Universidade de Lisboa

Faculdade de Farmácia

Hydrochlorothiazide Tablets formulated in a Ternary system with Cyclodextrin and Nanoclay

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Mestrado Integrado em Ciências Farmacêuticas

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Resumo

A solubilidade dos fármacos é uma propriedade essencial no desenvolvimento de novas formas farmacêuticas. Fármacos que apresentam baixa solubilidade em água, como os pertencentes às classes II e IV do *BiopharmaceuticsClassificationSystem (BCS),* exigem o desenvolvimento de estratégias que permitam melhorar a sua solubilidade para que possam ser formulados em formas farmacêuticas sólidas eficientes. Nos últimos anos, têm sido investigadas diversas estratégias das quais se destacam para o âmbito deste trabalho a complexação com ciclodextrinas (CDs) e a utilização de nano-argilas como sistemas de transporte de fármacos.

Este estudo tem como objetivo a produção de comprimidos de hidroclorotiazida (HCT), um fármaco de classe IV, utilizando um sistema ternário composto por uma CD, *Randomly Substituted Methyl-β-cyclodextrin* (RAMEβ), e uma nano-argila, Sepiolite (SV), com vista a melhorar as propriedades de dissolução do respetivo fármaco. Num primeiro passo, foram preparadas as misturas binárias HCT-RAMEβ, em vários rácios molares (1:1, 1:0,5, 1:0,25), através de duas técnicas diferentes, a Mistura Física e a Co-Evaporação, para selecionar a técnica de preparação e o rácio molar mais apropriados para a produção da mistura binária e, por conseguinte, dos comprimidos. Seguidamente, foram produzidos comprimidos constituídos pelo sistema ternário *HCT-RAMEβ-SV*, com o rácio molar de HCT-RAMEβ, anteriormente selecionado e utilizando três proporções de massa de HCT-SV (1:8, 1:4, 1:2) com vista a selecionar a proporção mais adequada para a produção de comprimidos e que simultaneamente seja eficaz na melhoria das propriedades de dissolução da HCT.

A mistura binária HCT-RAMEβ preparada por Co-Evaporação com o rácio molar de 1:1 demonstrou aumentar significativamente a dissolução da HCT, tendo sido considerada como a mais adequada para a preparação da mistura binária. Os comprimidos produzidos por Mistura Física com o rácio molar HCT-RAMEβ de 1:1 e a proporção de massa HCT-SV de 1:8 evidenciaram um aumento notável das propriedades de dissolução da HCT. Concluindo, o sistema ternário *HCT-RAMEβ-SV* representa uma tecnologia farmacêutica promissora na medida em que possibilita a melhoria da solubilidade da HCT através da conjunção dos benefícios de duas estratégias diferentes (inclusão com CD e conjugação com nano-argilas) num único sistema de administração de fármacos.

Palavras-chave: solubilidade, hidroclorotiazida, ciclodextrinas, sepiolite, comprimidos

Abstract

Solubility of drugs is an essential property in the development of new pharmaceutical forms. Drugs with low solubility in water, such as those belonging to classes II and IV of the Biopharmaceutics Classification System (BCS), require the development of strategies to improve their solubility, so that they can be formulated in efficient solid pharmaceutical forms. In recent years, several strategies have been investigated, of which the most important for the scope of this work are the complexation with cyclodextrins (CDs) and the use of nanoclays as drug delivery systems.

This study aims to produce tablets containing hydrochlorothiazide (HCT), a class IV drug, using a ternary system composed of a CD, Randomly Substituted Methyl-β-cyclodextrin (RAMEβ), and a nano-clay, Sepiolite (SV), in order to improve the dissolving properties of the respective drug. In a first step, the binary mixtures of HCT-RAMEβ were prepared with various molar ratios (1:1, 1:0,5, 1:0,25) using two different techniques, Physical Mixing and Co-Evaporation, to select the most appropriate preparation technique and molar ratio for the production of the binary mixture and, consequently, the tablets. Then, tablets composed of the ternary system HCT-RAMEβ-SV were produced with the previously selected molar ratio of HCT-RAMEβ, and using three different mass ratios of HCT-SV (1:8, 1:4, 1:2), in order to select the most suitable mass ratio for the production of tablets and, at the same time, be effective in improving the dissolution properties of HCT.

The binary mixture of HCT-RAMEβ prepared by Co-Evaporation with the molar ratio of 1:1 demonstrated to significantly increase the dissolution of HCT and was considered the most suitable for the preparation of the binary mixture. The tablets produced by Physical Mixture with the 1:1 HCT-RAMEβ molar ratio and the HCT-SV mass ratio of 1:8 showed a remarkable increase in the dissolving properties of HCT. In conclusion, the ternary system HCT-RAMEβ-SV represents a promising approach able to improve HCT solubility by combining the benefits of two different strategies (inclusion with CD and conjugation with nanoclays) in a single drug delivery system.

Keywords: solubility, hydrochlorothiazide, cyclodextrins, sepiolite, tablets

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Acronyms and Symbols

BCS - Biopharmaceutical Classification System

BM - Binary Mixtures

CD -Cyclodextrins

COE - Co-Evaporation

DE -Dissolution efficiency

FDA - Food and Drug Administration

HCT - Hydrochlorothiazide

PD - Percentage Dissolved

Ph. Eur. - European Pharmacopoeia

PM - Physical Mixture

PVP -Polyvinylpyrrolidone

RAMEβ - Randomly methylated-β-cyclodextrin

SBEβCD - Sulfobutyl ether-β-cyclodextrin

SV -Sepiolite

TB - Tablet

TM - Ternary Mixtures

1. Introduction

1.1. Oral Solid Dosage Forms

Oral solid dosage forms were created in the 19th century and since then, remain the most commonly used dosage forms in the pharmaceutical industry due to the several advantages they present [1]. From a manufacturing point of view, oral solid dosage forms require economy technology without sterile conditions, provide an accurate dosage and are usually the most stable forms of drugs [2,3]. From the therapeutic viewpoint, oral solid dosage forms are easy to administrate, allow a controlled and a systemic drug delivery without an invasive action and can be administrated by the patients themselves [1,4]. Thus, they are associated with high levels of patient compliance and are considered the primary option for drug formulation.

Considering all oral solid dosage forms, the tablets are the most used and present more advantages than others. Tablets can be produced on a large scale at low price by many different manufacturing technologies and using robust and quality-controlled production procedures [1,4]. Therefore, the tablets present a consistent quality along with a dosing precision, are chemical and microbiologically more stable and are easier to pack and storage comparing to the others [5]. Furthermore, the tablets are very versatile and can also be coated, providing many benefits such as protection of the drug from the external environment, enhancement the ease of swallowing and modification of the release rate of the drug [6,7]. For patients, these dosage forms are convenient to carry and administrate, are tamper-proof in contrast to capsules and are able to fulfill numerous therapeutic needs due to the various types of formulations [2,5].

1.2. Tablet Production

In order to prepare tablets with high quality, they must present an accurate dosage, be uniform in terms of diameter, mass and visual appearance and successfully undergo *in vitro* disintegration and dissolution tests before *in vivo* absorption in the gastrointestinal tract [2]. It is necessary to have extensive information before tablet production, either in small and large scale, to ensure its quality and effectiveness. On account of this, in the early drug development process, there are two crucial phases: pre-formulation and formulation [4].

1.2.1. Pre – formulation studies

Pre-formulation is the beginning phase of drug development process and it is based on the study of physicochemical, pharmacotechnical and biopharmaceutical properties of the drug, excipients and packaging materials [8].The main goal of this step is to streamline the process of converting a drug candidate into a drug product through the evaluation of the factors influencing development of the dosage form that could also affect the drug performance [8].

These studies are specifically focused on the physicochemical and pharmacotechnical properties of the drug and excipients with the aim of analyzing the compatibility between them before formulation phase, as seen in the Figure 1 [2].

All the data generated is later used to design a safe, effective, stable and bioavailable dosage form in formulation phase.

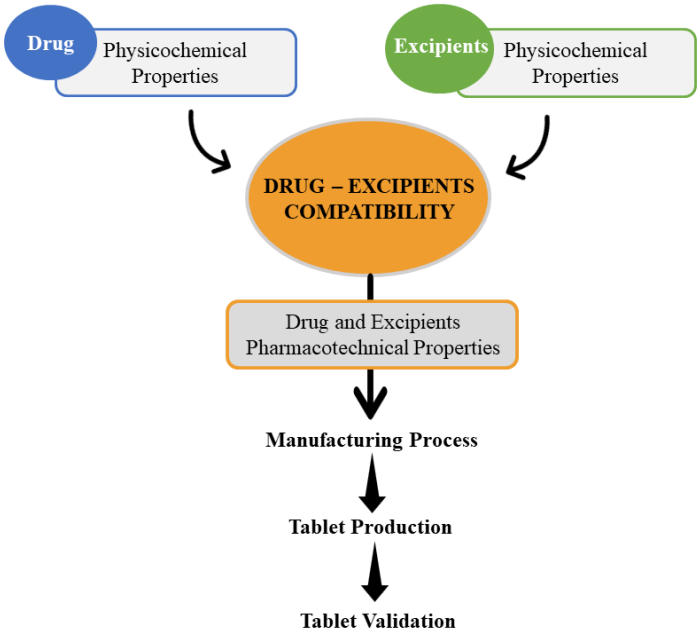

Figure 1: Pre-formulation studies for tablet production

Physicochemical and pharmacotechnical properties evaluated during pre-formulation include *organoleptic properties* as aspect, color, and odor. Others properties as crystallinity, polymorphism, hygroscopicity, particle size distribution, bulk density, powder flow and compression properties are also analyzed. *Stability* is also analyzed in solution and solid state and, finally, there is a *solubility* analysis by determination of intrinsic solubility, pKa and partition coefficient along with dissolution studies and evaluation of common ion effect [9].

1.2.2. Formulation studies

Formulation studies should consider the route of administration, planned usage, therapeutic target and posology of the drug candidate [10]. According to global *International Conference on Harmonisation (ICH)* guidelines [11], formulation studies should include an identification of the specific attributes essential to the quality of the drug substance and excipients, which should be carefully chosen in the right amount in order to produce a drug product with quality and efficacy. Manufacturing process should be appropriate and the characteristics crucial for the quality of the final product should also be pointed, in order to be evaluated and controlled during process so that the final product presents the desired quality.

After pre-formulation studies, formulation is designed with a consciously selection of excipients in combination with the drug substance. Each excipient has a specific a role in the formulation so that the final mixture presents suitable flowability, lubricity and compatibility characteristics required for the manufacturing process. These characteristics are crucial for the development of the manufacturing process in order to

produce tablets with good appearance and appropriate crushing strength, friability, disintegration and dissolution properties [12].

Regarding the type of excipients, the tablets formulation is commonly composed by a diluent, a disintegrating agent, a glidant, a binder and a lubricant [13]. The functions and examples of these excipients are listed in Table1-I:

Table 1-I:Excipients in Tablets Formulation

EXCIPIENT	FUNCTION	EXAMPLES
Diluent	Provide bulk volume of the powder and increase the size of the tablet. It is not necessary when the dose of drug substance per tablet is high	Lactose; Glucose; Silicates; Calcium phosphate; Calcium carbonate; Cellulose
Binder	Bind all the ingredients together, providing form and the required mechanical strength.	Gelatin; Polyvinyl pyrrolidone (PVP); Hydroxypropylmethyl cellulose; Polyethylene glycol; Starch; Cellulose
Disintegrating agent	Ensure that the tablet, when in contact with a liquid, breaks up into small fragments, releasing the drug substance and promoting its rapid dissolution.	Starch; Cellulose; Sodium starch glycolate
Glidant	Improve the flowability of the powder through reduction of the friction and adhesion between particles.	Silica; Magnesium Stearate; Talc
Lubricant	Guarantee that tablet formation and ejection occur with low friction between the solid and the die wall.	Magnesium stearate; Stearic acid; Polyethylene glycol; Sodium lauryl sulfate

1.2.3. Tablets Manufacturing Process

Tablets are produced by compression of particles or by another suitable manufacturing process as freeze-drying, molding or extrusion [14]. On industrial scale, whenever possible, the direct compression is the most used technology since it is the fastest, simplest and more effective method to produce tablets [5]. The process involves the compression of particles between two punches in a tablet machine. It can be performed either on uniform particles in powder or on particle aggregates previously obtained by granulation (wet or dry granulation), as shown in Figure 2 [4].

Granulation is a process of particle size enlargement in which small particles are grouped, forming permanent aggregates while maintaining the integrity of the original particles. There are two types of granulation based on the technology used to aid the agglomeration of powder particles: wet and dry granulation. Wet granulation uses an aqueous or solvent-based liquid solution, sometimes added with a binder, such as PVP, to facilitate agglomeration by creating a wet mass by adhesion, which is then dried. Dry granulation produces granules without a liquid solution since the powder particles can be sensitive to moisture and/or heat. This method uses either mechanical compression by slugging tooling in a tablet press machine or compaction by means of a roller compactor, to facilitate the agglomeration of particles, which are then milled and calibrated to form granules [15,16].

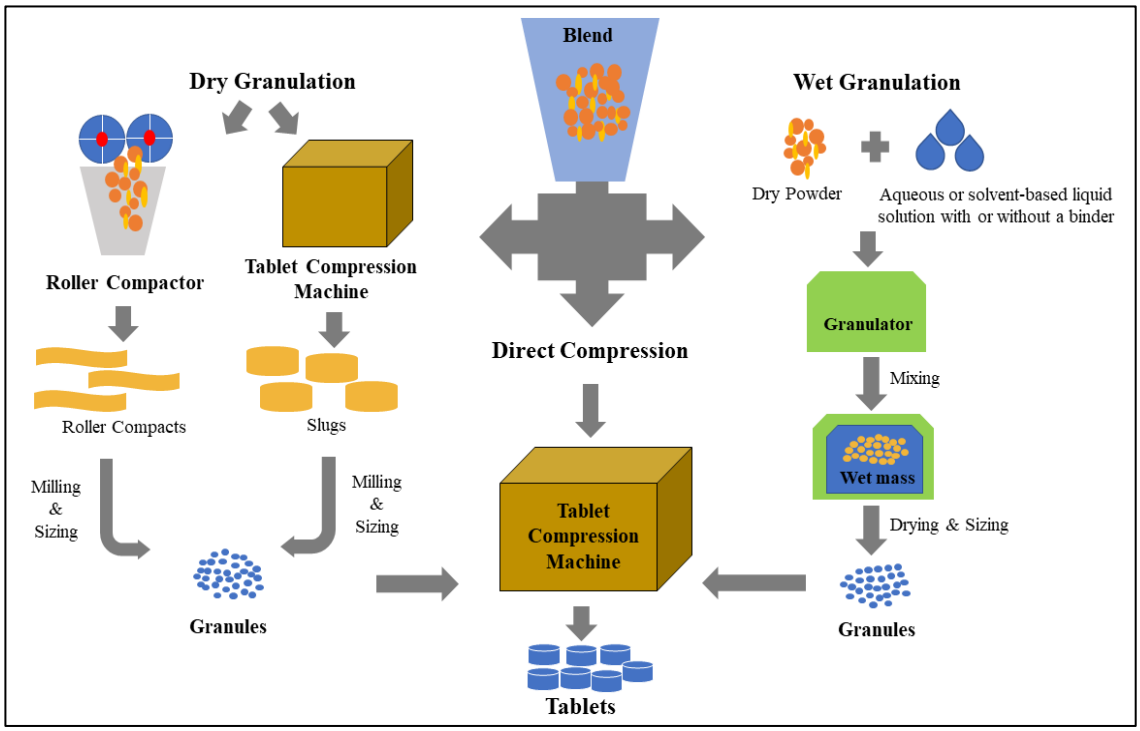

Figure 2:Schematic diagram of tablet manufacturing techniques

The main reason to perform the granulation before compression is to transform powder particles that were not suitable for direct compression into a granule that allows the process of tableting. Granulation provides an improvement in the flowability and mixing homogeneity of the powder, increasing the compressibility and the uniformity of content in the tablet. Thus, excessive amounts of fine particles are eliminated and the potential for segregation is reduced, allowing the production of tablets with fewer defects and better yields [16].

Nevertheless, granulation is a more time-consuming and expensive technology compared to direct compression and there is also a risk of product contamination and loss during the process [5].

1.3. Improvement of Drug Solubility and Permeability

Drug absorption from oral dosage forms is mostly influenced by two key parameters: solubility and permeability. Solubility is important since it is related to the drug dissolution rate and determines the speed of the drug in reaching its maximum concentration in the gastrointestinal fluid. Permeability is crucial because it is associated to the rate at which a drug crosses the intestinal wall to achieve the blood circulation [17].

Thus, determination of solubility and permeability properties of a drug provide useful information about its absorption. In addition, as a variety of drugs present low solubility or/and low permeability, strategies to improve these properties are increasingly important in new drugs development process.

1.3.1. Drug Solubility

To achieve a clinical response, a drug must be first dissolved to be then absorbed in the gastrointestinal tract [18].On account of this, solubility profile of drug candidate is the first parameter to be measured in pre-formulation phase as it determines the drug performance in reaching the therapeutic effect [4].

Nearly 40% of currently approved drugs and about 90% of the drugs candidates in development in the pharmaceutical industry present low aqueous solubility [18], [19].Consequently, these drugs have a low dissolution rate that frequently leads to inadequate bioavailability, resulting in poor clinical results [20].

1.3.1.1 Solubility Analysis

Amidon et al., (1995), created the Biopharmaceutics Classification System (BCS) to classify drugs in four classes based on their aqueous solubility and intestinal permeability, as seen in the Table 1-II [21].

Table 1-II: Biopharmaceutics Classification System (BCS)

CLASS	SOLUBILITY	PERMEABILITY
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

Class I drugs (high solubility and high permeability) are well absorbed. *Class II* drugs (low solubility and high permeability) are well absorbed too but are expected to be dissolution rate dependent due to their low solubility. *Class III* drugs (high solubility and low solubility) present a variable absorption profile limited by permeability rate although they dissolve rapidly. *Class IV* drugs(low solubility and low permeability) are poorly absorbed and bioavailability is dependent on both dissolution and permeability rate [22].

Regarding the classification system, Food and Drug Administration (FDA) considers a drug as a *highly soluble drug* when the highest dose strength is soluble in

250 ml or less of aqueous media in over the whole gastrointestinal pH range (from 1.2 to 7.4). A drug is *highly permeable* once its extension of absorption in humans is above 90% of an administrated dose, based on mass-balance or comparing to an intravenous dose reference [21].

1.3.1.2 Strategies to Improve Drug Solubility

The improvement of drug solubility remains one of the most challenging aspects of drug development process, especially for oral drug dosage forms. The need for effective formulations for BCS classes II and IV drugs led to the development of several technological strategies to overcome poor water solubility. These strategies are categorized in three different groups: physical modifications, chemical modifications and miscellaneous Methods, as shown in Figure 3.

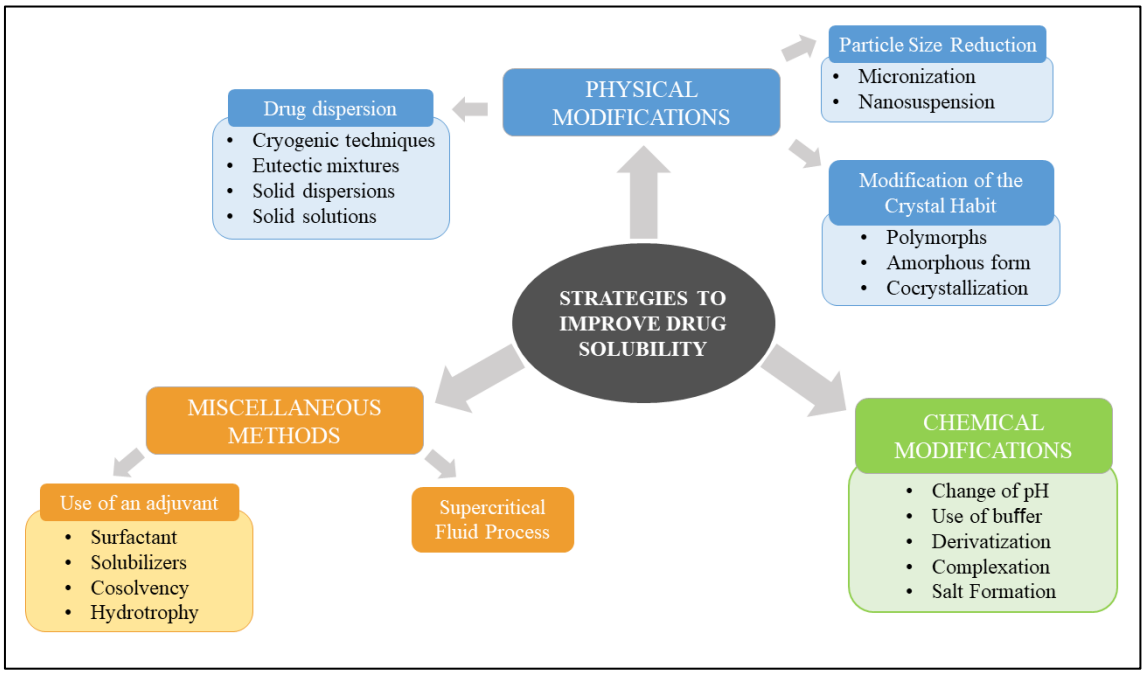

Figure 3: Strategies to Improve Drug Solubility

As for the physical modifications, they are based on the alteration of the physical characteristics of the drug to facilitate the solubilization process and increase the dissolution rate, resulting in a consequent increase in the solubility of the drug and its bioavailability [22]. Particle size reduction, like micronization and nanosuspension, is one of the oldest strategies since it is intrinsically related to the solubility of the drug. When the particle size decreases, the surface area to volume ratio increases and allows a greater interaction with the solvent, resulting in an improvement in the solubility of the drug [23]. Crystal habit modification involves several controlled processes of crystallization in drugs, such as the production of polymorphs, amorphous forms and co-crystals, in order to ease the solubilization process [20]. Drug dispersion is a molecular dispersion of a drug in a crystalline or amorphous biocompatible carrier that may be an eutectic mixture, a solid dispersion, a solid solution or a cryogenic technique [24].

About the chemical modifications, there is a group of techniques that can change the chemistry of the drug, increasing its own solubility. For ionizable drugs that exhibit a pH-dependent solubility, it is possible to alter the pH of the solution or produce a form of drug salt to increase its solubility [20]. In addition, it is also possible to use derivatization technique or use a buffer to change the conditions of the solution. On the other hand, inclusion complex technique provides a specific improvement in the aqueous solubility of poorly water-soluble drugs, since it allows the insertion of the nonpolar region of the molecule into the cavity of another molecule or group of molecules, whose surface is polar. The most commonly used molecules for inclusion complexes are cyclodextrins [23].

Regarding the miscellaneous methods, they allow an improvement in the solubility of the drug using adjuvants, which are soluble and can dissolve in water at a rapid rate, functioning as drug carriers to increase its dissolution. The most widely used adjuvants are surfactants, solubilizers, hydrotropic agents and co-solvents [25]. Another solubilization technique is the use of a supercritical fluid to recrystallize drug particles into very small sizes, increasing their solubility.

The various techniques described above, alone or in combination, can be used to increase the solubility of a drug. The selection of the technique to improve solubility depends on the properties of the drug, such as solubility, chemical nature, melting point, absorption site and physical nature, as well as the dosage form desired. Thus, the adequate selection of the solubility enhancement method is the key to ensure the purposes of an effective formulation, such as good oral bioavailability, reduced dosage frequency and patient compliance combined with low production cost.

1.3.2. Drug Permeability

In order to overcome incomplete absorption of the drug and reduced oral bioavailability, several strategies have been developed to improve the solubility of the drug, as described above. In fact, these techniques improve the solubility of drugs, but their effect on the overall absorption of drugs is not very significant and, in some cases, may remain unchanged or even decrease. Some recent research has proposed the importance of the interaction between solubility and the other fundamental parameter for greater absorption of oral dosage forms: intestinal permeability [26,27]. The development of strategies to also improve the permeability of drugs is mandatory so that the absorption of the oral dosage form can be completed with increased oral bioavailability.

1.3.2.1 Strategies to Improve Drug Permeability

BCS Class III and IV drugs have low absorption due to their low permeability, so it is necessary to increase it, as it is a rate-limiting phase in achieving appropriate bioavailability and consequent clinical efficacy. In recent years, several strategies have been widely studied to improve drug permeability, with the aim of increasing the oral bioavailability of poorly absorbed drugs. These strategies include traditional methods

such as prodrugs, permeation enhancers and ion pairing, as well as relatively modern methods such as nanotechnology-based techniques [28].

1.4. Cyclodextrins

Cyclodextrins (CDs) are a family of cyclic oligosaccharides obtained from the enzymatic reaction on starch or starch derivatives using CD glycosyltransferase. Chemically, CDs are composed of α-D-glucopyranose units linked by α-1,4 bonds in chair conformation, thus forming a truncated or doughnut-shaped cone with a central cavity. The hydroxyl functions of the glucopyranose units are oriented towards the outside of the cavity, with the primary and secondary hydroxyl groups located at the narrowest and widest edge, respectively, giving a hydrophilic character to the outer surface that provides solubility in water. In contrast, the inner cavity of CD is coated with hydrogen atoms and glycosidic oxygen brigdes, exhibiting a relatively hydrophobic character [29]. CD molecules are widely used in pharmaceutical formulations due to their ability to form inclusion complexes with drugs, consequently modifying crucial drug properties such as dissolution rate and bioavailability, among others [30].

1.4.1. Different types of cyclodextrins

There are three natural CDs depending on the number of glucopyranose units: α-CD, β-CD and γ-CD with six, seven and eight glucopyranose units, respectively. Their chemical structure, cavity diameter and height of the truncated-shaped cone are shown in Figure 4 [31].

Natural CDs exhibit limited water solubility due to the presence of strong intramolecular hydrogen bonds between secondary hydroxyl groups, which decrease their ability to interact with surrounding water molecules [32]. Thus, in recent years, several CD derivatives have been produced to convert natural CDs into amorphous and non-crystallizable derivatives in order to obtain high concentrations of CD, either in free form or as an inclusion complex, in aqueous solutions that remain physically and microbiologically stable for a reasonable period of time. Thus, CD derivatives provide an improvement in the properties of the three natural CDs, such as solubility, stability and inclusion capacity [33].

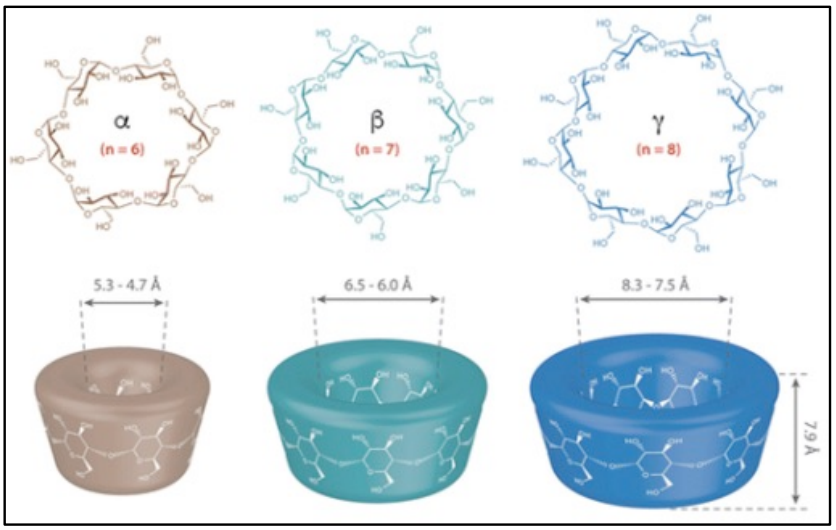

Figure 4: Strategies to Improve Drug Solubility[31]

CD derivatives can be classified according to their structure, depending on their substitutes, polarity and size. Thus, CD derivatives can be divided into three different groups: *hydrophobic*, as Acylated β-Cyclodextrin, *hydrophilic*, for example Randomly methylated-β-cyclodextrin (RAMEB) and *ionizable*, for instance Sulfobutyl ether-β-cyclodextrin (SBEβCD).Regarding the hydrophilic CD derivatives, the relevant ones for the pharmaceutical industry are methylated derivatives of β-CD, 2-hydroxypropylated β-CDs, sulfobutylated-β-CDs, branched CDs (glucosyl and maltosyl-β-CDs) and acetylated β-CDs [33]. In addition, the 2-hydroxypropylated-βCD and sulfobutylated-β-CD are specifically used to improve the solubility and dissolution rate of poorly water-soluble drugs [34].

1.4.2. Applications in the pharmaceutical industry

CDs are widely used in pharmaceutical industry as host molecules since they have the capacity to entrap in their cavity a variety of hydrophobic guest molecules of suitable size, leading to the formation of inclusion complexes. Hence, the CDs main application in the pharmaceutical industry is to increase the dissolution and bioavailability of poorly water-soluble drugs belonging to the BCS Class II and IV. Nevertheless, CDs can also have other applications such as:

1) Enhancing the drug physicochemical stability and increasing shelf-lives of medical products;
2) Modifying the release of the drug;
3) Reducing of preventing adverse drug reactions such as dermal, gastrointestinal and ocular irritation;
4) Preventing drug-drug or drug-excipients interactions;
5) Reducing or eliminating unpleasant taste or smell;
6) Converting oil and liquid drugs into microcrystalline or amorphous powders;
7) Protecting drugs from light, thermal and oxidative stress [20,30].

1.4.3. Characterization of the complex drug - cyclodextrin

CDs can act as “host molecules” by establishing specific interactions with several types of molecules by forming non-covalently bonded entities, either in the solid phase or in aqueous solution. In an aqueous solution, the relatively hydrophobic CD cavity is occupied by water molecules of high enthalpy. These water molecules can be quickly substituted by appropriate “guest molecules” that are less polar than them, such as poorly water-soluble drugs. Then, an inclusion complex between CD and the drug is formed, usually with a ratio of 1:1 [34]. Furthermore, the hydroxyl groups present on the external surface of the CD molecule allow the possibility of building hydrogen bonds with the drug molecules, resulting in the formation of non-inclusion complexes as well [35].

The drug-CD inclusion complexes are present in solution in dynamic equilibrium with free drug and CD molecules and are characterized by a well determined host:guest stoichiometry. Rates of formation and dissociation of drug-CD complexes are very near to the boundaries of controlled diffusion and they are being continuously formed and dissociated [36]. The drug molecules are then quickly released from the inclusion complex after media dilution or by competitive complexation so that they can be absorbed through the mucosa into the general bloodstream [37].

Drug-CD complexes have tendency to self-assemble in aqueous solutions to form aggregates. Moreover, at high concentrations of CD, these aggregates can become large and precipitated as solid microparticles. Natural CDs and their complexes have limited solubility in aqueous solutions, so that, based on the solubility dependence of the CD concentration, the formation of inclusion is represented by the phase solubility diagrams shown in Figure 5.

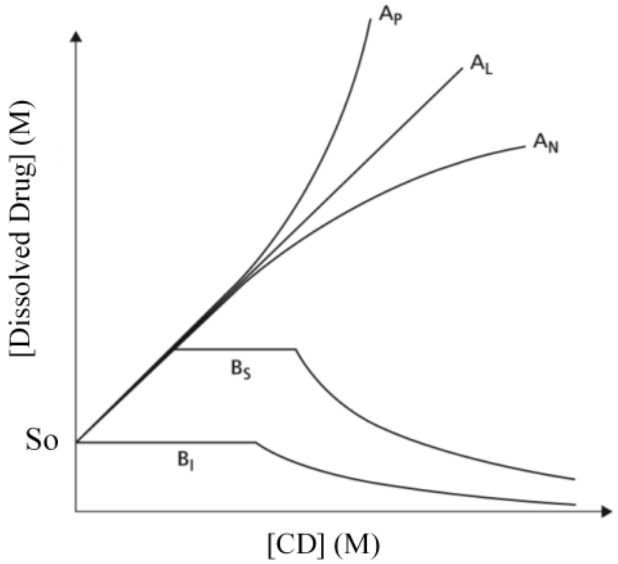

Figure 5:Types of phase-solubility diagrams according to Higuchi & Connors classification [36]*(So: intrinsic solubility of the drug in the aqueous complexation medium, AL: linear increase, AP: positive deviation from linearity, AN: negative deviation from linear*

A-type diagrams are formed when the drug-CD complex is soluble in the aqueous complexation media and are often associated with water-soluble CD

derivatives. B-type diagrams are observed when the complex has limited solubility in the media, and these are generally associated with natural CDs that have limited solubility in aqueous media [37].

In type A phase diagrams, the complex solubility increases with CD concentration and it is further classified according to the ratio of drug to CD in *AL*, a linear increase, *AP,* a positive deviation from linearity and *AN*, a negative deviation from linearity. In AL type diagrams, the total drug solubility increases as a function of the concentration of CD by the formation of soluble drug-CD complexes, indicating that the ratio between drug and CD is first order in relation to both, 1:1. The *AP* type diagram proposes the formation of higher order complexes in relation to CD, for example, the formation of drug-CD complexes with the ratio 1:2. In contrast, the *AN* type diagram suggests that the complex formed is probably second, or higher order, relative to the drug, but first-order relative to CD, for example, formation of drug-CD complexes with the ratio 2:1 [36].

In type B phase diagrams, the complex precipitates at a critical CD concentration and it is classified into *BS,* the complex has some but limited solubility, and *BI,* the complex is insoluble. In the plateau region of these diagrams, the solubility of the drug is constant even though the CD concentration is enlarged, demonstrating the formation of drug-CD complexes with limited solubility and that the solubility of CD is depressed by the presence of the drug. In the *BS* type phase diagram, the solubility of the drug increases first, followed by the plateau region, and then the total solubility of the drug (intrinsic drug solubility, S_o, plus the drug solubility in the form of CD-complexes) decreases in the presence of higher concentrations of CD due to the completion of the free drug in the aqueous complexation medium. *BI* type diagram is similar to the *BS* type but the drug–CD complexes formed are in soluble in the aqueous complexation media [35,37].

CD inclusion complexes can be prepared by various methods such as co-precipitation, kneading, damp mixing and co-evaporation, extrusion and dry mixing. The choice of the method of preparing the complex is crucial since it determines the extent of complexation and amorphicity [35].

1.5. Nanoclays

Clay minerals are a class of phyllosilicates composed by hydrous-layer mineral silicates of aluminum, sometimes containing magnesium and iron particles of reduced size. They are formed in geological environment as a result of the action of atmospheric agents on other silicate minerals on the Earth's surface, leading to a layer-type aluminosilicates [38,39].

Clay minerals present versatile properties such as ion exchange capacity, high adsorption capacity, large surface area to volume ratios, colloid and thixotropy, chemical inertness swelling property and low toxicity for oral administration [38]. Due to their unique properties and abundance, bio and eco-compatibility, low cost and

natural availability, clay minerals have several applications in many different fields, such as medicine, veterinary science, pharmaceuticals and cosmetics [40].

Nanoclays are clay minerals whose particles have at least one dimension in the nanoscale range, between 1 and 100 nm. They can be obtained by the ion exchange reaction of a clay with an organic cation, for example an alkyl ammonium or phosphonium ion. This ion exchange reaction changes the surface properties of clay minerals from hydrophilic to hydrophobic or organophilic in nanoclays and enables the movement of organic cations between their structure [41].

1.5.1. Structural characteristics

The physical and chemical properties of a specific nanoclay depend on its structure and composition. The basic structural unit of a nanoclay is a layer composed of a tetrahedral sheet and an octahedral sheet, as it can be seen in Figure 6 [39].

Both are oxide sheets, so that the *tetrahedral sheet* is composed of central atoms, often silica (Si^{4+}) and occasionally aluminum (Al^{3+}) coordinated with four oxygen (O^{2-}) atoms, and the *octahedral sheet* is formed of central cations, usually aluminum (Al^{3+}) and sometimes magnesium (Mg^{2+}) or iron (Fe^{2+}), coordinated with six oxygen (O^{2-})or bridging hydroxyl (OH^{-}) [42].

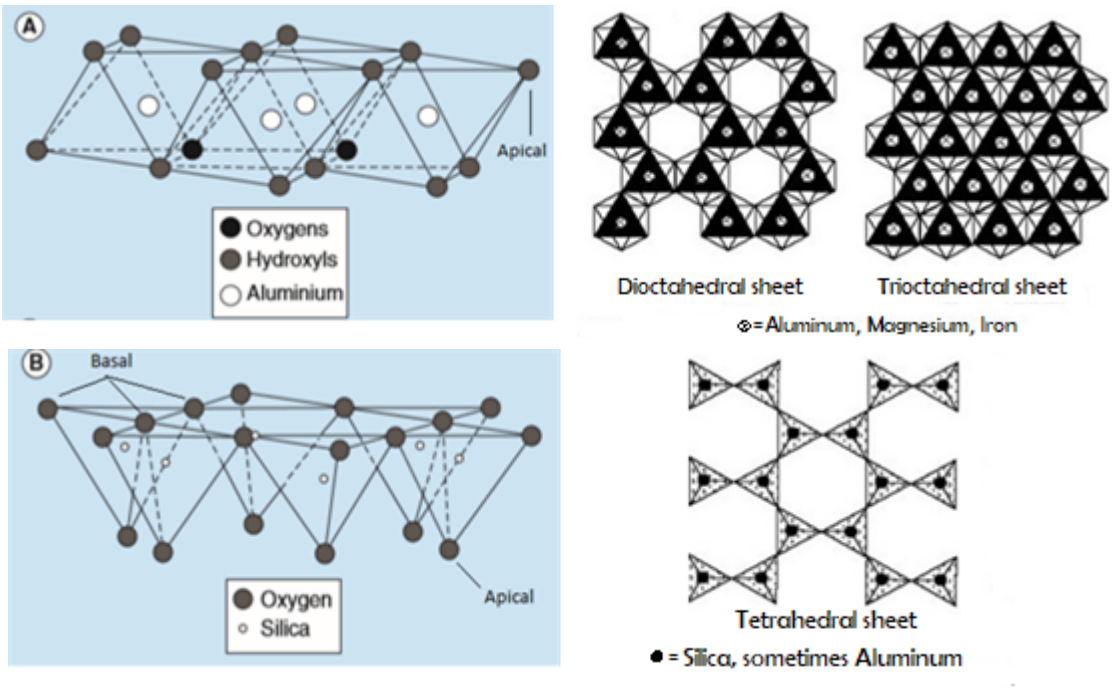

Figure 6:Nanoclay basic structure. A – Octahedral sheet B - Tetrahedral sheet

The tetrahedral and octahedral sheets are formed by sharing basal oxygens or hydroxyls, to form a hexagonal network infinitely repeated in two horizontal directions, as can be seen in figure 6.Clay minerals are made up of several layers formed by the aggregation of tetrahedral and octahedral sheets which are joined by sharing the apical oxygens or hydroxyls. Many clay minerals present cationic isomorphic substitutions

(replacement of cations of tetrahedral and/or octahedral units by others of approximate diameter) which allows maintaining the same dimensions but a smaller charge. The main substitutions are silica (Si^{4+}) by aluminum (Al^{3+}) in the tetrahedral sheets and aluminum (Al^{3+}) by iron (Fe^{2+}) or magnesium (Mg^{2+}) in the octahedral sheets [43].

Clay minerals are classified by type of layer, according to the ratio between the number of tetrahedral and octahedral sheets that were combined and divided into groups according to the types of cationic isomorphic substitution that occurred, which gives a charge per formula unit. In addition, clay minerals are divided into subgroups, differentiated by the composition of the octahedral sheets according to the present cation. When aluminum (Al^{3+}) is present, only two-thirds of the possible positions are filled in order to balance the charges and the clay mineral belongs to the *dioctahedral* subgroup (Figure 6A). On the other hand, when magnesium (Mg^{2+}) or iron (Fe^{2+}) are present, all three positions are filled to balance the charges, so that the clay mineral belongs to the *trioctahedral* subgroup (Figure 6B). The classification of clay minerals by layer type, charge per formula unit, group, subgroup and some examples of species is presented Table 1-III [39].

Table 1-III:Classification of Clay Minerals

LAYER TYPE	CHARGE PER FORMULA UNIT	GROUP	SUBGROUP	SPECIES (EXAMPLES)
1:1	0	Kaolinite - serpentine	Trioctahedral Dioctahedral Ditrioctahedral	Chrysotile, Kaolinite, Dickite, Halloysite
2:1	0	Pyrophyllite - talc	Trioctahedral Dioctahedral	Pyrophyllite, Talc
2:1	0.5 – 1.2	Smectite	Trioctahedral Dioctahedral	Saponite, Montmorrillonite
2:1	1.2-1.8	Vermiculite	Trioctahedral Dioctahedral	Dioctaheral and Vermiculite, Trioctahedral and Vermiculite
2:1	< 2	Mica/Illite	Trioctahedral Dioctahedral Trioctahedral	Muscovite, Biotite
2:1:1	1.1 - 3	Chlorite	Trioctahedral Dioctahedral Ditrioctahedral	Donbassite, Clinochlore, Cookeite
Similar to 2:1	Variable	Palygorskite - sepiolite	Trioctahedral Dioctahedral	Sepiolite, Palygorskite

For example, clay minerals belonging to the *kaolins and serpentines* group and those belonging to the *talc and pyrophyllite* group, have few or zero isomorphic substitution in their layers, therefore low or no charge per unit and, so that, their layers are only held together by weak electrostatic forces. The rest of clay minerals have isomorphic substitutions in their layers, so they have a permanent charge per formula unit which is balanced by exchangeable inorganic cations, like sodium (Na^{+}) and calcium (Ca^{2+}), occupying interlayer sites [42].

1.5.2. Applications in pharmaceutical technology

In the pharmaceutical industry, nanoclays are widely used in drug products both as excipients and active agents. As excipients, nanoclays can improve organoleptic properties of drugs, such as taste and color, enhance their physicochemical properties, for example viscosity, acting as emulsifying agents, and also ease their preparation and conservation by acting respectively as lubricants and opacifiers. As active agents, palygorskite, sepiolite and smectites act like antacids since they can neutralize protons in gastric acid along with the release of non-toxic ions (for example Mg^{2+} and Al^{3+}), thus reducing the gastric acidity. In addition, nanoclays such as kaolin, palygorskite, sepiolite and smectites are also used as gastrointestinal protectors due to their high specific area and adsorption capacity which allows adhesion to the gastric and intestinal mucosa and an increase in the thickness of the barrier, thus reducing gastric secretion and irritation. Other uses of nanoclays as active agents are related to their pharmaceutical activity as antidiarrheals, laxatives, dermatological protectors, anti-inflammatories and local anaesthetics [38,39].

In recent years, nanoclays have been used in the development of new drug delivery systems. Because of their biocompatibility and unique properties such as high specific area, high entrapment power, chemical inertness and negligible toxicity, nanoclays can facilitate the release of the drug substance in the specific therapeutic target [39]. They can interact with drug molecules and improve their dissolution rate, modify their release and increase their stability by protecting them against chemical and enzymatic degradation. Therefore, nanoclays can be used in drug products in order to provide targeting release and simultaneously reduce side effects and enhance the product shelf-life [44].

1.5.3. Interaction mechanisms drug – nanoclay

The clay–drug complexes are frequently prepared by mixing, in an appropriate volume ratio, an aqueous dispersion of clay with a solution (water/organic solvent) of drug. The resulting mixture can be equilibrated for a suitable time under favorable pH conditions, and then the solid phase is recovered via filtration. It is then washed several times with the appropriate solvent to ensure the removal of the physically adsorbed drug and finally dried under vacuum [44]. The preparation of complexes can be also done by entrapping bioactive molecules through induction of the coagulation into a clay dispersion, which has been experienced with montmorillonite [45]. Dry procedures have been reported as well, involving grinding of the drug and the clay together or placing them in contact at the melting temperature of the drug. These procedures are particularly useful for poorly water-soluble drugs belonging to BCS classes II and IV [46,47].

To form complexes, clay minerals can interact with drug molecules by several different mechanisms, depending on the characteristics of the clay mineral involved, as well as the functional groups and physical-chemical properties of drug molecules.

Therefore, the interactions between clay and drug molecules can be through hydrophobic interactions (van der Walls), hydrogen bonding, protonation, cation or ligand exchange and cation or water bridging [47].

1.5.4. The role of clay minerals in drug delivery systems

In order to maximize therapeutic activity, it is necessary to achieve and maintain the therapeutic plasma concentrations of the drug. Thus, the continuous development of new controlled drug delivery systems is driven by the need to control the drug release while improving its efficacy, specificity, tolerability and minimizing negative side effects and toxicity. Clay minerals play a crucial role in drug delivery systems, specifically, in modified drug delivery systems which includes delayed release, extended release, site-specific targeting and receptor targeting [48].

Table 4-XII, in Annexes III, summarizes some studies on the use of nanoclays as drug delivery systems and the respective mechanisms of interaction between drugs and nanoclays [49].

1.6. Brief description of the raw materials

1.6.1. Hydrochlorothiazide

Hydrochlorothiazide (HCT), (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide 1,1-dioxide) represented in Figure 7 is a thiazide diuretic first discovered in 1958 [50,51]. It's a drug extensively used in the treatment of hypertension and is also prescribed to treat edema secondary to heart failure or associated with renal or hepatic dysfunctions [50].

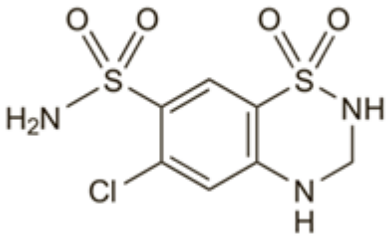

Figure 7: Chemical Structure of HCT [50]

HCT is a white or almost white, crystalline powder [52]. Chemically, HCT is slightly soluble in water (722 mg/L at 25 ºC) [53], sparingly soluble in ethanol (96 per cent), soluble in acetone, dilute ammonia, sodium hydroxide solution, *n*-butylamine, dimethylformamide and dissolves in dilute solutions of alkali hydroxides [14]. Its melting point is between 273 and 275 ºC [54] and it shows polymorphism [14]. Stability problems in aqueous solutions, such as hydrolysis, have also been reported [55,56]. HCT can be determined by ultraviolet spectrophotometry and its maxima absorption (λmax) is at 273 nm and 323 nm [14].

Additionally, HCT is not well absorbed across the intestinal mucosa [57]. Thus, due to its low solubility and also its low permeability, HCT is ranked as class IV according to the Biopharmaceutical Classification System (BCS) [21] presenting low and variable bioavailability, about 65 to 70% [50]. Therefore, HCT is difficult to formulate since its efficacy is compromised and is uncertain that it reaches the therapeutic target [57].

In order to overcome solubility and permeability problems of HCT, proper formulation is crucial to produce a suitable therapeutic effective drug [57]. Development of strategies to increase HCT bioavailability and optimize its delivery to the specific target are essential, since it prevents the use of high doses to obtain an appropriate drug exposure and, subsequently, enables a reduction of dose-related adverse effects [58].

1.6.2. RAMEβ

RAMEβ is a methylated CD approved as excipients for human pharmaceutical product that can be economically produced with constant quality. Due to the presence of the methyl substituents, this CD has a significantly higher water solubility compared to the parent β-CD, although it decreases with the rise of the temperature [33]. Solubility in organic solvents is also increased and the binding capacities to the "guest molecules" are improved, especially to poorly water-soluble drugs. In addition, RAMEβ presents reasonable stability in alkaline medium but is hydrolyzed by strong acids [59].

RAMEβ was selected for this study since it demonstrated high efficacy in improving drug solubility and dissolution properties in previous studies [60].

1.6.3. Sepiolite

Sepiolite (SV) represented in Figure 8 ($(OH_2)_4(OH)_4Mg_8Si_{12}O_{30}\cdot 8H_2O$) is a naturally occurring fibrous clay mineral in an elongate chain structure constituted by double silica tetrahedral chains linked by octahedral oxygen and hydroxyl groups containing aluminum and magnesium ions. Its layer type is similar to 2:1 but with inverted and discontinuous silica sheets, so that, it presents microporous channels running parallel to the length of the fibers [38]. These channels provide a higher internal surface area comparing to other clay minerals and may also present exchangeable cations [61].

SV contains two types of water: one coordinated to the octahedral cations and other which is loosely bonded in the channels named is termed zeolitic water. When the surface area is heated, zeolitic water is driven off and thus the chemical compounds with the proper size will fit into them and be readily absorbed.

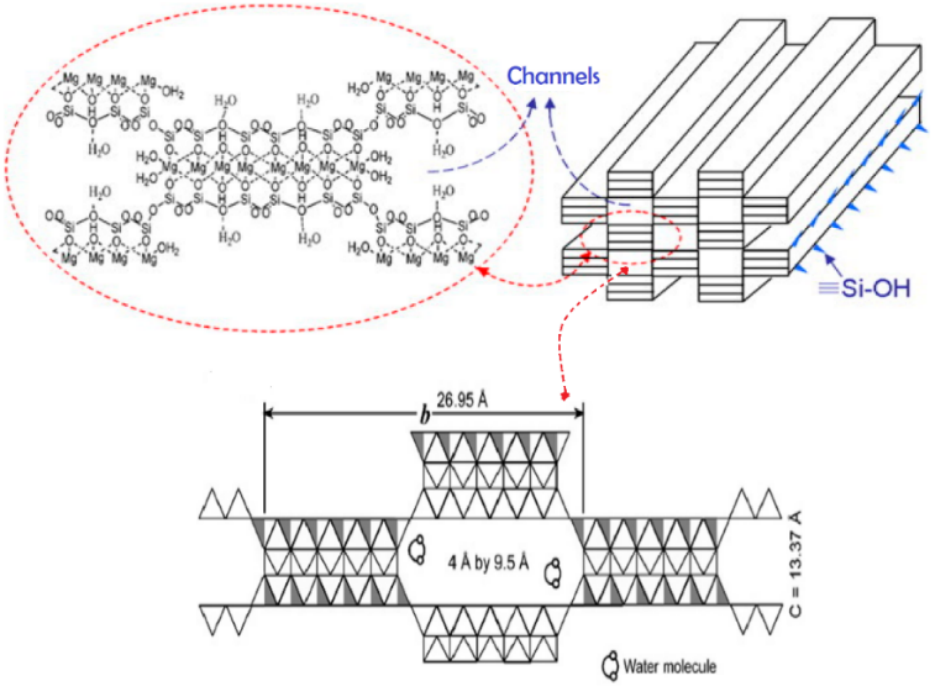

Figure 8: Structure of SV

Due to its fine particle size, high surface area (190 m^2/g), exchange capacity and presence of silanol (SiOH) groups on the external surface, SV has a high capacity to entrap drug molecules in their channels via cation exchange and hydrogen bounding [60]. Furthermore, its elongate shape increases the resistance to high concentrations of electrolytes and produces high viscosity when added to any liquid.

SV is a fine powder with a light brown colour. Its surface can be modified by heating and acid treatment and its maximum surface area is reached after heating to 150ºC, corresponding to 10% loss of water in the channels, while between 200 and 400ºC there is a reduction of the surface area due to destructive processes on the channels [62]. It has high flowability and it is a excipient suitable for direct compression, even at low doses [62,63].Because of its high adsorbing capacity and its large surface area, SV has also been used as a gastric protector [64]. Regarding its toxicity, the *International Agency for Research on Cancer(IARC)* reports that there is little data about *in vivo* and *in vitro* biocompatibility and carcinogenicity of SV, so that it is classified in *Group 3: The agent is not classifiable as to its carcinogenicity to humans* [65].

The SV was selected for this study due to its high drug adsorption capacity in relation to other clay minerals and the benefits of its complexation with CD, as demonstrated in previous studies [60].

1.6.4. Starch

Starch or classic starch is a complex naturally occurring material formed mainly by two polymers, amylose, which is linear and amorphous, and amylopectin, which is

branched and semi-crystalline [66]. It is generally cohesive and has poor flow characteristics which can be improved with drying. It is practically insoluble in cold ethanol (96%) and water, but swells instantly in water by about 5 to 10% at 38ºC [66].

Sodium starch glycolate is a tasteless, odorless and white or almost white free flowing hygroscopic powder. Due to its hygroscopicity, it must be closed and stored in such a way as to avoid caking caused by variations in temperature and humidity. It does not melt but burns at about 200°C and is classified as a non-toxic and non-irritating material. Acting primarily as a disintegrating agent, the efficacy of sodium starch glycolate is affected by the degree of cross-linking, carboxymethyl extension and purity. The tablets prepared with this excipient have good storage properties [66].

1.6.5. Polyvinylpyrrolidone

Polyvinylpyrrolidone (PVP) is a soluble, non-toxic, colourless, pH-stable, temperature-resistant binder powder that is chemically inert and physiologically compatible. This excipient is soluble in hydrophilic and hydrophobic solvents and can form water-soluble complexes with drugs poorly soluble in water, improving their solubility and dissolution rate. The K-value of PVP corresponds to the average molar mass and the PVP K25, PVP K30 and PVP K90 are widely used as binders in the formulation of tablets. The binder effect of PVP K90 is considerably greater, so that its concentration in tablets varies from 1 to 3%, while PVP K30 is between 2 to 5% of the concentration of the tablet [67].

1.6.6. Magnesium Stearate

Magnesium stearate is light white, very fine, precipitated or ground impalpable powder of low bulk density. It has a weak stearic acid odor, a distinct taste, poor flow properties and is classified as a cohesive powder. This non-toxic hydrophobic powder is widely used as a lubricant in oral solid dosage forms, but in the lowest possible concentration because it may delay the dissolution of the drug due to its hydrophobicity. Magnesium stearate is practically insoluble in ethanol (70 and 96%), ether and water and its melting temperature is between 117 and 150ºC [66].

1.7. Aim of the study

The aim of this study is to develop tablets composed by a ternary system *HCT - RAMEβSV* in order to improve the dissolution properties of HCT. The use of both, RAMEβ and SV, aims to unite and enhance the benefits of individual carriers in a single drug delivery system, reducing the amount and possible undesirable effects of each of them.

In order to achieve the main objective, the following steps will be taken:

1) Characterization and selection of the best preparation technique for the HCT-RAMEβ Binary Mixture (BM)

2) Selection of the best HCT-RAMEβ molar ratio for the preparation of HCT–RAMEβ BM
3) Selection of the best HCT-SV mass ratio suitable for the formulation of tablets
4) Characterization of the prepared tablets.

2. Experimental Procedure

2.1. Materials

Hydrochlorothiazide (Mw=297.728 g/mol) was kindly supplied by Menarini (L'Aquila), RAMEβ (Mw=1310 mol/g), average MS=1.8, was provided by Wacker-Chemie GmbH (Munich, Germany) and Sepiolite was from Vicalvaro (Spain). Magnesium (Mg^{2+}) Stearate was supplied by Sigma-Aldrich Chemistry (Switzerland), Sodium (Na^{+}) Starch Glycolate (EXPLOTAB) and Classic Starch were gently offered by Menarini and PVPK30 and PVPK90 were provided by Fluka AG (Switzerland). Ethanol 96%, distilled water.

Buffer solutions at different pH were prepared as described below.

- pH 1.2: 0.35g of Sodium Chloride (NaCl), 0.5g Glycine and 80mL of Hydrochloric acid (HCl) 1N; add distilled water up to 1L.
- pH 3.3: 24.087 of Sodium Citrate, 3.471g of Citric Acid and 800mL of distilled water. Adjustment of the pH with HCl 1N and add distilled water up to 1L.
- pH 4.5: prepared as described in 0.05M Phosphate buffer solution pH 4.5, according to Ph. Eur [14].

2.2. Methods

2.2.1. Preparation of Formulations

A) Binary Mixtures

A.1) Physical Mixture

The components of the physical mixture (PM) were weighed on the Mettler AE 166 digital laboratory scaleaccording to the molar ratio between HCT and RAMEβ, as shown in Table 2-I. Then, the components were manually mixed with a spatula in a porcelain mortar for 15 min, in order to obtain a homogeneous mixture of powders.

Table 2-I:Binary Mixtures prepared by PM (mg)

Formulation	HCT	RAMEβ
$BM_{PM}1$ (1:1)	500	2199.49
$BM_{PM}2$ (1:0.5)	500	1099.75
$BM_{PM}3$ (1:0.25)	500	549.87

A.2) Co-Evaporation

The components of the Co-Evaporation (COE) were weighted on the Mettler AE 166 digital laboratory scale according to the molar ratio between HCT and RAMEβ, as shown in Table 2-II. Then, HCT was solubilized in 83.5 mL ethanol and RAMEβ was solubilized in 18.5 mL water, both under magnetic stirring. Subsequently, the aqueous solution of RAMEβ was carefully added to the alcoholic solution of HCT with a Pasteur pipette and under magnetic stirring in order to avoid the formation of precipitates. After this step, the final solution was transferred to a round bottom flask with a ground neck. The solvent was then removed by vacuum evaporation in a rotavapor, with heating set at around 78 ºC (ethanol boiling point) and rotation speed of 200 rpm for approximately 1h. The product obtained was stored in an oven with 40 ºC for 12h in order to remove any solvent residues.

Table 2-II:Binary Mixtures prepared by COE (mg)

Formulation	HCT	RAMEβ
$BM_{COE}1$ (1:1)	500	2199.49
$BM_{COE}2$ (1:0.5)	500	1099.75
$BM_{COE}3$ (1:0.25)	500	549.87

B) Ternary Mixtures

Ternary mixtures (TM) were prepared by PM and COE methods. The BM (HCT: RAMEβ) powders with molar ratio 1:1 ($BM_{PM}1$ and $BM_{COE}1$) were selected for the preparation of the TM. The amounts (mg) to produce one tablet from TM powders are presented in Table 2-III, 2-IV and 2-V.

B.1) Physical Mixtures

PM was performed as described in A.1) and $BM_{PM}1$ obtained was added to SV followed by manual mixing for another 15 min. TM to produce one tablet was prepared with the $BM_{PM}1$ (Table 2-I) and SV powder in the mass ratio between HCT and SV, as shown in Table 2-III.

Table 2-III:Ternary Mixtures per tablet prepared by PM (mg)

Formulation	$BM_{PM}1$	SV
$TM_{PM}1$ (1:8)	135	200
$TM_{PM}2$ (1:4)	135	100
$TM_{PM}3$ (1:2)	135	50

B.2) Co-evaporation

- COE-I – The SV powder was added to the $BM_{COE}1$ obtained in A.2) following by manual mixing as described in A.1). TM to produce one tablet was prepared with the BM previously prepared ($BM_{COE}1$) and SV powder in the mass ratio between HCT and SV, as shown in Table 2-IV. $TM_{COE\text{-}I}$ with mass ratio 1:2 was not prepared.

Table 2-IV:Ternary Mixtures per tablet prepared by COE-I (mg)

Formulation	$BM_{COE}1$	SV
TM_{COE-I} (1:8)	135	200
TM_{COE-I} (1:4)	135	100

- COE-II – The SV powder was added to the other components of BM (HCT ethanolic solution and RAMEβ aqueous solution). HCT was solubilized in 4,175 mL of ethanol 96% as described in A.2) and RAMEβ was solubilized in 0,925 mL of distilled water as described in A.2). The final solutions obtained were transferred to the round bottom flask with a ground neck as described in A.2). After this step, the SV powder was added to the round bottom flask with a ground neck. The solvent was then removed by vacuum evaporation in a rotavapor as described in A.2) and the product obtained was stored as described in A.2). The TM components by tablet were weighted according to Table 2-V with SV powder in the mass ratio between HCT and SV. TM_{COE-II} with mass ratio 1:2 was not prepared.

Table 2-V:Ternary Mixtures prepared by COE-II (mg)

Formulation	HCT	RAMEβ	SV
TM_{COE-II} (1:8)	25	110	200
TM_{COE-II} (1:4)	25	110	100

2.2.1.1 Characterization of Binary Mixtures

The products obtained from PM and COE methods were classified according to their organoleptic characteristics, solubility and dissolution.

- Organoleptic characteristics

The powders obtained were characterized in terms of their appearance (homogeneous or heterogeneous), colour and smell.

- Evaluation of solubility

HCT and the different inclusion complexes solubilities were evaluated in a water bath at two different temperatures, 25ºC and 37ºC, and under magnetic stirring. Each sample (n = 2) was introduced into a 10 mL Erlenmeyer bottle in order to always have 50 mg of HCT, regardless of the type of preparation (HCT alone, PM or COE). Then, in each flask, 10 mL of a buffer solution with a certain pH (pH 1.2, 3.3 or 4.5) was introduced together with a cork stopper wrapped in parafilm. The flasks were placed in the bath for 24h. Then, 3 mL of each sample were withdrawn and filtered through Milliporea membrane nitrocellulose filter (pore size of 0,45 μm) in order to obtain clear solutions. The HCT concentration was obtained by reading the samples (quartz cells with 1 cm) in a UV spectrophotometer (UV-Vis 1601 Shimadzu spectrophotometer, Japan) using a suitable dilution.

- Dissolution studies

Dissolution rate studies of HCT as such and from its different binary and ternary systems as well as from the final tablets were performed according to the dispersed amount method. For this purpose, the solid all samples were weighed so as to always have 200 mg of hydrochlorothiazide. Each dissolution test for each sample (n = 2) was carried out in a 150 mL beaker containing 75 mL of one of the following buffer solutions (pH = 1.2, 3.3 or 4.5). The beaker was then immersed in water bath thermostated at a temperature of 37±0.5 °C. A stirring system consisting of 3-paddle stirrer with a 19 mm diameter was placed inside the beaker at approximately 1.5 cm from the bottom with 100 rpm of rotation speed. The solid sample was transferred to the beaker at time zero. Subsequently, at regular and predetermined time intervals (5, 10, 15, 30 and 60 min), samples of 3 mL each were withdrawn with a syringe. After each sampling, the same amount (3mL to 37 °C) of the selected buffer solution was added to the beaker to put the volume removed. The samples withdrawn were filtered with a Millipore membrane nitrocellulose filter (pore size of 0,45 μm) in order to obtain clear solutions and HCT concentrations of each sample (dilution factor of 1:200 or 1:300) were determined by UV spectrophotometer (UV-Vis 1601 Shimadzu spectrophotometer, Japan).In order not to overlook the effect of the progressive dilution caused by the replacement of the selected buffer after each sample withdraw, a correction factor was calculated according to the following formula:

$$C_{i\,corr} = C_i + \left(\frac{V_p}{V_0}\right) \sum C_i \quad (1)$$

where C_i *corr* corresponds to the corrected concentration, C_i to the concentration measured, V_p to the volume of sample, $V_0$to the total volume of dissolution medium and *Cn* to the sum of all concentration values until i-1 [68].

Dissolution efficiency (DE) was calculated from the area under the curve at time t (trapezoidal rule) and expressed as a percentage of the area described by 100% dissolution at the same time. The respective formula is presented below [69]:

$$DE = \frac{\int_0^t y.dt}{y_{100}.t} .100\% \quad (2)$$

2.2.2. Preparation of Tablets from Ternary Mixtures

Each tablet (TB) was produced from the TM previously prepared (TM_{PM}, $TM_{COE\text{-}I}$ and $TM_{COE\text{-}II}$ cited in B) after addition other excipients: Sodium Starch Glycolate or Classic Starch Glycolate (disintegrating agent), Magnesium Stearate (lubricant) and PVPK30 or PVPK90 (binder). The mixing of the various components was done by PM, as described in A.1). The tablets were prepared by direct compression, with a Perkin-Elmer hydraulic press, applying a force of 2.5 tons for 3 min.

A) Tablets prepared by Physical Mixture

Components amounts to prepare one tablet were weighted on the Mettler AE 166 digital laboratory scale according to Table 2-VI.

Table 2-VI:Formulation of Tablets prepared by PM (mg)

Formulation	$TM_{PM}1$	$TM_{PM}2$	$TM_{PM}3$	PVP K30	Classic Starch	Na^{+}Starch Glycolate	Mg^{2+} Stearate
TB1	335	-	-	33.5	13.4	-	3.35
TB2	335	-	-	33.5	-	13.4	3.35
TB3	-	235	-	23.5	9.4	-	2.35
TB4	-	235	-	23.5	-	9.4	2.35
TB5	-	-	185	18.5	7.4	-	1.85
TB6	-	-	185	18.5	-	7.4	1.85

B) Tablets prepared by Co-Evaporation I

Components amounts to prepare one tablet were weighted on the Mettler AE 166 digital laboratory scale according to Table 2-VII.

Table 2-VII:Formulation of Tablets prepared by TMCOE-I (mg)

Formulation	$TM_{COE-I}1$	$TM_{COE-I}2$	PVPK30	Na^{+} Starch Glycolate	Mg^{2+} Stearate
TB7	335	-	33.5	13.4	3.35
TB8	-	235	23.5	9.4	2.35

C) Tablets prepared by Co-Evaporation II

Components amounts to prepare one tablet were weighted on the Mettler AE 166 digital laboratory scale according to Table 2-VIII.

Table 2-VIII:Formulation of Tablets prepared by TMCOE-II (mg)

Formulation	$TM_{COE-II}1$	$TM_{COE-II}2$	PVPK30	PVP K90	Na^{+} Starch Glycolate	Mg^{2+} Stearate
TB9	335	-	33.5	-	13.4	3.35
TB10	-	235	23.5	-	9.4	2.35
TB11	335	-	-	33.5	13.4	3.35

2.2.2.1 Characterization of the tablets

Tablets were characterized according to Ph. Eur. [14], but with some adjustments.

- Organoleptic Characteristics: 20 tablets of each formulation were characterized in terms of their appearance (color and smell) and

dimensions (diameter and thickness); dimensions were measured with a Caliper represented in Figure 9.

Figure 9:Caliper

- Uniformity of Mass: 20 tablets of each formulation were tested, except for TB10 and TB11which were only tested 15 tablets were tested.

- Friability: 10 tablets of each formulation were tested using an Erweka TA.

- Hardness: 3 tablets of each formulation were tested using a Tablet Hardness Tester of type *Monsanto* as shown in Figure 10. Tablets were placed in vertical orientation related to the lower piston.

Figure 10:Monsanto Type Tablet Hardness Tester

- Disintegration: 6 tablets of each formulation were tested.

- Dissolution: 4 tablets of each formulation were tested according to the procedures described in the dissolution studies in section 2.2.1.1.

3. Results and Discussion

3.1. Binary Mixtures Characterization

3.1.1. Organoleptic Characteristics

The Binary Mixtures obtained were homogeneous, white and odourless powders. The BM_{COE}, in any of its molar ratio, had a finer powder texture compared to BM_{PM} and presented some crystals.

3.1.2. Evaluation of Solubility

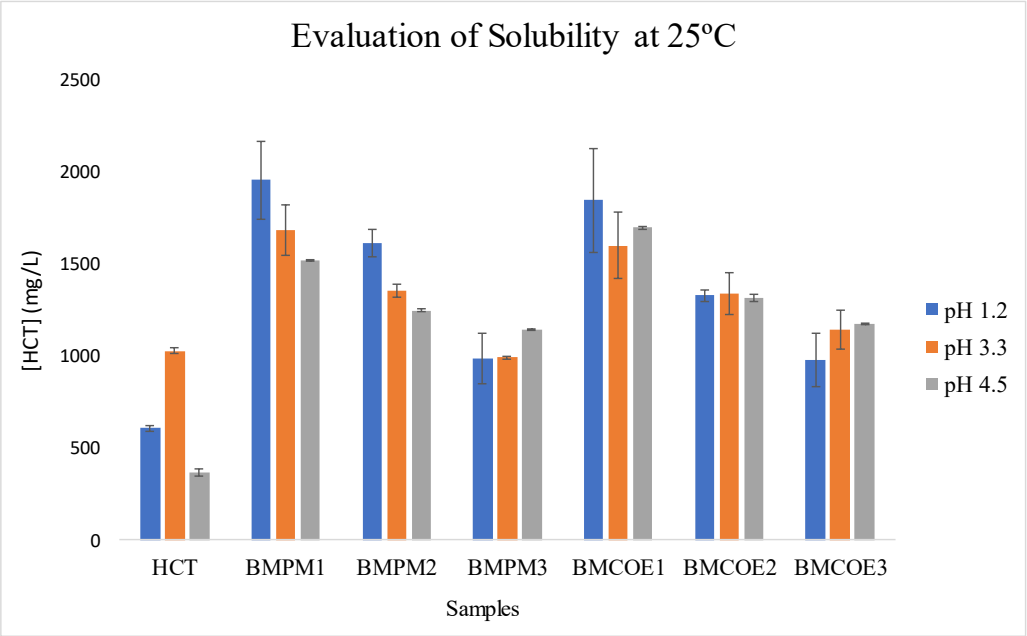

Figure 11:Evaluation of Solubility of HCT and BM at 25ºC

The presence of the CDs showed a remarkable increase in the solubility of HCT at all pH tested, as shown in Figure 11. The solubility of the untreated HCT is higher at pH 3.3 and lower at pH 4.5, so the greatest improvements were shown for this pH. Compared to PM, which is the reference for this study, COE presents similar results and proved to be a successful method for preparing the inclusion complex. Regarding the HCT:RAMEβ molar ratios, the HCT concentration decreases as the ratio decreases, which means that a greater amount of CD is necessary for a remarkable increase in HCT solubility. The BM that presented the most significant improvement was $BM_{COE}1$ at pH 1.2, which can be explained by the higher molar ratio HCT:RAMEβ, 1:1.

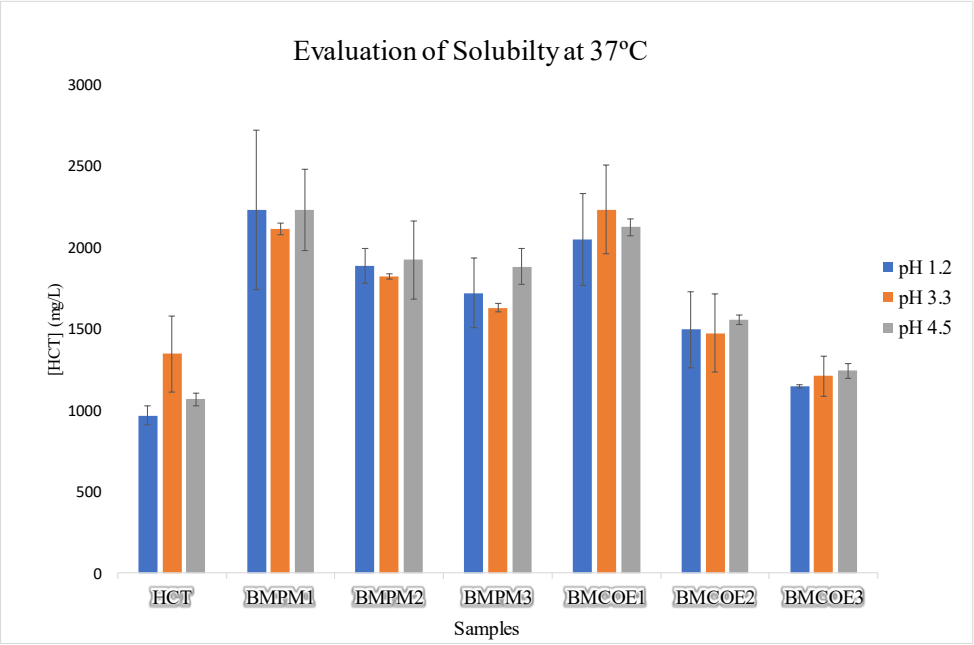

Figure 12:Evaluation of Solubility of HCT and BM at 37ºC

At 37°C, the solubility of HCT is considerably higher compared to 25°C for the entire pH tested, as shown in Figure 12. The presence of the CDs again showed a notable increase in the solubility of HCT throughout the pH tested, achieving greater increases in the solubility of HCT compared to 25°C. COE shows similar results to PM, particularly for $BM_{PM}1$ and $BM_{COE}1$. However, the HCT concentration decreases as the ratio decreases and a significant variation can be observed by comparing $BM_{PM}3$ and $BM_{COE}3$. $BM_{COE}1$ at pH 3.3 has proven to be the best BM for improving HCT solubility, which means that a higher HCT:RAMEβ molar ratio is beneficial for increasing HCT solubility.

3.1.3. Dissolution Studies

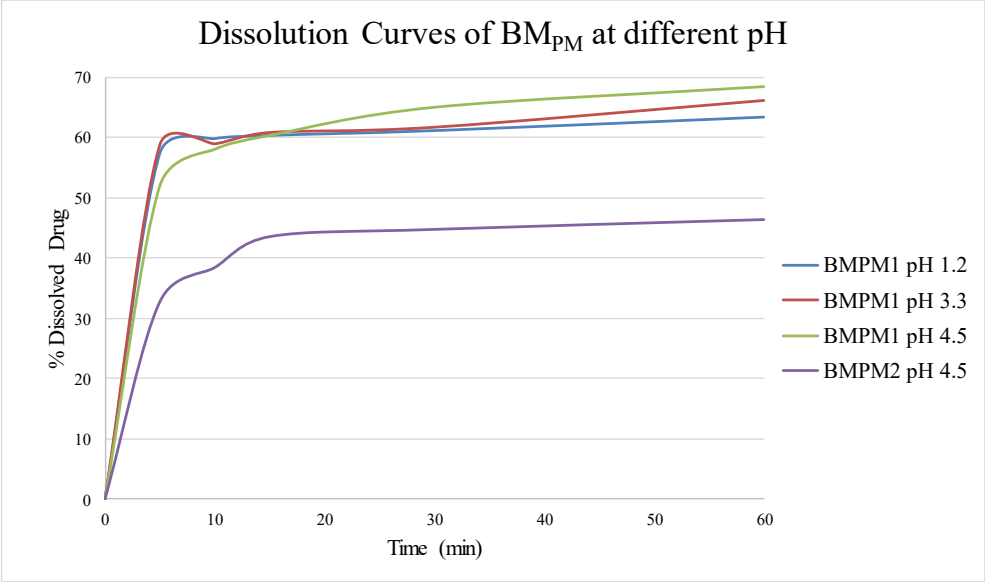

Figure 13:Dissolution Curves of BMPM at different pH

Figure 13 shows the dissolution curves of BM_{PM} with different molar ratios of HCT: RAMEβ and in the presence of different buffer solutions. For $BM_{PM}1$ at pH 1.2, pH 3.3 and pH 4.5 there were no relevant differences, and all presented similar PD at the end of the analysis: 63.375%, 66.185% and 68.518% for $BM_{PM}1$ at pH 1.2, 3.3 and 4.5, respectively (Table 4-I, Annexes I). Thus, the pH did not greatly influence the dissolution profile of the BM tested.

Comparing the two different molar ratios of HCT:RAMEβ, $BM_{PM}2$ presented a lower PD at the end of the analysis equal to 46.304% (Table 4-I, Annexes I). Therefore, the molar ratio of HCT:RAMEβ 1:1 proved to be the best, reaching a DE of 60.79% at pH 4.5 (Table 4-II, Annexes I).

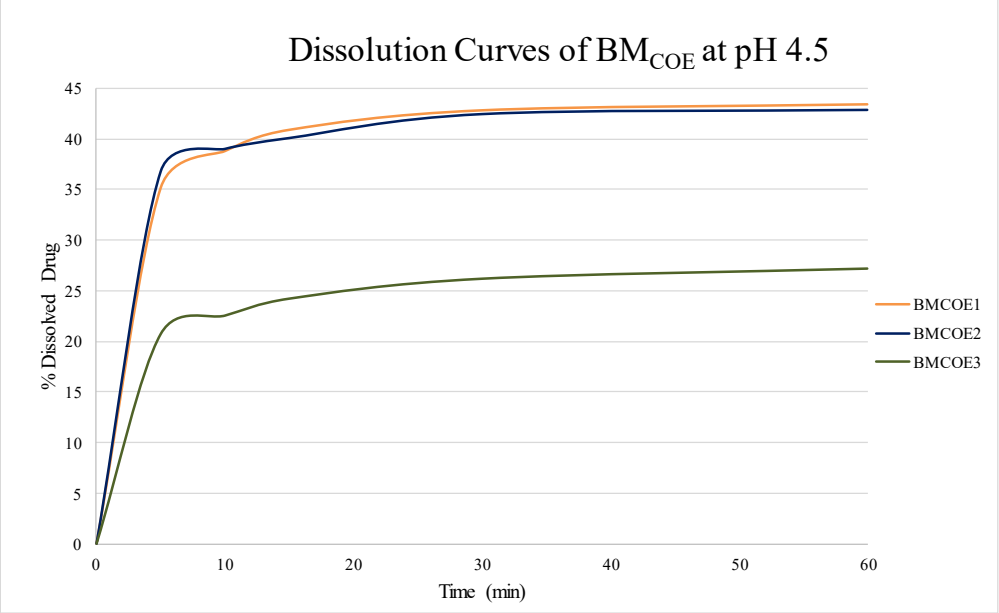

Figure 14:Dissolution Curves of BMCOE at pH 4.5

BM_{COE} was also tested in different molar ratios of HCT: RAMEβ at pH 4.5 and it was necessary to use an amount of BM_{COE} equivalent to 700 mg of HCT to work in presence of a precipitate (Figure 14). The dissolution curves in Figure 14 showed that $BM_{COE}1$ and $BM_{COE}2$ presented superimposable dissolution profiles, reaching a PD equal to 43.441% and 42.807%for $BM_{COE}1$ and $BM_{COE}2$, respectively, within 1 h test (Table 4-III, Annexes I). However, $BM_{COE}3$ had a worse dissolution profile, reaching a PD of 27.207% within 1 h test (Table 4-III, Annexes I).

To conclude, the molar ratio of HCT: RAMEβ 1:0.25 is not adequate for the preparation of the binary mixture while the ratios 1:1 and 1:0.5 are suitable and showed good dissolution curves.

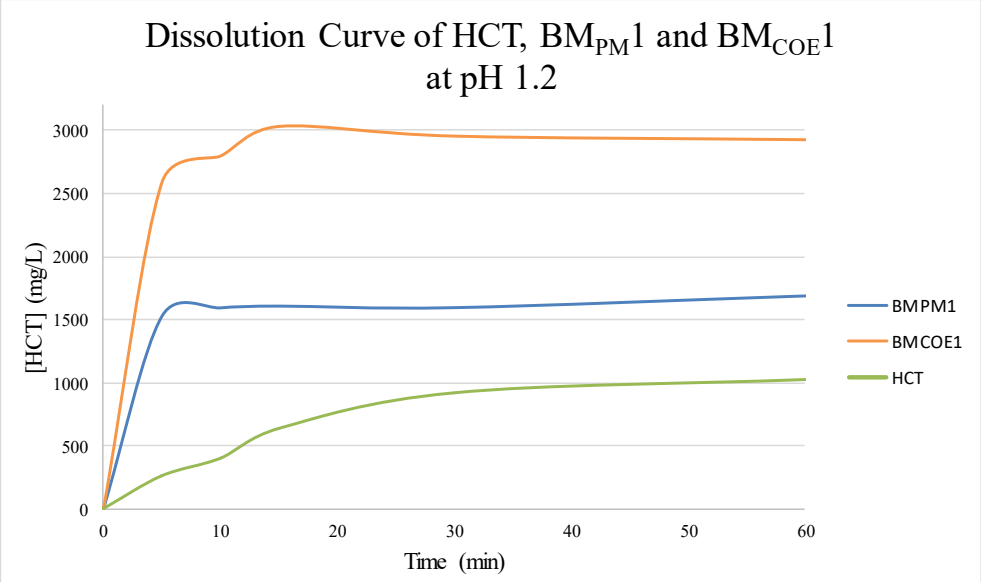

Figure 15:Dissolution Curves of HCT untreated, $BM_{PM}1$ and $BM_{COE}1$

The two binary mixtures which presented better dissolution curves in previous studies, $BM_{PM}1$ and $BM_{COE}1$, were tested at pH 1.2 in order to find the best preparation technique (Figure 15). $BM_{COE}1$ showed the best dissolution profile proving that RAMEβ was able to significant increase HCT solubility, considering that it was necessary to use an amount of BM_{COE} equivalent to 700 mg of HCT. PD in the end of the test was 38.365% for HCT untreated (Table 4-V, Annexes I), corresponding to a DE of 28.58% (Table 4-VI, Annexes I), while $BM_{COE}1$ presented a PD of 39.613% (Table 4-V, Annexes I), corresponding to a DE of 33.87%, with 700 mg of HCT (Table 4-VI, Annexes I).

To summarize, COE is the most suitable preparation technique for BM, with the molar ratio HCT:RAMEβ equal to 1:1.

3.2. Tablet Characterization

3.2.1. Organoleptic Characteristics

The tablets prepared were cylindrical, flat, odourless and light brown in colour, spotted with dark brown. All prepared tablets were 1.3 cm in diameter and the thickness varied according to the SV mass ratio: 0.25 cm, 0.15 and 0.10 for the ratio of 1:8, 1:4 and 1:2, respectively.

Figure 16:Tablets 1

3.2.2. Uniformity of Mass

All the tablets of each formulation comply with test and the respective mean masses are presented in Table 3-I.

Table 3-I:Uniformity of Mass of Tablets

Formulation	Mean mass (mg)	Satisfy	No satisfy
TB1	381.5 ± 0.001	X	
TB2	385.7± 0.001	X	
TB3	267.7± 0.002	X	
TB4	268.7± 0.003	X	
TB5	211.4 ± 0.004	X	
TB6	212.0± 0.009	X	
TB7	380.0 ± 0.002	X	
TB8	265.4± 0.006	X	
TB9	384.1± 0.002	X	
TB10	268.9± 0.001	X	
TB11	384.3± 0.001	X	

3.2.3. Friability

The initial mass, final mass and percentage mass loss are presented in Table 3-II for each tablet formulation. TB1, TB2, TB4 and TB11 comply with friability test with a mass loss of less than 1%.

Table 3-II:Friability of Tablets

Formulation	Initial Mass (mg)	Final Mass (mg)	MassLoss (%)
TB1	3847.2	382.11	0.678
TB2	3856.7	381.89	0.980
TB3	2692.1	-	-
TB4	2687.0	266.53	0.808
TB5	2124.6	-	-
TB6	2091.5	-	-
TB7	3792.0	-	-
TB8	2664.2	-	-
TB9	3829.8	-	-
TB10	2687.6	-	-
TB11	3834.6	3818.8	0.412

Tablets with HCT:SV mass ratio of 1:2 (TB5 and TB6) were very thin, so that all the tablets were broken at the end of the test. In contrast, the tablets with HCT:SV mass ratio of 1:8 (TB1, TB2 and TB11) were thick and comply with test. For the rest of the tablets tested, only TB4 with HCT:SV mass ratio of 1:4 comply with test. In general, tablets prepared by COE (I and II): TB7, TB8, TB9 and TB10 were more fragile than those prepared by PM: TB1, TB2, TB3, TB4, TB5 and TB6.

However, TB11 was the exception, as it was prepared by the COE, was more rigid than all the others and comply with test. Thus, its performance could be justified with the presence of PVP K90 instead of PVP K30 used in the other tablet formulations.

3.2.4. Hardness

Hardness test was performed in all the tablet formulations and the minimum force, maximum force and mean force for each TB formulation are presented in Table 3-III. TB1 and TB2 were the hardest with a mean force of 10.33 and 12 N, respectively, and TB8 and TB10 were the less harsh with a mean force of 3.33 and 2.67 N, respectively.

Tablets prepared by PM: TB1, TB2, TB3, TB4, TB5 and TB6 were harder than the others prepared by COE: TB7, TB8, TB9, TB10 and TB11. Tablets with the highest HCT:SV mass ratio, 1:8, showed to be harder (TB1, TB2 and TB11), predicting that the SV confers hardness to the tablets.

Table 3-III: Hardness of Tablets (N)

Formulation	Mean Force	Minimum Force	Maximum Force
TB1	10.33	12	9
TB2	12	13	11
TB3	6.17	7.5	5
TB4	7.83	9	7
TB5	6.33	7	6
TB6	7.83	8	7.5
TB7	4.33	5	4
TB8	3.33	4	3
TB9	4.33	4.5	4
TB10	2.67	3	2
TB11	5.33	6	5

3.2.5. Disintegration

The disintegration test was performed on tablets that comply with friability test (TB1, TB2, TB4 and TB11) and the results are presented in Table 3-IV. For TB1, TB2 and TB4, all 6 dosage units tested for each tablet formulation were completely disintegrated, and the respective disintegration times are presented in Table 3-IV. However, TB11 did not satisfy the test and no tablet was disintegrated after 15 min. Probably, TB11 did not satisfy the test due to the high amount of PVP K90 that was not adequate for the amount of Na^+ Starch Glycolate.

Table 3-IV:Disintegration of Tablets (s)

Formulation	Satisfy	No satisfy	Disintegration Time (sec)
TB1	X		236
TB2	X		238
TB4	X		239
TB11		X	

3.2.6. Dissolution

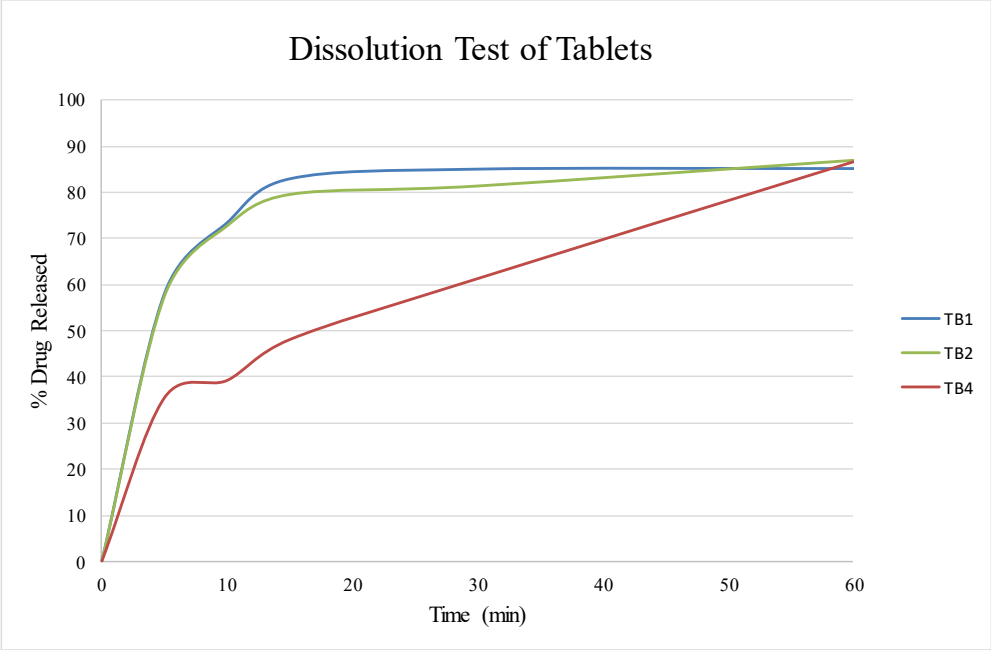

Figure 17:Dissolution Test of Tablets

The dissolution test was performed in tablets that comply with disintegration test (TB1, TB2 and TB4) and the respective dissolution profiles are shown in Figure 17. TB1 and TB2 presented a very similar dissolution profile, reaching a PD of 85.15% and 87.07%, respectively, after 1h of analysis (Table 4-VII, Annexes I). The higher PD achieved by TB2 could be due to the use of sodium starch glycolate instead of classic starch (TB1). In contrast, TB4 presented an irregular dissolution profile with a large increase in PD from 30 to 60 min, 61.33% and 86.75%, respectively (Table 4-VII, Annexes I). Thus, TB4 didn´t proved to be an adequate tablet formulation and DE at 60 min was lower than the others: 77.95% for TB1, 76.47% for TB2 and 58.91% for TB4 (Table 4-VIII, Annexes I).

4. Conclusions and Further Studies

This study aimed to find the best method to produce tablets containing HCT formulated in a ternary system composed of CD, RAMEβ, and nanoclay, sepiolite (SV). Thus, it is possible to increase the solubility and bioavailability of HCT, with consequent reduction of undesirable effects and obtaining high therapeutic efficacy. After studying and characterizing the HCT-RAMEβ binary systems, the most appropriate preparation technique (PM or COE) and the best molar ratio of HCT-RAMEβ (1:1, 1:0.5, 1:0.25) were selected. The tablets were then produced with the previously selected HCT-RAMEβ molar ratio and using different HCT-SV mass ratios (1:8, 1:4, 1:2). The characterization of the tablets was performed according to Ph. Eur. 9.0, 2017 and the most appropriate HCT-SV mass ratio was selected to find the best formulation for the preparation of the tablets.

Regarding the BM, HCT-RAMEβ BM prepared by COE presented the best results in the evaluation of solubility test and dissolution studies, proving to be the most adequate technique for these mixtures preparation. HCT-RAMEβ molar ratio equal to 1:1 and 1:0.5 presented very similar results, but the greatest increase in PD and DE of HCT was observed with 1:1 ratio, so that it was selected for tablet preparation.

Concerning the tablets, the ones prepared by PM with the HCT-SV mass ratio equal to 1:8 presented the best results, especially in dissolution studies, in which they presented the best performance in relation to tablets produced by PM with the HCT-SV mass ratio equal to 1:4. Thus, 1:8 was the selected HCT-SV mass ratio for tablets formulation. Considering the two types of starch used, tablets produced with sodium starch glycolate were harder and presented a better dissolution profile. Therefore, tablets produced by PM with HCT-SV 1:8 (mass ratio) and using sodium starch glycolate showed the best results and proved to be more suitable for tablets formulation.

About the tablets prepared by COE, either COE-I or COE-II, were very fragile and did not comply with all tests, with the exception of those prepared by COE-I with PVP K90 instead of PVPK30. These tablets were harder than the others and were the only ones that comply with friability test. The disaggregation test in this formulation was probably not successful due to the amount of PVP K90 used, since its binding effect is considerably greater and perhaps the amount of sodiumstarch glycolate was not sufficient to disintegrate the tablets.

The next step to be taken should be the preparation of tablets by PM and COE, either by COE-I and COE-II, using PVP K90 instead of PVP K30 as it made the tablets harder and robust. The amount of sodiumstarch glycolate should be modified according to the amount of PVP K90 so that tablets can disintegrate properly into the solution. After this step, the most appropriate technique for tablets production between PM, COE-I and COE-II should be selected for the production of tablets with a HCT-RAMEβ 1:1 mass ratio of and a HCT-SV mass ratio of 1:8. Then, tablets could also be prepared using HCT-RAMEβ equal to 1:0.5 molar ratio) and HCT-SV mass ratio equal to 1:4, taking into account the results obtained and with the aim of reducing the amount of these constituents in tablets formulation.

Afterwards, the prepared tablets should be tested according to the Ph. Eur. 9.0, 2017, and comparative study should be performed with *Esidrex*, an HCT formulation already present on the Italian market. Later, *in vitro* and *in vivo* studies could be carried out to characterize pharmacodynamics, pharmacokinetics and toxicity of these tablets.

To conclude, the steps taken during this study demonstrated the potential to merge the benefits of two distinct strategies (CD and nanoclay) into a single ternary drug delivery system. This ternary system represents a promising strategy to improve bioavailability of HCT, increasing its therapeutic effectiveness while reducing its dose and possible side effects. In the future, this ternary system may be used to improve solubility and permeability of other drugs, especially the BCS class IV drugs.

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Annexes

Annex I

Table 4-I:Percentage Dissolved of BMPM at 5,10,15,30 and 60 min

Time (min)	$BM_{PM}1$ pH 1.2 (%)	$BM_{PM}1$ pH 3.3 (%)	$BM_{PM}1$ pH 4.5 (%)	$BM_{PM}2$ pH 4.5 (%)
5	57.379	58.819	52.046	32.695
10	59.812	59.003	58.117	38.372
15	60.343	60.871	60.413	43.480
30	61.137	61.748	65.089	44.680
60	63.375	66.185	68.518	46.304

Table 4-II: Dissolution Efficacy of BMPM at 10, 30 and 60 min

Formulation	DE 10	DE 30	DE 60
$BM_{PM}1$ pH 1.2	43.64	54.93	58.59
$BM_{PM}1$ pH 3.3	44.16	55.36	59.67
$BM_{PM}1$ pH 4.5	40.55	54.77	60.79
$BM_{PM}2$ pH 4.5	25.94	37.51	41.50

Table 4-III: Percentage Dissolved of BMCOE at 5,10,15,30 and 60 min

Time (min)	$BM_{COE}1$ pH 4.5 (%)	$BM_{COE}2$ pH 4.5 (%)	$BM_{COE}3$ pH 4.5 (%)
5	35.200	36.752	20.745
10	38.822	38.959	22.558
15	40.918	40.030	24.233
30	42.848	42.393	26.204
60	43.441	42.807	27.207

Table 4-IV:Dissolution Efficacy of BMCOE at 10, 30 and 60 min

Formulation	DE 10	DE 30	DE 60
$BM_{COE}1$ pH 4.5	28.21	36.69	39.92
$BM_{COE}2$ pH 4.5	28.12	36.56	39.58
$BM_{COE}3$ pH 4.5	16.01	21.85	24.28

Table 4-V:Percentage Dissolved of HCT, BMPM1 and BMCOE1 at 5,10,15,30 and 60 min

Time (min)	HCT pH 1.2 (%)	$BM_{PM}1$ pH 1.2 (%)	$BM_{COE}1$ pH 1.2 (%)
5	9.644	57.379	29.357
10	14.975	59.812	30.379
15	23.724	60.343	33.008
30	34.618	61.137	35.970
60	38.365	63.375	39.613

Table 4-VI:Dissolution Efficacy of HCT, BMPM1 and BMCOE1 at 10, 30 and 60 min

Formulation	DE 10	DE 30	DE 60
HCT pH 1.2	8.57	20.67	28.58
$BM_{PM}1$ pH 1.2	43.64	54.93	58.59
$BM_{COE}1$ pH 1.2	22.53	29.95	33.87

Table 4-VII:Percentage Dissolved of Tablets at 5,10,15,30 and 60 min

Time (min)	TB1 (%)	TB2 (%)	TB4 (%)
5	58.084	57.606	35.476
10	73.414	72.842	39.210
15	82.925	79.644	48.081
30	85.025	81.480	61.326
60	85.147	87.069	86.714

Table 4-VIII:Dissolution Efficacy of Tablets at 10, 30 and 60 min

Formulation	DE 10	DE 30	DE 60
TB1	47.40	70.81	77.95
TB2	47.01	68.66	76.47
TB4	27.54	43.81	58.91

Annexes II

Standard Curves of HCT

The UV spectrum of the HCT mother solutions (MS) with different concentrations were recorded with various buffer solutions by scanning the wavelengths between 200 and 400 nm. The different buffer solutions and HCT MS were the following:

- pH 1.2 buffer solution: [HCT MS] = 14.140 mg/L
- pH 3.3 buffer solution: [HCT MS] = 12.876 mg/L
- pH 4.5 buffer solution: [HCT MS] = 13.300 mg/L

The absorption peak was identified at 272.2 nm using pH 1.2 buffer solution and HCT MS with the concentration equal to 14.140 mg/L. After this step, for each pH, five solutions with different dilutions of the respective HCT MS were prepared, according to the Table 4-IXfor pH 1.2, Table 4-X for pH 3.3 and Table 4-XI for pH 4.5 buffer solution. The five prepared solutions were subjected to UV readings at the wavelength of 272.2 nm and the respective absorbances are presented in Table 4-IX for pH 1.2, Table 4-X for pH 3.3 and Table 4-XI for pH 4.5 buffer solution. Standard curves of HCT were obtained using the concentration values of the prepared solutions and the respective absorbances. Standard curve of HCT at pH 1.2, pH 3.3 and pH 4.5 are presented in Figure 18, Figure 19 and Figure 20 respectively.

Table 4-IX:Standard Curve of HCT at pH 1.2

Solution	Volume withdrawn from the MS (mL)	Volume pH 1.2 buffer solution (mL)	[Solution] (mg/L)	Absorbance
1 (2:10)	2	10	2.828	0.212
2 (4:10)	4	10	5.656	0.373
3 (5:10)	5	10	7.070	0.471
4 (6:10)	6	10	8.484	0.589
5 (8:10)	8	10	11.312	0.765
MS	10	-	14.140	0.822

Table 4-X: Standard Curve of HCT at pH 3.3

Solution	Volume withdrawn from the MS (mL)	Volume pH 1.2 buffer solution (mL)	[Solution] (mg/L)	Absorbance
1 (2:10)	2	10	2.575	0.180
2 (4:10)	4	10	5.150	0.329
3 (5:10)	5	10	6.393	0.418
4 (6:10)	6	10	7.726	0.495
5 (8:10)	8	10	10.301	0.670
MS	10	-	12.876	0.839

Table 4-XI: Standard Curve of HCT at pH 4.5

Solution	Volume withdrawn from the MS (mL)	Volume pH 1.2 buffer solution (mL)	[Solution] (mg/L)	Absorbance
1 (2:10)	2	10	2.660	0.181
2 (4:10)	4	10	5.320	0.350
3 (5:10)	5	10	6.650	0.424
4 (6:10)	6	10	7.980	0.501
5 (8:10)	8	10	10.640	0.664
MS	10	-	13.300	0.864

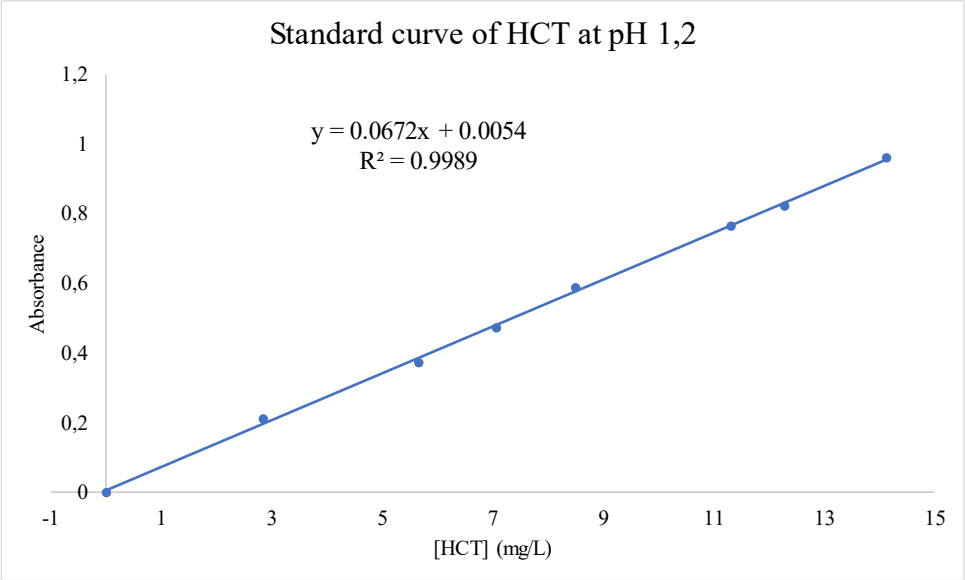

Figure 18:Standard Curve of HCT at pH 1.2

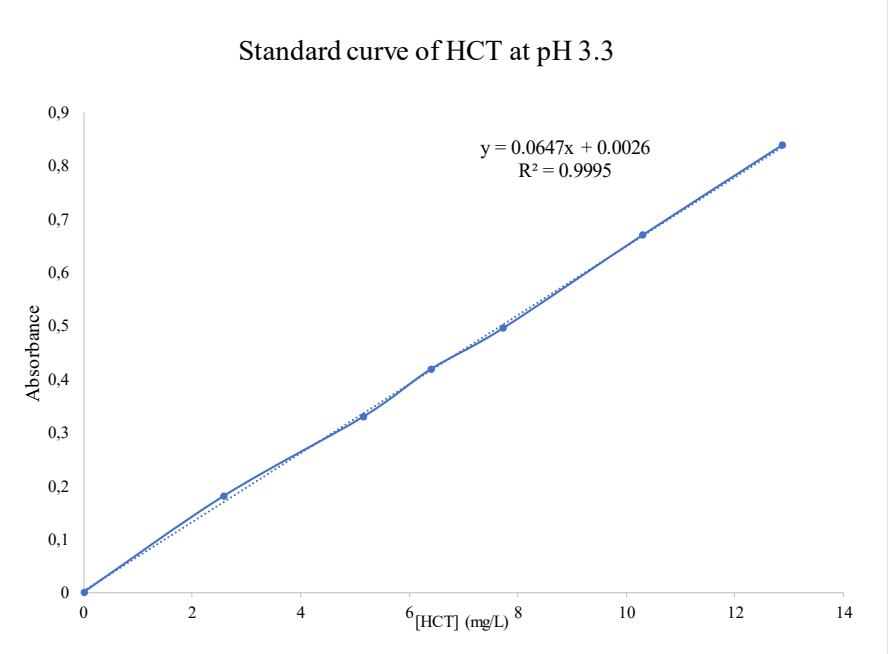

Figure 19:Standard Curve of HCT at pH 3.3

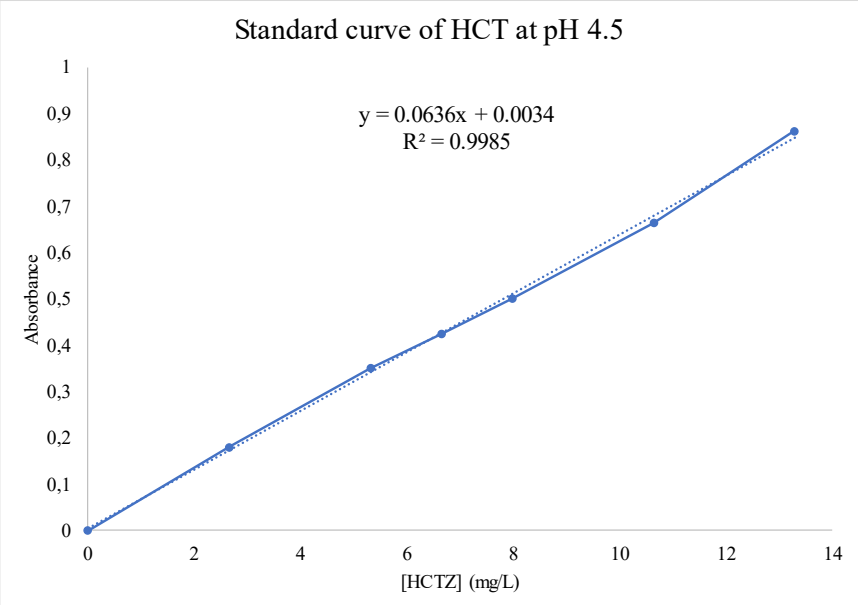

Figure 20:Standard Curve of HCT at pH 4.5

Annexes III

Table 4-XII: Studies on Nanoclays as Drug Delivey Systems

Nanoclay	Drug Molecule	Interactions Drug-Nanoclay	Type of Release
Montmorrillonite (MMT)	Ibuprofen (IBU) [70]	Hydrogen bonding between COO- groups of IBU with OH- groups of the MMT layers	In vitro drug release in: • Simulated gastric acid fluid (pH 1.2) was 11% after 120min • Simulated intestinal fluid (pH 7.4) was 30,7% after 120min MMT can be used as an extended release carrier of IBU in oral administration.
	Timolol maleate [71]	Electrostatic interactions between NH+ group and clay surface	In vitro release in simulated gastric (pH 1.2) and intestinal fluids (pH 7.4) • pH 1.2: 43% release after 9h • pH 7.4: 48% release after 9 h
Montmorrillonite (MMT), Saponite (SA), Laponite (LA)	Donepezil [72]	Cation exchange	In vitro release in simulated human gastric media (pH 1.2): • Release from clay without coating with Eudagit E-100 depends on CEC. Larger CEC created stronger interactions between drug molecules and clays and sustained release (7% for MMT, 15% for SA and 37% for LA after 3h) Donepezil-nanoclayhybrids coated with Eudragit E-100 showed a fast drug release during a short period of time
Magnesium alluminium silicate (MAS) (mixture of Montmorrillonite and Saponite)	Complexes of Propranolol HCl (PPN) [73]	PPN-MAS complexes formed via cation Exchange, hydrogen bonding and water bridging interactions	Calcium alginate (CA) beads loaded with intercalated complexes of PPN and MAS in various concentrations (PPN-MAS complex-loaded CA beads). In vitro release in simulated gastro-intestinal conditions (HCl 0,1 M for 2h and then phosphate buffer pH 6.8): • HCl 0,1M: release after 10 hs was 89.8% and 51.1% respectively with lower and higher amount of MAS • Phosphate buffer pH 6.8: release after 10h was 82.4% and 46.2% respectively with lower and higher amount of MAS Results indicate that a higher amount of clay lead to lower amount of released drug. Continuous release of propranolol was performed when the PPN-MAS complex-loaded CA beads was transferred to phosphate buffer 6.8, which simulates the small intestine.

Nanoclay	Drug Molecule	Interactions Drug-Nanoclay	Type of Release
Sepiolite (SV)	Oxaprozin (OXA) (poorly water-soluble drug) [60]	Cation Exchange	Ternarysystem of OXA, cyclodextrin (RAMEB) and SV. Co-ground products (GR) of OXA with RAMEB in combination with SV by physical mixture (PM), (OXA-RAMEB GR) – SV PM or by confused products (COF), (OXA-RAMEB GR) – SV COF. *In vitro* drug release in buffer solution of pH 5.5: • OXA was 5.5% dissolved after 15min with a DE at 60min of 7% • OXA in (OXA-RAMEB GR) – SV PM was 48.5% dissolved after 15min with a Dissolution Efficacy at 60min of 47.8% • OXA in (OXA-RAMEB GR) – SV COF was 93.6% dissolved after 15min with a DE at 60min of 93.5%. Synergistic effect between complexation with CD and nano-entrapment in clay in improving OXA solubility and dissolution rate.
Mesoporous synthetic hectorites (MSH): MSH1 (without organic template) and MSH2 (with organic template)	Quinine (QUI) [74]	Electrostatic interactions between QUI and MSH	The clay-drug nanocomposites were coated with sodium alginate (AL) to slow down the drug release in gastric media. The composites were tested in HCl 0,1M (pH 1.2) for 2h and then in a phosphate buffer (pH 6.8). *In vitro*release in HCl 0.1 M andphosphate buffer: • Without coating: 63% and 94% release after 8h from MSH1-QUI and MSH2-QUI, respectively • With AL coating: 25% and 45% release after 8h from MSH1-QUI/AL and MSH2-QUI/AL, respectively MSH-QUI and MSH-QUI/AL nanocomposites exhibited sustained delivery of QUI in *in vitro* conditions and AL coating led to a retarded release of QUI in gastric media.