and industrial scale while the ultrasonic method can only be used at laboratory scale (1).

Although high energy operations make scale-up possible, they might be unsuitable for heat sensitive drugs. When this happens, it is possible to recur to low energy and temperature

methods, such as self-emulsification phase transition and phase inversion (26). Drugs can be dissolved with or without co-solvents by extemporaneous addition or *de novo*. *De novo* is a method where a hydrophobic drug is solubilized in the oil before the development of the nanoemulsion itself. Extemporaneous addition has the drug added to a pre-formed nanoemulsion. Whereas drugs with high lipophilic character are directly dissolved in this phase, slightly lipophilic drugs have the possibility of being assisted by co-solvents: the hydrophobic drug is added to an organic solvent that will be mixed with the oil phase and later removed (27). Another possibility is concerned with the drug and phospholipids being dissolved in an organic solvent later removed by vacuum evaporation. From here results a lipidic film that is going to be added to an aqueous phase and sonificated, forming a dispersion. This dispersion will be added to oil phase and emulsified, resulting in the emulsion. Though it helps the process, the use of organic solvents, resulting in drug precipitation, might affect the stability of the formulation and raise the cost of the process 28). A new technology, SolEmul, is a solvent-free method for incorporation, placing the drug in the interface. The hydrophobic drug is in powder or nanocrystal form and is mixed with a pre-formed nanoemulsion. This mixture is homogenized until the powder or cristals are dissolved in the interface (29).

When drugs have low solubility in water, adding fatty acids or raising the temperature can help the process (30). Propofol and halotane are liquid substances at room temperature and are widely soluble in water, which turns them into a perfect option of substances to be added to emulsions where the drug tends to be placed in the oil phase (1).

1.1.5. Metabolism

After administration, the formultation is subject to interaction with a variety of compounds in the bloodstream, such as erythrocytes, monocytes, leukocytes, platelets, opsonins, macrophages, dendritic cells, B cellsand lymph nodes. The probability of interaction between the formulation and erythrocytes is very high as these are the widest fraction of blood cells so, if the nanoemulsion ends up generating hemolysis, it will be removed by macrophages 31). As this kind of dosage form has a long half-life in plasma, the chance of interacting with blood cells is even greater and it might induce thrombogenicity, resulting in blood vessel occlusion (32). Opsonins are able to adsorb to the surface of nanoemulsions, promoting the uptake by macrophages, leading to reduction of drug delivery to the wanted place. This immune process might lead to anaphylactic, allergic and hypersensitivity reactions (31). Also influencing the interaction with blood components are comprised size, charge and surface properties of the nanoformulation. Large cationic or anionic particles are more likely to go

through phagocytosis. Adding to this, cationic surfaces tend to damage erythrocytes producing hemolysis (33), Addition of PEG (33), poloxamer (34) or poloxamine (35) to the surface of nanoemulsions creats a shield wich leads to a reduction in opsonization.

Phagocytosis, macropinocytosis or endocytosis are responsible for the cell uptake of nanoparticles which later accumulate in lysosomes, vacuoles or cytoplasm (1). The Brain-Blood Barrier (BBB) constitutes a remarkable obstacle to drug delivery that might be exceeded recurring to targeted nanoemulsions in order to reach receptors expressed in the cells of interest (36).

The main pathway for this formulations metabolism is the hepatic clearance, exposing them to hepatocytes, sinusoidal hepatic endothelial cells, Kupffer cells and hepatic stellate cells (37). Those taken by hepatocytes are eliminated by the biliary system and the ones taken by Kupffer cells go through phagocytosis, degradation and elimination. Renal clearance stands as an eminent step of metabolism of nanoformulations. Glomerural filtration is influenced by the particle size: particles smaller than 6 nm are filtered into the kidney and the particles with a larger size return to systemic circulation (38).

1.1.6. Nanoemulsions in cancer treatment

The great surface area, superficial charge, elevated half-time of circulation, specific targeting and imaging capacity of the formulation studied may represent a bonus in what refers to complex diseases therapy, like cancer.

As discussed ahead on this paper, cancer cells are surrounded by week vascularization and, once nanoemulsions possess biocompatible proporties with high stability, they can easily accumulate in these cells with their size as an advantage in passing through barriers. Not only this, but they can also be designed to define their function and encapsulate different types of drugs (2).

The main focus to achieve better therapeutic outcomes in cancer illness is to attain a targeted and efficient delivery, avoiding toxicity in healthy tissues. As chemotherapeutic drugs are developed to destroy rapidly proliferating cells, in case of lack of high selectivity, the side effects may be lethal. The employment of nanoformulations can also increase the solubility of substances that, in normal conditions, have low solubility, which is one of the main reasons why many drugs do not get approval in clinical trials (39).

1.2. Cancer and therapeutics

1.2.1. Microenvironment of tumor stroma

Tumor stroma is composed by Extracellular Matrix (ECM), fibroblasts, epithelial cells, immune cells, pericytes, adipocytes, glial cells (only present in the nervous system), proteins, vascular cells and lymphatic cell, constituting the so-called Tumor Microenvironment (TME) (40). ECM supports the growth, structure, migration, invasion and metastasis of the tumors possessing specific surface receptors which can be targeted by drugs. The delivery of oxygen and other basic cell supplies in small tumors is possible by simple diffusion. When the tumor gets bigger than 2.0 mm^3, the hypoxia conditions increase as a consequence of the lack of oxygen, leading to angiogenic development of new blood vessels (41). This means that inhibition of angiogenesis can affect cancer cells growth. In the last decade, a wide portion of anti-angiogenic drugs have been developed: bevacizumab (VEGF-neutralizing antibody), sorafenib (VEGF signaling pathway blockers), sunitinib and pazopanib. The problem is that these angiogenesis inhibitors have noticeable toxicity, increased development of therapeutic resistance and delivery issues (42). Nanoemulsions can encapsulate the drug in its core leading to diminished events of toxicity and enhancing payload delivery.

Cancer cells generate their energetic balance through glycolysis. Still, due to the hypoxic environment, the final metabolite (pyruvate) is yielded as lactacte. Lactate is eliminated by a monocarboxilate transporter recurring to H^+ (protons) on a phenomenon known as tumor acidification. Also, when hypoxia is present, the expression of carbonic anhydrase IX increases, generating bicarbonate from carbone dioxide, which leads to tumor cells uptake of weak bases producing a gradient between extracellular and intracellular tumor environment (1). Therefore, the value of pH is varied in cancer tissues: healthy tissues have pH values of 7.4 while tumors have values ranging from 6.0 to 7.0. This is the focus of pH-responsive lipids, which might play an interesting role since they are stable at a pH of 7.4 but can change their behaviour when placed in an acidic pH and release the therapeutic load. Several studies clarify that these particuled systems hold enhanced anti-cancer efficiency and reduced toxicity (1). Figure 4 represents the different variables interacting with TME.

1.2.2. Passive targeting

Tumor microvasculature is unorganized and heterogeneous, features leading to leaks and high pressure on interstitial fluid, producing modifications on the blood flow, presence of oxygen, drug and essential molecules in TME (43). In behalf of this, hypoxia of tumor stroma becomes more evident and, therefore, metastastic events have keener probability to occur. The collapse of lymphatic vessels leads to an unstable lymphatic system, which enhances the

retention time of drugs in tumor due to the decreased clearance of macromolecules. These events create high vascular permeability and low lymphatic drainage - this goes by the name of Enhanced Permeability and Retention (EPR) (44). EPR effect is, nowadays, a main point when developing anti-cancer drugs and can be a tool for passive targeting. Macromolecular and hydrophobic drugs are the ones taking most advantage of EPR, and their main mechanism is based on the capacity of retention as low molecular weight molecules are not taken and return to blood circulation (4).

Drugs with sizes between 20 nm and 100 nm have the capacity of being encapsulated as a method to ensure accumulation in tumor tissue without toxicity on healthy cells, as their size is too small to pass through blood vessels but big enough to avoid fast renal clearance. Nevertheless, this size enhances the circulation time and, therefore entrapment by MPS (45). To avoid opsonization by MPS, the surface of the nanoemulsion can be coated with hydrophilic polymers (PEG). This procedure has been tested and shown to increase time of circulation and accumulation in cancer cells (46).

The surface charge of the formulation is also relevant. It was demonstrated that positive charged particles are more likely to be taken by the tumor cells and retained through longer time periods. The reason behind this is a negatively charged molecule in tumor cells surface, phosphatidyl-serine (47).

Passive targeting methodologies can use a combination of size, charge and coating procedures to increase their therapeutic effect. The dark side relies on the fact that this kind of targeting does not differentiate a healthy tissue from a cancerous one and the fenestration and vascularization depends on the stage and type of tumor (48). There is also a problem with the use of PEG-coated nanocarriers since they can reduce interactions with cancer cells (49). The nanoemulsions which are currently being under investigatation for use in passive targeting are mentioned in Table 2.

1.2.3. Active targeting

Nanoemulsions can present ligands at their surfaces which recognize a certain molecule on the tumor tissue - this process is named active targeting. Like passive targeting, active targeting takes advantage of the environment surrounding the tumor, but it also finds a path to deliver the drug specifically to cancer cells. This process is only efficient if the molecules (usually, receptors) are homogeneously and only expressed it target cells (50). The bond formed can be like ligand-receptor or antigen-antibody (51). Targeting moieties link to over-

expressed receptors in cancer cells like folate (11), transferrin (52), epidermal growth factor (EFGR) (53) or prostate-specific membrane antigen (PSMA) (54).

Targeted delivery results in a specific toxicity to tumor cells and less side effects. It can also resort to changes in surface charge and increased sensibility to stimuli to enhance cellular uptake (55). Nanoemulsions using active targeting that are currently under investigation can be consulted on Table 3.

1.2.4. Multidrug resistance

Cancer cells find a variety of alternatives to avoid accumulation of drugs, resulting in multidrug resistance (MDR). The main mechanisms of MDR are based in the increase of the expression of multidrug transporters and modifications on the apoptosis course (56).

Transporter-dependent MDR is responsible for an over-expression of drug efflux pumps of ATP- binding cassette (ABC) family. This family of molecules pumps drugs out the cell and acts on a large group of anti-cancer drugs as substract. P-glycoprotein (P-gp), a transporter from the ABC family, often over expressed in liver, pancreatic, gastrointestinal and ovarian cancer, can pump doxorubicin (DOX), vinblastine and paclitaxel (PCX) (57). MDR influences the apoptotic pathway enhancing the expression of anti-apoptotic genes, namely Bcl-2 and nuclear factor kappa B (NF-kB) (58). MDR might be reduced recurring to P-gp inhibitors, decreasing Bcl-2 and NF-kB expression and using nanocarriers (passive or active targeting) (2).

Small interfering RNA, or siRNA molecules, were developed to aid chemotherapy since these molecules reduce MDR proteins expression and downregulate anti-apoptotic genes. Nevertheless, there are few compatible vectors to co-deliver siRNA together with drugs. Nanoemulsions might be of great utility to solve this problem, combining them with P-gp modulators or Bcl-2 inhibitors to surpass MDR. Hoping to correct apoptotic pathways and siRNAS and suppress efflux bombs to stop MDR genes, synthetic molecules are constantly being developed and investigated (59).

Ceramides are part of a group containing apoptotic molecules and are produced in situations of environmental stress, playing the role of programed cell death messenger. Some MDR cells avoid apoptosis through over expression of glucosylceramide, responsible for turning ceramide into an inactive glycosylated form. Ceramide might be delivered through nanomeulsion formulation to target a certain moiety in order to increase apoptotic effects in the tumor tissue (2).

1.2.5. Nanoemulsions multifunctionalization

A list of multifuncional nanoemulsions under research is represented in Table 4.

Nanoemulsions and antibodies

The bond between antigen and antibody (Ab) is quite specific and selective mostly because Ab possesses two binding places which also contributes for its selectivity. Nanoemulsions have the possibility to being conjugated with antibodies (Abs) or their fragments, with the purpose of targeting a certain molecule (60). The following monoclonal antibodies have been approved by the FDA for cancer treatment: trastuzumab, bevacizumab, rituximab and cetuximab (61). Distinct studies show that this conjugation results in internalization by cancer cells and successful drug delivery. The complex Ab-nanoemulsion can be stimuli-responsive making it even more specific to a certain tissue (60).

Nanoemulsions and aptamers

The SELEX method works by providing a library of ssDNA and ssRNA able to form DNA or RNA oligonucleotides, originating aptamers (62). Aptemers are smaller then Abs, do not possess immunogenicity, are hard to develop and have a low rate of penetration. They perform they role by binding efficiently to the compound of interest and then they fold into secondary and tertiary DNA/RNA structures. SELEX provides aptamers against tumor cells based on receptors and biomarkers recognition (63). A large variety of aptamers was developed against known cancer proteins like prostate-specific membrane antigen (PSMA), tenascin-C, nuclear factor kB (NFkB), human epidermal growth factor receptor-3 (HER3), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) (64). The conjugation of aptamers with nanoemulsions results in a more stable formulation in comparison with antibodies and, therefore, more efficient.

Nanoemulsions and Folic Acid

Folic acid possesses high affinity to folate receptor which makes it an ideal targeting moiety (65). Several types of cancer reveal high expression of folate receptor such as brain, lung, pancreas, breast, ovary, cervix, endometrium, prostate and colon (66). In healthy tissues it is only located on polarized epithelia in the apical surface (67). Several studies reveal that the number of folate receptors increases with the development of cancer cells mass (68). Folic acid is not expensive, not toxic, not immunogenic, easy to pair with nanocarriers, possessing a high binding affinity and is stable in circulation and storage (69). It is useful in combination with liposomes, toxins, immune system stimulants, chemotherapeutic drugs, imaging agents,

oligonucleotides, dendrimers and nanoparticules to selectively deliver them to cancer cells which present folate receptor (70).

Nanoemulsions and oligonucleotides

Oligonucleotides are unstable, with a very short half-life in biological fluids and weak intracellular penetration, making them one rare choice for conjugation with nanoemulsions. However, changing phosphodiester to phosphorothionate can enhance defenses against enzymatic degradation (71). Conjugation with PEG (72), cationic lypossomes (73), micelle polyelectrolyte complex (PEC) (74), lipidic and plasmidic DNA complexes (75), and pH-sensitive nanocarriers (76) can surpass the remaining problems, helping to enhance the success of cancer therapy.

Nanoemulsions and fluorescent conjugation for imaging purposes

Figure 5 shows a representation of a multifunctional nanoemulsion for cancer imaging. Imaging techniques allow several procedures for monitorization of cancer treatment which are, by turn, none or very little invasive with reduced tissue damage. The traditional anatomical methods are ultrasound, magnetic resonance imaging (MRI), bioluminescence imaging (BLI) and computed tomography (CT) (77) while molecular imaging employs methods like positron emission tomography (PET), molecular magnetic resonance imaging (mMRI), single-photon emission computed tomography (SPECT), optical bioluminescence and optical fluorescence, and contrast enhanced ultra-sound (CEU) (78). These often recur to conjugation of nanoemulsions with fluorophores and, seldom, radioisotopes, as they are toxic compounds (2).

2. Nanoemulsions in therapy of different types of cancer

2.1. Nanoemulsions applied to colon cancer therapy

2.1.1. Introduction

Colon cancer results in a large percentage of deaths related to cancer in the world (79). This type of cancer is divided in three distinct classifications: familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, sporadic colon cancer and cancer associated with colitis (80). These days, the mais choices in colon cancer therapy are operation combined with herbs, immunotherapy, radiotherapy and chemotherapy. However, about five years after surgery the survival rate starts to decrease, mainly due to metastasis and recurrence leadind to the belief that the cause of death goes beyond the tumor (81). The process where cells transition into mesenchymal cells with a change in their structure and increase in the addition and migration is know as mesenchymal transition (EMT) (82).

Lycopene (LP) is a molecule present in tomatoes with a wide variety of cancer therapy features, such as protection against chronic diseases, antiproliferation activity for leukemia and colon cancer cells and inniciating cell cycle arrest of some cancer cells (83). The problem with LP is that it is very unstable in circulation and has low bioavailability (84). A recent study focused on developing a nanoemulsion formulation containing LP, in order to solve the problem mentioned. The encapsulation of gold nanoparticles (AN) was also a resource as this particles can act as drug carrier and incorporate cell receptor ligands to achieve a targeted therapy (85). The issue with AN is that, in high doses, it promotes migration of human fibroblast cells, becoming toxic (88). The toxicity of AN might be reduced with the incorporation of lipid-based assemplies, such as a nanoemulsion containing LP (87).

This way, the ideal formulation would be composed of an oil phase containing AP, an aqueous AN solution and and emulsifier like Tween 80. The referred research used a human colon cancer line known as HT-29 with the purpose of evaluating the effect of AN, LP and the combination of AN and LP. Several parameters were evaluated to determine the efficiency of both components and their combined use: drug loading, particle size, polidespersity index, zeta-potencial, encapsulation efficiency, stability, cell viability and concentration. The formation of apoptotic cells and necrotic cells was also accessed with AV-positive (green AV from AV-fluos) and PI-negative (pidium iodide) fluorescence defined as apoptotic and AV-negative and PI-postivie (PI permeable) as necrotic. In order to calculate cell migration, several periodic photos were taken by a microscope to an incubation of a HT-29 cell line. The interaction with tumor markers antybodies NFkB and Akt, E-cadherin, Vimetin, β-catenin, procaspase-8, procaspase-9, Bcl-2, PARP, Bax, MMP-2 and MMP-9 was also accessed (79).

2.1.2. Results and conclusions

Analysis on shape, particle size, zeta potencial, polydispersity index, encapsulation efficiency, stability and identification were satisfying. AN placed itself around each nanoemulsion particle due to van der Walls forces as LP is nonpolar and lopophilic. Although the use of Tween 80 and LP should result in a neutral or slightly negative surface charge, AN grants a negative surface charge value to the formulation (79).

Figure 6 shows the changes in cytotoxic effect depending on NA particle size, revealing the highest grade of toxicity is achieved in 3-5 nm range. It also shows that LP-nanogold nanoemulsion in lower dose has higher cytotoxicity in HT-29 cells, with high percentage of reduction of cell viability. All treatments ended up inducing apoptotic changes.

Nanoemulsion treatment led to greater shrinking and more aggregation of apoptotic cells. To guarantee the specific target of HT-29 cells, a cell line called MCR-5 reaction to the same treatments was observed not showing any significant changes. Combined treatment induced early apoptotic cells development 15-fold more in comparison to control, with this rait raising when enhancing the concentration of LP. However, the nanoemulsion formulation increased early apoptotic cells with concentrations 100-fold smaller than the combined treatment (79).

Figure 7 shows the Western Blot results. Treatment with nanoemulsion encapsulating AN and LP reduces procaspase 3 and 8 by 90%, while combined treatment showed little decline. This means that treatment with nanoemulsion can activate death signal pathways more successfully that the combined therapy. PARP-1 – responsible for necrotic cell death - seems to be activated by the nanoemulsion, probably because the formulation enters HT-29 cells' nucleus inducing singnaling pathways. The combined treatment did not induce this effect. Figure 8 reveals that nanoemulsion decreases Bcl-2 levels more than combined treatment and increase Bax (pro-apoptotic protein) more, lowering the quantity of procaspase more as well. Treatments with AN alone and LP only change the migration behavior in high doses. The nanoemulsion formulation reduces migration rate more than any other treatment evaluated, achieving zero migration rate with high doses (79).

The absence of E-cadherin (epithelial marker) might induce mesenchymal cell transition (manifested by Vimetin marker), can promote proteloytic enzymes (MMP-2 and MMP-9) and induce EMT progression. The treatment with nanoemulsion raised the expression of E-cadherin and limited the expression of Akt and NFkB (both responsible for promoting cell survival), not affecting vimetin and β-catenin (EMT transition markers). The increase in migration might result from a week cell adhesion due to cell connection distruction during EMT. While this happens, more MPPs are produced, resulting in proliferation and angiogenesis by action in the extracellular matrix. AN alone and nanoemulsion reduced pro-MMP-2 expression 0.4-fold more than control and the combined treatment 0.5-fold. Nevertheless, pro-MPP-2 expression with LP treatment alone and active MMP-2 expression with all treatments didn't reveal any major change (79).

TEM was performed to understand how the different treatments interacted with cellular targets and microcompartments. In figure 9, control shows normal nuclear structure and homogeneous euchromatin fibers. The mitochondria has its normal round shape with cristae layers, some vacuoles in cytosol and filaments produced by plasma membrane. AN treatment also showed the same morphology and shape. The combined treatment revealed vacuoles

surrounding AN in the cytosol. The vacuoles were taken inside nuclear envelope folds in HT-29 cells and emerged from the mitochondria exhibiting abnormal morphology. The treatment with nanoemulsion at low dose resulted in more vacuoles then the combined treatment and unusual mitochondrial shape with loss of cristae. Cristae are inner membrane folds enhancing surface area which means its loss retards electron transport chain and chemiosmosis, resulting in low production of adenosine triphosphate – less production of cellular energy (79).

2.2. Nanoemulsion applied to ovarian cancer therapy

2.2.1. Introduction

A wide variety of cancer treatments resourt to Platinum (Pt) chemotherapeutics. Two known molecules belonging to Pt family, carboplatin and cisplatin have properties that help to increase survival more than any other existent ovarian cancer treatment (88). Pt mechanism is based on the distruction of the DNA structure through the formation of intrastrand and interstrand links (89). Nevertheless, this mechanism affects both cancer and healthy tissues and tumors might develop mechanisms to resist Pt treatment, such as promotion of pumps in the membrane, induction of enzymes or development of pathways to repair DNA. With his in mind, it is also important to consider if the tumor is Pt-sensitive or Pt-resistant (90). Furthermore, Pt possesses a high lipophilic profile (91).

Once again, nanoemulsions can solve delivery issues and enhance efficiency of Pt drugs by incorporating considerable quatitites of hydrophobic drugs and bind specific ligands to their surface, resulting in a targeted delivery (92). A study developed to access this theory involved the encapsulation of myrisplastin (recently discovered Pt based drug) and C_6-ceramide (pro-apoptotic substance) with the surface receptor EGFR binding peptide and gadolinium (imaging agent). The effect of the nanoemulsion was evaluated in three different cell lines: SKOV3 (human ovarian adenocarcinoma cells), A2780 (human ovarian cancer cells) and $A2780_{CP}$ (human ovarian cancer cells resistant to cisplastin). The lipidic core was composed by C_6-ceramide while the surface possessed PEG, Gd^{3+} (gadolinium) and EGFR. The emulsigying agent was egg lecithin (90).

To evaluate the formulation and stability obtained several tests were perfomed in order to attain size distribution, zeta potencial, cell morphology, Pt concentration and free Gd^{3+} and EFGR concentrations. The cellular uptake of both targeted and not-targeted nanoemulsions is SKOV3 cells was observed through their labeling with rhodamine. Citotoxicity was studied with MTT assay and interaction betewwn Pt effect and proapoptotic properties of ceramide

was accessed by the calculation of combination index (CI): CI<1 reveals the interaction is synergistic, CI=1 relates an additive interaction and CI>1 refers to an antagonistic interaction (90).

2.2.2. Results and conclusion

The characterization of the formulation indicated that neither myrisplatin or ceramide encapsulation affect size. Size distribution, concentrations, morphology and zeta potencial results were within the values expected. The aqueous phase did not contain free Pt revealing high encapsulation efficiency which was expected since the lipophilic profile of myrisplatin contributes for the retention in the oil core. It also did not contain Gd or EGFR (90). The particle size was evaluated in different environments to access stability: plasma, parenteral infusion fluids (dextrose and sodium chloride) and phosphate buffered saline to confirm there were no significant changes and the stability of targeted and not-targeted nanoemulsions. (Figure 10) (90).

The fluorescent labeling of formulations revealed that both the targeted and non-targeted formulations were taken efficiently by SKOV3 cells with targeted nanormulsions showing faster uptake and accumulation of the drugload. This is a result of the specific bond to EGFR peptide which means this moiety affectively targets ovarian cancer cells (Figure 11) (90).

The screening of cytotoxicity showed SKOV3 cells with EGFR were resistant to cisplastin but not to myrisplastin. Targeted nanoemulsions had 2-fold more toxicity than non-targeted formulations. The biggest change in cytotoxicity occurred when ceramide was also encapsulated, as the combination confirmed a synergistic behaviour. The targeted nanoemulsion containing ceramide and myrisplatin was 50.5-fold more effective than cisplastin. A2780 and $A2780_{CP}$, not expressing EGFR, presented more toxic effects with myrisplatin than with cisplatin. These results are detailed on table 5 and 6 (90).

2.3. Nanoemulsion applied to prostate cancer

2.3.1. Introduction

Deaths related to prostate cancer (PrC) have increased in the last few years and 70% of the affected population faces recurrence and further transition to untreatable situations (93). Prostate cancer development, resistance to therapies and metastasis iniciate through stem cells (CSCs) or tumor initiating cells (TICs) (94). Researches reveal that cancer cells presenting CSC markers, mostly CD133 and CD4, not only contribute to drug resistance mechanisms, but also proliferate after therapy (95). The presence of resistance to drugs in CSCs might have its origin in the up-regulation of drug efflux transporters, promotion of

anti-apoptotic pathways, destruction of apoptotic mechanisms, development of new processes to avoid DNA damage and repair processes (96).

The issue with known prostate cancer therapies is that they target groups of fast-growing tumor cells but do not detect subpopulations like CSCs. Furthermore, researches on anti-prostate cancer drugs use cell lines possessing elevated passage number which results in the cells acquiring properties with low or no similarity to the original tumor tissues (97). A new investigation focused on using a cell line from a patient of prostate cancer (PPT2) containing a small passage number. These cells also possess genes related to anti-apoptotic signaling and drug resistance, constituting an ideal model to study CSC-targeted therapies (98).

Abraxane®, a pro-drug of paclitaxel, is being studied for its use in prostate cancer therapy as it increases the solubility of paclitaxel, bounding him to human serum albumin. Nevertheless, paclitaxel therapies often result in the development of MDR (96). SBT-1214 is a new generation taxoid efficient against drug-resistance mechanisms that might be conjugated with docosahexaenoic acid (DHA) which is a natural PUFA with high affinity to a bloodstream transporter, human serum albumin, helping in the development of a targeted toxicity. Combination of DHA with paclitaxel originates a weak substract for Pgp and ABC transporters (99). A DHA-SBT-1214 nanoemulsion including phospholipids and fish oil was developed in the research referred. Drug encapsulation will be enhanced due to the affinity of the drug to fish oil. The main goal is that the nanoemulsion acts on PPT2 cell line, through the EPR effect and induces cancer cells apoptosis (96).

PPT2 cell lines were developed as monolayer and spheroids. To access the success of the formulation obtained, stability, drug loading and encapsulation were tested. Fluorescence recurring to rhodamine evaluated cell uptake. Cell viability was accessed by the measurement of Absorbance of DHA-SBT-1214 solutions with different concentrations. Suspensions of cloned cells expressing CD133, CD44, CD44v6, CD166 and EpCAM (tumor cell markers) injected to mice in order to determine the effect of NE-DHA-1214 and Abraxane® treatment in different organs (96).

2.3.2. Results and conclusion

Figure 12 confirms that the nanoemulsions produced presented spherical structure and size between 100-220 nm and that the encapsulation of DHA-SBT-1214 encapsulation does not affect the particle size significantly. HPLC revealed that DHA-SBT-1214 nanoemulsion had a value of 97% for drug loading, probably due to the lipophilicity of the drug. All particles

maintained surface charge and size during storage and the formulations were stable for 6 months at 4°C. The level of toxins was minimum in all formulations (96).

Figure 13 reveals an elevated uptake by PTT2 cells and spheroids. The replacement of rhodamine with DHA-SBT-1214 can confirm the nanoemulsion effect on cell viability. The administration of DHA-SBT-1214 resulted in more cytotoxicity, more so in the nanofomultation than the aqueous solution. Equal number of spheroids were also treated with different concentrations of nanoemulsion revealing healthy spheroids that get darker as the formulation concentration increases (96).

When analyzing tumor development in Figure 14, even low concentrations of NE-DHA-SBT-1214 had tremendous effect on reduction of tumor growth, contrary to treatment with Abraxane®. Tumour cells were transparent with no vascularization after treatment. Although reduction on tumor growth seemed to increase as the dose of the nanoemulsion did as well, the higher dose of the formulation did not result in augment of tumor reduction. The optimal dose of Abraxane ended up causing little supression during the 4 weeks of treatment and then the tumor started to grow similarly to untreated mice control group. All mice treated with nanoemulsion lost 17% of body weight by the third week but, started to regain the weight on the second week after treatment. On the other hand, Abraxane® didn't induce significant changes in weight (96).

Figure 15 reveals the analysis of tumor xenografts stained with hematoxylin and eosin and tissue sections of kidney, liver, pancreas and intestines from the mice, showing that the control group (untreated) had tissue sections with low degree of adenocarcinoma differentiation and visible nuclear atypia. On the other hand, the treated tumor tissues had cellular abnormalities, hyalurization, vacuolization, necrosis. Within the organs observed, liver tissue from treated mice was the only one revealing nuclear changes in hepatocytes, suggesting injury. The seeded cells evaluation of control group showed dense vascularization and NE-DHA-SBT-1214 treated mice revealed tumors with with no visible capillaries, lack of adherent holoclone production, small size and transparent aspect (96).

2.4 Nanoemulsions applied to lung cancer

2.4.1. Introduction

An anticancer drug know as Paclitaxel (PTX) is usually used in lung cancer, breast cancer, pancreatic cancer and ovarian cancer. This drug possesses the capacity to alter the process of breakdown of microtubules during cellular division which results in apoptosis, mitotic arrest and inhibition of cells activity (100). However, PTX has low water solubility, which

lead to the development of formulations, like Taxol® composed by Cremophor-EL® and ethanol. The problem with Cremophor-EL® is its toxicity (101).

Hyaluronic acid (HA) might be useful on the active delivery of PTX to cancer cells as it possesses a negative charge, binding efficiently to cluster of differentiation 44 (CD44), highly expressed in tumor cells (102). A study recently developed a nanoemulsion carrier to encapsulate PTX and HA (HA-complexed PTX nanoemulsion- HPNs) with the objective of testing its efficiency in the therapie of tumor cells expressing CD44 in non-small lung carcinoma cell line (NCI-H460) (103).

This study involved the assessment of zeta potencial, size, polydispersity index, morphology, drug encapsulation and concentration of PTC in PNs and HPNs. Mice were injected with NCI-H460 xenograft overexpressing CD44 for 6 weeks to evaluate the effect of different treatments on tumor size and bodyweight. A week after administration mices were divided into 4 groups with similar tumor sizes: one control group, another group treated with Taxol®, another treated with PNs and, finally, a group treated with HPNs (103).

2.4.2. Results and conclusion

Results on zeta potencial, charge, polydispersity index, size, morphology and encapsulation efficiency were statisfying (103).

PN and HPN induced more suppression in cancer cell growth than Taxol® (Figure 16), probably because both PN and HPN target cancer cells, taking advantage of the EPR effect. However, while PN targeted delivery is passive, HPN is able to actively target tumor cells because of HA's presence on the nanoemulsion surface, resulting in a higher cancer growth suppression. This means, HPNs not only target cells through EPR effect, but also by specific binding of HA to CD44. On Figure 17, tumor weight also reduces significantly in PN and HPN groups compared to Taxol® treated mices. HPNs group shows the lowest tumor weight, once again, demonstrating the formulation success in cancer cells suppression (103).

Figure 18 shows body weight loss in Taxol® treated group was greater than the control group, due to the drug's toxicity in all body tissues. Groups treated with PN and HPN have lost less than 10% of their body weight. Figure 19 confirms no significant change in lung, heart, liver, spleen and kidney weights (103).

3. Limitations of nanoemulsions

Nanoemulsions might reveal themselves as a unique resource to deliver drugs to cancer cells. Nevertheless, a large amount of critical considerations and parameters must be evaluated so the formulation ends up being safe and efficient. Although nanoemulsions represent an emerging option for cancer therapy no formulation has been approved by FDA or went past clinical trials. A wide range of issues may decrease the success of these systems (2).

The process of production of nanoemulsions often involves the resource to feverish temperature and pressure conditions. This difficults the choice of starting materials as a significant part are not suitable for these manufacturing processes. Such thing demands the design of an appropriate production method or, if necessary, its optimization, which might take some time to achieve. It is essential to use a method that protects labile drugs in large scale. This fact might be particularly hard as there is a fair number of variables to consider. [(2)] Choosing and developing a suitable method demands the consideration of several parameters as the safety of the material, scale-up and quality control (1).

All the founding components of the nanoemulsion, as well as its possible moieties and even the *in vivo* metabolism behaviour of the formulation need to be carefully assessed. Each compound or substance acts according to a very characterist mode and, therefore, their metabolism also develops in its own singular manner. Absorption, distribution and excretion directly influences drug safety which results in the requirement for continuous evaluation (2). When a new material is considered in a formulation, it is necessary to evaluate its long-term stability and safety. However, the models used to evaluate toxicity are frequently questionable since the absence of a dynamic environment with real organic interactions taking place on a real human body might create invalid results.

Studies and researches often start by using cellular models or animal species whose characteristics and metabolisms differ in a significant way from the human organism. Also, the human body behaves differently from person to person and, the same individual, may present varying health conditions depending on their state. Human metabolism is dependent on sex, race, age, environmental features and a lot of other terms. These topics thwart the translation from *in vitro*/*in vivo* to actual cancer treatment (1).

4. Future Perspectives

Nanoemulsions are formulations which aim the delivery of drugs, possessing also the capacity of encapsulating hydrophilic and hydrophobic molecules and being designed to satisfy a variety of requests (2).

The major target to achieve in the future is to find a mechanism that potentiates the selective and effective deliver of drugs with nanoemulsions, highlighting this formulation in comparison to others. This demands a continuous research, always baring in mind the interactions of the formulation with the other components in the organism, the influence of the manufacturing mechanism and the stability of the substance. In the future the interaction of nanoemulsions with target cells will also constitute a chief field for studies, exploring new paths to induce drug release and successful uptake. Hopefully, the future will also carry the discovery of new routes of administration for nanoemulsions encapsulating anti-cancer drugs. It is important to generate new insights on nanoformulations in order to come up with ideas for anticancer drug delivery (104).

Lately, nanoemulsions have revealed to be useful as imaging agents, providing real time monitoring of cancer cells and tissues with minimal or any degree of both destruction and invasion. These days, imaging techniques concern X-Ray tomography, magnetic resonance and ultrasound. All these methods work by marking a targeted nanoemulsion with a radioactive isotope or a fluorophore (1).

In the last years, vaccine-carriers in nanoemulsion formulations to target tumor cells have also been a topic. Nanoemulsions, as previously explained, possess the capacity of delivering macromolecules, such as antigens, which might lead to a useful antigen specific response from the immune system. This formulation allows longer circulation times and efficient uptake by cells with the specific antibody for that antigen on their surface, or vice-versa, creating a very specific interaction (2).

5. Conclusion

Nanoemulsions currently stand as one of the most promising strategies to develop new cancer therapies. Due to their hydrophobic core, it is possible to encapsulate hydrophobic drugs and create an option to unsoluble ones. They are also composed of an emulsifying agent as GAS excipients which proportionate the modeling and engineering of a stable and safe alternative. Furthermore, they can stay in circulation for a long time as they are constituted by very small particles.

Nanoemulsions differentiate themselves from other formulations because they can be designed to target specific moieties on the surface of tumor cells and avoid MDR. This is a remarkable discovery in the development of cancer therapeutics, since most of the existing ones fail due to their toxicity in healthy tissues or due to the fact that cancer cells develop mechanisms of drug resistance.

Passive targeting uses ERP effect to act, typically, in tumor tissues. Nevertheless, active targeting might comprise more positive features using not only EPR effect, but also specific targeting moieties directed to cancer cells. Multifunctional nanoemulsions can co-encapsulate or bind to compounds that overcome MDR mechanisms.

The examples described prove that there are distinct methodologies to design nanoemulsions in order to achieve successful therapeutic results in several types of cancer.

The positive features referred become useless if the manufacturing process and the metabolism of the formulation are not carefully evaluated. These parameters constitute the chief barriers in the development of nanoemulsions. To obtain this type of formulation, surpass every phase of clinical trials and achieve approval, all variables must be assessed, and innovative solutions are required to be evaluated in order to achieve safe and efficient anticancer drugs. The development of these formulations might be an answer to cancer therapy dilemmas as this multifactorial illness contributes to a substantial portion of deaths with no completely viable therapy been described to this day.

SECTION II

CURRICULAR INTERNSHIPS

PART I

Internship in Pharmaceutical Industry

Introduction

The *curriculum* provided by the course Integrated Master in Pharmaceutical Sciences (MICF) held at Faculty of Pharmacy, University of Coimbra (FFUC) is constituted, among others, by a curricular unit named *Curricular Internship*. This unit requires the attendance of one or more internships during the second semester of the fifth year of the considered course. The referred performance must be evaluated through a report describing the work attained during the internship. The present report will be presented as a SWOT (Strengths, Weaknesses, Opportunities and Threats) analysis.

The FFUC allows students of MICF to go through an internship in the sector of Pharmaceutical Industry. This field is getting more relevant in the pharmaceutical market, modelling it in a very competitive sector. This internship not only contributes to achieve formation and experience, but also transmits good curricular advantages. The internship was developed at Basi® Indústria Farmacêutica S.A. (Mortágua) - part of FHC group - in the Quality Assurance department.

The internship was supervised by João Oliveira, Pharm. D., and was integrated in the Qualification of Extern Entities. It began in the 8th January of 2018 and ended in the 29th March of 2018.

Through this report I will describe the work developed, the articulation between the information acquired from college and the tasks proposed as well as the way this experience impacted my professional profile.

Basi® Laboratories

Basi® is a pharmaceutical industry enterprise integrated in the group "FHC | Farmacêutica". FHC is constituted by a group of industries which develop their activity in the production, distribution, promotion, logistic management, consulting and technology services associated to the manufacturing of drugs in different dosage forms (105).

Basi® Laboratories has a portfolio containing 200 drugs for 50 different therapeutic finalities and its market is now extended to other European countries than Portugal, Africa, Middle East, Latin America and South Asia (106).

This industry has been a reference in the pharmaceutical sector with more than 60 years of success. Its goal is the development, manufacture, commercialization and distribution of drugs all over the world, always bearing in mind the European standards and the Quality Assurance and Control. These tasks are daily developed under flexibility, innovation, competition and efficiency values. The authorized activities executed in the factory concern the production of external use liquids, production on internal use liquids, production of semi-solid preparations, production of suppositories, primary packaging, secondary packaging, quality control and package release. Some of these activities are subjected to contracts with other manufacturers to acquire active pharmaceutical ingredients, excipients, finished products and packaging material (106).

SWOT analysis

The SWOT analysis stands for Strengths, Weaknesses, Opportunities and Threats for a determinate procedure, labelling it as a great tool of analysis, commonly used by organizations in the management departments.

STRENGTHS

- ✓ **Internal formation in the beginning of the internship**
- ✓ **Knowledge acquired in university applied**
- ✓ **Development of tasks with autonomy but with the necessary supervision**
- ✓ **Visualization and recognition of extern entities documents**
- ✓ **Development of new computer skills**
- ✓ **Contact with other languages**

WEAKNESSES

- ✓ **Short duration of the internship**

OPPORTUNITIES

- ✓ **FFUC with agreements with industries for the realization of internships**
- ✓ **Participation in the elaboration of a QMP model**
- ✓ **Contact with different departments**
- ✓ **Elaboration of complaint reports**

THREATS

- ✓ **The Pharmaceutical Industry is a very competitive area**

MICF doesn't provide enough knowledge on the industrial area

Strenghts

Internal formation in the beginning of the internship

On the first day of the internship, João Oliveira, Pharm. D., was responsible for a little formation regarding the Quality Management System (QMS). The goal of this formation was to give a general idea on the practices of the factory: access areas, equipment, blueprint of the system, organigram with the function of staff, management of documents, conditions of quality for correct manufacture, control and batch release. After this theoretic introduction to the factory, a guided tour ensued. The tour went through different units: administrative, laboratorial, production, packaging and storage. This tour contributed to collect knowledge in what comes to the distinct operations that take place in the factory and made up for a first contact with the collaborators from all the areas. At last, I was provided with all the theorical information, kept in dossiers, which was useful during the three months of internship. These data included: Quality Manual, Site Master File (SMF), Validation Master Plan, Quality Management Procedures (QMP) and Technical Operational Procedures (TOPs).

The knowledge acquired in this first moment was essential for all the activities performed during the internship and helped me to perform all the tasks at hand according to the quality politics followed by the company.

Application of knowledge acquired in college

A large part of the tasks performed during this internship demanded the constant attention to the distinct parameters of quality. The subject thought during the course, *Pharmaceutical Administration and Organization*, was crucial, since many of the concepts introduced by hand of my training supervisor were already familiar. All my work had to agree with the Good Manufacturing Practices (GMP), described in the volume 4 of Eudralex. These guidelines refer to the pharmaceutical management system, staff, equipment, documents, production, quality control, contracted activities, complaints and returns, and internal audits. During this internship I was responsible for checking, through received documentation, if the external entities complied with the parameters described in the GMPs, such as: fabrication authorization, European GMP certificate, local GMP certificate, ISO 9001 certificate, ISO 14001 certificate, ISO 18001 certificate and ISO 22000 certificate, assuring they were valid and within the due expiration date (107).

The subject *Regulatory Issues of Medicinal Products* was also quite useful, especially in the assessment of external entities, since some documents which I was beginning to evaluate

were taught in the lectures of this subject. Some examples are Drug Master File (DMF), Material Safety Data Sheet (MSDS), Certificate of Analysis (CoA) and Certificate of Suitability (CEP). My work included the analysis of these documents with further checking addresses, dates, names, among other issues.

Autonomy in the development of tasks

Each task was explained to me in detail. This never invalidated the fact that I could recur to my supervisor, or other element of the department, to clarify any doubt that occurred during the realization of my work. Nevertheless, I had full autonomy to perform the tasks at hand, using my common sense, which was essential in the development of my work with trust and knowledge on the area. Every time I finished the tasks proposed, my supervisor would review the documents or changes that I inserted aiming to warrant a correct result.

Visualization and recognition of documents from extern entities

A great part of the work I developed included the analysis of documents sent by suppliers and external producers to help with the qualification process. This allowed me to develop a critical opinion and vision in the moment of accessing the veracity of a given document. The permanent contact with these documents also aid me to realize the detailed process behind the qualification of external entities.

To qualify manufacturers of finished product (FP), Basi® does require a variety of documents: Manufacture Authorization (MA), European GMP certificate, national GMP certificate, Technical Agreement (where the manufacturer compromises to warn the client if any condition of production changes), audit report elaborated less than three years ago, CoA, SMF, Qualified Person (QP) declaration, manufacture instructions, stability data, among others.

The qualification of Active Pharmaceutical Ingredient (API) depends on: MA, European GMP certificate, Written Confirmation (compromising to follow European guidelines if the factory is located out of the European Union), audit report elaborated less than three years ago, *Curriculum Vitae* of the auditor, Technical Agreement, local GMP certificate, CEP, DMF, MSDS, TSE/BSE declaration (*Transmissible Spongiform Encephalopathy*/*Bovine Spongiform Encephalopathy*), Residual Solvents declaration, stability data, manufacture flowchart, among others.

To access certain documents and their respective emission and expiration dates, it was necessary to consult the website of *EudraGMP,* which provides the most recent versions of European GMP certificates (108) and Manufacture Authorization (109) of several entities.

Development of new computer skills

Along these three months, a substantial portion of my work demanded the knowledge of Microsoft Excel®. This tool was vital when it came to organize charts to qualify API and FP suppliers, allowing the elaboration of lists with different APIs, FPs, excipient packaging materials with the objective of classifying documents, especially their veracity and utility.

The management of information related with released batches, suppliers, complaints and deviations reports, among other important activities, is mainly achieved using two distinct informatic systems: Primavera® and Q-pulse®. The Primavera Software® holds information needed to manage stocks, such as alterations in status of stocks or information coming from suppliers or inside the factory. Q-pulse® is applied to perform risk analysis on data, assess audits/inspections, as well as Corrective and Preventive Actions (CAPA) and Change Control (CC) and even analyze complaints. Some of the proposed activities allowed me to work with both systems and understand their functioning.

Contact with other languages

The evaluation of documents from suppliers all around the world (Portugal, Spain, France, England, Italy, Germany, Netherlands, United States of America, Mexico, India and China) enhanced the contact with other languages, mostly English, as the generality of the documents was written in this language. This permanent contact developed my knowledge of the English.

Weaknesses

Short internship

Although the opportunity to go through this internship was unique and extremely fulfilling, both professionally and personally, three months do not seem enough to reach minimally all sectors which are concerned by pharmaceutical industry - even when referring to only one department. The pharmaceutical industry, particularly the Quality Management department, covers a wide range of variables, demanding continuous and long-time formation. The short duration of the internship limits the trainee, since there is no time to reach the full potential that would allow the development of tasks with full efficacy. In addition, it also hinders the supervisor in the creation of a well-balanced internship plan.

Opportunities

Arrangements stablished between FFUC and industries for internships

The establishment of agreements with several pharmaceutical industries is unprecedent and to praise to the FFUC, as this type of internship is not possible in other universities in Portugal. Since this area is achieving more and more attention by pharmacists, the competition gets stronger every day. This internship constitutes, therefore, an advantage to students of FFUC in comparison to other students from other universities.

Participation in the elaboration of a QMP model

Within the classification of external entities, I was asked to access one GMP guideline relative to the good manufacturing practices of excipients with the purpose of performing a Risk Analysis to the suppliers using the Gap Analysis method (110). Gap analysis is based on a comparison between a present process and a desired process that the company desires to achieve. After the evaluation of the guideline I proceeded to create a list with all the excipients used in production and all the risks (described in the guideline). This list would later be useful to assign an individual score to each parameter and, finally, a total score to the entity. The final score would define the need, or not, of an audit. This model would be included in the QMP relative to the qualifications of entities.

Contact with different departments

The dynamic of a factory demands that every department is in permanent contact and cooperation. The Quality Management department is dependent on several areas such as Quality Control, Logistics, Product Management and Production. Therefore, it also affects all these sectors. Using this method, the collaborators from the departments are constantly interacting, enhancing the spirit of collaboration. As a consequence of such, I had the opportunity to not only interact with collaborators from the Quality Assurance department, but also from other departments.

In periods where I was less busy with the tasks on my internship plan, I was given the opportunity to help other departments in their work. As an example, I elaborated lists of nonconformities associated with the internal production of medicines or with substances from suppliers. These lists contained the respective characterization, root-cause identification, preventive and/or corrective actions.

Elaboration of complaint reports

During the period when I collaborated with other departments I had the possibility to participate in the elaboration of complaint reports, under the orientation of José Teixeira, Pharm.D. The development of reports describing the process of research of a non-conformity is essential in any pharmaceutical industry and the related procedure allowed me to realize that the elaboration of this document demands knowledge about every single sector within the factory, mainly the production one. To formulate the document, it is usually necessary to analyze the samples relative to the complaint, retention samples, access the documentation from the specific batch, consider past complaints, determine probable root causes, evaluate the range of the occurrence, perform a risk analysis and describe corrective and preventive actions. The process is, therefore, crucial for the development of information about a wide variety concerning the activity of production.

Threats

Pharmaceutical Industry as a very competitive area

Even though this internship is a strongly competitive advantage in the working market of the Pharmaceutical Industry, this field turns more competitive every day. Pharmacists are becoming more interested about different sectors other than community pharmacy and, so, the pharmaceutical industry is, without any doubt, on of the most appealing. The further quest for work in this area makes the process harder for anyone who wants to labor in it, even with the experience arising from this internship.

MICF does not provide enough knowledge about industry

Community Pharmacy is the chief professional area for pharmacists and, therefore, MICF is seriously directed for that purpose. Only a few subjects during the course are truly related to the industry sector. Such reality does not favor the professional practice in industry for fresh professionals.

Conclusion

The internship in Basi® was an extraordinary, challenging and enriching experience. The industrial field was already of enormous interest to me and going through these three months gave me a wider vision of the sector and an expanded will to work, in the future, in this sector. Being able to apply all the theoretical knowledge acquired in college enhanced my critic views, my confidence and, most of all, gave me the possibility to learn and absorb new notions and perspectives.

Keeping up with the process of qualifying entities, among other functions, lead me to emerge with ideas I already had but never actually witnessed or performed, which tremendously facilitates the learning process.

I would like to finish this report expressing gratitude to the entire team from Basi® for the way they received me and helped me through every step of this short (but intensive) path and, above all, acknowledge with special regard my supervisor, João Oliveira, Pharm. D., for his time, patience and availability. Another special note of gratitude to José Teixeira, Pharm. D., for always challenging me with new tasks and granting me with the chance to keep up with his amazing work.

PART II

Internship in Community Pharmacy

Introduction

The following report was developed to describe the second part of the work established within the subject *Curricular Internship* belonging to the course Integrated Master in Pharmaceutical Sciences (MICF) held at Faculty of Pharmacy, University of Coimbra (FFUC), which demands the realization of an internship in the field of Community Pharmacy. The attendance of this internship enables the contact with the professional practice and, therefore, allows the students to apply the knowledge acquired during the course.

The Community Pharmacy stands for the most visible activity of a pharmacist and represents the milieu where pharmacy professionals can be in touch with the customer. This is a function of great responsibility since turns the pharmacist one of the main characters in the promotion of adhesion to therapeutics, correct use of medicines and prevention of illnesses.

The internship started on the 2nd April of 2018 and lasted until the 3rd August of 2018. It took place at Pharmacy Sanal in Oliveira do Bairro, Águeda, under the orientation of Maria Ângela Lima, Pharm. D.

The present internship report will be presented in the form of a SWOT analysis in order to describe the work developed and access its utility for my future professional career.

Pharmacy Sanal

The pharmacy where the internship took place, Pharmacy Sanal, is located at Oliveira do Bairro, Águeda. The owner and technical director is Maria Ângela Lima, Pharm. D. The pharmacy is considered in a group of two pharmacies which includes Pharmacy São José, in Sangalhos. Both pharmacies work together and collaborate to provide the best service to their customers. Pharmacy Sanal is associated with National Pharmacies Association.

The schedule of the pharmacy is settled every day of the week from 9 a.m. until 21 p.m., taking night shifts on alternate days of the week, once the town only has two pharmacies. The night shifts work on an availability regimen assured by the presence of a pharmacist or an auxiliary pharmacist according to the directive Law-Decree number 172/2012, August 1st (111). On Saturdays, the pharmacy is open from 9 a.m. until 13 p.m., except on a weekend of service. In such case it is open on Saturday and Sunday all day. The weekends of service also rotate between the two pharmacies in town.

The professional team is composed by two pharmacists and two pharmacy technicians cooperating to satisfy the necessities of community. Regarding the installations, they are composed by the room where the service to the public takes place, storage, laboratory, sanitary compartments, personalized care office, area of receiving orders and technical direction office, according to the deliberation number 1502/2014, July 3rd (112).

SWOT analysis

The SWOT analysis allows the identification of Strenghts, Weeknesses, Oppurtunities and Threats of a determinate procedure, which makes it a great tool of analysis, commonly used by organizations in the management departments.

STRENGTHS

- ✓ **Introduction to the team, pharmacy politics and work areas**
- ✓ **Sifarma 2000®**
- ✓ **Orders gadget from Cooprofar**
- ✓ **Integration of theoretical knowledge**
- ✓ **Development of compounded drugs**

WEAKNESSES

- ✓ **Difficulties in associating a trade name with the name of the active substance**

OPPORTUNITIES

- ✓ **Participation in several formations**
- ✓ ***Valormed* awareness raisig action**
- ✓ **Professional development**

THREATS

- ✓ **Lack of practice**
- ✓ **Possibility of acquisition of non-prescription medicines in other commercial facilities and online**
- ✓ **Precarious economic situation in Portugal**

Strengths

Introduction, pharmacy politics and work areas

On the first day of this internship, the team and the pharmacy areas were introduced to me as well as the services provided: pharmaceutical counseling, biochemical analysis (cholesterol, glucose and blood pressure determination), weighing-machine, nutrition and osteopathy.

The organization of stocks was also explained to me in detail, as there were specific places for generics, extemporaneous preparations, powders, emergency contraceptive pills, transdermal systems, nasal preparations and oral solutions. Non-prescription medicines would stay behind the counter and cosmetics, animal, baby and diet products were exposed in shelfs. (Figure 20)

This first contact was essential to understand the everyday developed work and how I would be able to help in the future.

Sifarma 2000®

The software used by Pharmacy Sanal was the Sifarma 2000® system, a well-known managing program in Portuguese pharmacies. This system allows the management of stocks (reception, returns, expiration dates, invoicing, among others), provides detailed scientific and commercial information on each medicine and enables the elaboration of files for each client. This way, the counseling provided can be more accurate and efficient.

The FFUC provided their students with a formation on this software. This and the fact that I had already gone through a summer extracurricular internship on a pharmacy before facilitated the use of this tool.

Cooprofar® ordering tool

Pharmacy Sanal belonged to a group of pharmacies named Firstpharma Group. These pharmacies worked mainly with Cooprofar®, a pharmaceutical distributor. The prior provided them with the advantage of ordering certain products, through the Cooprofar® tool, with discount. Not only this, but the gadget had detailed information about the orders marking them as "in placement", "in transit" and "delivered". The gadget also allowed the recognition of instantaneous orders, for products with priority to arrive as soon as possible to the pharmacy.

Integration of theoretical knowledge

Along four months of internship, I was able to apply many of the concepts acquired in the subjects I had studied for, mainly from *Clinical Pharmacy, General Pharmacology, Pharmacology I* and *Pharmacology II*. These four curricular subjects provided information on the metabolism, adverse reactions, interactions and dosage of the main pharmacological groups. The background achieved from these subjects furnished me with the confidence required to be in contact with the customer and advise him correctly.

Development of combined drugs

According to the directive Law-Decree number 95/2004, April 22nd, responsible for the prescription and preparation of combined drugs. A combined drug is any masterful formulation or officinal preparation arranged and dispensed under the responsibility of a pharmacist (113). On several occasions I was given the opportunity to prepare extemporaneous preparations such as sulfur at 6.0 % on sterilized Vaseline base (Figure 21). This was relatively easy for me since I had prepared this type of dosage form before, during the practical component of the curricular subject *Galenic Pharmacy*.

The preparation had to be made in the adequate physical and chemical conditions and space. When the preparation of the formulation is finished it is also important to alert the customer about the conditions of conservation and dosage in the moment of delivery of the preparation.

Weaknesses

Difficulties with association of medicines trade names and active substances

Although some commercial names were already familiar, on an initial phase of the internship the lack of knowledge in what comes to the trade names of drugs was clear. The subjects belonging to MICF do explore a large variety of pharmacotherapeutic groups. Nevertheless, they are referred to as active substance name and rarely are accompanied by their commercial name. The prescription that is delivered on a pharmacy often comes with the commercial name which turns it hard to recognize the substance needed on a first impression.

Opportunities

Participation in several formations

One of the positive aspects of working in community pharmacy is that a person is always getting in touch with the last news about medicines. A variety of laboratories and companies contribute for formations, both outside and inside of pharmacy, on their products and associated treated diseases. This method turns possible to pharmacists to be in constant updating about critical information to provide the most correct counseling possible.

Valormed awareness action

Valormed project results from the cooperation between the pharmaceutical industry, distributors and pharmacies with the goal of separating medicines out of use from the population to avoid their residues to stay in contact with the community (114). Therefore, it is relevant to sensitize the community to bring these medicines to the pharmacy so that they can be properly collected. With this purpose in mind, Pharmacy Sanal organized awareness raising actions prepared for kids in kindergarten. I was responsible for assembling a presentation with the purpose of explaining the importance of Valormed project as well as the course of the medicines since they are produced until they are collected by Valormed. (Figure 22)

Professional development

During the internship the evolution reflected on my professional knowledge was clear. I started by helping in the organization of medicines, placing them in the right place, according to their expiration date (first in, first out). This helped me to recognize different dosage forms, brands and functions of the products. When comfortable with this task, I started to work more with Sifarma 2000® in the reception of orders (Figure 23), regularization of devolutions (Figure 24) and managing of products near their expiration date (Figure 25). Lastly, in the third and fourth month of the internship I started activities which demanded contact with the public: dispensing of medication, pharmaceutical counseling, reading of manual (Figure 26) and electronic medical (Figure 27) prescriptions, measurement of blood pressure and of biochemical tests.

The organized and oriented development of my work was essential for a correct apprehension of important concepts and for the accomplishment of confidence that would be really important on the contact with the clients.

Threats

Lack of practice

During the five curricular years of MICF there is no demanded official practice in a community pharmacy but the curricular internship. The students can apply to a summer internship, but it lasts very little to allow a viable adaptation to any pharmacy. As this practice is one of the main activities developed by pharmacists, it would be expected an extended the time devoted to this practice. This way the curricular internship would be easier and there would not be needed such a long time of adaptation.

Possibility of acquisition of non-prescription medicines in other commercial facilities

According to the Law-Decree number 238/2007 June 19th, non-prescription medicines can be sold out of community pharmacies(115). The role of pharmacists is undeniable and essential when it comes to advises on medicines and illnesses. The problem is related with the fact that this installations practice very competitive prices, attracting more customers and, therefore, promoting the irresponsible self-medication.

Precarious economic situation in Portugal

By these days, Portugal faces a clear economic crisis that reflects on the pharmaceutical activity. Customers have less money due to their low salaries, reduction of co-participation of some medicines and the unaffordable prices of some products. The existence of parallel trade originates rupture of stocks in laboratories, resulting in some customers to stay without their medication for months. This economic situation not only harms clients but also the professionals, with lack of favorable conditions to contribute for community health.

Conclusion

The realization of this internship is required to complete the MICF course and allows the application of knowledge previously acquired. Its completion was essential to make me grow as future health professional and to make me closer to the public, developing my personal interaction capacities.

Finishing this internship made me realize of the significant role of a pharmacist in the community pharmacy since this health professional might be the first to contact with a diseased person and, therefore, will provide the first advice and impressions the patient. Therefore it is of immense importance that the pharmacist provides the correct and adapted information, contributing for the good health of the community.

I want to finish this report thanking to all the team from Pharmacy Sanal for welcoming me to their everyday routine and environment. I was immensely lucky and fortunate to have worked with such wonderful professionals that taught me so much about the craft of a pharmacist and contributed in such a caring way to make me a better professional. I want to thank to Maria Ângela Lima, Pharm. D., for being so patient with me and for being constantly worried about my attaining of the necessary knowledge she transmitted. I also want to thank Ana Margarida Melo, Pharm. D., for making me much more aware of details and organize my work correctly, as a true pharmacist should, and Paula Ribeiro who made this internship much more gratifying and interesting. Finally, I want to acknowledge Luísa Lima, who made me learn with joy and interest and always accompanied my internship with all the help she could provide.

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Annexes

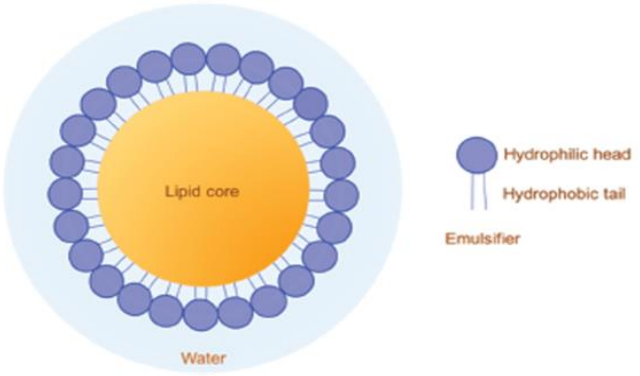


Figure 1: Basic composition of a nanoemulsion [2)]

Table 1: Commercialy available drug delivery systems in nanoemulsion formulation [1)]

Drug	Commercial Name
Alprostadil Palmitate	Liple®
Clevidipine	Cleviprex
Dexamethasone palmitate	Limethasone
Diazepam	Diazemubs
Flurbiprofen axetil	Lipfen
Flurbiprofen axetil	Ropion
Propofol	Diprivan
Vitamins A, D, E, K	Vitalipid®

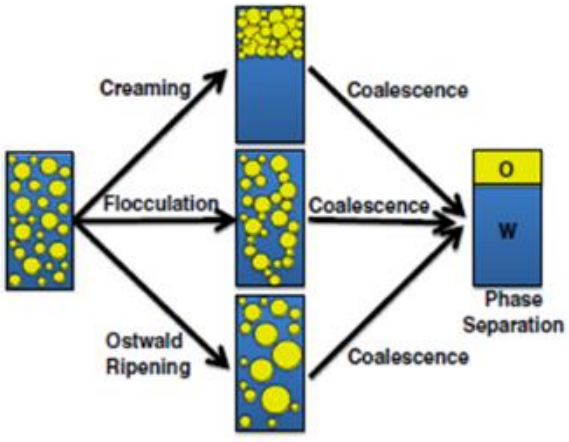


Figure 2: Mechanisms of nanoemulsion breakdown [1)]

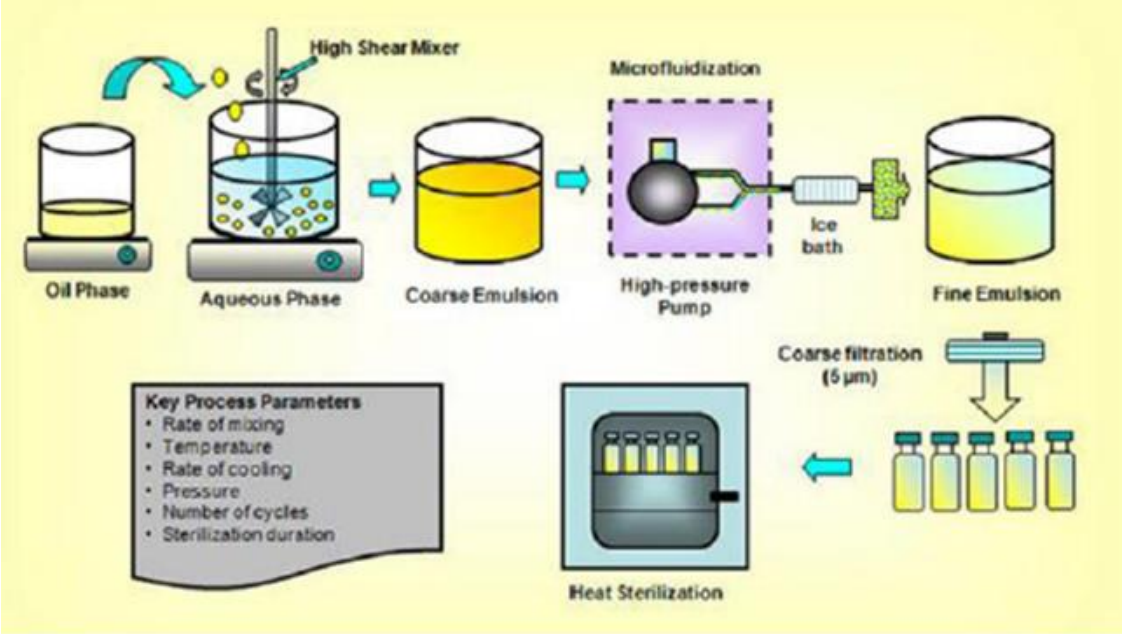


Figure 3: Production of nanoemulsions [1)]

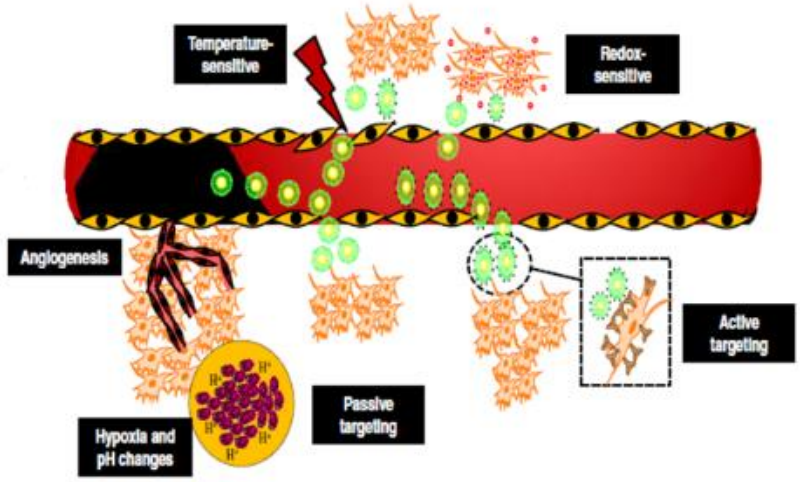


Figure 4: Tumor microenvironment [1)]

Table 2: Nanoemulsions using passive targeting in cancer treatment [2)]

Drug	Commercial Name	Type of Cancer
Paclitaxel	NK-105 Genexol®	Metastic breast cancer, breast cancer, recurrent breast cancer, lung cancer, pancreatic cancer, urothelial cancer, gynecologic cancer
Aminolevulinic acid	Ameluz®	Lentigo maligna, multiple actinic keratoses, superficial basal cell cancer
Curcumin	NEC	Breast Cancer in obese women
Docetaxel	Docetaxel	Recurrent/Metastic head and neck squamous cell carcinoma
Cisplatin	NC-6004 + GEM	Metastatic Pancreatic Cancer

Table 3: Nanoemulsions using active targeting in cancer treatment [2)]

Target	Targeting Ligand	Drug	Type of Cancer
Folate receptor	Folate	Paclitaxel Doxorubicin	4T1 breast cancer A2780 ovarian cancer
Integrins (α, β_3)	RGD peptide	Paclitaxel	MDA-MB-435 breast cancer
Transferrin receptor	Tranferrin	Docetaxel	MDA-MB-231 breast cancer
PSMA	TDO5 aptamer	-	(K562) myelogenous leukemia
Biotin Receptor	Biotin	Epirubicin	HL-60 marrou leukemia
EGFR	Anti-EGFR mab (cetuximab)	Cisplatin (NC-6004)+5-FU	Head and neck squamous cell cancer
HIF-1α	Anti-HIF-1α mab	Paclitaxel	MGC-803 stomach cancer

Table 4: Multifunctional nanoemulsions used in cancer treatment [2)]

Drug/siRNA	Targeting Ligand	Stimuli	Imaging agent
PIK75 (PI3K inhibitor)	EGFR sepcific folate ligand	-	NBD-C6-ceramide
Doxorubicin	Folate	pH	DHPE (fluorencence)
-	Anti-myosine mab, 2G4 temporarly shielded TATp	-	Rhodamine (fluorescence)
Doxorubicin	Anti-Her2 Fab	Thermo responsive	QG fluorescente probe
Doxorubicin	Folate	pH	SPION for MRI
Paclitaxel	Anti-PSMA mab, J591	-	Supermagnetic iron planinum NPs (SIPP) for MRI Rhodamine (fluorescence)
Prednisolone acetate valerate (PAV)	$Av\beta_3$-specific RGDp	-	(FeO) nanocrystals for MRI Cy7 (fluorescence)
Doxorubicin Mdr1-siRNA	$Av\beta_3$-specific RGD4Cp TATp	pH	Cys5.5 (fluorescence)
Doxorubicin VEGF-siRNA	Folate	pH	FAM labeled siRNA (imaging)
Docetaxel Plk1-siRNA	Herceptin mab	pH	Nile red (fluorescence) Picogreen labeled siRNA

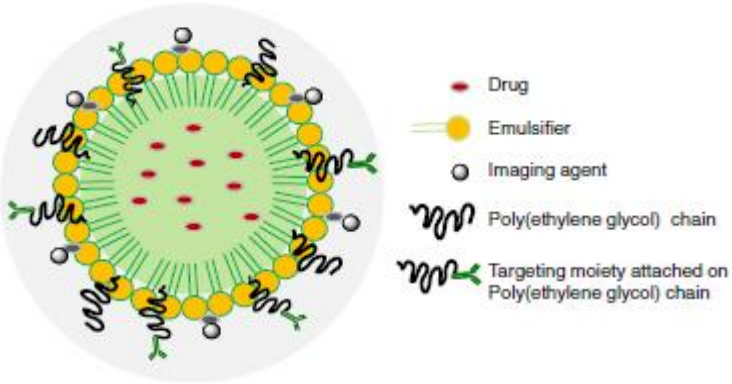


Figure 5: Multifunctional nanoemulsion for cancer imaging [1)]

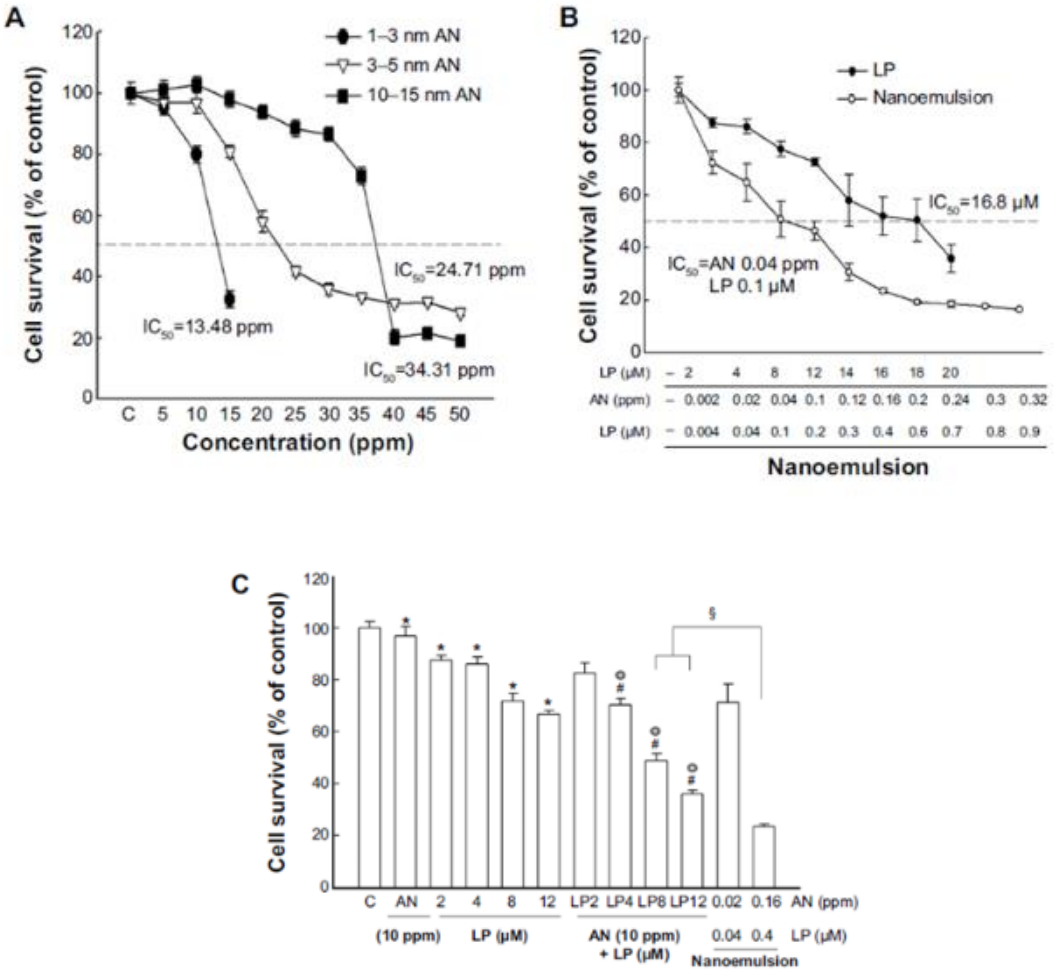


Figure 6: AN alone, LP alone, AN+LP (combined treatment) and LP-nanogold nanoemulsion's effect on citotoxicity of HT-29 cells [79)]

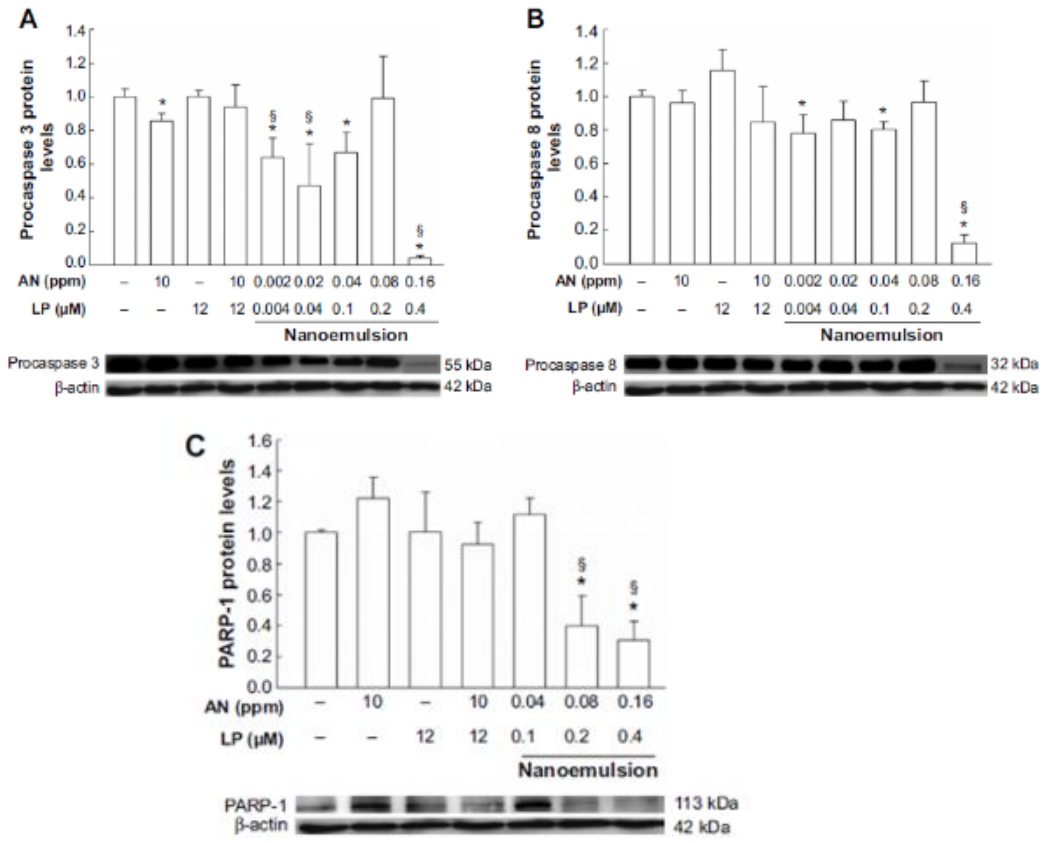


Figure 7: AN alone, LP alone, AN+LP (combined treatment) and LP-nanogold nanoemulsion's effect on procaspase 3, procaspase 8, PARP-1 activation of HT-29 cells [79)]

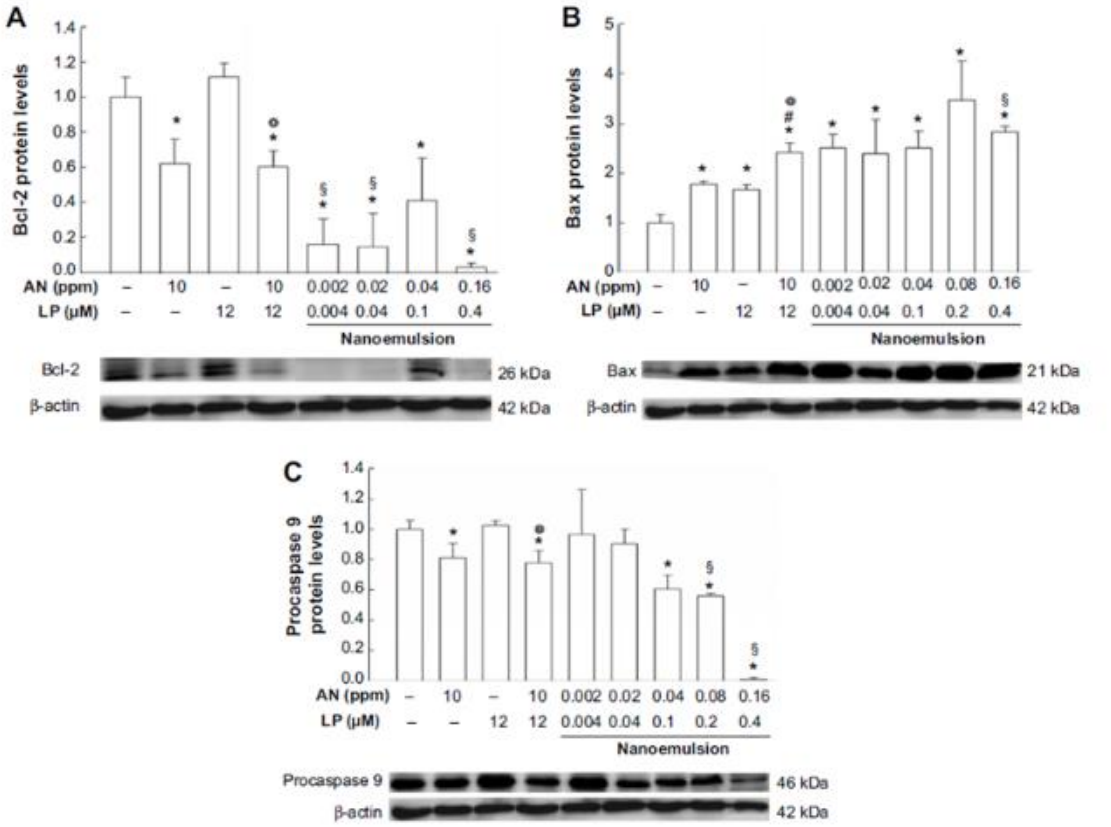


Figure 8: AN alone, LP alone, AN+LP (combined treatment) and LP-nanogold nanoemulsion's effect on mithocondrial death signaling of HT-29 cells [79)]

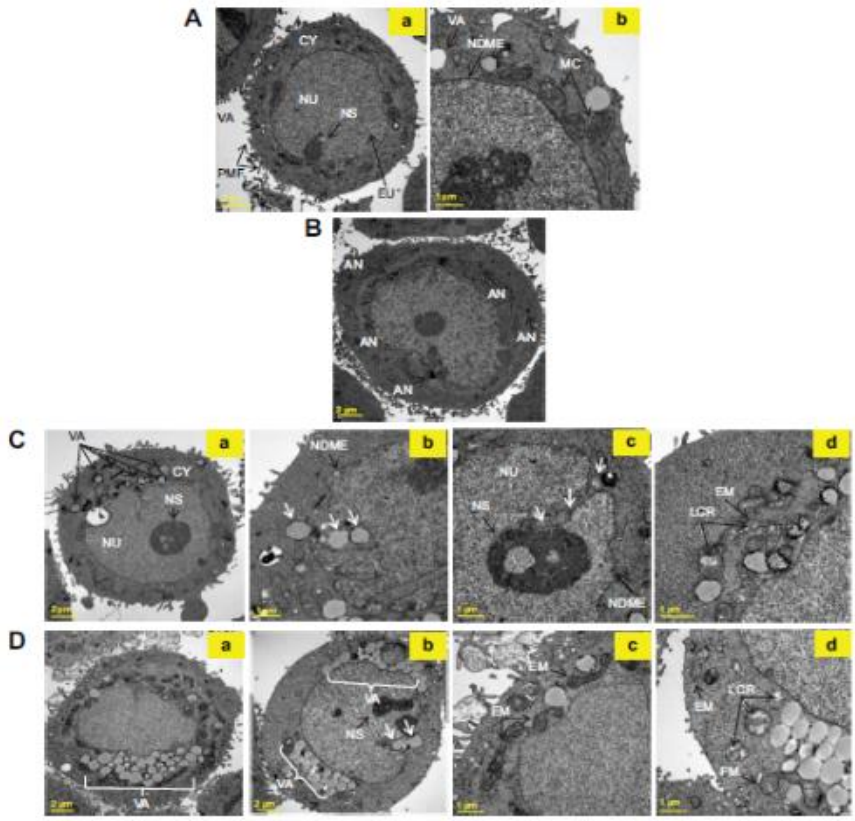


Figure 9: TEM results of cellular uptake – A represents control groups, B represents groups treated with AN alone, C represents groups treated with NA+LP (combined treatment) and D represents groups treated with LP-nanogold nanoemulsion [79)]

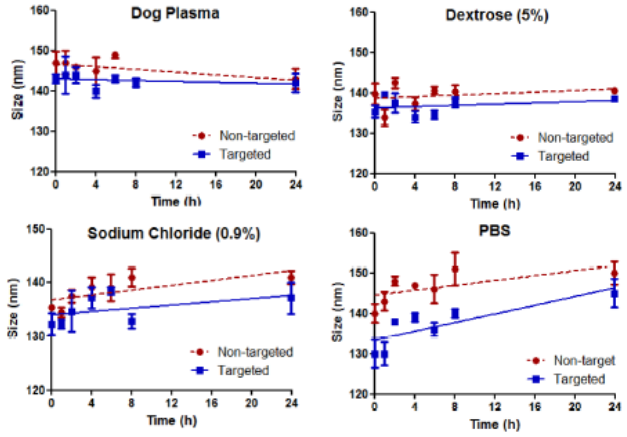


Figure 10: Stability of formulations in plasma, parenteral infusion fluids (dextrose and sodium chloride) and phosphate buffered saline [90]

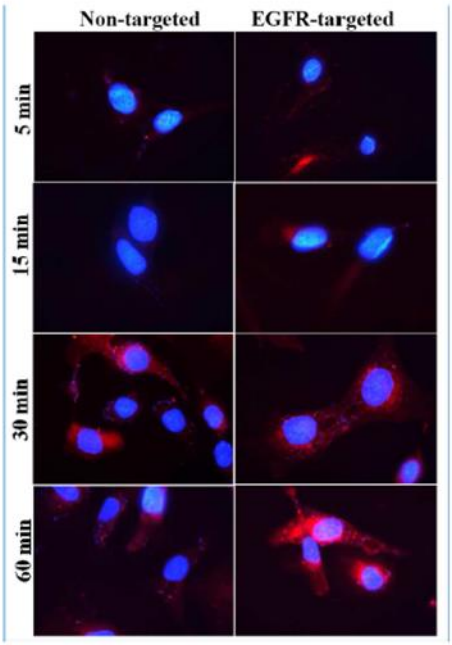


Figure 11: Results of fluorescent microscopy on SKOV3 cells uptake of Rh-PE (in red) labeled non-targeted nanoemulsion and EGFR targeted nanoemulsion (DAPI, in blue, stains SKOV-3 cells nucleus) [90]

Table 5: Combination index (CI) of platinum and ceramide against SKOV3 cells [90]

Combination Type	SKOV3	
	CI	Interaction type
Cisplatin/C_6-ceramide	0.46	Synergetic
Myrisplatin/C_6-ceramide NE (NT)	0.86	Synergetic
Myrisplatin/C_6-ceramide NE (T)	0.80	Synergetic

CI<1, synergetic; CI = 1, additive; CI > 1, antagonistic

Table 6: IC_{50} of platinum and ceramide alone and in combination [90]

Treatment Type	SKOV3	Pt-potency Fold Enhancement	A2780	Pt-potency Fold Enhancement	A2780cp	Pt-potency Fold Enhancement
Cisplatin in PBS	18.2 ± 0.1 μM	--	3.6 ± 0.1 μM	--	96.5 ± 0.1μM	--
Ceramide in DMSO	10 ± 0.1 μM	--	9.6 ± 0.1μM	--	30 ± 2 μM	--
Ceramide NE (NT)	9.1 ± 0.1 μM	--	1.5 ± 0.01 μM	--	1.3 ± 0.02 μM	--
Ceramide NE (T)	8.3 ± 0.1 μM	--	ND	ND	ND	--
Myrisplatin NE (NT)	5.5 ± 0.1 μM	3.3	0.24 ± 0.01 μM	15	0.8 ± 0.01μM	120.6
Myrisplatin NE (T)	2.4 ± 0.1μM	7.6	0.42 ± 0.01 μM	8.6	ND	ND
Myrisplatin/ceramide NE (NT)	0.46 ± 0.01 μM	39.6	0.16 ± 0.01 μM	22.5	0.4 ± 0.02 μM	241.3
Myrisplatin/ceramide NE (T)	0.36 ± 0.01 μM	50.5	ND	ND	1.1 ± 0.01μM	87.7

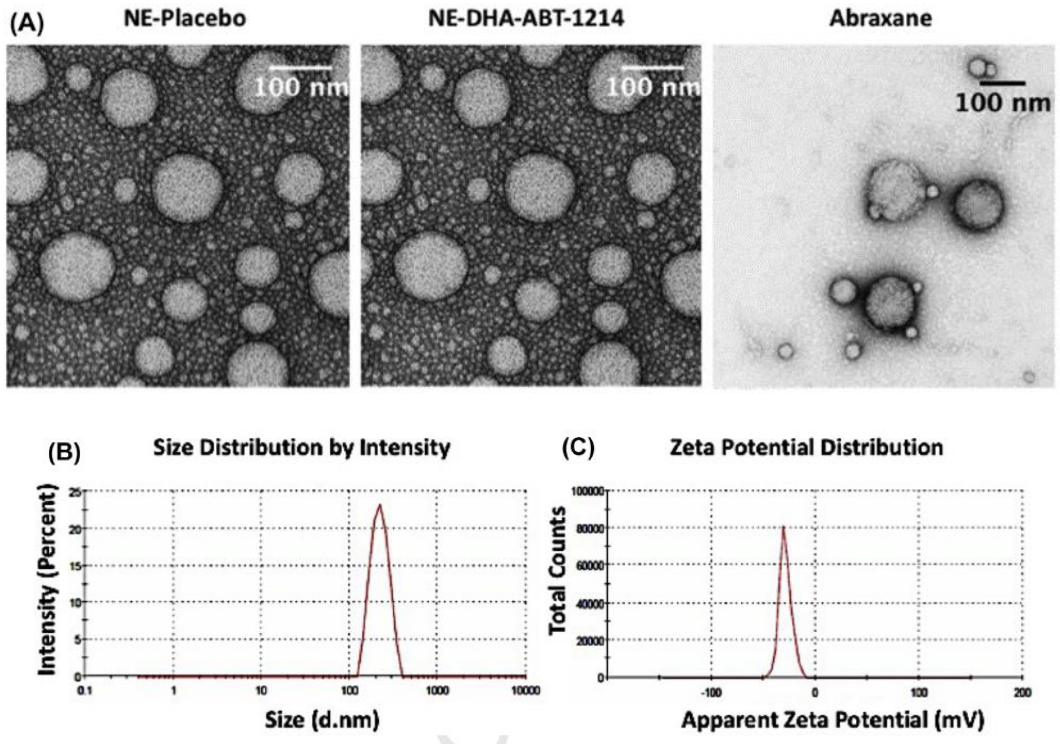


Figure 12: Characterization of DHA-SBT-1214 nanoemulsion [96]

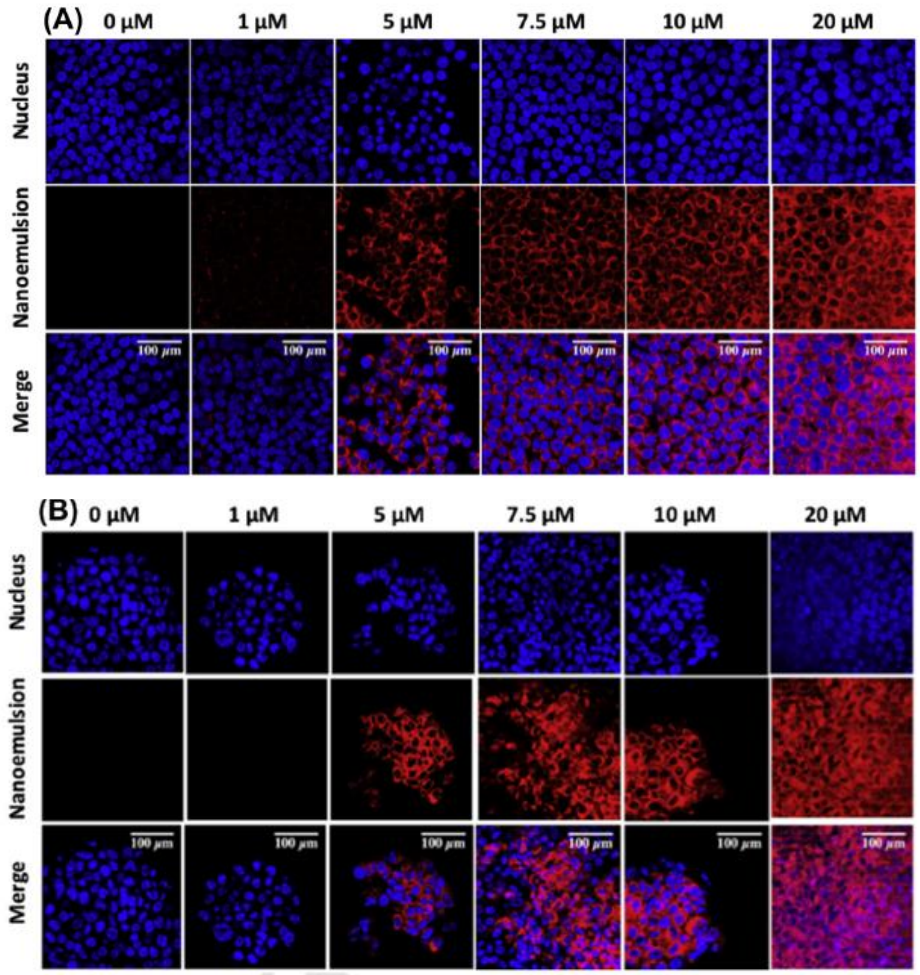
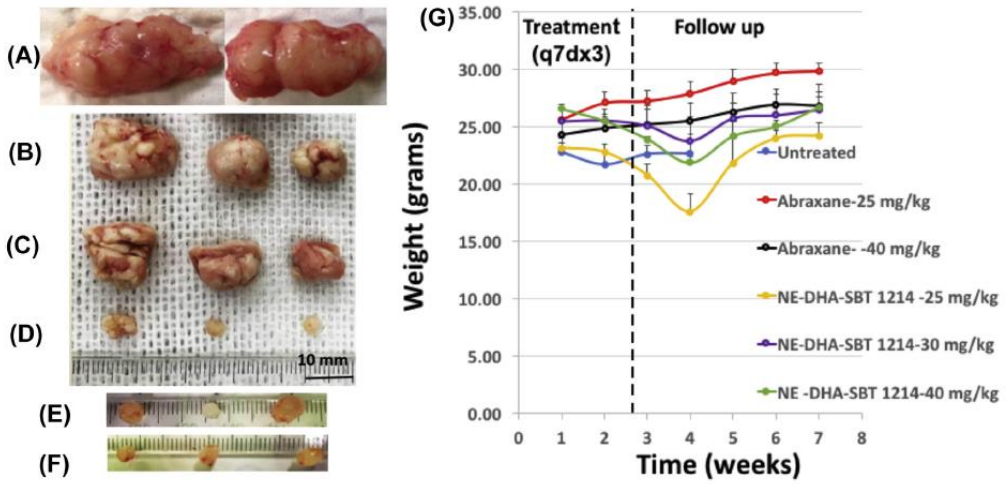


Figure 13: Rhodamine nanoemulsion uptake in monolayer (A) and spheroid (B) by PPT2 cells [96]



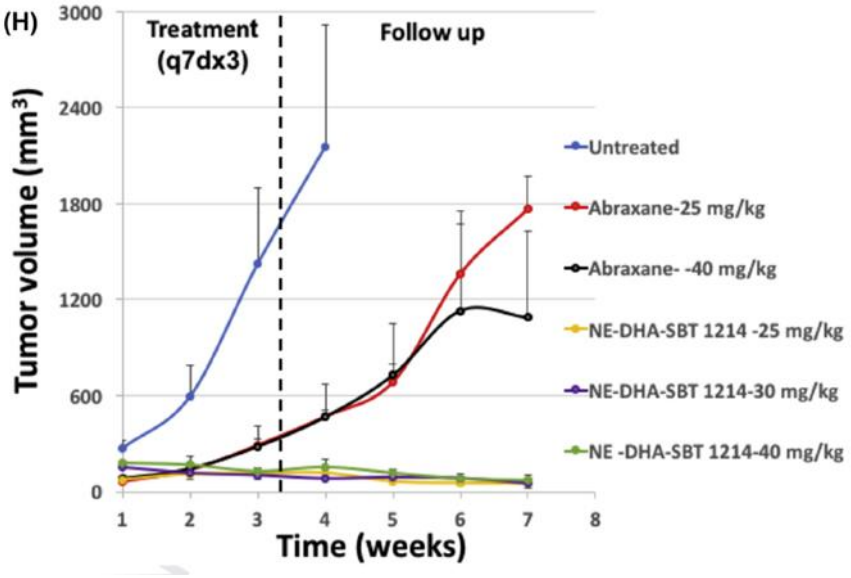


Figure 14: In vivo efficacy of NE-DHA-SBT and Abraxane® against PPT2 cells [96]

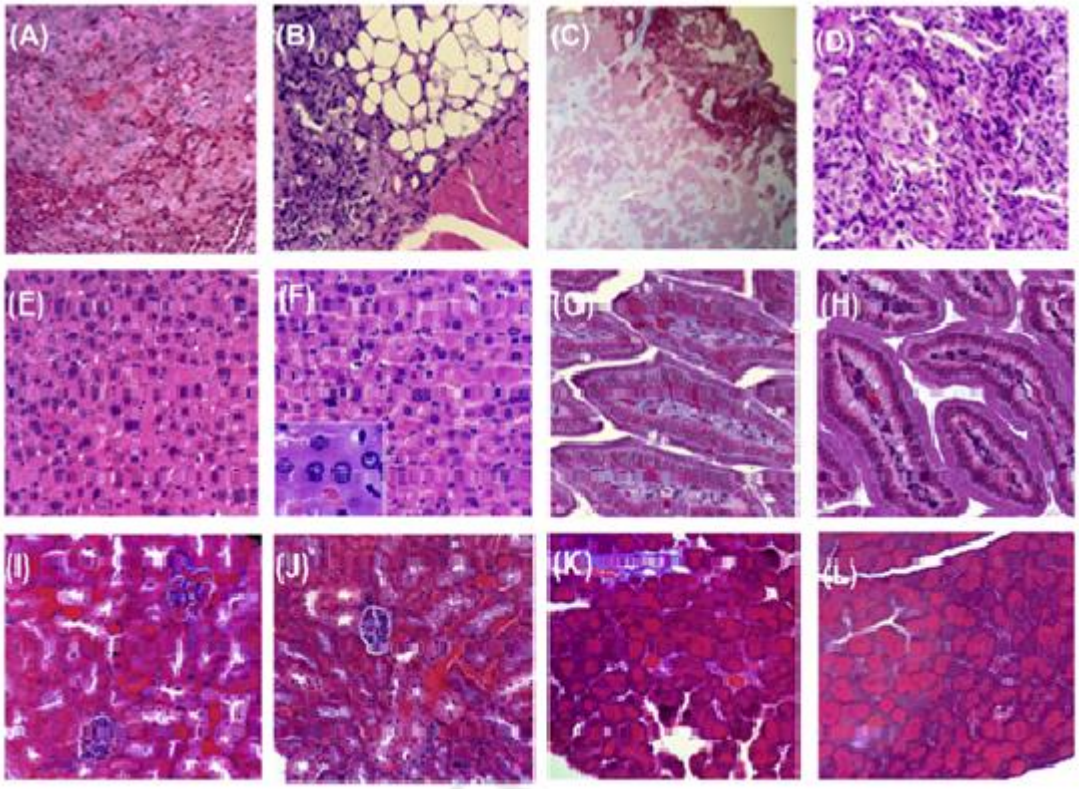


Figure 15: Evaluation of induced tumor - A represents control, B to D are groups treated with nanoemulsion, E is untreated liver tissue, G is untreated intestine tissue, I is untreated kidney tissue, K is untreated pancreas tissue, F is treated liver tissue, H is treated intestine tissue, L is treated kidney tissue and L is treated pancreas tissue [96)]

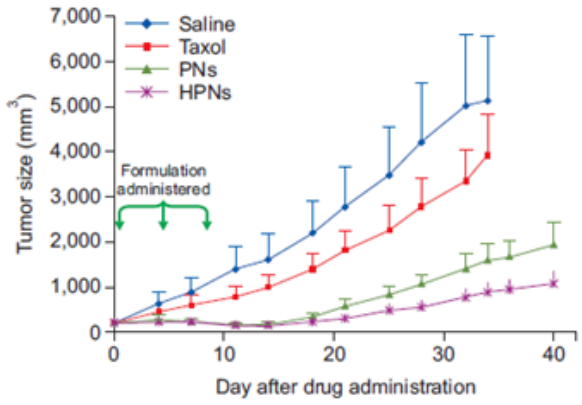


Figure 16: In vivo anti-tumor efficacy of saline, Taxol®, PNs and HPNs [103)]

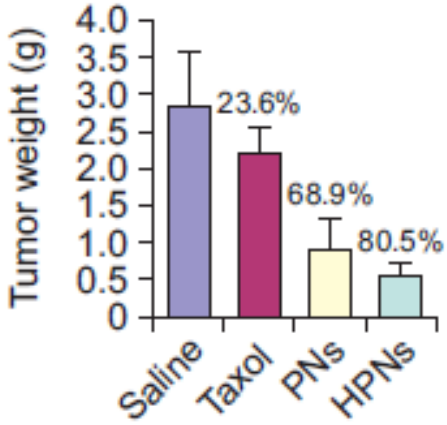


Figure 17: Tumor tissue weight 6 weeks after administration of saline, Taxol®, PNs and HPNs [103)]

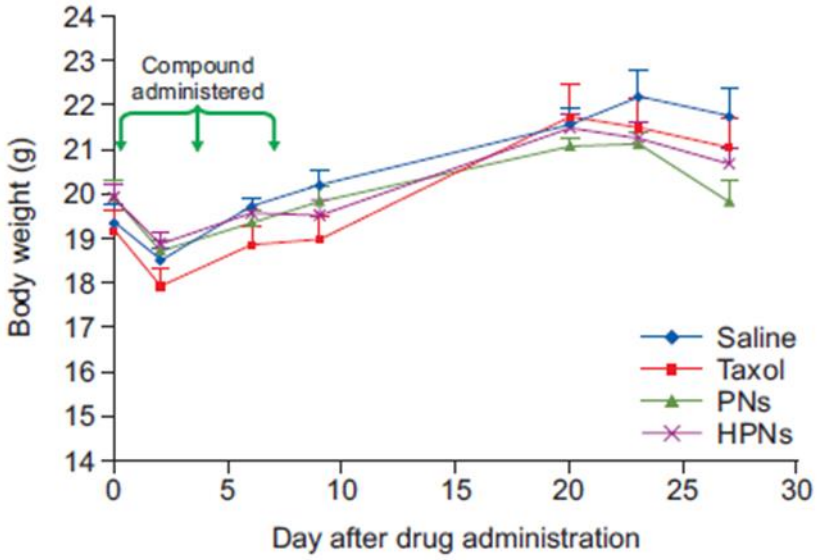


Figure 18: Changes in bodyweight in mice treated with saline, Taxol®, PNs and HPNs [103)]

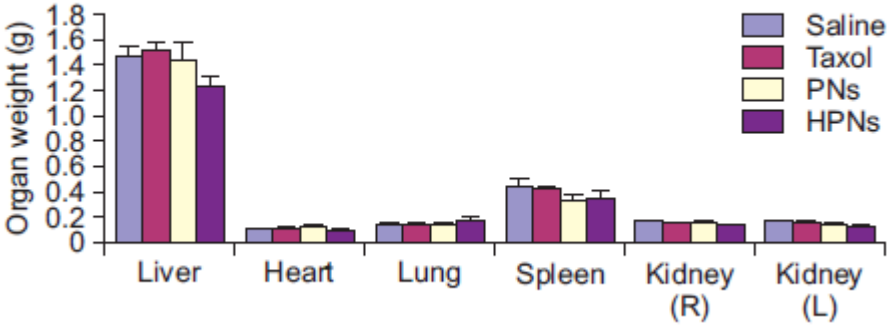


Figure 19: Variations in organ weight of mices treated with saline, Taxol®, PNs and HPNs [103]

Figure 20: *Organization of the products in Pharmacy Sanal*

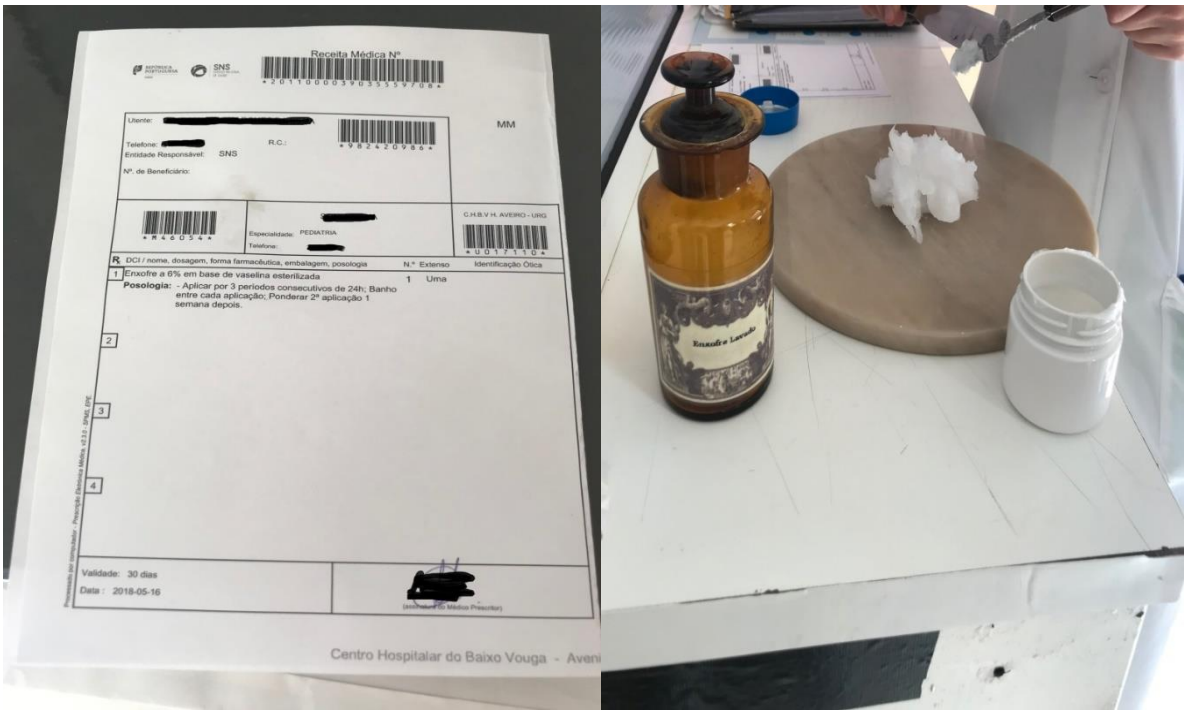
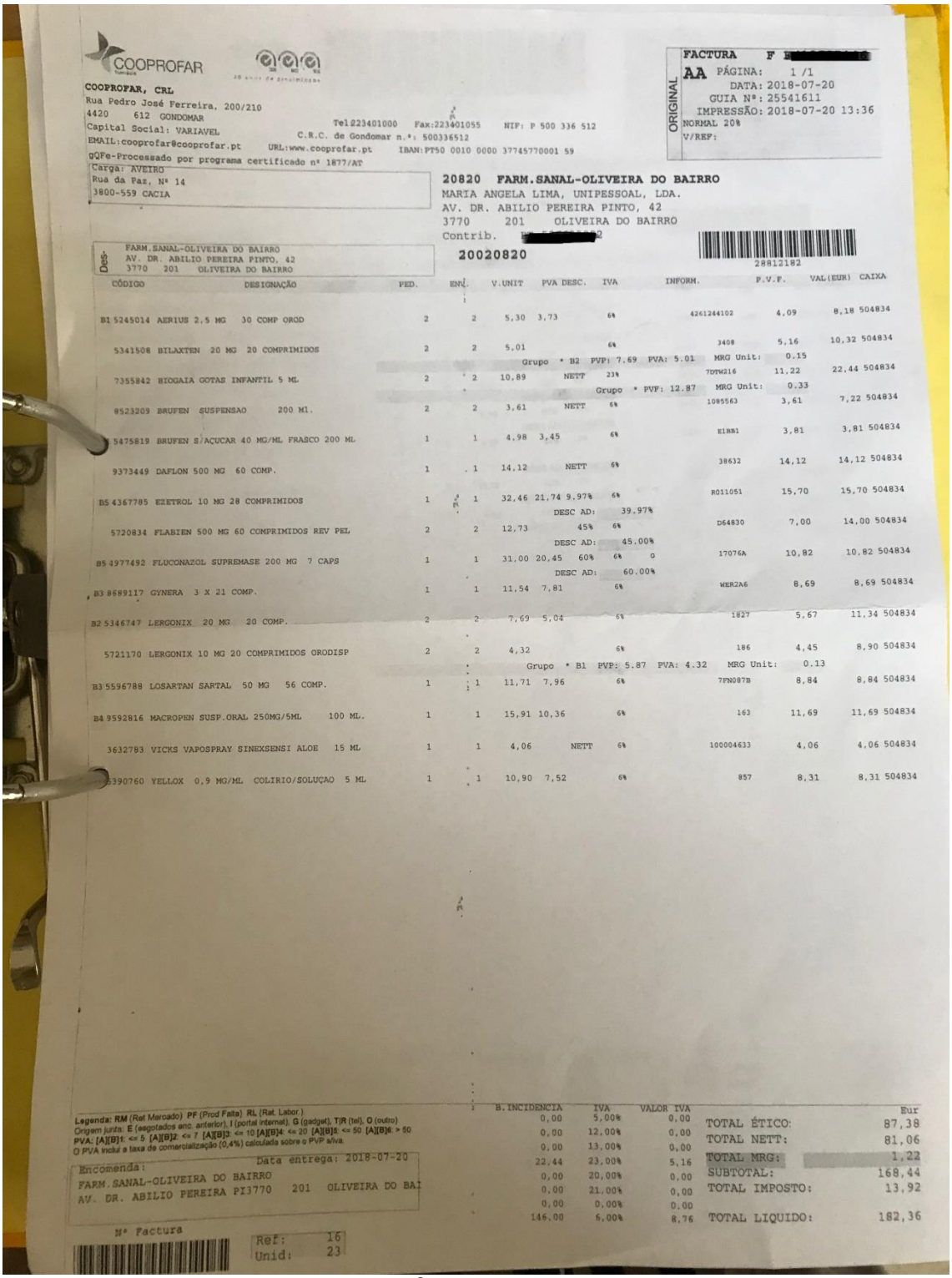


Figure 11: *Prescription and development of a compounded drug*

Figure 22: *Awareness action on Valormed*



COOPROFAR

COOPROFAR, CRL
Rua Pedro José Ferreira, 200/210
4420 612 GONDOMAR
Capital Social: VARIAVEL
EMAIL:cooprofar@cooprofar.pt
Tel:223401000 Fax:223401055 NIF: P 500 336 512
C.R.C. de Gondomar n.º: 500336512
URL:www.cooprofar.pt IBAN:PT50 0010 0000 37745770001 59
gQFe-Processado por programa certificado nº 1877/AT

ORIGINAL

FACTURA F [redacted]
AA PÁGINA: 1 /1
DATA: 2018-07-20
GUIA Nº: 25541611
IMPRESSÃO: 2018-07-20 13:36
NORMAL 20%
V/REF:

Carga: AVEIRO
Rua da Paz, Nº 14
3800-559 CACIA

20820 FARM.SANAL-OLIVEIRA DO BAIRRO
MARIA ANGELA LIMA, UNIPESSOAL, LDA.
AV. DR. ABILIO PEREIRA PINTO, 42
3770 201 OLIVEIRA DO BAIRRO
Contrib. [redacted]

Dest FARM.SANAL-OLIVEIRA DO BAIRRO
AV. DR. ABILIO PEREIRA PINTO, 42
3770 201 OLIVEIRA DO BAIRRO

20020820

28812182

CÓDIGO	DESIGNAÇÃO	PED.	ENV.	V.UNIT	PVA DESC.	IVA	INFORM.	P.V.P.	VAL(EUR) CAIXA
B1 5245014	AERIUS 2,5 MG 30 COMP OROD	2	2	5,30	3,73	6%	4261244102	4,09	8,18 504834
5341508	BILAXTEN 20 MG 20 COMPRIMIDOS	2	2	5,01		6%	3408	5,16	10,32 504834
				Grupo * B2 PVP: 7,69 PVA: 5,01			MRG Unit:	0,15	
7355842	BIOGAIA GOTAS INFANTIL 5 ML	2	2	10,89	NETT	23%	7DTW216	11,22	22,44 504834
						Grupo * PVF: 12,87	MRG Unit:	0,33	
8523209	BRUFEN SUSPENSAO 200 Ml.	2	2	3,61	NETT	6%	1085563	3,61	7,22 504834
5475819	BRUFEN S/AÇUCAR 40 MG/ML FRASCO 200 ML	1	1	4,98	3,45	6%	E1BB1	3,81	3,81 504834
9373449	DAFLON 500 MG 60 COMP.	1	1	14,12	NETT	6%	38632	14,12	14,12 504834
B5 4367785	EZETROL 10 MG 28 COMPRIMIDOS	1	1	32,46	21,74 9,97%	6%	R011051	15,70	15,70 504834
					DESC AD:	39,97%			
5720834	FLABIEN 500 MG 60 COMPRIMIDOS REV PEL	2	2	12,73	45%	6%	D64830	7,00	14,00 504834
					DESC AD:	45,00%			
B5 4977492	FLUCONAZOL SUPREMASE 200 MG 7 CAPS	1	1	31,00	20,45 60%	6% G	17076A	10,82	10,82 504834
					DESC AD:	60,00%			
B3 8689117	GYNERA 3 X 21 COMP.	1	1	11,54	7,81	6%	WER2A6	8,69	8,69 504834
B2 5346747	LERGONIX 20 MG 20 COMP.	2	2	7,69	5,04	6%	1827	5,67	11,34 504834
5721170	LERGONIX 10 MG 20 COMPRIMIDOS ORODISP	2	2	4,32		6%	186	4,45	8,90 504834
				Grupo * B1 PVP: 5,87 PVA: 4,32			MRG Unit:	0,13	
B3 5596788	LOSARTAN SARTAL 50 MG 56 COMP.	1	1	11,71	7,96	6%	7FN087B	8,84	8,84 504834
B4 9592816	MACROPEN SUSP.ORAL 250MG/5ML 100 ML.	1	1	15,91	10,36	6%	163	11,69	11,69 504834
3632783	VICKS VAPOSPRAY SINEXSENSI ALOE 15 ML	1	1	4,06	NETT	6%	100004633	4,06	4,06 504834
5390760	YELLOX 0,9 MG/ML COLIRIO/SOLUÇAO 5 ML	1	1	10,90	7,52	6%	857	8,31	8,31 504834

Legenda: RM (Rel Mercado) PF (Prod Falta) RL (Rel. Labor.)
Origem junta: E (esgotados enc. anterior), I (portal internet), G (gadget), T/R (tel), O (outro)
PVA: [A][B]1: <= 5 [A][B]2: <= 7 [A][B]3: <= 10 [A][B]4: <= 20 [A][B]5: <= 50 [A][B]6: > 50
O PVA inclui a taxa de comercialização (0,4%) calculada sobre o PVP s/iva.

	B.INCIDENCIA	IVA	VALOR IVA
	0,00	5,00%	0,00
	0,00	12,00%	0,00
	0,00	13,00%	0,00
	22,44	23,00%	5,16
	0,00	20,00%	0,00
	0,00	21,00%	0,00
	0,00	0,00%	0,00
	146,00	6,00%	8,76

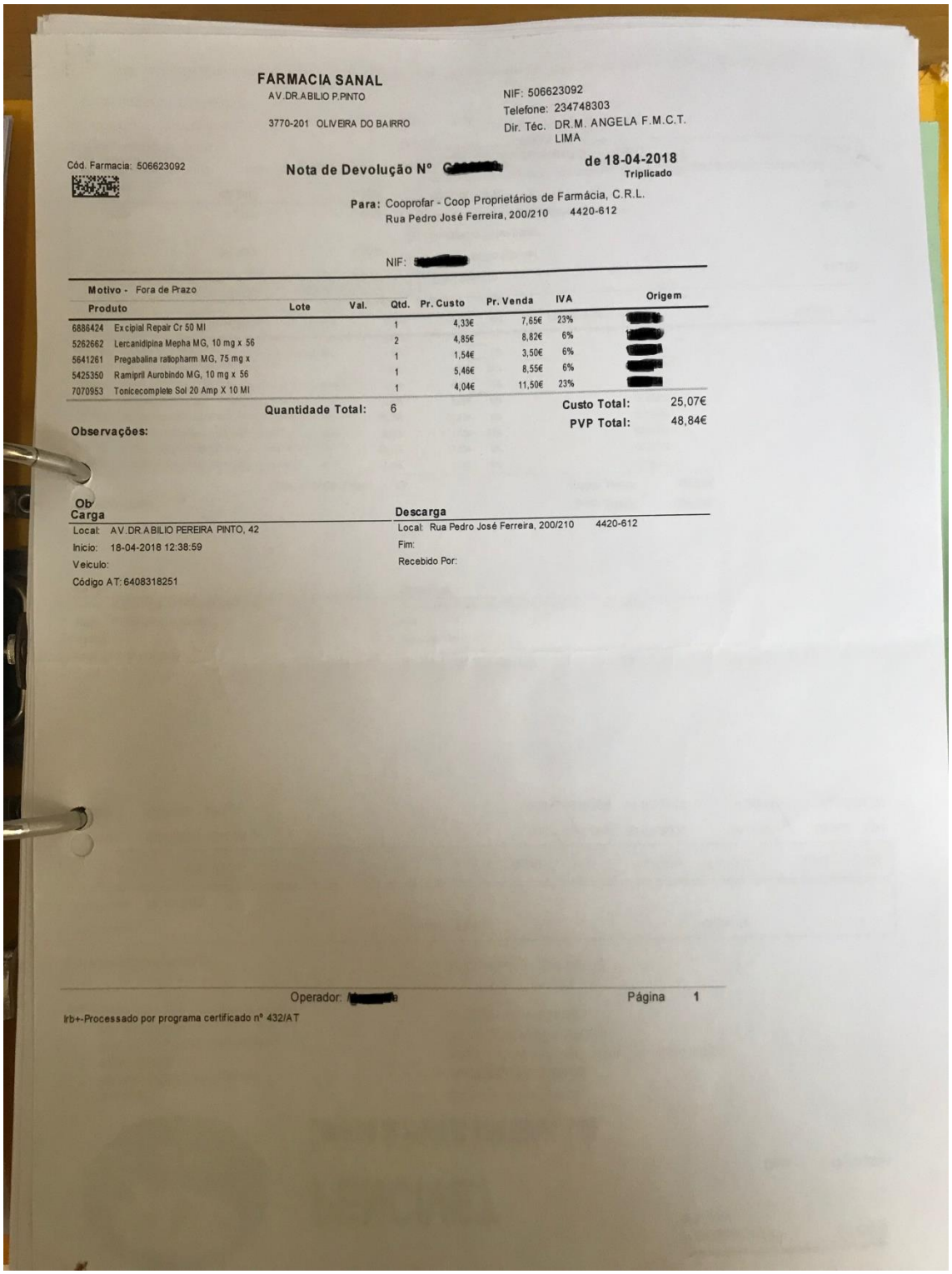
	Eur
TOTAL ÉTICO:	87,38
TOTAL NETT:	81,06
TOTAL MRG:	1,22
SUBTOTAL:	168,44
TOTAL IMPOSTO:	13,92
TOTAL LIQUIDO:	182,36

Encomenda: Data entrega: 2018-07-20
FARM.SANAL-OLIVEIRA DO BAIRRO
AV. DR. ABILIO PEREIRA PI3770 201 OLIVEIRA DO BAI

Nº Factura

Ref: 16
Unid: 23

Figure 23: *Invoice from Cooprofar® placed it a dossier after the reception of an order*



FARMACIA SANAL
AV.DR.ABILIO P.PINTO
3770-201 OLIVEIRA DO BAIRRO
NIF: 506623092
Telefone: 234748303
Dir. Téc. DR.M. ANGELA F.M.C.T. LIMA

Cód. Farmacia: 506623092

Nota de Devolução Nº [redacted] de 18-04-2018
Triplicado

Para: Cooprofar - Coop Proprietários de Farmácia, C.R.L.
Rua Pedro José Ferreira, 200/210 4420-612

NIF: [redacted]

Motivo - Fora de Prazo

Produto		Lote	Val.	Qtd.	Pr. Custo	Pr. Venda	IVA	Origem
6886424	Excipial Repair Cr 50 Ml			1	4,33€	7,65€	23%	[redacted]
5262662	Lercanidipina Mepha MG, 10 mg x 56			2	4,85€	8,82€	6%	[redacted]
5641261	Pregabalina ratiopharm MG, 75 mg x			1	1,54€	3,50€	6%	[redacted]
5425350	Ramipril Aurobindo MG, 10 mg x 56			1	5,46€	8,55€	6%	[redacted]
7070953	Tonicecomplete Sol 20 Amp X 10 Ml			1	4,04€	11,50€	23%	[redacted]

Quantidade Total: 6

Custo Total: 25,07€
PVP Total: 48,84€

Observações:

Ob

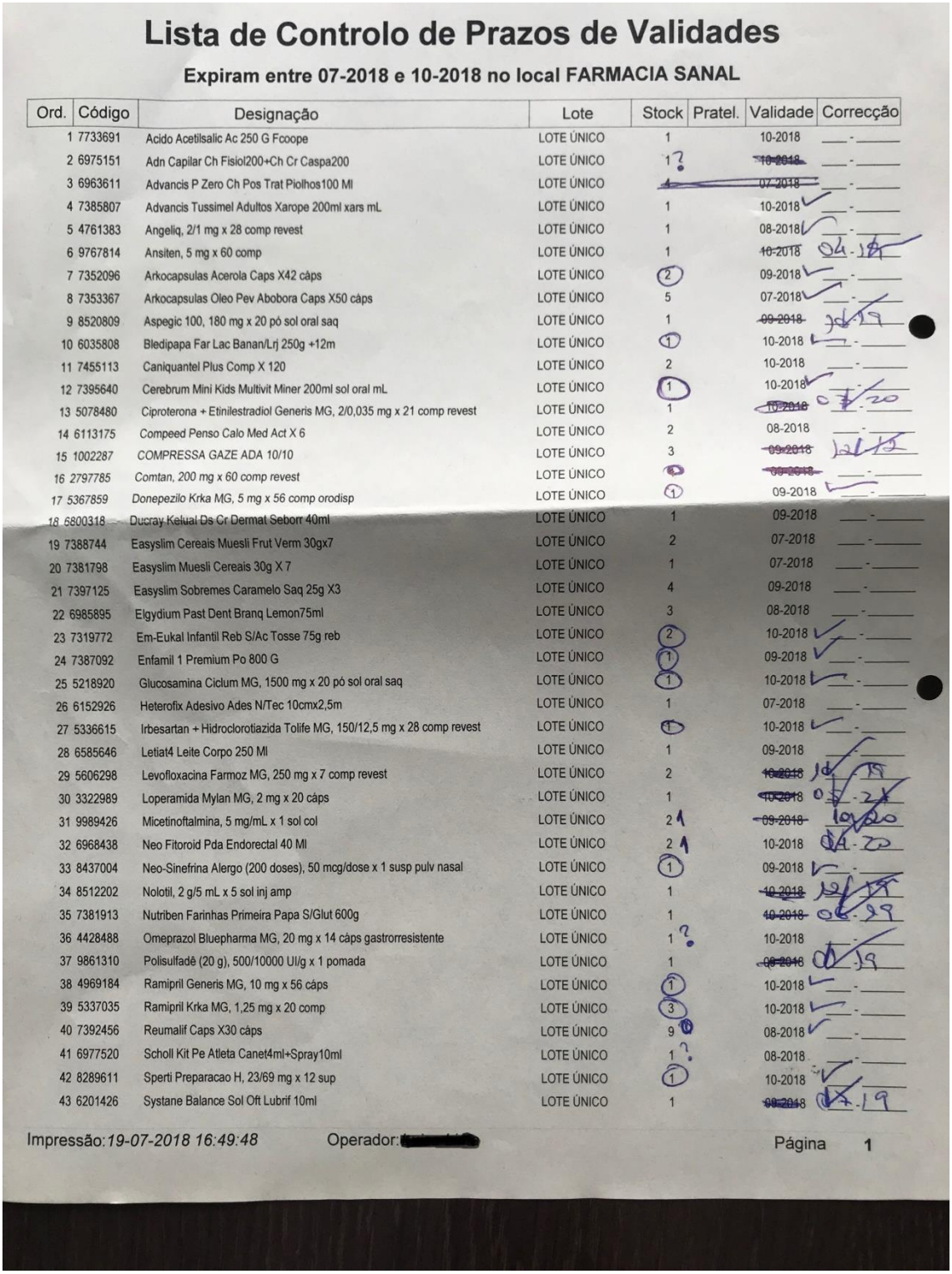
Carga
Local: AV.DR.ABILIO PEREIRA PINTO, 42
Inicio: 18-04-2018 12:38:59
Veiculo:
Código AT: 6408318251

Descarga
Local: Rua Pedro José Ferreira, 200/210 4420-612
Fim:
Recebido Por:

Operador: [redacted] Página 1

Irb+-Processado por programa certificado nº 432/AT

Figure 24*: Devolution note*



Lista de Controlo de Prazos de Validades

Expiram entre 07-2018 e 10-2018 no local FARMACIA SANAL

Ord.	Código	Designação	Lote	Stock	Pratel.	Validade	Correcção
1	7733691	Acido Acetilsalic Ac 250 G Fcoope	LOTE ÚNICO	1		10-2018	
2	6975151	Adn Capilar Ch Fisiol200+Ch Cr Caspa200	LOTE ÚNICO	1		~~10-2018~~	
3	6963611	Advancis P Zero Ch Pos Trat Piolhos100 Ml	LOTE ÚNICO	~~4~~		~~07-2018~~	
4	7385807	Advancis Tussimel Adultos Xarope 200ml xars mL	LOTE ÚNICO	1		10-2018	
5	4761383	Angeliq, 2/1 mg x 28 comp revest	LOTE ÚNICO	1		08-2018	
6	9767814	Ansiten, 5 mg x 60 comp	LOTE ÚNICO	1		~~10-2018~~	04-18
7	7352096	Arkocapsulas Acerola Caps X42 cáps	LOTE ÚNICO	2		09-2018	
8	7353367	Arkocapsulas Oleo Pev Abobora Caps X50 cáps	LOTE ÚNICO	5		07-2018	
9	8520809	Aspegic 100, 180 mg x 20 pó sol oral saq	LOTE ÚNICO	1		~~09-2018~~	
10	6035808	Bledipapa Far Lac Banan/Lrj 250g +12m	LOTE ÚNICO	1		10-2018	
11	7455113	Caniquantel Plus Comp X 120	LOTE ÚNICO	2		10-2018	
12	7395640	Cerebrum Mini Kids Multivit Miner 200ml sol oral mL	LOTE ÚNICO	1		10-2018	
13	5078480	Ciproterona + Etinilestradiol Generis MG, 2/0,035 mg x 21 comp revest	LOTE ÚNICO	1		~~10-2018~~	07-20
14	6113175	Compeed Penso Calo Med Act X 6	LOTE ÚNICO	2		08-2018	
15	1002287	COMPRESSA GAZE ADA 10/10	LOTE ÚNICO	3		~~09-2018~~	
16	2797785	Comtan, 200 mg x 60 comp revest	LOTE ÚNICO			~~09-2018~~	
17	5367859	Donepezilo Krka MG, 5 mg x 56 comp orodisp	LOTE ÚNICO	1		09-2018	
18	6800318	Ducray Kelual Ds Cr Dermat Seborr 40ml	LOTE ÚNICO	1		09-2018	
19	7388744	Easyslim Cereais Muesli Frut Verm 30gx7	LOTE ÚNICO	2		07-2018	
20	7381798	Easyslim Muesli Cereais 30g X 7	LOTE ÚNICO	1		07-2018	
21	7397125	Easyslim Sobremes Caramelo Saq 25g X3	LOTE ÚNICO	4		09-2018	
22	6985895	Elgydium Past Dent Branq Lemon75ml	LOTE ÚNICO	3		08-2018	
23	7319772	Em-Eukal Infantil Reb S/Ac Tosse 75g reb	LOTE ÚNICO	2		10-2018	
24	7387092	Enfamil 1 Premium Po 800 G	LOTE ÚNICO	1		09-2018	
25	5218920	Glucosamina Ciclum MG, 1500 mg x 20 pó sol oral saq	LOTE ÚNICO	1		10-2018	
26	6152926	Heterofix Adesivo Ades N/Tec 10cmx2,5m	LOTE ÚNICO	1		07-2018	
27	5336615	Irbesartan + Hidroclorotiazida Tolife MG, 150/12,5 mg x 28 comp revest	LOTE ÚNICO	1		10-2018	
28	6585646	Letiat4 Leite Corpo 250 Ml	LOTE ÚNICO	1		09-2018	
29	5606298	Levofloxacina Farmoz MG, 250 mg x 7 comp revest	LOTE ÚNICO	2		~~10-2018~~	
30	3322989	Loperamida Mylan MG, 2 mg x 20 cáps	LOTE ÚNICO	1		~~10-2018~~	07-21
31	9989426	Micetinoftalmina, 5 mg/mL x 1 sol col	LOTE ÚNICO	2		~~09-2018~~	10-20
32	6968438	Neo Fitoroid Pda Endorectal 40 Ml	LOTE ÚNICO	2		10-2018	04-20
33	8437004	Neo-Sinefrina Alergo (200 doses), 50 mcg/dose x 1 susp pulv nasal	LOTE ÚNICO	1		09-2018	
34	8512202	Nolotil, 2 g/5 mL x 5 sol inj amp	LOTE ÚNICO	1		~~10-2018~~	
35	7381913	Nutriben Farinhas Primeira Papa S/Glut 600g	LOTE ÚNICO	1		~~10-2018~~	
36	4428488	Omeprazol Bluepharma MG, 20 mg x 14 cáps gastrorresistente	LOTE ÚNICO	1		10-2018	
37	9861310	Polisulfadê (20 g), 500/10000 UI/g x 1 pomada	LOTE ÚNICO	1		~~08-2018~~	
38	4969184	Ramipril Generis MG, 10 mg x 56 cáps	LOTE ÚNICO	1		10-2018	
39	5337035	Ramipril Krka MG, 1,25 mg x 20 comp	LOTE ÚNICO	3		10-2018	
40	7392456	Reumalif Caps X30 cáps	LOTE ÚNICO	9		08-2018	
41	6977520	Scholl Kit Pe Atleta Canet4ml+Spray10ml	LOTE ÚNICO	1		08-2018	
42	8289611	Sperti Preparacao H, 23/69 mg x 12 sup	LOTE ÚNICO	1		10-2018	
43	6201426	Systane Balance Sol Oft Lubrif 10ml	LOTE ÚNICO	1		~~08-2018~~	

Impressão: *19-07-2018 16:49:48* Operador: [redacted] Página 1

Figure 25: *Expiring dates control sheet*

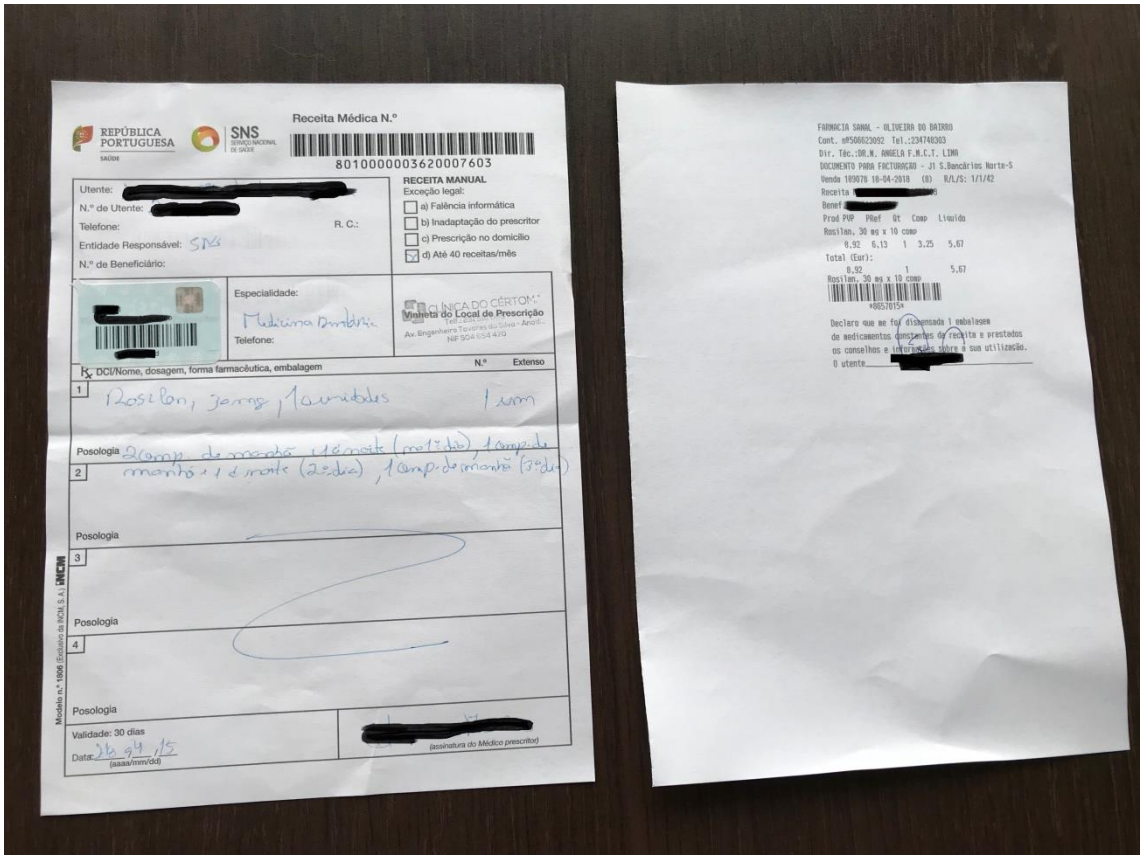
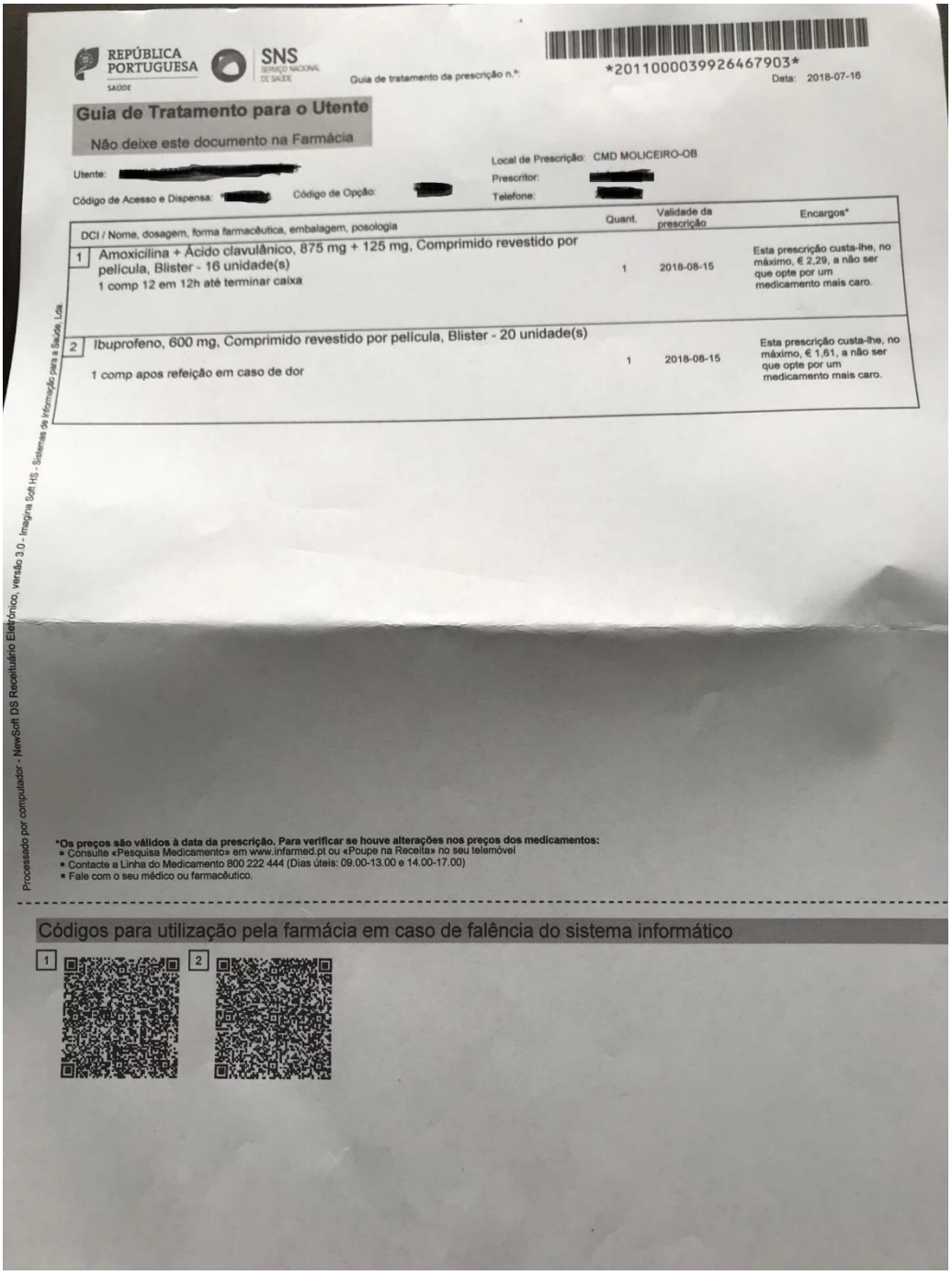


Figure 26*: Manual prescription*



REPÚBLICA PORTUGUESA
SAÚDE

SNS
SERVIÇO NACIONAL DE SAÚDE

Guia de tratamento da prescrição n.º: *201100003992646790 3*

Data: 2018-07-16

Guia de Tratamento para o Utente

Não deixe este documento na Farmácia

Utente: [redacted]

Local de Prescrição: CMD MOLICEIRO-OB

Prescritor: [redacted]

Código de Acesso e Dispensa: [redacted] Código de Opção: [redacted]

Telefone: [redacted]

	DCI / Nome, dosagem, forma farmacêutica, embalagem, posologia	Quant.	Validade da prescrição	Encargos*
1	Amoxicilina + Ácido clavulânico, 875 mg + 125 mg, Comprimido revestido por película, Blister - 16 unidade(s) 1 comp 12 em 12h até terminar caixa	1	2018-08-15	Esta prescrição custa-lhe, no máximo, € 2,29, a não ser que opte por um medicamento mais caro.
2	Ibuprofeno, 600 mg, Comprimido revestido por película, Blister - 20 unidade(s) 1 comp apos refeição em caso de dor	1	2018-08-15	Esta prescrição custa-lhe, no máximo, € 1,61, a não ser que opte por um medicamento mais caro.

Processado por computador - NewSoft DS Receituário Eletrónico, versão 3.0 - Imagina Soft HS - Sistemas de Informação para a Saúde, Lda.

*Os preços são válidos à data da prescrição. Para verificar se houve alterações nos preços dos medicamentos:
- Consulte «Pesquisa Medicamento» em www.infarmed.pt ou «Poupe na Receita» no seu telemóvel
- Contacte a Linha do Medicamento 800 222 444 (Dias úteis: 09.00-13.00 e 14.00-17.00)
- Fale com o seu médico ou farmacêutico.

Códigos para utilização pela farmácia em caso de falência do sistema informático

1 2

Figure 27: *Electronic prescription*