DEGREE FINAL PROJECT
2nd Call
Faculty of Pharmacy
University of Barcelona

CYCLOPHOSPHAMIDIDE
SUGAR-COATED
TABLETS

Main Field: Pharmaceutical Technology
Secondary Fields: Physical Chemistry, Biopharmacy and Pharmacokinetics, History of Pharmacy

Emma Sanpere Amat
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Special thanks to my mentor, Joaquim Tejero, for sharing your knowledge with me, for encouraging me throughout this project and for the advice and the conversations.
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ABSTRACT

Cyclophosphamide is a cytotoxic anti-tumor pro-drug belonging to the alkylating agents which is indicated for the treatment of many cancers as well as some immune-related disorders. In Spain, it is marketed as powder for injection and as sugar-coated tablets by Baxter Oncology. Cyclophosphamide is well absorbed from the gastrointestinal wall, with an oral bioavailability of 85-100%. This justifies the existence of an oral medication comprising the drug, like sugar-coated tablets. Cyclophosphamide structure is prone to undergo hydrolysis and other degradation reactions in aqueous solution, and is labile to high temperatures, changes in moisture levels, and light. Instability to the latter conditions might accelerate the degradation of the active ingredient during the manufacture of the drug product, and is one of the reasons for tablet coating. Accordingly, drug sensitivity to water and temperature are especially critical, since most manufacturing processes are carried out in aqueous medium and involve heating systems like hot air drying. Indeed, commercially available cyclophosphamide coated tablets are produced by means of a wet granulation process followed by coverage with the sugar-coating technique. Both of these methods are subjected to the use of water and to several drying stages, thus compromising the stability of the active ingredient. Hence, the main objective of this project has been to develop a novel formulation of cyclophosphamide coated tablets that minimized drug exposure to water and heat during the manufacturing process. As a result, sorbitol film-coated tablets have been proposed. These consist of a tablet core obtained by direct compression without the need of water, and a film-coating containing glycerol instead of water as the main coating solvent together with sorbitol as a plasticizer.

RESUM

La ciclofosfamida és un profàrmac antitumoral citotòxic pertanyent al grup dels agents alquilants que està indicat en el tractament de nombrosos càncers i algunes malalties del sistema immunitari. A Espanya es troba comercialitzada per Baxter Oncology en forma de pòlvores per a solució injectable i com a dragees. La ciclofosfamida s'absorbeix bé al tracte gastrointestinal, presentant una biodisponibilitat d'entre el 85 i el 100%, la qual cosa justifica l’existència d'una forma farmacèutica d’administració
oral del fàrmac com són les dragees. L’estructura de la ciclofosfamida és propensa a patir hidròlisis i altres reaccions de degradació en solució aquosa, i és làbil a les altes temperatures, als canvis d’humitat i a la llum. La inestabilitat del fàrmac enfront a aquestes condicions podria accelerar la degradació del principi actiu durant la fabricació del medicament i és una de les raons per a recobrir els comprimits. En aquest sentit, la inestabilitat a l’aigua i a la temperatura són especialment crítiques, ja que la majoria de processos de fabricació es realitzen en medi aquós i impliquen l’escalfament del fàrmac per mitjà de sistemes com l’assecament amb aire calent. De fet, les dragees de ciclofosfamida disponibles al mercat es fabriquen a través d’un procés de granulació per via humida seguit de drageat o recobriment amb sacarosa. Aquests dos mètodes requereixen l’ús d’aigua i de variedes etapes d’assecat, la qual cosa pot comprometre l’estabilitat del principi actiu. En conseqüència, el principal objectiu d’aquest treball ha estat desenvolupar una nova formulació de comprimits de ciclofosfamida recoberts que minimitzés l’exposició del fàrmac a l’aigua i la calor durant el procés de fabricació. Com a resultat, s’han proposat uns comprimits amb recobriment pel·lícular de sorbitol. Aquests estan formats per un nucli obtingut per compressió directa sense necessitat d’aigua, i una cobertura pel·lícular amb glicerol enlloc d’aigua com a solvent principal juntament amb sorbitol com a plastificant.

**INTEGRATION OF THE DIFFERENT FIELDS**

This work integrates several pharmaceutical disciplines. The main one is pharmaceutical technology, which is ubiquitous throughout the whole project and takes on especial relevance in the results/discussion section, where an existing pharmaceutical formulation is analyzed and a novel one is presented.

In order to address the mentioned task as well as understand the reasons underlying the route of administration and the pharmaceutical formulation of the drug, prior attention is drawn to the physicochemical, biopharmaceutical and pharmacokinetic properties of the active ingredient. Hence, physical chemistry and biopharmacy and pharmacokinetics are important fields comprised in the project.

On the other hand, history of pharmacy is tackled when reviewing the sugar-coating technique, a traditional coating process that dates back to the 1st century AD but has survived the passing of time.

The structure of cyclophosphamide is also discussed, thus introducing some basic concepts of pharmaceutical chemistry. Finally, the introduction brings in several aspects of the drug related to the pharmacology and therapeutics area, such as the mechanism of action, the indications and the adverse effects.
1. INTRODUCTION

1.1. What Is Cyclophosphamide?

1.1.1. Indications

Cyclophosphamide is an alkylating anti-tumor drug that shows anti-tumor activities against a broad range of cancers including Hodgkin’s disease and non-Hodgkin lymphoma, multiple myeloma, leukaemia, mycosis fungoides, neuroblastoma, carcinoma of the ovary, retinoblastoma, carcinoma of the breast[1][2][3], germinal tumors[1], small cell cancer of the lung[3], and sarcoma[3]. In the treatment of cancer it can also be used at low doses as either an anti-angiogenic or an immune-stimulatory agent in combination with other immunotherapies[4].

Cyclophosphamide is as well an immunosuppressive agent. Thus, it can prevent graft rejection after organ and bone marrow transplantation[1], and might be used in the treatment of autoimmune disorders[1][5], such as Wegener’s granulomatosis[5], rheumatoid arthritis[5], lupus erythematosus[5], and nephrotic syndrome[2][5].

1.1.2. Mechanism of Action

Cyclophosphamide is a cytotoxic alkylating agent which exerts its mechanism by forming covalent bonds between its alkyl groups and different nucleophilic molecules in cells. Although many cellular entities are alkylated by the drug, its interaction with DNA, known as DNA cross-linking, is the most important one in relation to anti-tumor activity. Cyclophosphamide action is said to be cell cycle nonspecific, since it can react with cells at any time. Nevertheless, due to the fact that nucleotides are more likely to be alkylated during the replication process, alkylation is more effective against rapidly proliferating (in cycle) cells than against non-cycling cells[5].
The cross-linking effect applies not only for cancer cells, but also for overactive immune competent cells that are found in various autoimmune diseases. Indeed, cyclophosphamide is an effective inhibitor of cell mediated immune response, it leads to a depletion of lymphocytes in the peripheral blood and tissue, and it affects monocyte function leading to a decrease in IL-1 and TNF production[6].

Metabolic Activation

Cyclophosphamide is a pro-drug, so, in order to exhibit its anti-tumor activity, it has to be converted to its active metabolite. This conversion takes place mainly in the liver by the enzymes of the mixed function oxidase system (cytochrome P450 enzyme system, CYP450), specially CYP450 2B. The first step in this activation involves the ring hydroxylation of cyclophosphamide to yield 4-hydroxycyclophosphamide, which is spontaneously converted to phosphoramid mustard and acrolein once in cells. Phosphoramid mustard is a bifunctional alkylating metabolite responsible for the biologic activity, whereas acrolein is the main toxic metabolite of cyclophosphamide. Thereby, phosphoramid mustard produces multiple monofunctional and bifunctional adducts with guanine. A part from phosphoramid, there are other metabolites involved in the cross-linking process, like nonnitrogen mustard (a carboxyphosphamide metabolite) and acrolein itself[5].

DNA Alkylation

The bischloroethylamino group of phosphoramid mustard bound to a tertiary nitrogen is responsible for the biologic activity. In the first step, one of the chlorides of the drug is lost and the beta carbon reacts with the nucleophilic nitrogen to form a cyclic, positive charged and very reactive aziridine molecule. The second step is characterized by the formation of the primary alkylating product, as a result of the reaction between the aziridium ring and a nucleophilic group in the DNA molecule (usually the N7 position of guanine). This process is repeated when the second chloroethyl group of the molecule losses its chloride, generating once again an aziridine electrophyl radical that will alkylate another nitrogen base. The overall reaction can take place sequentially thanks to the bifunctional alkylating character of the drug, which allows it to react with the N7 groups of two different guanine residues. This is evidenced by a covalent cross-linking between two alkylated nucleophilic groups in the same DNA chain or between the two strands of the double helix[5].

Figure 2. DNA alkylation mediated by mechlorethamine, an alkylating agent similar to cyclophosphamide.
Consequences of DNA Alkylation

In the guanine adduct, the iminol tautomer is favored. This changes the base-pairing preference from cytosine to thymine, causing mutations during DNA replication and other alterations which ultimately result in inhibition of the replication process and cell death by apoptosis. Although the specific cause of cell death induced by cyclophosphamide is not yet well known, mechanisms that lead to apoptosis, like p53, may be activated in response to DNA alteration[5].

1.1.3. Adverse Effects and Contraindications

Cyclophosphamide mechanism of action is associated with many severe adverse effects which may require dose monitoring, dose reduction or even discontinuation of treatment[6].

Like most cytotoxic drugs, cyclophosphamide can cause myelosupression (leukopenia, neutropenia, thrombocytopenia and anemia), bone marrow failure, and severe immunosuppression which may lead to serious infections. On the other hand, the genotoxic and mutagenic character of the drug can give rise to male and female infertility, while embryo-fetal toxicity or teratogenicity of the embryo or fetus may be seen if it is administered to a pregnant woman. Hemorrhagic cystitis and secondary bladder cancer, as well as other forms of urinary and renal toxicity, have also been reported. Other possible side effects of cyclophosphamide are cardial toxicity, pulmonary toxicity, secondary malignancies, veno-occlusive liver disease (VOD), impairment of wound healing, hyponatremia, anaphylactic reactions, nausea and vomiting, and alopecia[2].

Cyclophosphamide is contraindicated in patients with a history of severe hypersensitivity reactions to it, and in urinary outflow obstruction. Patients with severe renal impairment should be monitored, since decreased renal excretion may result in increased plasma levels of the drug and its metabolites, leading to increased toxicity. In addition, pregnancy and nursing should be avoided during treatment with cyclophosphamide, and female and male patients of reproductive potential should use contraception after completion of treatment[2].

1.1.4. Methods of Administration and Dosages

Cyclophosphamide is administered orally or by intravenous injection or infusion in several different dosage regimens[2].

In patients with malignant diseases receiving cyclophosphamide monotherapy, induction therapy is usually initiated with an intravenous cyclophosphamide loading dose of 40–50 mg/kg administered in divided doses over 2–5 days, whereas the usual oral dose for induction or maintenance therapy is 1–5 mg/kg daily. These doses must
be adjusted in accord with evidence of antitumor activity and/or leukopenia and may be reduced in combination with other cytotoxic agents[2].

In the treatment of inflammatory rheumatic diseases, lower intravenous (e.g. 15 mg/kg every 2-3 weeks) or oral doses (e.g. 2 mg/kg/day) are used[6], while in patients who undergo transplantation cyclophosphamide can be given in very high doses (e.g. 60 mg/kg for two days)[3].

1.2. In What Dosage Forms is Cyclophosphamide Currently Marketed?

Cyclophosphamide is used in most countries around the world, where it is marketed by several laboratories in different dosage forms and strengths. In this project, attention has been drawn to the cyclophosphamide medications that are currently commercialized in Spain, on the one hand, and in the United States of America, on the other.

1.2.1. Dosage Forms Marketed in Spain

In Spain, two dosage forms of cyclophosphamide are currently available under prescription[7]:

- Powder for solution for injection: Marketed by Baxter Oncology under the brand name Genoxal® in two different doses (200 mg/vial and 1 g/vial).
- Coated Tablets: Marketed by Baxter Oncology under the brand name Genoxal® in a dose of 50 mg.

1.2.2. Dosage Forms Marketed in The United States of America

In contrast, in the United States, coated tablets have been recently withdrawn by the marketing authorization holder and replaced by cyclophosphamide capsules, which have the same composition and indications as those of the prior commercialized oral form[8]. In addition, lyophilized powder for injection has been introduced. Overall, currently marketed forms of cyclophosphamide in the USA are[9]:

- Powder for solution for injection: Marketed as a generic drug by Sandoz in collaboration with Jiangsu Hengrui Med[10] (the owner of the Abbreviated New Drug Application (ANDA)) and by Baxter Healthcare. In both cases, the drug is accessible in three doses (500 mg/vial, 1 g/vial and 2 g/vial).
- Lyophilized powder for solution for injection: Marketed by Baxter Healthcare under the brand name Cytoxan® in three doses (500 mg/vial, 1 g/vial and 2 g/vial).
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- Capsules: Marketed as a generic drug by Roxane in two available doses (25 mg and 50 mg).

1.2.3. Overview of Cyclophosphamide Medications Available in Spain and in The United States

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<tr>
<th>PRODUCT</th>
<th>STRENGTH</th>
<th>EXCIPIENTS</th>
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<tr>
<td>Genoxal grageas® (Baxter Oncology)</td>
<td>50 mg</td>
<td>Tablet core: Maize starch, lactose monohydrate, calcium hydrogen phosphate dihydrate, talc, magnesium stearate, gelatine, glycerol (85%) Coating: Sucrose, titanium dioxide, calcium carbonate, talc, macrogol 35000, silica colloidal anhydrous, povidone, sodium carboxymethylcellulose, polysorbate 20, montan glycol wax, FD&amp;C Blue No 1, D&amp;C Yellow No. 10 aluminum lake</td>
</tr>
<tr>
<td>Cyclophosphamide capsules (Roxane Laboratories)</td>
<td>25 mg, 50 mg</td>
<td>Capsule: Pregelatinized starch and sodium stearyl fumarate Capsule shell: FD&amp;C Blue No 1, FD&amp;C Red No 40, gelatin and titanium dioxide</td>
</tr>
<tr>
<td>Genoxal inyectable® (Baxter Oncology)</td>
<td>200 mg/vial, 1 mg/vial</td>
<td>None</td>
</tr>
<tr>
<td>Cyclophosphamide for injection (Baxter Oncology)</td>
<td>500 mg/vial, 1 g/vial, 2 g/vial</td>
<td>None</td>
</tr>
<tr>
<td>Cyclophosphamide for injection (Jiangsu hengrui med – Sandoz)</td>
<td>500 mg/vial, 1 g/vial, 2 g/vial</td>
<td>None</td>
</tr>
<tr>
<td>Cytoxan® (Liophylized)</td>
<td>500 mg/vial, 1 g/vial, 2 g/vial</td>
<td>Mannitol</td>
</tr>
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</table>

Table 1. List of cyclophosphamide medications available in Spain and in the USA (different doses and excipients are detailed for each medication).[9][7]
2. OBJECTIVES

The objectives of this work are the following:

- Study cyclophosphamide focusing on its structure, physicochemical properties, stability, pharmacokinetics, and other aspects which condition the route of administration and the dosage form in which is delivered.

- Review the traditional sugar-coating technique and introduce some of the changes that it has faced over time.

- Justify oral administration of cyclophosphamide and underline the advantages of coated tablets in comparison with other oral forms in which the drug can be encountered.

- Analyze currently commercially available cyclophosphamide sugar-coated tablets in terms of pharmaceutical technology.

- Propose a novel formulation of cyclophosphamide coated tablets.
3. MATERIAL AND METHODS

The sources referred in this book are a combination of summaries of product characteristics, research papers, reviews, books, and websites.

The summary of product characteristics of Genoxal Grageas was not available at CIMA (Centro de Información Online de Medicamentos de la Agencia Epañola de Medicamentos y Productos Sanitarios), so the equivalent ones from the United States of America and from the United Kingdom have been used. These documents have been obtained from the FDA (Food and Drug Administration) and the Electronic Medicines Compendium (eMC) websites, respectively, and have been key to introduce the most relevant aspects of the medication and to find out the different excipients comprising currently commercialized cyclophosphamide coated tablets.

Most articles and reviews have been found through Scifinder, a database of chemical and bibliographic information that allows to make searches by keyword and to select the most interesting information. Then, if possible, they have been downloaded from online libraries and databases like Springer or Pubmed, or partially consulted online in case of restriction. Research papers and reviews have been especially important to outline the pharmacokinetics of the active ingredient, as well as to justify both the route of administration and the dosage form of cyclophosphamide sugar-coated tablets.

To describe the traditional sugar-coating process, some books have been borrowed from the library of pharmacy. On the other hand, the Handbook of Pharmaceutical Excipients consulted online through CRAI (Centre de Recursos per a l'Aprenentatge i la Investigació) has been the main information source when reviewing the formulation of cyclophosphamide sugar-coated tablets and when choosing the excipients of the novel sorbitol film-coated formulation.
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4. DEVELOPMENT OF THE PROJECT

4.1. Important Cyclophosphamide Properties Regarding Pharmaceutical Formulation

4.1.1. Structural Properties

Cyclophosphamide, an organic compound with chemical name 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide and the molecular formula C_7H_{15}Cl_2N_2O_2P, is a tertiary amine which belongs to the nitrogen mustard compounds. These are compounds that have two beta-haloalkyl groups bound to a phosphorus atom[11].

Several substituents can be found in the cyclophosphamide structure: phosphorodiamide, phosphoric acid ester, oxazaphosphinine, polyamine, organochloride, and alkyl halide, approaching the substance to many alternative chemical parents[11].

The presence of an asymmetric phosphorus atom in the structure of cyclophosphamide is the feature that explains its chirality. This results in two enantiomers, R-(+)-cyclophosphamide, and S-(-)-cyclophosphamide, which have the same structure but different configuration and thus are not interchangeable[12]. In spite of this, the racemic mixture of S-(-)- R-(+) -cyclophosphamide enantiomers is the one usually used in the clinical practice, although preclinical data show that S-(-)-cyclophosphamide exhibits a greater antitumor effect than the R-(+) -enantiomer[12][13].

Cyclophosphamide can be found in its anhydrous form or as cyclophosphamide monohydrate. The last one contains a single molecule of water of crystallization per molecule of drug substance and is the most stable form of the drug. Thus, it is usually
the one used to manufacture both oral and intravenous cyclophosphamide preparations.

4.1.2. Physicochemical Properties

Physical and chemical properties of cyclophosphamide monohydrate, referred to as cyclophosphamide, are described in this section.

Cyclophosphamide is an odorless slightly bitter white or almost white fine crystalline powder at room temperature. It is soluble in water (40 g/l), ethanol (~750 g/l), chloroform, dioxane, and glycols, and slightly soluble in benzene, carbon tetrachloride, ether, and acetone[14].

With a predicted logP of 0.63, a molecular weight of 279.10 g/mol, 2 hydrogen bond donors and 5 hydrogen bond acceptors, cyclophosphamide complies with Lipinski’s rule of five (logP < 50, molecular weight < 500 g/mol, hydrogen bond donors ≤ 5, and hydrogen bond acceptors ≤ 10)[14].

Cyclophosphamide is a weak acid, with an experimental pKa of 6.0[15]. Consequently, it is to be expected that the drug will absorb well from the gastrointestinal tract, although no data on the specific site of absorption is available. On the other hand, preclinical data suggest that weakly acidic drugs such as cyclophosphamide enhance intratumor uptake, since the acidic extracellular pH in human tumors facilitates their diffusion through membranes[16].

Finally, it must be said that the melting range of the crystal form is low (49.5-53°C)[14] and that the drug has low flowability and compressibility.

4.1.3. Stability

Cyclophosphamide is sensitive to oxidation, moisture, light[17], temperature, and pH[18]. It has also been reported that mechanical treatment of the monohydrate form can affect its stability[19].

One the one hand, degradation of cyclophosphamide monohydrate in aqueous solution is due to hydrolysis (cyclophosphamide structure has several hydrolysable groups, like phosphorodiamide) loss of a chloride ion, or both. An increase in temperature, as well as the presence of benzyl alcohol, accelerates the rate of breakdown[18]. Decomposition of the drug in aromatic elixir (33% water, 33% sucrose, and 33% alcohol) is slower, what may reflect the decreased water concentration, while at extreme pHs it is susceptible to undergo specific acid and specific base catalysis[20].

On the other hand, the monohydrate form of cyclophosphamide is converted to the anhydrous one through a metastable phase that gives rise to a sticky gel which decreases the rate of release and the bioavailability of the drug. These forms can be
detected at about 39ºC, and appear when the drug is desiccated, as well as with mechanical treatment. Indeed, cyclophosphamide monohydrate is only stable if the relative humidity is higher than 70% and the temperature lower than 30ºC[19].

### 4.1.4. Pharmacokinetics

Many studies regarding the pharmacokinetics of cyclophosphamide have been conducted. Nevertheless, the relations between pharmacokinetics and pharmacodynamics of cyclophosphamide are not fully established yet. This might be due to interindividual variations in the metabolism and distribution of the drug, since cyclophosphamide itself is inactive. Hence, the pharmacokinetics of its active metabolites should be the one to be considered for predicting drug efficacy[21].

Difficulty in understanding exposition to the active compounds is increased by the fact that cyclophosphamide induces its own metabolism after repeated administration. This decreases its elimination half-life and increases its total body clearance, thus reducing drug exposure, expressed as the area under the plasma concentration-time curve (AUC). However, it is controversial whether the subsequent increase in the rate of formation of the active metabolites correlates with an increase in their AUC[21].

In addition, nonlinear elimination of high-dose cyclophosphamide, which is likely to cause saturability of 4-hydroxilation reducing the formation of 4-hydroxycyclophosphamide, a metabolite involved in the biological activity, has been described[21].

Finally, since cyclophosphamide is largely metabolized, drug-drug interactions that cause modifications in its activation, inactivation and detoxification processes have a major impact in its pharmacokinetics[21].

#### 4.1.4.1. Absorption

In order to reach systemic circulation, orally administered drugs need to be absorbed through the gastrointestinal wall. Absorption is only possible if drugs are in solution; thus, prior to crossing the physiological membranes solid forms like tablets must be able to disintegrate and deaggregate (during the process known as liberation). Consequently, water solubility is of vital importance[22].

The other fundamental parameters controlling drug absorption are drug permeability, which is based on the drug n octanol/water partition coefficient P (a measure of drug hydrophobicity), and drug ionization[22].

Technological (e.g. particle size), and physiological characteristics (e.g. absorption membrane, gastric pH, bowel transit time) module the above parameters. However, only technological properties affecting the dissolution process can be modified by
physical procedures. Thus, the absorption rate of a specific drug can only be modified by controlling its dissolution process by means of pharmaceutical technology.

Finally, it must be said that, although a high absorption does not necessarily imply a high bioavailability, a high oral bioavailability correlates with a high gastrointestinal absorption.

The Biopharmaceutics Classification System (BCS) Guidance classifies drug substances in 4 different classes according to their solubility and permeability (Class I - High Permeability, High Solubility, Class II - High Permeability, Low Solubility, Class III - Low Permeability, High Solubility, Class IV - Low Permeability, Low Solubility)[23]. According to BCS, cyclophosphamide, with an aqueous solubility of 40 g/L, is highly soluble in water (highest dose strength soluble in < 250 ml water over a pH range of 1 to 7.5), as well as highly permeable (extent of absorption in humans > 90% of an administered dose, based on mass-balance or in comparison to an intravenous reference dose), thus pertaining to class I[24]. Consequently, neither dissolution nor permeability of cyclophosphamide should be limiting factors for its gastrointestinal absorption.

The latter classification is in line with in vivo studies regarding the pharmacokinetics of orally administered cyclophosphamide, which have demonstrated that the drug is well absorbed in humans, with peak concentration (C$_{max}$) occurring after 1-3 hours (t$_{max}$) following oral administration and an oral bioavailability of 85-100%[21]. This small decrease in bioavailability in comparison with the intravenous formulation, which by definition is 100%, is due to the first pass effect in the liver and gut, where a fraction of drug is metabolized[25]. In addition, Navid et. al.[26], in their recent study of low-dose cyclophosphamide administered in children and young adults, reported an oral absorption rate constant (K$_a$) of 0.17 h$^{-1}$ (0.15-0.21 h$^{-1}$).

4.1.4.2. Disposition

Disposition is a dynamic process where distribution, metabolism and excretion take place at the same time. Elimination half-life is the main parameter representing cyclophosphamide disposition, and ranges between 5 and 9 hours over a large concentration range. However, it appears to be shorter for children and young adults compared with adults as a result of increased CYP activity[21].

Distribution

After oral and intravenous administration, cyclophosphamide is rapidly distributed throughout the body with a low degree of plasma protein binding and a volume of distribution of 30-50L, which approximates to total body water[21]. In contrast, the ability of protein binding is higher for its metabolite, 4-hydroxycyclophosphamide (<67%)[25].
Cyclophosphamide enters the cells, such as hepatocytes, via passive diffusion and active transport, where it is converted into 4-hydroxycyclophosphamide. This compound diffuses into the plasma and, because it is relatively nonpolar, enters target cells via passive diffusion. Here it is trapped (weakly acid drugs such as cyclophosphamide are more likely to be in the ionized form inside the tumor cells due to its less acidic pH compared to the extracellular one) and converted into phosphoramidine mustard. Although phosphoramidine mustard is also produced extracellularly, it is highly polar and thus cannot diffuse through the lipid bilayer of cells.

Apart from entering hepatocytes, cyclophosphamide is extensively bound by erythrocytes, which may be a carrier of 4-hydroxycyclophosphamide to the tumor site. Cyclophosphamide may also enter into cerebrospinal fluid through the blood brain barrier. Penetration in the brain is limited for its metabolites, which undergo higher plasma protein binding and are more polar[25].

**Metabolism**

Approximately 70-80% of the administered dose of cyclophosphamide is activated in the liver by CYP450, such as CYP2A6, 3A4, 3A5, 2C9, 2C18, 2C19, and especially 2B6, to form 4-hydroxycyclophosphamide. This compound is in equilibrium with its ring-open tautomer aldophosphamide and spontaneously decomposes into phosphoramidine mustard by β-elimination of acrolein. Phosphoramidine mustard is a bifunctional DNA alkylating agent considered to be the ultimate metabolite responsible for the alkylating effect of the drug, whereas acrolein is a highly reactive aldehyde and may enhance cyclophosphamide-induced cell damage by depletion of cellular glutathione by conjugation[21].

Cyclophosphamide may be directly detoxified by chain oxidation, leading to the formation of chloroacetaldehyde, while 4-hydroxycyclophosphamide and aldophosphamide are irreversibly deactivated by an oxidative reaction to 4-ketocyclophosphamide and carboxyphosphamide, respectively. Detoxification of 4-hydroxycyclophosphamide, phosphoramidine mustard and acrolein may also occur via intracellular conjugation with glutathione[21].

![Figure 4. Metabolism of cyclophosphamide.[21]](image-url)
Finally, the appearance of the metabolite nor-nitrogen mustard in plasma and urine is the result of decomposition of cyclophosphamide and its metabolites, particularly phosphoramid mustard and carboxyphosphamide[21].

**Excretion**

Between 30-60% of the total cyclophosphamide dose is eliminated renally as cyclophosphamide (less than 20% of the administered dose) or metabolites, while a very small fraction is eliminated via faeces and expired air. The major metabolite found in urine is carboxyphosphamide, although large interindividual variability in its urinary excretion has been seen. Urinary elimination of cyclophosphamide and its metabolites is almost complete 24 hours after the start of treatment[21].

Total systemic clearance of cyclophosphamide ranges from 4-5 L/h, of which the greater part is nonrenal clearance, probably due to extensive renal tubular reabsorption. However, it has been shown that renal clearance of cyclophosphamide is dependent on urine flow[21].

### 4.2. The Sugar-coating Technique

Tablets may be coated for a variety of reasons, including:

- Masking unpleasant color, flavor or odor.
- Enhancing administration by presenting a softer and slider surface.
- Protecting the active ingredient from atmospheric agents such as air, moisture or light.
- Avoiding incompatibilities by incorporating separately (coat and nucleus) non-compatible active ingredients.
- Modifying bioavailability (sugar-coating usually does not pursue this goal, in contrast to film-coating)[27].
- Protecting an acid labile drug from the gastric environment[28].
- Protecting the gastric mucosa from the action of the active ingredient.
- Differentiating among pharmaceutical formulations during the manufacturing process.
- Improving product appearance
- Improving product robustness because coated products generally are more resistant to abrasion and attrition[29].

There are several sugar-coating techniques, but the essential four major ones today are the following: Sugar-coating, film-coating, microencapsulation, and compression coating[29]. Recent trends in tablet coating techniques also include electrostatic dry
coating, magnetically assisted impaction coating (MAIC), vacuum film-coating, and dip coating[28]. In this section we will focus on tablet sugar-coating.

4.2.1. Origins and Evolution of the Sugar-coating Technique

Coating of medicinal products is one of the most ancient pharmaceutical processes. According to this, in the 1st and 2nd centuries AD the coating process was already common in Egypt to cover pills which tasted bad. One of the first written references of coated forms appeared in the Islamic literature by the hand of Rhaces (850-923)[30].

In the middle ages, the popularity of coated baked goods gave rise to the introduction of the first coated medicines to the market. In the 14th century, dragée manufacturing as it is known today started, but sugar-coatings were not fully developed until the mid-19th century (two of the first patents of the sugar-coating technique date back to 1837 and 1840)[30].

Until the forties, evolution of the sugar-coating process remained static[30], but soon the development of the film-coating technology and the advances that came with it, such as the design of new equipment, benefited the sugar-coating process, creating fully automated processes that can produce a batch in less than one day[29].

4.2.2. What Is Tablet Sugar-coating?

Tablet is a pharmaceutical solid dosage form comprising a mixture of active substances and excipients pressed or compacted into a solid. Tablet is one of the most preferred dosage forms all over the world, and most drug molecules can be formulated in a tablet[31].

Coating is a process by which an essentially dry, outer layer of coating material is applied to the surface of a dosage form to achieve specific benefits. Coating may be applied to a wide range of oral solid dosage forms, including tablets, capsules, particles, powders, pellets, and drug crystals[28].

Tablet sugar-coating, also known as “dragée”, involves the application of a continuous and homogeneous sugar (sucrose) coat on the tablet (nucleus or core)[30]. Unlike film-coating, sugar-coating is a multistep process which requires a fair degree of skill; although some approaches to shorten the traditional technique have recently been made[32].

The tablet sugar-coating process has many disadvantages in terms of process length (it takes up to several days), process difficulty (it is characterized by delicate operations), and need for highly skilled operators. Another possible drawback is the increased bulk of the finished tablet. Despite these undoubted disadvantages, it can have certain advantages: Well tolerated and widely accepted non-expensive
excipients, simple equipment, controlled non-critical process which can meet modern GMP (Good Manufacturing Practices) standards, availability to work with softer and more friable nucleus than those required in the film-coating process, and obtainment of more stable tablets compared to the ones resulting from the film-coating process[32].

4.2.3. The Sugar-coating Process

4.2.3.1. Requirements of the Tablet Cores to Be Coated

Tablets that are to be coated must possess the proper physical characteristics. Otherwise, the coating process might not succeed[33].

In the coating process, the tablets roll in a coating pan as the coating composition is applied. To tolerate the intense attrition of tablets striking other tablets or walls of the coating equipment, the tablets must be resistant to abrasion. So, mechanical resistance (hardness and friability) must be high[27].

Ideally, the tablets used should have a biconvex shape with minimal edges[27], since the more convex the surface is the fewer difficulties will be encountered with tablets agglomeration[33]. In addition, the corners of biconvex tablets are easier to coat.

The sugar-coating process involves high temperatures. Because of this, it is important to keep moisture at a low level[27]. Furthermore, tablets must be compatible with the coating materials and the tablet surface must be smooth so that the imperfections are covered.

4.2.3.2. Equipment

Historically, the sugar-coating process has been performed in conventional coating pans. These pans consist of an ellipsoid metal drum[27] of stainless steel with a unique opening angularly mounted on a stand[33]. Throughout the coating process, the core bed is moved in the container, which rotates on an inclined axis by motor at the same time that the coating liquid is fed through a spraying nozzle which is installed at the front of the opening. Cores get coated as they enter the spray zone prior to cascading down and merging into the bulk of the core bed[34]. Additionally, the coating pan is fitted with a means of supplying drying air to the tablets and an exhaust to remove moisture and dust-laden air from the pan[29].
Early sugar-coating pans used in the pharmaceutical industry came from those originally used for the production of candies[34]. Conversely to present conventional coating pans, the latter ones were made of copper instead of stainless steel[35]. On the other hand, they used to be attached to a blower vacuum to facilitate evaporation of moisture, and its container was rotated manually by means of a gear directed by a strap. The coating syrup was also applied manually on the core bed[36].

In general, conventional pan coaters suffer from two disadvantages: Inefficient particle movement resulting in the appearance of so-called “dead zones” that impair homogeneous mixing of the core bed, and inadequate air transport causing insufficient drying of the core bed[34].

To overcome these drawbacks, over the 20th century, with the raise of the film-coating technology, several new coating machines reached the market. Although some of them were specifically designed for film-coating, such as fluidizer beds, others have been widely used for sugar-coating as well[32]. This new equipment differs mainly from the standard coating pans described above in the position of the rotating axis, which is horizontal[34].

In this regard, the introduction of pan coaters rotating on horizontal axis provided with baffles or blades which contributed to improvements in the particle movement and so to more uniform mixing is remarkable. The Pellegrini pan, which provides an enclosed coating system, was one of the first to incorporate such novelties[34].

On the other hand, with the aim to improve the drying efficiency of the conventional system, where only the surface of the core bed is exposed to the drying air, two different drying gadgets were developed: The immersion tube and the immersion sword, in which drying air is introduced through a perforated tube or metal sword, respectively, immersed in the tablet bed[34].

Another approach to increasing the drying efficiency is the invention of perforated pans. According to this, a major advance in pan coating technology was the introduction of the side-vented pan concept[29]. This innovation was developed by Eli Lilly and formally designated as the Acela-cota[29], an enclosed coating pan in which drying air is directed into drum, passes through bed, and is exhausted through perforations in to drum[33].

Acela-cota was such a revolution that it has formed the basis for a wide range of partially or fully perforated pans known as side-vented pans[29], which are currently used almost exclusively for sugar-coating. Some typical examples of this modern pan coating equipment include: Hi-Coater, Premier Coater, HTF/150, IDA, and Glatt, which also provide means for automating the sugar-coating process[37].
4.2.3.3. Traditional Sugar-coating Process: Stages

A typical sugar-coating process takes some days and encompasses five stages, with a final optional stage: Sealing, subcoating, smoothing, color coating, polishing, and printing (optional)[30]. Throughout these stages, tablet cores are successively treated with aqueous sucrose solutions which, depending on the stage of coating reached, may contain other functional ingredients. Typically, in each stage heated air is blown across the drum to warm the cores up. Then, a single liquid application which will spread over the entire tablet bed is made. At this point, drying air is used to dry the application. The whole cycle is then successively repeated until the desired coating is obtained[32]. The overall process takes place in a coating pan, and usually results in a final product weight gain of 30-100% of the weight of the original tablet core[27].

Sealing

The purpose of sealing is to protect the cores from the water of the successive layers and from the abrasion they suffer throughout the process[27], as well as to prevent some tablet core ingredients from migrating into the coating[35].

To accomplish this, the cores are exposed to water insoluble film-forming polymers dissolved in organic solvents (ethanol, acetone, ethyl acetate), which enable the formation of a waterproof insulating film[27]. Examples of polymers that might be used include shellac[27][32], cellulose acetate phthalate (CAP)[27][35][32], hydroxypropyl methylcellulose (HPMC)[35], polyvinyl acetate phthalate (PVAP)[35][32], zein[35][32], high-molecular weight polyethylene glycol[27], and polymethacrylates[27]. These solutions also include small amounts of plasticizers such as castor oil and alkyl phthalates to provide film elasticity and ensure waterproofing[35]. The use of dusting powders such as talc to prevent the tablets from sticking together or to the pan has also been described[35][32].

During the sealing stage, the general process of blowing air, applying the sealing solution and drying is repeated until reaching a weight increase of 1-3 %[27].
**Subcoating**

Subcoating is the first major step of the sugar-coating process, which provides the means for rounding off the tablet edges[27][35] and for reaching the definitive tablet shape[27] while building up the core weight[35] (in this stage the weight increase is around 30%-50%[32]). To get effective coverage of the cores and avoid twinning, tablet shape is especially important in this stage[27]. According to this, biconvex tablets are preferable, as discussed in section 4.2.3.1.

Effective subcoating is achieved through a lamination process[35], which consists of the application of a concentrated gummy syrup (binder solution) containing sucrose and small amounts (3-5%) of binders such as gelatin, polyvinylpyrrolidone, acacia gum, etc., followed by addition of powders (fillers and detackifiers)[27]. Since in the lamination process overdusting can create tablets with brittle coatings, a suspension subcoating process in which a suspension of the gummy syrup and the powder is applied over the tablets is frequently used. In addition, the latter approach increases the quality of the coated-tablets, facilitates automation and reduces the complexity of the process in comparison with the first one[35].

**Smoothing (Grossing)**

The purpose of the smoothing or grossing stage is to achieve a smooth surface at the same time that the nucleuses reach the desired size (approximately 40% of the initial weight)[30]. This is possible by successive applications of a diluted sucrose syrup (70% w/w[35][29]), depending on the degree of smoothness acquired in the subcoating stage[35]. In some cases, the smoothing coating can also contain titanium dioxide, an opacifier/whitening agent, or other colorants. In addition, large degrees of unevenness might require some subcoating solids in low concentrations in the initial smoothing coats, such as talc, calcium carbonate or corn starch[35].

**Color Coating**

Color coating is one of the most important steps in the sugar-coating process as it has immediate visual impact[35][32], but it is often the most critical one[29]. Color coating can be tackled by the use of appropriate coloring agents dissolved or dispersed in a simple syrup[35]. Depending on whether these agents are water-soluble or water insoluble, two different color coating techniques exist. The most ancient one relies on the application of water soluble dyes, whereas the second one uses modern predispersed suspensions of water-insoluble pigments, including inorganic pigments such as the opacifier titanium dioxide or iron oxides[35][32].

<table>
<thead>
<tr>
<th>SOLUBLE DYES</th>
<th>WATER INSOLUBLE PIGMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>The final color is determined by the overall thickness of the successive color layers, so irregularities in the surface of these layers result in an uneven color</td>
<td>The final color is not dependent on the thickness of the color layer thanks to the use of opacifiers</td>
</tr>
</tbody>
</table>
Coating syrups are applied in increasing specific concentrations until reaching the target color, what can involve 30-50 color applications. Coating syrups are applied in a single relatively high concentration of color.

The color layer is easily disrupted due to migration of the color to the tablet surface in case of underdrying or quick drying. Color migration problems are not usual thanks to the water insoluble nature of the pigments.

Color uniformity is not maintained from batch to batch if the number of applications in each batch varies, since it depends on the thickness of the color layer. Color uniformity is maintained from batch to batch even if the number of applications in each batch differs slightly, since the final color is not dependent on the thickness of the color layer.

The process is time-consuming and requires highly skilled operators. The process is shorter thanks to the fact that the coating syrups are applied in a single concentration and that quick drying is possible.

| Coating syrups are applied in increasing specific concentrations until reaching the target color, what can involve 30-50 color applications | Coating syrups are applied in a single relatively high concentration of color |
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| The process is time-consuming and requires highly skilled operators | The process is shorter thanks to the fact that the coating syrups are applied in a single concentration and that quick drying is possible |

Table 2. Differences between soluble dyes and water insoluble pigments[35][32].

Overall, although advantages of the pigment coating process tend to prevail, making it the process of choice, it must be said that coatings derived from pigments are generally not as bright as those obtained with soluble colorants[35].

Polishing (Glossing)

After the color-coating process the tablets have a matt appearance which requires a separate polishing step to give them the adequate degree of gloss. Polishing methods vary considerably, but it is generally important that the tablets are dry prior to polishing[35]. Some examples of polishing methods include:

- Application of an organic solvent solution/suspension of waxes, for example carnauba and beeswax[35]. An available variant of this technique provides an emulsion of waxes in an aqueous continuous phase stabilized by a surfactant, with the advantage of aqueous processing[32].
- Finely powdered wax application[35].
- Mineral oil application[32].
- Pharmaceutical glazes containing shellac in alcohol with or without waxes[35].

The equipment available for carrying out the polishing stage includes polishing pans such as wax-lined pans and canvas-lined pans, although the procedure can also be performed in the sugar-coating pans where the prior steps take place, especially in automated approaches[35].

Printing

The aim of the printing process is to enable the product to be easily identified[30]. This might be done by engraving a product name, dosage strength or company name or logo on the tablet coating[35]. Indeed, some regulatory authorities demand or
encourage that tablets should possess some detailed identifying mark as part of the overall GMP requirements\[32\].

Typically, such printing involves the application of a pharmaceutical ink to the coated tablet surface by means of a process known as offset rotogravure\[35\]. A typical edible pharmaceutical ink formulation suitable for this process consists of: Shellac, alcohol, pigment, lecithin, antifoam and other organic solvents. Shellac is the lacquer most commonly utilized, but is slowly giving ground to cellulose derivatives as it can pose severe stability problems. Lecithin is frequently included to maximize the quantity of pigment that can be utilized, while antifoam is necessary to prevent the foam build up\[32\].

Recently, other technologies which are less sensitive to minor changes in procedure than the offset gravure process, such as the ink-jet printing technique, are being introduced\[32\].

4.2.3.4. **Automated and Fast Coating Systems**

Over the course of time, the pharmaceutical industry has witnessed a general transition away from manually operated sugar-coating processes to film-coating processes, where operator intervention is infrequent. Nevertheless, the sugar-coating process is still used by many companies that have invested in its complete modernization, which has allowed reduction of processing time (traditionally, the process could take up to 5 days, whereas nowadays the time has been reduced to less than one day) and has lowered the number of operators needed in traditional sugar-coating. This has been possible through thin sugar-coating procedures (such as the uniform or fast sugar-coating process known as Tucker) and process automation (in which coating application is accomplished using automated dosing techniques)\[29\].

**Uniform or Fast Sugar-coating (Tucker)**

In the Tucker sugar-coating method, the subcoating and grossing stages are carried out simultaneously. This gives rise to a thinner cover than that resulting from the classical sugar-coating technique. The quality of the tablets obtained is also compromised compared to the ones of the traditional sugar-coating method. However, positive aspects of this technique are speed and possibility of automation\[30\].

**Automated Sugar-coating**

Dependence of operators in the sugar-coating process can be minimized through automation. However, the natural sequencing of events that are the basis of the sugar-coating process adds a level of complexity when considering the implementation of automation. Recent initiatives such as Quality by Design (QbD), which aims to control all aspects of the process involved in the release of pharmaceuticals by designing fully optimized processes, creating an effective design space, and implementing in Process Analytical Technologies (PAT), simplify the challenges of automation\[29\]. Thus,
automation involves a series of regulating devices for temperature, airflow, spray rate and pan speed which enable to maintain a feedback control of the process[33]. An example of this is effective use of nIR (near infra-red) techniques such as the nIR sensor[29].

4.2.4. Quality Problems with Sugar-coated Tablets

Finished sugar-coated tablets can present several quality problems, such as chipping of coatings, cracking of the coating, inability to dry sugar-coatings properly, twinning, uneven color, blooming and sweating, and marbling[35].

Chipping can be avoided with the inclusion of small quantities of polymers, whereas it is exacerbated with excessive use of fillers and pigments which increase the brittleness of the sugar-coating. Cracking might be due to moisture absorption by the tablet core, and can be minimized by appropriate use of a seal coat. Inability to dry sugar-coatings properly is often an indicator that excessive levels of invert sugar are present. This might happen when sucrose syrups are exposed to elevated temperatures under acidic conditions for extended periods of time. Twinning usually occurs because of the sticky nature of sugar-coating formulations, and becomes a problem when the tablets being coated have flat surfaces. Uneven color can be caused by many factors, such as color migration of water-soluble dyes, excessive drying between color applications, etc. Blooming and sweating occur when residual moisture of the finished sugar-coated tablets diffuses out, causing appearance alterations in the tablet surface and sticking of the tablets. Finally, failure to achieve the requisite smoothness often results in a marbled appearance on polishing[35].
5. RESULTS AND DISCUSSION

5.1. Justification of the Route of Administration

Is Oral Administration of Cyclophosphamide Pharmacokinetically Equivalent to Intravenous Administration?

As reviewed in the pharmacokinetics section, cyclophosphamide has a high oral bioavailability. Thus, it is reasonable to assume that oral administration of the drug might be a great alternative to intravenous administration. However, the fact that cyclophosphamide itself is not responsible for the final biological activity should not be overlooked. For instance, the pharmacokinetics of cyclophosphamide metabolites which contribute to the cytotoxic activity must also be studied.

Struck et al.[38], in the first study to compare plasma levels of the two main cytotoxic metabolites of cyclophosphamide (phosphoramid mustard and 4-hydroxycyclophosphamide), reported that exposure to these compounds, measured as the mean AUC values of the participants in the study, was similar after administration of an oral liquid formulation and an intravenous preparation of the same cyclophosphamide dose. Conversely, exposure to cyclophosphamide was higher when administered intravenously due to the first past effect of the oral preparation, which decreases the bioavailability of the parental compound without compromising clinical efficacy (see figure 7).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean AUC ( ^a )</th>
<th>Mean difference ( ^b )</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.v.</td>
<td>p.o.</td>
<td>(i.v. – p.o.)</td>
</tr>
<tr>
<td>CPA</td>
<td>203.958 (55.872)</td>
<td>154.174 (48.151)</td>
<td>49.783 (23.027)</td>
</tr>
<tr>
<td>HOCPA ( ^b )</td>
<td>0.531 (0.289)</td>
<td>0.513 (0.212)</td>
<td>0.017 (0.166)</td>
</tr>
<tr>
<td>PM</td>
<td>0.507 (0.309)</td>
<td>0.490 (0.232)</td>
<td>0.017 (0.222)</td>
</tr>
</tbody>
</table>

\( ^a \) Numbers in parenrs, ± SD.

\( ^b \) HOCPA, 4-hydroxycyclophosphamide.

Figure 7. Comparison of mean AUC values for cyclophosphamide, 4-hydroxycyclophosphamide, and phosphoramid mustard after oral and intravenous administration.[38]
These data support that the intravenous and oral routes are interchangeably in the clinical practice in terms of pharmacokinetic/pharmacodynamic relationships and explain the current availability of both intravenous and oral forms.

5.2. Justification of the Dosage Form

5.2.1. Pharmacokinetic Justification

Are Cyclophosphamide Sugar-coated Tablets Pharmacokinetically Equivalent to Capsules and Oral Liquid Forms?

Few studies regarding oral bioavailability of cyclophosphamide have been conducted. In some of these studies patients were administered cyclophosphamide tablets, while in others oral liquid formulations prepared using cyclophosphamide powder for injection were preferred. No study regarding the pharmacokinetics of cyclophosphamide capsules, recently introduced in the United States, has been found. However, this new dosage form has demonstrated bioequivalence with the tablet formulation[39].

The mean AUC values for cyclophosphamide after oral administration obtained in some of the studies regarding the pharmacokinetics of orally administered cyclophosphamide are summarized in table 3.

<table>
<thead>
<tr>
<th>NO. OF PATIENTS</th>
<th>DOSE REGIMEN</th>
<th>FORMULATION</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (μmol/L*h)</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>18&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>50 mg/m² once daily for 21 days</td>
<td>Liquid formulation/tablet</td>
<td>62.5 (49.2–80.9)</td>
<td>[26]</td>
</tr>
<tr>
<td>7</td>
<td>600 – 1000 mg/m²</td>
<td>Elixir syrup</td>
<td>590.50 ± 184.42</td>
<td>[38]</td>
</tr>
<tr>
<td>12&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>175 mg/m² once daily on 4 consecutive days</td>
<td>Endoxan®, gastric juice resistant dragees</td>
<td>141.5 ± 53.6</td>
<td>[40]</td>
</tr>
<tr>
<td>12&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>175 mg/m² once daily on 4 consecutive days</td>
<td>ASTA 82134 I, gastric juice soluble dragees</td>
<td>138.7 ± 34.7</td>
<td>[40]</td>
</tr>
</tbody>
</table>

Table 3. Mean AUCs for cyclophosphamide after oral administration in different studies.

<sup>a</sup>Pediatric patients; <sup>b</sup>Patients also receiving bevazizumab and sorafenib; <sup>c</sup>Female patients; <sup>d</sup>Patients undergoing Cyclophosphamide Methotrexate Fluorouracil (CMF) therapy.

Data are presented as mean ± SD or median and the range.

In a recent study in which low-dose cyclophosphamide was administered in children and young adults with refractory/recurrent solid tumors in combination with bevazizumab and sorafenib, Navid et. al.[26] reported that the AUC difference of 4-hydroxycyclophosphamide administered as an oral liquid formulation or in tablet form
was not statistically significant, suggesting that oral liquid formulations, despite not being marketed as such, might be an alternative when managing pediatric patients.

On the other hand, Brujin et al.[41], when studying the pharmacokinetics of intravenous and oral cyclophosphamide in the presence of methotrexate ant fluorouracil, found that lag-times and mean absorption times of the drug given in tablets were prolonged in comparison with those resulting from administration of oral liquid preparations, although extent bioavailability of the drug administered by tablets was enough.

Years ago, cyclophosphamide coated tablets were available as gastric juice resistant or enteric tablets and as soluble or immediate release tablets, as shown in table 4 with Endoxan® (gastric juice resistant dragee) and ASTA (juice soluble dragee). Significant differences in the rate of oral bioavailability (t<sub>max</sub>) between these two types of formulations were reported by Wagner et. al.[40] when studying the bioavailability of cyclophosphamide from three oral formulations. In this study, the time for the enteric formulation Endoxan® to reach the maximum concentration (t<sub>max</sub>) was higher (2,5 ± 1,81 h) than that needed for the immediate release tablets (1,13 ± 0,83 and 1,82 ± 0,59). Nowadays, in most countries around the world cyclophosphamide tablets are commercialized by Baxter Oncology and are no longer enteric tablets but immediate release ones.

In conclusion, available pharmacokinetic data indicate that cyclophosphamide can be administered orally in different pharmaceutical forms, such as tablets, capsules and liquid preparations, with minimal changes in the pharmacokinetic profile. In some cases, rate bioavailability might be decreased for tablets, especially if these are gastric juice resistant. However, as said before, when considering if two or more pharmaceutical forms or routes of administration are interchangeable, the AUCs of the active metabolites, rather than the ones of the prodrug, should be taken into consideration.

### 5.2.2. Stability Justification

*Are Cyclophosphamide Coated Tablets Better Than Capsules and Oral Liquid Forms from a Stability Point of View?*

As stated before, oral commercially available dosage forms of cyclophosphamide include coated tablets (in Spain) and capsules (in the United States). Although in young children it is preferable to administer the drug as an oral liquid formulation, and so is frequently done in the clinical practice, this preparation is not marketed. The explanation for this finding is likely to lie in the compromised stability of cyclophosphamide in aqueous vehicles and aromatic elixirs, as introduced in 4.1.3.

In 1973, Brook D. et al[42] showed that extemporaneous oral suspensions of cyclophosphamide powder for injection in aromatic elixir were stable for 2 weeks at 5°C. A recent study of R. Kennedy et al[43] has proven that cyclophosphamide is
stable at 4°C in both simple syrup (85 g sucrose/100 ml water) and Ora-Plus (97% Water, <1% sodium phosphate monobasic, <1% sodium carboxymethylcellulose, <1% microcrystalline cellulose, <1% xanthan gum, <1% carrageenan) for approximately two months (56 days) in a concentration of 10 mg/ml. In fact, the formulations proposed in both studies are likely to be stable for longer if the mentioned storage conditions are maintained, but later time points have not been investigated. On the contrary, the shelf lives of cyclophosphamide in simple syrup and in Ora-Plus at room temperature are 8 and 3 days, respectively. These data explain the reason why oral liquid commercial preparations of cyclophosphamide are not available, since they would have a very short shelf-life and would require special conservation conditions.

Unlike liquid oral preparations, commercialized cyclophosphamide coated tablets have an expiring period of 36 months if stored below 25°C[44], which enables distribution of the medication, safekeeping in the pharmacy and storage in the patients' home. In the same line, Roxane’s cyclophosphamide capsules also have an acceptable shelf-life (24 months if stored at 20 to 25°C[39]). However, the shelf-life difference of one year between the two solid forms helps conclude that, from a stability point of view, cyclophosphamide coated tablets is the most convenient dosage form among those used in the clinical practice.

Why Are Cyclophosphamide Tablets Coated?

Cyclophosphamide is sensitive to oxidation, moisture, light[17], temperature, pH[18], and mechanical treatment[19], as reviewed in 4.1.3. Hence, it could be assumed that the compromised chemical and physical stability of the active ingredient is one of the main reasons for tablet coating, since in this way it can be protected from the atmospheric agents that are likely to accelerate its degradation. However, avoidance of direct contact with the active ingredient is another major reason for tablet coating due to it carcinogenicity.

5.3. Cyclophosphamide Sugar-coated Tablets: Review of the Pharmaceutical Formulation and Shortcomings

The aim of this section has been to classify the different excipients of cyclophosphamide sugar-coated tablets according to the role they probably play in the pharmaceutical formulation. Hence, the excipients which appear in the summary of product characteristics of Baxter cyclophosphamide formulation have been used. By doing this, the manufacturing method undergone by the drug product has been deduced. Finally, attention has been drawn to the shortcomings of Baxter’s cyclophosphamide sugar-coated tablets.
5.3.1. **Review of the Pharmaceutical Formulation**

Baxter Oncology cyclophosphamide sugar-coated tablets are white rounded tablets with blue flecks containing 25 or 50 mg of cyclophosphamide monohydrate equivalent to 25 or 50 mg of the anhydrous form[2].

<table>
<thead>
<tr>
<th>TABLET CORE EXCIPIENTS</th>
<th>Excipient[2][44]</th>
<th>Function</th>
<th>Description[45]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize starch</td>
<td>Disintegrant</td>
<td>Matt, white to slightly yellowish, tasteless, very fine powder derived from the corn (maize) grain. It is practically insoluble in cold ethanol and in cold water, it swells instantaneously in water by about 5–10% at 37°C, and becomes soluble in hot water at temperatures above the gelatinization temperature.</td>
<td></td>
</tr>
<tr>
<td>Lactose monohydrate</td>
<td>Diluent</td>
<td>White to off-white, odorless, slightly sweet-tasting, crystalline powder soluble in water and practically insoluble in ethanol, ether and chloroform.</td>
<td></td>
</tr>
<tr>
<td>Calcium hydrogen phosphate dehydrate</td>
<td>Binder/Diluent</td>
<td>White, odorless, tasteless powder or crystalline solid characterized by being nonhygroscopic and practically insoluble in ethanol, ether, and water, but soluble in dilute acids.</td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td>Antiadherent</td>
<td>Purified, hydrated, magnesium silicate which may contain small, variable amounts of aluminum silicate and iron. Talc occurs as a very fine, white to grayish-white, odorless, unctuous, crystalline powder which adheres readily to the skin and is soft to the touch and free from grittiness. It is practically insoluble in water and organic solvents.</td>
<td></td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>Lubricant</td>
<td>Magnesium stearate is a very fine, light white, precipitated or milled, impalpable powder of low bulk density, having a faint odor of stearic acid and a characteristic taste. The powder is greasy to the touch and readily adheres to the skin. It is practically insoluble in ethanol, ether and water; and slightly soluble in warm ethanol.</td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>Binder</td>
<td>Gelatin is a mixture of purified protein fractions which occurs as a light-amber to faintly yellow-colored, vitreous, brittle solid. It is practically odorless and tasteless. In water, it swells and softens, gradually absorbing between five and 10 times its own weight of water. Above 40°C it is...</td>
<td></td>
</tr>
</tbody>
</table>
Cyclophosphamide Sugar-coated Tablets – Final Degree Project

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>Coating agent</td>
<td>Sweet, odorless crystalline sugar obtained from sugar cane, sugar beet, and other sources. It is hygroscopic, soluble in water, and slightly soluble in ethanol.</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>Opacifier</td>
<td>White, amorphous, odorless, and tasteless nonhygroscopic powder which is practically insoluble in water and organic solvents.</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>Bulking agent</td>
<td>Odorless tasteless white powder or crystals practically insoluble in ethanol and water.</td>
</tr>
<tr>
<td>Talc</td>
<td>Antiadherent</td>
<td>Purified, hydrated, magnesium silicate which may contain small, variable amounts of aluminum silicate and iron. Talc occurs as a very fine, white to grayish-white, odorless, unctuous, crystalline powder which adheres readily to the skin and is soft to the touch and free from grittiness. It is practically insoluble in water and organic solvents.</td>
</tr>
<tr>
<td>Macrogol 35000</td>
<td>Plasticizer/Binder</td>
<td>Free-flowing high molecular weight (HMW) polyethylene glycol powder with a faint, sweet odor which is soluble in water, ethanol, acetone, and insoluble in fats and mineral oil.</td>
</tr>
<tr>
<td>Silica colloidal anhydrous</td>
<td>Glidant</td>
<td>Light, fine, white or almost white amorphous powder, not wettable by water, which is practically insoluble in water and insoluble in organic solvents.</td>
</tr>
<tr>
<td>Povidone</td>
<td>Binder</td>
<td>Synthetic polymer consisting essentially of linear 1-vinyl-2-pyrrolidinone groups, characterized by its viscosity in aqueous solution. It occurs as a fine, white to creamy-white colored, odorless or almost odorless, hygroscopic powder. It is soluble in water, ethanol, ketone, and chloroform, and insoluble in ether and</td>
</tr>
</tbody>
</table>

Table 4. Tablet core excipients of Baxter Cyclophosphamide coated tablets, probable functionality in the pharmaceutical formulation and description.
Table 5. Coating excipients of Baxter Cyclophosphamide coated tablets, probable functionality in the pharmaceutical formulation and description.

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Functionality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium carboxymethylcellulose</td>
<td>Film-forming agent</td>
</tr>
<tr>
<td>Polysorbate 20</td>
<td>Stabilizer/Plasticizer</td>
</tr>
<tr>
<td>Montan glycol wax</td>
<td>Polishing agent</td>
</tr>
<tr>
<td>FD&amp;C Blue No. 1 (Brilliant blue FCF)</td>
<td>Coloring agent</td>
</tr>
<tr>
<td>D&amp;C Yellow No. 10 Aluminum lake (Quinoline yellow WS)</td>
<td>Coloring agent</td>
</tr>
</tbody>
</table>

From the use of glycerol in the tablet core formulation, it can be deduced that the tablets are obtained through an aqueous wet granulation process. This method of tablet manufacturing consists of a first granulation step in which the active ingredient, diluents, binders, disintegrants, and other excipients, are mixed and formulated into granules by means of water as vehicle. This is followed by the compression of the granules with the help of lubricants, glidants, and antiadherents[29].

Glycerol is a viscous, hygroscopic, water soluble liquid. Gelatin is a solid which is soluble in water only at relatively high temperatures (above 40ºC) and gels on cooling, whereas calcium hydrogen phosphate dehydrate is non-hygroscopic and has a low water solubility[45]. Consequently, glycerol might enhance the solubilization of gelatin and calcium hydrogen phosphate dehydrate in water by acting as a humectant, thus allowing these ingredients to form a binder solution that will be applied over the other excipients in the formulation. In conclusion, the use of a humectant brings in the need for an aqueous wet granulation process.
In relation to the coating process, sucrose and all the other excipients in the coating formulation indicate that the tablet nucleuses are covered by means of the sugar-coating technique, described in 4.2.3.

5.3.2. Shortcomings

As already mentioned in previous sections, cyclophosphamide physical and chemical properties can be easily compromised when the drug is exposed to certain agents and conditions. This should be taken into account not only during the distribution and storage of the drug product, but also, and very importantly, during its manufacture. Thus, the excipients used in the formulation of the tablets should be carefully chosen, as well as the manufacturing process itself.

Although Baxter’s formulation of cyclophosphamide sugar coated tablets provides good results (perfect white blue sugar-coated tablets with blue flecks are obtained), some of the excipients used might not be the most adequate in terms of drug stability. This is mainly due to the fact that they are subjected to certain manufacturing processes which require water as the principal vehicle and drying or heating steps, overlooking that cyclophosphamide is chemically labile in aqueous solution and that it is sensitive to humidity changes and high temperatures.

This is the case of gelatin, a binder that yields strong granules and tablets of intermediate hardness, providing the tablet cores with the mechanical resistance needed to overcome the subsequent coating process[46]. However, in Baxter’s formulation, gelatin must be dissolved in water at high temperatures (above 40ºC) with the help of glycerol and cooled in order that gelation can occur. This requires a wet granulation process in which water is essential, exposing cyclophosphamide labile structure to hydrolysis. In addition, gelatin needs to be heated in order to solubilize, while wet granulation encompasses a drying stage. These latter processes could be detrimental for a thermolabile drug like cyclophosphamide, which undergoes gelation when heated and has a low melting range (49.5-53ºC), as introduced in sections 4.1.2. and 4.1.3.

The use of sucrose as a coating agent by means of the sugar-coating technique is also subjected to utilizing water as coating vehicle, what, again, can result in the hydrolysis of cyclophosphamide. In addition, sugar-coating requires several cycles of drying that,
if not carried out at cool temperature, might accelerate the degradation of cyclophosphamide monohydrate. Finally, sugar is likely to react with many functional groups, undergoes inversion and hydrolyzation at high temperatures and with the presence of acids, and may attack aluminum closures[45].

5.4. A New Approach to Cyclophosphamide Coated Tablets: Design of Cyclophosphamide Sorbitol Film-coated Tablets

Given that currently commercialized cyclophosphamide coated tablets undergo a complex and tedious manufacturing process that might compromise the stability of the active ingredient, it seems reasonable to assume that the need exists for a simple preparation of an oral solid dosage form comprising cyclophosphamide that diminishes the exposure of the active ingredient to stability compromising processes. In this section, a sorbitol film-coating formulation that could cover this necessity is disclosed.

5.4.1. Formulation

<table>
<thead>
<tr>
<th>INGREDIENT</th>
<th>TABLET CORE/ COATING %</th>
<th>QUANTITY (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet core</td>
<td></td>
<td></td>
</tr>
<tr>
<td>API</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide monohydrate</td>
<td>22</td>
<td>53,45 (50 mg anhydrous)</td>
</tr>
<tr>
<td>DC vehicle (Diluent/Binder)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcrystalline cellulose (Vivapur®)</td>
<td>43</td>
<td>102,00</td>
</tr>
<tr>
<td>DC vehicle (Diluent/Binder)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anhydrous dibasic calcium phosphate (Anhydrous Encompress®)</td>
<td>31</td>
<td>75,00</td>
</tr>
<tr>
<td>Disintegrant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium starch glycolate (Explotab®)</td>
<td>2,5</td>
<td>6,00</td>
</tr>
<tr>
<td>Glidant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colloidal anhydrous silica (Aerosil®)</td>
<td>0,5</td>
<td>1,15</td>
</tr>
<tr>
<td>Lubricant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>1</td>
<td>2,40</td>
</tr>
<tr>
<td>Coating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasticizer</td>
<td>17</td>
<td>5,60</td>
</tr>
<tr>
<td>Solvent/Plasticizer</td>
<td>68</td>
<td>22,42</td>
</tr>
<tr>
<td>Film-forming agent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Povidone (Kollidon®)</td>
<td>5,2</td>
<td>1,72</td>
</tr>
<tr>
<td>Coloring agent</td>
<td>FD&amp;C Blue No. 1 (E133) Aluminum lake</td>
<td>0,09</td>
</tr>
<tr>
<td>Opacifier</td>
<td>Titanium dioxide</td>
<td>0,7</td>
</tr>
<tr>
<td>Solvent</td>
<td>Water</td>
<td>9</td>
</tr>
</tbody>
</table>

Figure 9. Formulation of cyclophosphamide sorbitol film-coated tablets.
The formulation proposed consists of sorbitol film-coated tablets comprising a tablet core obtained by direct compression and a sorbitol/glycerol coating achieved with the use of the film-coating technique. The tablet core weighs 240 mg and accounts for the major part of the dosage form, whereas the coating increases the bulk by an 11%, bringing in 30 additional mg. Accordingly, the final tablet weight is 270 mg.

Like commercially available cyclophosphamide coated tablets, this formulation contains the required dose of cyclophosphamide monohydrate equivalent to 50 mg of anhydrous cyclophosphamide. The necessary quantity of cyclophosphamide monohydrate has been calculated as follows:

\[
0.05 \text{ g CPA} \times \frac{1 \text{ mol CPA}}{261.086 \text{ g CPA}} \times \frac{1 \text{ mol CPA} \cdot \text{H}_2\text{O}}{1 \text{ mol CPA}} \times \frac{279.10 \text{ g CPA} \cdot \text{H}_2\text{O}}{1 \text{ mol CPA} \cdot \text{H}_2\text{O}} \times \frac{1000 \text{ mg}}{1 \text{ g}} = 53.45 \text{ mg CPA} \cdot \text{H}_2\text{O}
\]

Hence, the tablet core contains 53.45 mg of cyclophosphamide monohydrate in conjunction with several excipients. These are microcrystalline cellulose and anhydrous dibasic calcium phosphate, as direct compression vehicles, colloidal anhydrous silica and magnesium stearate, as glidant and lubricant, respectively, and sodium starch glycolate, as super disintegrant.

Microcrystalline cellulose is widely recognized to be one of the most common vehicles for direct compression, since it is not only free-flowing but also sufficiently cohesive to act as a binder[29]. Anhydrous dibasic calcium phosphate has been chosen because, when unmilled, has good compactation and flow properties which are ideal for direct compression[45]. In addition, the low hygroscopicity[45] of anhydrous dibasic calcium phosphate allows controlling the moisture of the preparation. Sodium starch glycolate at a low concentration is included in order to facilitate the liberation of the active ingredient.

Microcrystalline cellulose and anhydrous dibasic calcium phosphate make up an important part of the tablet core. Incorporation of these excipients in elevated quantities is essential for assuring an adequate compression and achieving proper tablet cores, since cyclophosphamide itself does not possess the cohesive strength and flowability required to undergo direct compression on its own.

On the other hand, the coating accounts for approximately 11% of the overall formulation, and is made up of povidone (film-forming agent), sorbitol (plasticizer) and glycerol (solvent/plasticizer) as the principal ingredients. Sorbitol and povidone are soluble in glycerol[45], which is used as the main coating solvent, allowing a significant reduction in the use of water. Nevertheless, given that it has a high boiling point[45], sorbitol is not expected to be fully eliminated in the drying stage. Consequently, a certain concentration of this ingredient will remain in the final tablets, thus enhancing the plasticizing action of sorbitol.

A water insoluble colorant and an opacifier (FD&C Blue No. 1 aluminum lake and titanium dioxide, respectively) are also added in the coating suspension. FD&C Blue
No. 1 aluminum lake is encountered in a very low concentration in order to provide the tablets with the characteristic light blue shade of the traditional cyclophosphamide sugar-coated tablets, while titanium dioxide increases the film coverage and helps achieve the desired final color.

The presented coated tablets are conceived to be produced by a simple, non-laborious manufacturing process. Hence, since direct compression requires fewer unit operations, only the blending of the tablet core excipients and the active ingredient will be necessary prior to compactation. Blending should be carried out in a mixer (e.g. a double-cone mixer) whereas compression must be executed by means of a tablet machine (e.g. a rotatory tablet machine). Since the active ingredient is heat sensitive, it might be recommendable to use Teflon® coated punches during compression, as they are resistant to heat.

Once the tablet cores have been obtained, the coating suspension is ready to be sprayed over the tablet bed. An Acela Cota® pan, in which the spraying nozzle is positioned within a drum consisting of perforated walls and the drying air flows through an air supplying inlet into the pan and fluidizes the core bed[34], could be suitable for carrying out the film-coating process.

5.4.2. Advantages

The presented pharmaceutical formulation has certain advantages in comparison with the commercially available product. The most relevant one is the reduction in the use of water, which is important in order to minimize the degradation of cyclophosphamide that is likely to take place when the drug is exposed to an aqueous medium.

As already seen in section 5.3.1., Baxter’s cyclophosphamide sugar-coated tablets undergo an aqueous wet granulation process and are coated by means of the traditional sugar-coating method. Wet granulation requires the use of water in order to obtain the granules that will then be compressed, while in sugar-coating, sucrose is diluted in water to form the simple syrup that will be repeatedly applied over the tablet bed. In contrast, thanks to the variation of the excipients in the formulation, the presented cyclophosphamide coated-tablets can be obtained by direct compression followed by coverage through the film-coating technique.

This significantly reduces the amount of water needed in the traditional process, since direct compression is water-free, while film-coating offers the possibility to use other solvents rather than water. Indeed, in the novel cyclophosphamide sorbitol film-coated tablets, water is reduced to approximately 9% of the overall coating suspension thanks to the use of glycerol as the main solvent. In addition, the fact that one application of the coating suspension is enough to achieve the desired coverage also minimizes exposure of the active ingredient to water.
Beyond enabling the avoidance of water, the use of direct compression eliminates the drying step required in wet granulation, while the film-coating technique diminishes the number of drying steps compared to those needed in sugar-coating. This is beneficial because temperature can lead to the melting of cyclophosphamide and to its conversion to a metastable phase, as already mentioned.

Finally, the presented formulation is much simpler, what can significantly reduce the length and complexity of the traditional sugar-coating process.
6. CONCLUSIONS

- Cyclophosphamide has been used as an antineoplastic drug in a broad range of cancers for quite a long time. This explains the fact that it is marketed under different names by different laboratories around the globe, either as a brand name drug or, most commonly, as a generic.

- Cyclophosphamide is a pro-drug with a high oral bioavailability. In addition, when administered orally, the AUC of its active metabolite is similar to that obtained with intravenous administration. Because of this, oral administration of cyclophosphamide is equivalent to intravenous administration from a pharmacokinetic point of view.

- From the reduced stability of cyclophosphamide under certain conditions, especially in aqueous solution and at high temperatures, some conclusions can be elucidated:
  
  - The existence of a commercially available oral liquid formulation of cyclophosphamide would represent a major contribution to certain patients. However, it is reasonable to assume that simple syrups will continue to be prepared extemporaneously from powder for injection due to cyclophosphamide instability in aqueous solution.
  
  - Cyclophosphamide tablets can be protected from moisture, light, temperature, oxidation, etc. by applying a coating over the tablet core. Hence, available cyclophosphamide tablets are sugar-coated.
  
  - The traditional sugar-coating method provides excellent coatings. However, a part from being tedious, time-consuming and expensive, it requires important amounts of water and several drying stages. Utilization of such processes is controversial when the active ingredient is unstable in aqueous solution, sensitive to moisture or prone to degradation when heated. Thus, sugar-coating, as well as other manufacturing processes that might compromise the stability of the active ingredient, should be replaced with procedures that avoid water and drying at high temperatures.
The proposed formulation, consisting of sorbitol film-coated tablets obtained by direct compression of the tablet cores followed by coverage with the film-coating technique, seems a good alternative for preserving cyclophosphamide stability during the manufacturing of the medication. Nevertheless, the formulation presented in this project is only a first approach to the development of cyclophosphamide sorbitol film-coated coated tablets. Consequently, several laboratory studies and quality control testing should be conducted in order to confirm the viability of the formulation and to establish a detailed manufacturing scheme that led to the optimal results.
7. REFERENCES


[23] U.S. Food and Drug Administration (FDA) [Internet]. The Biopharmaceutics Classification System (BCS) Guidance [Internet]. [Cited 2015 Apr 02]. Available from: http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ucm128219.htm


