UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

TÍTULO: Effect of the excipients on the solubility of Ibuprofen and Fluconazol

Trabajo de integración curricular presentado como requisito para la obtención del título de Ingeniero Biomédico

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Dedication

I dedicate this work: To my dear parents, Maria and Eugenio, the cornerstone of my family, who have taught me the true meaning of sacrifice and dedication. Their unconditional love and constant support have been the foundation that has guided my path and made each of my achievements possible, I will always be indebted to them. To my older siblings, Josue and Maria Eugenia, examples of strength in my life. Their perseverance and achievements are constant sources of inspiration for me. Their unconditional support has been essential in every step I have taken. To my beloved little ones, Dianita and April, who have brought boundless joy into my life. Their presence, brimming with love and tenderness, is truly invaluable, and my affection for them transcends what words can express.

This is for and thanks to you, my beloved family.
May your eternal love and unwavering support
continue to be my anchor and strength in
every moment of my life!

Ana Julia Andrade Reyes

Acknowledgements

First and foremost, I thank God for granting me the strength, wisdom, and perseverance needed to complete this thesis. My sincere gratitude also extends to my thesis advisor, Diego Lucero, whose expert guidance, constant support, and valuable suggestions have significantly enriched this work. His tireless dedication and profound knowledge in the field have been the compass that guided every step of this journey. Furthermore, his unparalleled patience has allowed me to overcome obstacles and develop my work with confidence and clarity, leaving a lasting impact on my formation as a researcher.

Similarly, I express my deepest gratitude to my family (Eugenio A., Maria R., M. Eugenia A., Josué A., Diana A., and April), who have been my unwavering anchor throughout this academic journey. Their unconditional love, constant support, and willingness to stand by me during moments of stress have been fundamental pillars that made the completion of this work possible. I highlight the invaluable financial support provided by my parents and siblings, who ensured that I lacked nothing throughout my entire academic career. Their generosity and sacrifice were not only a financial backbone but also a constant reminder of their unconditional commitment to my success.

Likewise, I want to express my immense gratitude to Dayanna B. and Marlene P., my loyal friends and classmates. Throughout this journey, we have shared not only moments of joy and success but also conflicts and tears. However, despite disagreements and difficult times, their unwavering support never wavered. In exhausting study nights and days of frustration, their comforting presence and sincere encouragement were a true balm for the soul. To Dayanna and Marlene, I thank for every shared laugh, comforting hug, and shed tear together. Their friendship has been a beacon of light in dark moments and a constant source of strength. Without their companionship, this path would have been much more challenging to traverse. Thanks for always being there, even in the toughest times.

I also want to express my gratitude to Erick T. for his unwavering support throughout my university journey. His consistent assistance and willingness to help, whether it be with academic matters or providing moral support, have been invaluable in making the university life more manageable.

Additionally, I would like to thank my friends “Falsetas”, Jordan A. and Alexander U., who despite having met them recently, have proven to be close friends who have never left me alone in my moments of relaxation. I deeply appreciate their companionship and care for me, and I am profoundly grateful for their presence in my life.

Similarly, I want to express my deepest appreciation to Arianna A., Diana C., Sixto C., and Rosemary D. for the wonderful memories we've shared throughout my university journey. Thank you for allowing me to get to know each of you; you are truly incredible individuals!

Last but not least, I want to express my gratitude to my best friend Rubén S., who was the only one who stood by my side when I went to study far from home and was willing to follow my craziness despite the distance. His true friendship is something I greatly value.

Truly, without the help and presence of all of them, this achievement would not have been possible. Infinite thanks to all for being part of this significant achievement in my life!

Ana Julia Andrade Reyes

Resumen

La comprensión de cómo los excipientes interactúan con un ingrediente farmacéutico activo (API), ya sea ácido o básico, es crucial para optimizar propiedades fisicoquímicas como la solubilidad del API, lo que conduce a mejoras en los procesos de fabricación y la calidad de las formulaciones farmacéuticas. Por esta razón, este estudio se centró en evaluar el impacto de varios excipientes, incluyendo beta-ciclodextrina (BCD), carbómero (CBER), hidroxipropil metilcelulosa (HPMC), polivinilpirrolidona (PVP) y almidón de Oxalis tuberosa (OCAS), en la solubilidad del ibuprofeno (IBU) como API ácido y el fluconazol (FLU) como API básico en soluciones acuosas a temperatura ambiente (25°C). En este contexto, se evaluaron mezclas con diferentes proporciones de excipiente y API, y la solubilidad de cada combinación se determinó utilizando el Método del Agitado en Matraz. Se consideraron valores de referencia de pKa y logS0 de IBU y FLU para establecer un pH que favorezca la forma neutral de los compuestos, y se construyó el modelo Henderson Hasselbalch (HH) para comparar la solubilidad intrínseca de referencia con la solubilidad experimental. Además, se emplearon herramientas estadísticas como análisis de correlación, ANOVA y prueba de Tukey para validar los resultados. Los hallazgos indican que BCD y ciertos porcentajes de PVP aumentan la solubilidad del ibuprofeno, mientras que OCAS, CBER y HPMC no muestran efecto positivo. Por otro lado, la solubilidad del fluconazol no se incrementa por estos excipientes. Además, se observaron diferencias al comparar el comportamiento de IBU y FLU al interactuar con los excipientes durante el estudio. Por lo tanto, estos resultados destacan la importancia de emplear estrategias personalizadas que consideren las características moleculares específicas de cada compuesto, lo que podría resultar en el desarrollo de formulaciones a medida para optimizar la eficacia de cada medicamento.

Palabras Clave: Solubilidad, excipiente, API, interacciones, ácido, base.

Abstract

Understanding how excipients interact with an active pharmaceutical ingredient (API), whether acidic or basic, is crucial for improving physicochemical properties such as API solubility, thereby leading to enhancements in manufacturing processes and the quality of pharmaceutical formulations. For this reason, this study focused on evaluating the impact of various excipients, including beta-cyclodextrin (BCD), carbomer (CBER), hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone (PVP), and Oxalys tuberosa starch (OCAS), on the solubility of Ibuprofen (IBU) as an acidic API and Fluconazole (FLU) as a basic API in aqueous solutions at room temperature (25°C). In this context, mixtures with different proportions of excipient and API were evaluated, and the solubility of each combination was determined using the Flask Shaking Method. Reference values of IBU and FLU pK_a and logS_0 were considered to establish a pH favoring the neutral form of the compounds, and the HH model was constructed to compare reference intrinsic solubility with experimental solubility. Additionally, statistical tools such as correlation analysis, ANOVA, and Tukey's test were employed to validate the results. Findings indicate that BCD and certain percentages of PVP enhance the solubility of ibuprofen, while OCAS, CBER, and HPMC show no positive effect. On the other hand, the solubility of fluconazole is not increased by these excipients. Furthermore, differences were observed when comparing the behavior of IBU and FLU when interacting with the excipients during the study. Thus, these results underscore the importance of employing personalized strategies that consider the specific molecular characteristics of each compound, which could result in the development of tailored formulations to optimize the efficacy of each drug.

Key Words: solubility, excipient, API, interactions, acid, base.

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CHAPTER I: General Introduction

1. INTRODUCTION

1.1 Overview of Pharmaceutical Formulation

The formulation, development and commercialization of a drug involves several stages that must be carried out rigorously. The process begins from the discovery of a new active pharmaceutical ingredient (API) to the identification of various of its aspects such as the mechanism of action, toxicity, side effects, among others; guaranteeing that this new API effectively fulfills its purpose of treating diseases and ensuring that it works as desired. This is then followed by the conduct of clinical studies and concludes with the availability of the drug on the market for prescription and the use of the medication by the public.

In this process, there is an initial stage centered on acquiring knowledge about the potential drug, known as pre-formulation [1, 2]. During this phase, thorough evaluations are carried out to define the physicochemical properties of the drug [3, 4]. The primary objective of this stage is to obtain essential information that will direct the selection of appropriate excipients [5] and establish the required conditions for the design and formulation of a suitable pharmaceutical form tailored to the characteristics of the potential drug identified in the pre-formulation stage [1, 4, 5]. The physicochemical properties commonly analyzed in the pre-formulation stage encompass ionization constant (pK_a), solubility, partition coefficient, dissolution rate, stability, among other key aspects [3, 6–8].

Solubility is one of the most important physicochemical properties examined during the pharmaceutical pre-formulation phase [9], as it plays a fundamental role in the bioavailability and effectiveness of drugs [3, 9]. That is, the drug must have a certain degree of solubility, or it must be able to dissolve in water to achieve therapeutic efficacy upon entering the bloodstream and efficiently distributing throughout the body [3].

Compounds with low solubility, those that do not dissolve easily in water, often experience incomplete absorption [3]. This issue is widespread, particularly among many active pharmaceutical ingredients

(APIs) that share the specific traits of low solubility and a sluggish dissolution rate in water-based solutions [10]. As a result, the bioavailability of these APIs with limited solubility tends to be relatively reduced [10].

Therefore, the standard approach to address this issue is to include an excipient, a supplementary ingredient to enhance the drug's water solubility. This addition facilitates the drug's more efficient dissolution in water-based solutions, improving absorption and increased bioavailability [10]. This practice is essential in designing pharmaceutical formulations to ensure that medications are more stable, effective and efficient.

1.2 Key physicochemical parameters in pharmaceutical formulations

1.2.1 Ionization Constant (pK_a)

In the oral dosing of an active pharmaceutical ingredient (API), the effectiveness of its absorption is conditioned by the proportion of the API that manages to dissolve in the fluids of the gastrointestinal tract. From this perspective, as the solubility of the API increases, its absorption capacity also experiences a notable increase. Additionally, the API's solubility and absorption capacity correlate to its degree of ionization. Ionization is the formation of positively or negatively charged ions in an aqueous solution [11]. Generally speaking, the ionized form of a compound is more soluble in water, which suggests a greater absorption capacity. However, according to available studies [12–16], the ionized form exhibits reduced absorption compared to the non-ionized (or neutral form), which has lower solubility. This phenomenon is explained by the greater capacity of the non-ionized form to cross both biological membranes and those of the gastrointestinal tract. So, by enabling the passage of non-ionized molecules, these membranes contribute to a more efficient absorption of the non-ionized form.

Furthermore, once the active pharmaceutical ingredient (API) has been absorbed and is in the bloodstream, it moves throughout the body toward its specific site of action or target organ, interacting

with plasma proteins. In practical terms, the neutral form of the drug is the most suitable for this transport since, despite its low solubility, it demonstrates more effective absorption [17].

It is essential to highlight that the interactions of the drug with plasma proteins must be strong enough to facilitate its transit. However, this binding should not be so solid, as this could prevent the effective release of the drug in the target organ, leading to a decrease in pharmacological efficacy and, potentially poisoning [18].

On the other hand, if the interaction with proteins is not strong enough, the drug could accumulate in a specific area, also causing toxicity. This is because the neutral form of the drug has a low degree of ionization and an inability to dissolve in water, which leads to its accumulation in fatty tissues instead of being rapidly eliminated [18].

Thus, the inefficient transport of non-ionized or neutral forms of the drug, characterized by its low water solubility and its affinity for fats (lipophilia), could cause difficulties in excretion through urine. This suggests that a low degree of ionization and insufficient interaction of the API with plasma membranes could complicate drug elimination, increasing the risk of toxicity [19].

In this sense, metabolization is a crucial process to prevent toxic accumulation since it facilitates the elimination of foreign substances from the body. The beginning of this process involves a series of chemical changes in the drug before it enters the bloodstream, leading to the formation of ionic metabolites that the body can easily discard through urine. From this point of view, a higher degree of ionization is needed for metabolization to occur more fluidly.

It is crucial to note that, during the drug release process in the body, a free part of the active ingredient does not undergo initial metabolization. After playing its role, this fraction usually fulfills its therapeutic function and is eliminated unchanged upon reaching the kidneys, where it is expelled through the urine. Therefore, determining the pK_a of the active pharmaceutical ingredient (API) is essential to understanding its degree of ionization in various biological fluids and at different pH values. This value provides information on the drug's proportion of ionized or neutral species under varying conditions [17]. In this context, exploring the dissociation equilibrium equations for monoprotic acids and bases is

essential, as it offers a solid theoretical framework that quantifies the relationship between pK_a and API dissociation. This exploration not only reinforces the practical importance of pK_a in the interaction of the drug with the organism but also highlights the need to understand the theoretical bases that support this concept in the pharmacological field.

Equations of the dissociation equilibrium for a monoprotic acid and base [14]:

- For a monoprotic acid (HA):

$$AH \rightleftharpoons [A^-] + [H^+] \qquad (Eq.1)$$

$$K_a = \frac{[A^-]\,[H^+]}{[AH]} \qquad (Eq.2)$$

- For a monoprotic base (BH):

$$BH^+ \rightleftharpoons [B] + [H^+] \qquad (Eq.3)$$

$$K_a = \frac{[B]\,[H^+]}{[BH^+]} \qquad (Eq.4)$$

- And:

$$pK_a = -\log(K_a) \qquad (Eq.5)$$

Equations 1 and 2 illustrate the dissociation equilibrium for a monoprotic acid (HA), while equations 3 and 4 describe this process for a monoprotic base (B), both linked by the acid-base equilibrium constant (K_a). In the case of monoprotic acid (HA), its dissociation is represented by equation 1, where the acid is ionized, releasing protons into the solution. On the other hand, the monoprotic base (BH) undergoes ionization according to equation 3, in which the base captures a hydrogen ion to form a conjugate acid.

These ionization processes affect the balance between acidic and basic species in solution. The associated constants, K_a and pK_a, quantify these tendencies, where K_a indicates tendency towards dissociation, both for acids and bases, indicating the ease with which these substances release or accept

protons in an aqueous solution. While pK_a, being the negative logarithm of K_a (see Eq. 5), provides a more precise quantitative measure of a substance's propensity to ionize as an acid or a base [18].

It is essential to mention that the constants in equations 2 and 4 focus exclusively on evaluating concentrations, assuming an ideal solution scenario and omitting possible interactions with other substances present in the solution. This simplified approach starts from the idea that the solution follows a theoretically perfect behavior without considering the natural complexities that could arise from the interactions between the different substances in the solution. These interactions, which are not considered in the ideal model, could significantly impact the effective concentration of the species involved in the constant. So, to address this challenge, the importance of resorting to activities instead of concentrations is highlighted, especially in non-ideal situations, which are expressed in the following equation:

$$K_a = \frac{a_{A^-}.a_{H^+}}{a_{AH}} \qquad (Eq.6)$$

Activities (a) provide a means to determine effective concentrations considering interactions between species, especially when the solution does not follow ideal behavior due to factors such as the presence of additional ions. Furthermore, the activities are intrinsically linked to the ionic strength of the solution: as the ion concentration increases, so does the ionic strength, intensifying the interactions between chemical species. Then, this method not only improves the precision of chemical constants under non-ideal conditions, but also provides a deeper understanding of how ionic interactions affect chemical reactions in solution [18, 22].

1.2.2 Intrinsic Solubility (S_0) and Thermodynamic Solubility (S)

Intrinsic solubility (S_0) and thermodynamic solubility (S) play fundamental roles in how compounds dissolve in aqueous solutions. While thermodynamic solubility (S) is the maximum amount of solid sample that can dissolve in a given amount of solvent at a certain pH and temperature [19]; occurring whenever an equilibrium is established between the solid and solution phases; intrinsic solubility (S_0)

is the capacity of an ionizable compound to dissolve at a pH where the compound exists as a free acid or base [20].

In this context, the Henderson-Hasselbalch (HH) equations emerge as invaluable tools. These equations, derived from equilibrium processes, allow for the calculation of the solubility profile of a monoprotic compound using only two experimental determinations: the compound's pK_a and its intrinsic solubility [21]. This simplicity and effectiveness make the Henderson-Hasselbalch model an essential tool in chemistry and biochemistry, facilitating the understanding and prediction of acid-base behavior and compound solubility in aqueous solutions.

Thus, from equations 1 and 2, the following equations are deduced, detailing the specific solubility equilibria and the HH equation for **monoprotic acids** [14]**.**

$$[AH]_{(s)} \rightleftharpoons [AH]_{(aq)} \qquad (Eq.7a)$$

$$S_0 = [AH]_{(aq)} \qquad (Eq.7b)$$

$$S = [AH]_{(aq)} + [A^-] \qquad (Eq.8)$$

$$logS = logS_0 + log(1 + 10^{pH-pK_a}) \qquad (Eq.9)$$

Similarly, from equations 3 and 4, the following equations are deduced, which detail the specific solubility equilibria and HH equation for **monoprotic bases** [14]**.**

$$[B]_{(s)} \rightleftharpoons [B]_{(aq)} \qquad (Eq.10a)$$

$$S_0 = [B]_{(aq)} \qquad (Eq.10b)$$

$$S = [B]_{(aq)} + [BH^+] \qquad (Eq.11)$$

$$logS = logS_0 + log(1 + 10^{pK_a-pH}) \qquad (Eq.12)$$

In this sense, Eq. 7 - Eq. 9 describe how the solubility of an acidic solute changes as a function of the pH of the medium. When the pH of the medium is higher than the pK_a of the acidic solute, the concentration of hydrogen ions ([H^+]) is low. This low concentration favors the chemical equilibrium

to shift to the right, which means that more molecules of the acidic solute dissolve in the aqueous medium because of an increased ionization [21, 22]. In contrast, when pH is lower than the pK_a, the ionization decreases, a neutral form is produced, and solubility decreases [21]. Although the pH may vary, the solubility remains unchanged since the molecules do not undergo ionization. This constant level of solubility is known as the intrinsic solubility (S_0) of the compound, reflecting a state in which the molecules have not undergone any ionization process.

Figure 1 illustrates the relationship between the solubility (S) and pH for a typical monoprotic acid given by Eq. 9. The graph shows two key segments: a plateau representing S_0 and a linear one with a slope of 1, which indicates how ionized species contribute to solubility. The intersection of the extrapolation of these two segments reveals the pK_a of the molecule [27].

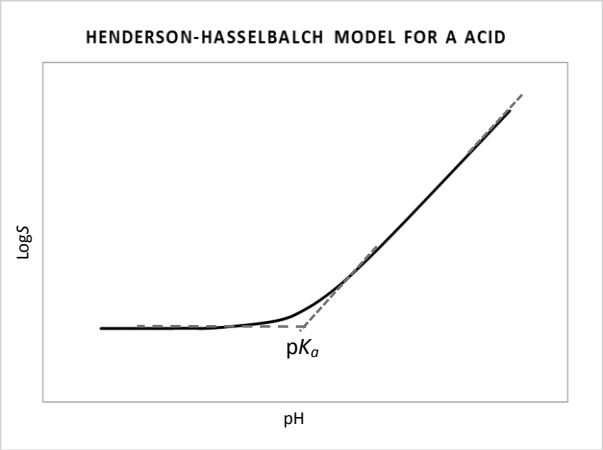

***Figure 1**. A schematic illustration of the pH-dependent solubility profile for a monoprotic acidic drug.*

Likewise, the solubility equilibrium associated with a monoprotic base is described by equations (Eq. 10 – Eq. 12) and is presented graphically in Figure 2. In this sense, it is crucial to emphasize that when the pH of the solution is below the pK_a of the base, an increase in the concentration of hydrogen ions ([H+]) is evident [24]. This increase leads to a rise in the base's ionization degree [24]. The underlying reason for this phenomenon lies in the shift of the equilibrium to the right, thus promoting the formation of BH^+ and increasing the solubility of the base in the solution [25]. On the contrary, when the pH exceeds the pKa of the base, the concentration of hydrogen ions decreases, minimizing the formation of BH^+ [21, 24]. As a result, the base tends to remain primarily in its non-ionized form, leading to a

decrease in its solubility [24, 25]. In this particular state of the compound, the solubility is denoted as S_0.

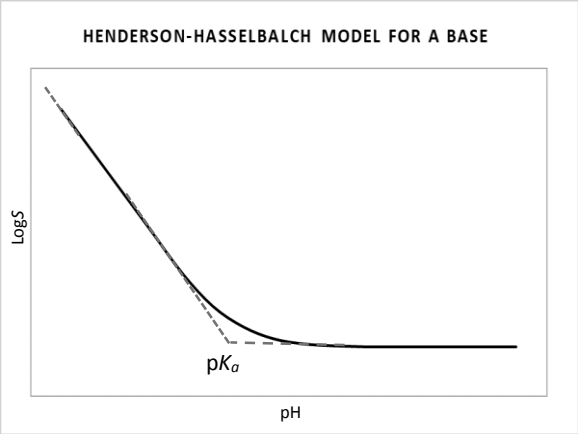

Figure 2. *A schematic illustration of the pH-dependent solubility profile for a monoprotic basic drug.*

It is relevant to highlight that the representations of Henderson-Hasselbalch (HH) models for both acids and bases are provided in figures 1 and 2 where the solubility (S) of the compound is conceived as the cumulative molar concentrations of both the neutral and charged species within the solution, offering a more detailed understanding of solubility processes as a function of pH. Generally, in these models, solubility is mainly influenced by acid-base equilibria, assuming there are no other significant parallel reactions. However, it is essential to emphasize that buffer components used in solubility studies and body fluid constituents can interact with the sample. Furthermore, the compound under study could interact with itself due to its chemical structure, giving rise to aggregation reactions [27].

Adjusted models based on the Henderson-Hasselbalch equations are used in aggregate formation situations. These models are specifically designed to consider the impact of other equilibria on the solubility process. In this context, equation (nAH)⇌(AH)n describes a chemical equilibrium for a monoprotic acid before deprotonation [18]. The notation (AH)n indicates the formation of molecular aggregates after the loss of a proton, and the term n allows to model how different amounts of acid molecules (nAH) participate in creating these aggregates.

Similarly, the equation nB⇌(B)n, which represents a monoprotic base before protonation, allows us to determine by the term n how many units of the base (nB) are involved in the equilibrium [18]. This

flexibility in the choice of n allows the equation to be adjusted to accommodate various numbers of molecules, demonstrating the versatility and applicability of the HH model.

In this same sense, equations emerge that detail the interactions of the sample with the additional components present in the solution. In this context, the equations ($nA^- + nAH \rightleftharpoons (AH \cdot A^-)n$) and ($nBH^+ + nB \rightleftharpoons (BH^+ \cdot B)n$) outline the formation of aggregates between the negatively or positively charged form of the sample and its neutral form [18]. These equations illustrate how species in the sample can interact with each other, adding complexity to solubility processes. Additionally, following this perspective, another equation stands out, represented by $(AH+X) \rightleftharpoons [AH \cdot X]$, which models the interaction between the sample and any external component "X" (it can be a component of the buffer, or another reagent added), and which also offers a clear example of how external interactions influence the significant in the solubility of the compound [18]. Besides, this can also happen with negatively charged components, depending on the charged state of the analyte, as can be seen in Figure 3. In this figure also can be appreciated pHmax, that marks the point where the neutral form of the compound coexists with the solid salt formed by the compound and other component present in the medium, thus reaching the maximum solubility of the neutral species and starting the formation of salt.

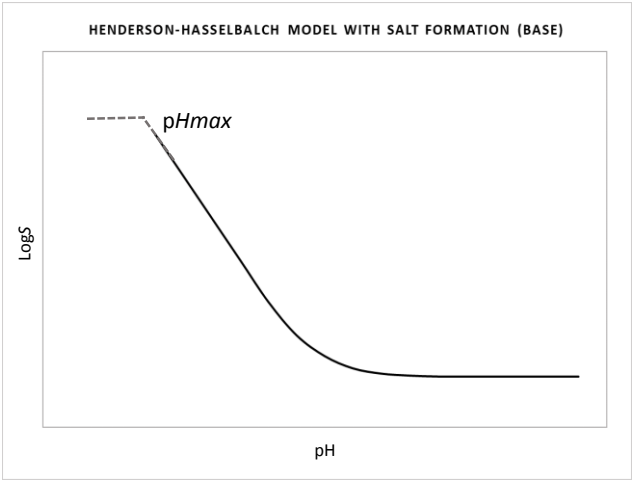

Figure 3. *Graphic representation of the pH solubility profile for a base, emphasizing the presence of a maximum pH when a salt is formed.*

1.3 Biopharmaceutical Classification System

Delving into pharmacology requires a deep understanding of how compounds achieve optimal bioavailability. This intricate process relies on the delicate interplay between a compound's solubility and permeability. Once dissolved and reaching its zenith concentration in gastric or intestinal fluid, the compound faces the critical task of traversing cell membranes within the intestinal wall to access the bloodstream. The diverse solubility and permeability levels exhibited by molecules shape their ability to dissolve and navigate these membranes, thus directly influencing their absorption [30].

The bioavailability of orally administered drugs is always less than 100%, unlike those administered intravenously, where all the solute is completely dissolved. The proximity between oral and intravenous bioavailability indicates bioequivalence. Bioequivalent studies, in vivo to analyze the concentration in blood plasma, are complemented with in vitro or in silico models to evaluate the permeability and bioavailability of the drug, comparing results with those obtained in vivo. This relationship is linked to the Biopharmaceutical Classification Systems, which classifies medications based on their solubility and permeability characteristics, impacting the bioavailability and bioequivalence of pharmaceuticals [31].

Biopharmaceutical Classification System (BCS) has been an essential pillar in pharmaceutical research and development, providing a theoretical framework to understand and optimize the bioavailability of medications, especially those administered orally. This classification, which categorizes drugs into four different classes (Class I to IV) (see Figure 4), is based on the interrelation of two fundamental properties: the solubility of the drug in biological media and its permeability through cell membranes [17]. Thus, the BCS, which guides decisions in drug formulation and development, will be described in detail below.

Class I: High Solubility and High Permeability

Class I of the biopharmaceutical classification (BCS) includes drugs with exceptional characteristics of high solubility in biological fluids and a significant ability to cross cell membranes. These compounds,

being highly soluble, achieve a bioavailability close to 100%. The practical implications of this class are substantial since oral administration of these drugs tends to be highly efficient. They do not require extensive correlation between in vitro and in vivo studies [32], simplifying the drug development and formulation process [33]. The high bioavailability and therapeutic efficacy make Class I highly desirable for formulators and drug developers.

Class II: Low Solubility and High Permeability

In Class II, drugs exhibit low solubility in biological media but show a high ability to cross cell membranes. The bioavailability of this class is highly dependent on the solubility of the drug, implying that the formulation must address this challenge. Practical implications suggest the need for specific formulations to improve solubility and bioavailability. These drugs may require detailed in vivo and in vitro studies to demonstrate bioequivalence, adding complexity to the development process [27, 29]. Class II highlights the importance of finding innovative solutions to improve solubility and therapeutic efficacy.

Class III: High Solubility and Low Permeability

Class III includes drugs highly soluble in biological fluids but with a low ability to cross cell membranes. Although the solubility is high, the bioavailability is not conditioned by this factor. Practical implications suggest that, although absorption may not be efficient due to low permeability, improvements in this property could benefit bioavailability. Formulation can focus on strategies to improve permeability, which could lead to greater therapeutic efficacy [27]. Class III highlights the complexity of balancing high solubility with improving permeability for efficient oral administration.

Class IV: Low Solubility and Low Permeability

Class IV groups drugs with low solubility in biological media and low ability to cross cell membranes. These compounds present substantial challenges for oral administration [28, 30, 31], as their bioavailability is low, and efficient absorption requires significant improvements in both properties. In cases where these improvements are not feasible, the formulation for parenteral administration is

recommended. Practical implications highlight the need for innovative approaches and specific formulation strategies to overcome the intrinsic limitations of low solubility and permeability. Class IV underscores the importance of considering administration alternatives when significant improvements are challenging to achieve orally.

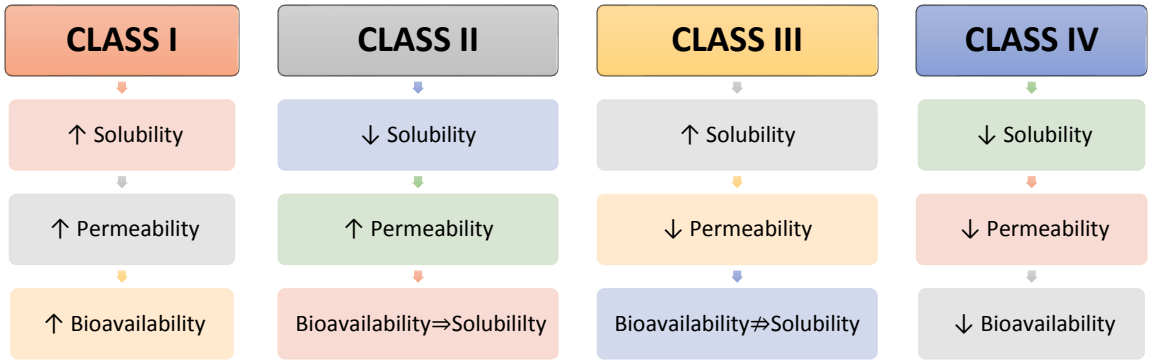

Figure 4. *Biopharmaceutical Classification System (BCS) based on the solubility and the permeability.*

It is important to note that in the 1970s and 1980s, solubility was not a significant problem in drug development. At that time, most therapeutic molecules exhibited adequate physicochemical properties, including good solubility, which facilitated their formulation into tablets without requiring substantial adjustments.

However, as time has passed, drug-candidate molecules have evolved towards more complex structures with more challenging chemical properties, often characterized by greater lipophilicity. This change has led many of these molecules to be classified in Class II, indicating a tendency to have lower solubility [32]. Therefore, it becomes crucial to adjust in the molecule itself or the formulation to address the challenges associated with low solubility.

Adaptation to Class II refers to pharmaceutical molecules that find it difficult to dissolve properly, which could compromise their effectiveness as medicines. Various strategies address this challenge, with excipients standing out as one of the most promising alternatives.

The first strategy consists of adjusting the molecule's chemical structure, seeking to improve its dissolution capacity. However, this approach can be limited, especially if the original structure is designed to interact specifically with a biological site.

Another option is the formation of salts, a process that can improve the solubility of the drug. However, it has limitations since some molecules do not easily form salts, and the choice of counterion can affect the stability and release of the drug.

The third strategy involves the development of specific formulations designed to improve drug solubility. This approach, which may include the addition of excipients or the application of advanced techniques in the preparation of the drug, emerges as an effective and practical alternative.

In this context, using excipients is a particularly prominent strategy since it makes it possible to improve solubility without altering the drug's molecular structure, thus preserving its therapeutic effectiveness. Excipients offer flexibility in choice, adjusting to the specific properties of the drug and formulation needs. Additionally, they facilitate large-scale production and have well-established safety profiles, mitigating regulatory risks. Therefore, using excipients is presented as an effective and practical option to address solubility challenges in developing and formulating new pharmaceutical products.

1.4 Active Pharmaceutical Ingredient (API) in the drug formulation

The Active Pharmaceutical Ingredient (API) represents the essential component in the formulation of any medication, being the primary chemical substance that gives the drug its specific therapeutic activity. This central role of the API in drug efficacy underscores the critical importance of its selection and formulation in the pharmaceutical development process [38].

API quality is essential to ensure that a final pharmaceutical product or drug is safe and effective. Any variation in the API's quality, purity, or identity can have significant consequences on the therapeutic response and safety of the drug. Therefore, rigorous quality standards and controlled manufacturing practices are imperative in API production. In this sense, the implementation of standardized procedures and adherence to specific regulations are essential to ensure the consistency and reliability of the API, thus, contributing to the integrity of pharmaceutical products.

Furthermore, drug formulation faces fundamental challenges, such as the limited water solubility of APIs and the need to achieve controlled release in the body [33]. The solution to these challenges is carefully selecting excipients and understanding their interaction with the API. Appropriate excipients can improve the solubility of the API, facilitating its absorption in the body [34]. Moreover, the adequate selection of excipients can enable a regulated and prolonged API release, maintaining consistent therapeutic concentrations [35]. This precise interaction between the API and excipients directly affects the quality of the drug and its safety and effectiveness.

Therefore, in this work, the choice of Fluconazole (FLU) and Ibuprofen (IBU) as APIs to study the effect of excipients on the solubility of different APIs is based on several reasons. Firstly, both molecules have diverse physicochemical properties, which facilitates the evaluation of the impact of the excipients on different molecules. Furthermore, the extensive clinical application of both drugs adds immediate relevance to research focused on their solubility. Likewise, the therapeutic diversity between Fluconazole (FLU) (antifungal) and Ibuprofen (IBU) (non-steroidal anti-inflammatory) allows for investigation of how the excipients affect APIs with different clinical functions, addressing challenges in solubility and contributing to the understanding of the excipient-API interaction. In summary, the strategic choice of Fluconazole (FLU) and Ibuprofen (IBU) enriches research by addressing various aspects of solubility and interaction with excipients in the pharmaceutical field.

- **Ibuprofen (IBU)**

Ibuprofen (IBU), categorized as a nonsteroidal anti-inflammatory drug (NSAID) and renowned for its analgesic and antipyretic effects, has found extensive application in addressing conditions like rheumatoid arthritis, joint degeneration, ankylosing spondylitis, and acute gouty arthritis [36]. Its chemical structure, represented in Figure 5, is a propionic acid derivative characterized as an amphipathic molecule with a carboxyl group and an aromatic ring [37].

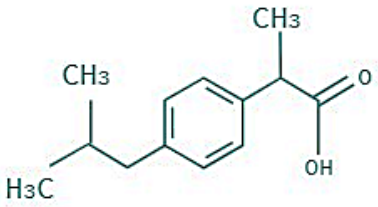

Figure 5. *Chemical Structure – Ibuprofen (IBU).*

Under the systematic (IUPAC) name of (RS)-2-(4-(2-methylpropyl)phenyl)propanoic acid [36], Ibuprofen (IBU), a weak monoprotic acid, belongs to the group of drugs that are in Class 2, according to the Biopharmaceutical System Classification (BSC) due to its low solubility, especially at low pH levels, but high permeability [39].

- **Fluconazole (FLU)**

Fluconazole (FLU), whose chemical name is (2-(2,4-difluorophenyl)-1,3-bis(1H-1,2,4triazole-1-yl)-propan-2-ol), is a premier triazole antifungal agent generation [43]. Its application covers preventing and treating various fungal infections, such as candidiasis, blastomycosis, coccidioidomycosis, pityriasis versicolor, cryptococcosis, histoplasmosis, and dermatophytosis [37].

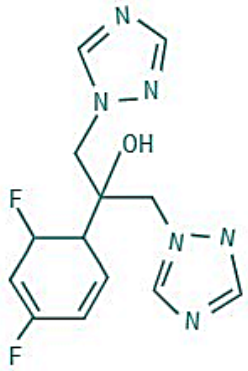

Figure 6. *Chemical Structure – Fluconazole (FLU).*

Figure 6 provides a visual representation of the chemical structure of Fluconazole (FLU), particularly highlighting the existence of a triazole ring in its chemical structure [43]. This triazole ring is a distinctive feature crucial in making Fluconazole (FLU) especially suitable for systemic treatments. Also, Fluconazole (FLU) is a weak base belonging to the drug's class I group according to the Biopharmaceutical Classification System (BCS), characterized by its high solubility and high permeability [39].

1.5 Excipients in the drug formulation

In the context of the formulation of medicines and other pharmaceutical preparations, an excipient refers to any substance intentionally incorporated into the formulation, apart from the active ingredient, to fulfill various functions that contribute to the drug's quality, stability, administration, and acceptability of the final product. These inert and non-therapeutic components play fundamental roles in the manufacturing and performing pharmaceutical forms, improving physical, chemical, and organoleptic properties.

Excipients can cover various functions, such as coating agents, stabilizers, thickeners, emulsifiers, colorants, preservatives, among others. Each excipient is carefully chosen based on its ability to fulfill specific functions in the formulation, ensuring drug efficacy and patient safety. Excipients used in this work are described below:

- **β-Cyclodextrin (BCD)**

β-cyclodextrins (BCD) are cyclic oligosaccharides consisting of seven D-glucose units joined by α-1-4 glycosidic bonds (Figure 7) [45]. They have a unique ring-shaped molecular structure consisting of an external region that interacts well with water (hydrophilic) and an internal cavity with an affinity for water-insoluble (lipophilic) substances [45]. This dual characteristic enables beta-cyclodextrins to form inclusion complexes by encapsulating non-aqueous molecules through non-covalent bonds.

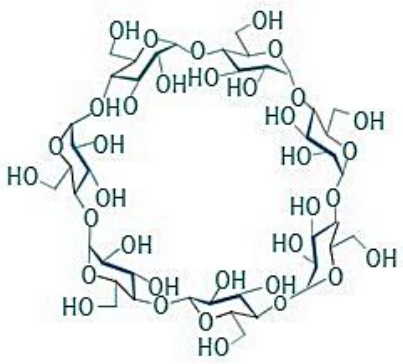

Figure 7. *Chemical Structure – Beta-Cyclodextrin (BCD).*

In the pharmaceutical industry, BCD, classified as a cyclodextrin, plays a crucial role. It functions as a complexing agent by forming inclusions with hydrophobic molecules, enhancing their solubility and absorption capacity [46]. Likewise, it serves as a crucial stabilizer agent and taste and odor masker, providing flexibility in the formulation of pharmaceutical products [42–44]. Furthermore, BCD stands out for its ability to regulate drug release, making it a valuable component to refine specific release profiles.

- **Polyvinylpyrrolidone (PVP)**

Polyvinylpyrrolidone (PVP) is a synthetic polymer obtained by polymerizing vinylpyrrolidone in an aqueous solution, where hydrogen peroxide acts as a catalyst. Its chemical structure (Figure 8) includes functional groups such as carbonyl (C=O), methylene (CH_2), and single bonds between carbon and nitrogen atoms [45]. This unique configuration encompasses a highly hydrophilic section, represented by pyrrolidone, and another significantly hydrophobic section, composed of the alkyl group [46]. Combining these elements gives PVP distinctive properties, highlighting its biocompatible, inert, non-ionic, and non-toxic nature [52]. These characteristics facilitate its efficient interaction with hydrophilic and hydrophobic drugs, consolidating its position in various applications.

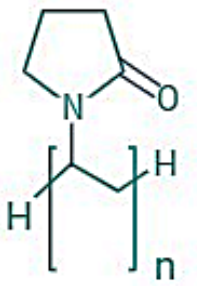

Figure 8. *Chemical Structure - Polyvinylpyrrolidone (PVP).*

Similarly, as an excipient, PVP acts as a stabilizing agent to prevent degradation, a binder for tablet cohesion, a thickener in liquid formulations, a dispersant for uniform particle dispersion, and a solubilizer to enhance bioavailability [45]. Thus, its diverse functions highlight its essential role in ensuring pharmaceutical formulations' stability, effectiveness, and quality.

- **Carbomer (CBER)**

Carbomer (CBER) (Figure 9) is a solid hydrophilic polymer, distinguished by its white color, resulting from the combination of allyl sucrose or allyl pentaerythritol with acrylic acid [48]. Notably, it contains a significant number of carboxyl groups [48, 49]. Among its most common variants, this compound includes Carbomer 934 (carboxy polymethylene), ideal for pharmaceutical formulations with controlled drug release; Carbomer 940, used in topical and ophthalmic products due to its ability to form clear, viscous gels; Carbomer 941, strategically formulated to enhance the stability of emulsions with an oil-in-water composition; and Carbomer 980, which stands out for the formation of transparent gels and its good suspension properties for insoluble particles.

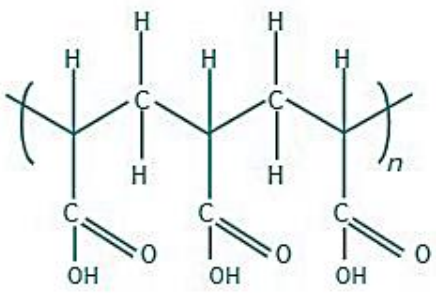

Figure 9. *Chemical Structure – Carbomer (CBER).*

It is essential to highlight that Carbomer is safe for human application since it does not have carcinogenic, toxic, or allergenic properties. Its primary function is acting as a thickening, dispersing, and stabilizing agent in various formulations [49]. As a thickening agent, it can increase the viscosity of aqueous solutions, being beneficial in producing gels, creams, and lotions to improve their application and adhesion to the skin. Likewise, it performs functions as a dispersing agent, facilitating the uniform distribution of other ingredients and preventing the aggregation of solid or liquid particles. In addition, Carbomer is a stabilizing agent by preventing phase separation and improving the cohesion of ingredients, contributing to the homogeneity and stability of products such as emulsions and suspensions. In summary, Carbomer is revealed as a versatile component compatible with various ingredients, playing an essential role in improving the texture, stability, and effectiveness of a wide range of products.

- **Hydroxypropylmethylcellulose (HPMC)**

Hydroxypropylmethylcellulose (HPMC) is characterized as a semisynthetic, inactive, adhesive, and nonionic polymer derived from cellulose and made up of methyl and hydroxypropyl groups (Figure 10). Its appearance varies between white, yellowish-white, or grayish-white powders or granules, showing hygroscopic properties. The versatility of HPMC is reflected in various functions, playing essential roles as a binder in wet and dry granulations and as a coating agent. In addition, it acts as a stabilizing agent by functioning as a viscosity enhancer in suspensions and emulsions.

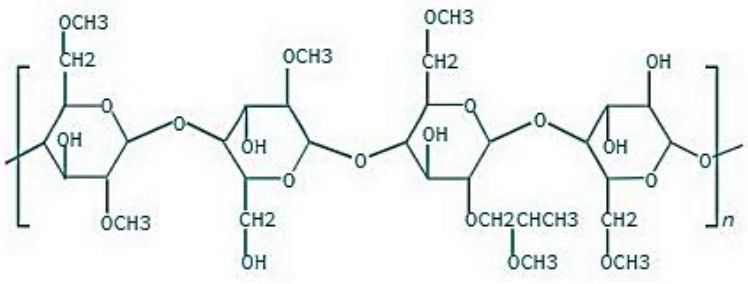

Figure 10. *Chemical Structure – Hydroxypropylmethylcellulose (HPMC).*

In addition, Hydroxypropylmethylcellulose (HPMC) plays a crucial role as a suspending agent, preventing insoluble particles from settling. In addition, it acts as a dispersing agent, contributing significantly to achieving a uniform distribution of particles and improving homogeneity in different formulations, which optimizes the overall quality of the products.

- **Oxalys tuberosa Starch (OCAS)**

Starch from Oca is a new type of starch derived from a tuber that has shown much promise in pharmaceutical research. Although detailed information about its chemical structure and specific function in therapeutic formulations is not yet available, the possibility is raised that it shares crucial properties, such as wettability, swelling, and mechanical strength, with cellulose. This promising excipient could play a relevant role, potentially influencing wettability and affecting the processability and performance of formulations. Additionally, it could have a significant impact on controlled drug release and mechanical properties crucial for tablet compression and disintegration.

Given this uncertainty, the urgency of conducting additional research and experiments to validate and expand knowledge about this novel excipient is emphasized. Thus, this work focuses explicitly on exploring how starch from Oca could influence the solubility of pharmaceutical active ingredients, opening new perspectives in developing therapeutic formulations.

1.6 Literature Review: Excipients and API Solubility.

The scientific literature highlights the low solubility of numerous active pharmaceutical ingredients (API) in aqueous solutions, negatively affecting oral bioavailability. Various formulation strategies seek to improve the aqueous solubility of these APIs, with excipients being crucial components in this context.

A previous study highlights the effective use of β-cyclodextrin, an excipient considered in this research, to enhance the solubility of APIs such as enzalutamide and apalutamide in buffer solutions with pH 7,4 [50]. Furthermore, another study showed that β-cyclodextrin is an efficient solvent for apigenin in various concentrations and physiological pH levels [51].

In another investigation, PVP and HPMC, were used to measure the solubility of aspirin in aqueous systems [52]. These polymeric excipients, especially PVP, showed an outstanding solubilizing capacity [52], a characteristic supported by previous studies [53–55]. Furthermore, further investigation using PVP evaluated the solubilities of indomethacin and naproxen in water [10]. The results revealed a notable increase in the solubility of both compounds [10].

In the case of Ibuprofen (IBU), one of the active pharmaceutical ingredients (APIs) examined in this work, various substances have been explored as excipients to improve its solubility. Studies involving co-milling of Ibuprofen (IBU) with hydroxypropylmethylcellulose (HPMC), using different proportions of IBU and HPMC, revealed a notable increase in the solubility of Ibuprofen (IBU) in these mixtures [56].

Furthermore, HPMC, accompanied by other excipients such as mannitol and leucine, was used in an evaluation of the solubility of Ibuprofen (IBU) in a co-solvent composed of water (W) and ethanol (E),

evidencing a low solubility of Ibuprofen (IBU) without excipients in water, and a significantly improved exponential solubility in a co-solvent system incorporating said excipients [57].

Correspondingly, another study focused on forming inclusion complexes between Ibuprofen (IBU) and β-cyclodextrin to improve solubility at different aqueous media (pH 7,4 and pH 1,5) revealed that these inclusion complexes exhibited an optimal increase in solubility compared to pure IBU [58].

It is crucial to emphasize that, throughout the literature review, no prior studies were found that explicitly investigate the solubility of Fluconazole (FLU) in the presence of excipients or the Carbomer's impact on the solubility of active pharmaceutical ingredients (APIs). Likewise, it is essential to mention that OCAS, a newly created excipient in the Yachay Tech University laboratory, still needs previous research due to its recent nature.

Furthermore, the scarce presence of studies investigating the interaction of the excipients and the APIs selected for this work in a mixture to study solubility is highlighted. This limitation in the scientific literature underlines the relevance and originality of this study, as it aims to address and fill this knowledge gap. By addressing this lack, this work is positioned as a valuable contribution by providing detailed information on how presence of excipients influences on the solubility of the selected APIs solubility. This approach will contribute significantly to advancing pharmaceutical formulation strategies, offering a unique and specific perspective on the components studied in this thesis.

1.7 Justification and relevance of this work

The justification of this work is based on the urgent need to deepen the study of the factors that directly affect the solubility of different APIs in an aqueous media at room temperature (25°C). The presence of enhancers, commonly known as excipients, and the proper identification of excipient-API ratios for the formulation are critical aspects that must be explored in detail to optimize processes in pharmaceutical formulation.

In this context, careful and proportional selection of excipients acquires substantial relevance. Research on the effect of these enhancers on API solubility not only adds to a better knowledge of the chemical

processes involved but also gives practical information for the development of more effective and safe pharmaceutical formulations.

The need to explore solubility at 25°C is essential, considering that this temperature reflects the standard environmental conditions during drug handling, storage, and administration. This specific approach contributes to a more precise and applicable understanding of how excipients can influence the solubility of APIs in real-world situations.

As a common intermediate point in environmental conditions, this strategic choice serves as a preliminary phase before delving into more advanced studies that simulate physiological conditions. This approach optimizes resource management and provides a solid basis for the initial effects of excipients on the solubility of APIs.

It is essential to mention that although absorption under physiological conditions cannot be directly deduced from studies at 25°C, it can be anticipated that excipients that improve solubility will benefit drug absorption. Although not fully representative of physiological conditions, this strategic approach allows for an efficient initial selection of excipients before conducting more detailed studies.

Based on what has been said above, it can be suggested that this work is positioned as a valuable contribution to the pharmaceutical field by directly and in detail addressing the challenges and opportunities associated with the solubility of APIs under ambient conditions, thus providing a solid foundation for the development of advanced pharmaceutical formulations.

1.8 Objectives

1.8.1 General Objective

- Comprehensively investigate the impact of various excipients on the solubility of acidic and basic active pharmaceutical ingredients (APIs) in an aqueous medium at room temperature (25°C).

1.8.2 Specific Objectives

- Determine the pKa and logS0 values of the APIs (Ibuprofen (IBU) and Fluconazole (FLU) through exhaustive bibliographic references, selecting those that optimally resemble the specific conditions of our study.
- Investigate the impact of the addition of different proportions of excipients (beta-cyclodextrin, carbomer, hydroxypropylmethylcellulose, polyvinylpyrrolidone, and O. tuberosa starch) on the solubility of Ibuprofen (IBU) and Fluconazole (FLU) in aqueous solutions at 25°C.
- Compare the solubility of APIs, considering theorical HH models of Ibuprofen (IBU), an acid, and Fluconazole (FLU), a base, when mixed with the excipients.
- Perform a detailed correlation analysis to identify the relationship between the solubility of the APIs (IBU and FLU) and the percentage of each excipient used in the aqueous solutions at 25°C. Appropriate statistical tools will be employed to quantify and validate the strength and direction of the correlation.

CHAPTER II: Materials and Methods

2. MATERIALS AND METHODS

2.1 Molecules, reagents and solutions.

The Pharmaceutical Active Ingredients (APIs) used in this study, Ibuprofen (IBU) and Fluconazole (FLU), were obtained through the purification of capsules or tablets containing these APIs. Regarding the excipients selected for the study, they include Beta cyclodextrin (BCD) from ChemCenter, USA; Polyvinylpyrrolidone (PVP) from SIGMA-ALDRICH, USA; Hydroxypropylmethylcellulose (HPMC) from THERMO Fisher Scientific, USA; Carbomer 980 (CBER) from La Casa de los Químicos, Ecuador; and a new excipient, a starch extracted from Oxalys tuberosa at Yachay Tech University. On the other hand, the chromatographic-grade methanol (MeOH) used as a cosolvent in mobile phase is sourced from Thermo Chemical, Germany.

The buffers and solutions used in the study method included Water ISA (Ionic Strength Adjustment), pH 1.6 Acetate/Phosphate Buffers (Ac/P), and Monobasic Potassium Phosphate Buffers (BPP). A detailed description of their preparations is provided in the "Procedures" section. Additionally, 0.5 M NaOH and HCl solutions were used as pH adjusters to maintain stable and constant pH values in both samples and buffers, following the specific requirements of the procedure.

2.2 Instruments

The necessary inputs for the general assays are detailed as follows: micropipettes, Pasteur-type glass pipettes, plastic tips for calibrates quantification pipettes, 1.5 mL glass vials for HPLC, 5 mL glass test tubes with caps, 5 mL amber vials, and volumetric flasks.

The HPLC column used was a 150x4.6 mm, 5 µm particle size, reverse-phase C18 column from Phenomenex (Torrance, USA), employed for quantifying the Active Pharmaceutical Ingredients (APIs) in various solutions and for the standard solution of FLU and IBU.

Furthermore, a METTLER TOLEDO InLab Ultra Micro glass electrode (Barcelona, Spain) was used to measure pH in small volumes and containers.

2.3 Equipment

For the quantification of the various samples in this study, it is necessary to follow several steps that involve the use of specific equipment, which are described below:

- Cobos Analytical Balance, model HR-150A (Barcelona, Spain): Used for precise mass measurements.
- Orion Star-A111 pH meter (Waltham, USA): Employed to measure the pH of buffers and sample solutions.
- Thermo-scientific orbital shakers (Waltham, USA): Utilized for sample agitation.
- Thermo Scientific SORVALL Legend XTR centrifuge with temperature control (Waltham, USA): Used to expedite the solid-liquid decantation process in the sample.
- Thermo Scientific brand high-resolution or high-efficiency Liquid Chromatography (HPLC) equipment, model Ultimate 3000 (Waltham, USA), with UV/Vis diode array detector: Used for quantifying components in a sample.

2.4 Software

The control of liquid chromatography systems was carried out through Chromeleon®v.7.0, a comprehensive data acquisition software developed by Thermo Fisher Scientific (Waltham, USA).

Additionally, for calculations, data processing, generation of charts, and statistical analysis, Microsoft® Excel® for Microsoft 365 MSO (version 2312 build 16.0.17126.20126) 64-bit was employed. This software facilitated the execution of various tasks, ensuring precision and efficiency in managing and analyzing experimental data during the study.

2.5 Procedure

2.5.1 Preparation of Solutions and Buffers

For Ibuprofen (IBU) samples with different excipients, a pH 1.6 Buffer Acetate/Phosphate (Ac/P) was used, where 4.2528 g of KH2PO4 and 4.2525 g of trihydrated sodium acetate were weighed. Both were dissolved in 250 mL of purified water and placed in a volumetric flask. The pH was then adjusted to the desired level with 0.5 M HCl, specifically the pH at which Ibuprofen (IBU) is completely neutral (pH = 2.05±0.02). This solution has a 0.125 M ionic strength.

For Fluconazole (FLU) samples with different excipients, a Monobasic Potassium Phosphate Buffer (BPP) was used, where 1.0214 g of KH2PO4 was weighed and dissolved with ISA water in a 500 mL volumetric flask, resulting in an ionic strength equivalent to 0.015 M. It is worth noting that the ISA Water for this buffer was prepared by dissolving 11.1842 g of KCl in 1L of purified water, obtaining a solution with a 0.15 M ionic strength. The pH for working with Fluconazole (FLU) in its non-ionized form was also set at 4.5±0.3.

On the other hand, for the buffer used in the mobile phase for the High-Performance Liquid Chromatography (HPLC) separation technique, a Monobasic Potassium Phosphate Buffer (BPP) was used for both Ibuprofen (IBU) and Fluconazole (FLU), using 11.184 g of KCl in 1 L to prepare ISA water for both APIs (Ionic strength of ISA water = 0.15M). Furthermore, a BPP buffer was prepared for each API, where 1.0197 g of KH2PO4 was used for Ibuprofen (IBU) and 1.0226 g of KH2PO4 for Fluconazole (FLU). Both were dissolved with 500 mL of ISA water in a volumetric flask, obtaining a 0.015 M ionic strength in both solutions.

2.5.2 Determination of pK_a and logS_0 for Ibuprofen (IBU) and Fluconazole (FLU):

A thorough review of multiple existing studies was conducted to accurately determine the pK_a and logS_0 values for the active principles of Ibuprofen (IBU) and Fluconazole (FLU). The search focused on those

sharing similar conditions, prioritizing a temperature of 25°C. During this phase, special attention was given to the quality and reliability of the data, ensuring its relevance to the specific study conditions.

Subsequently, a critical analysis of the selected pK_a and logS_0 data from the literature was performed. This analysis focused on scrutinizing the methodology of the chosen studies, ensuring its rigorous applicability to the study's specific context. It is important to note that the logS_0 value was later used to construct the reference Henderson-Hasselbach (HH) model for both acid and base. The graph enables deducing the percentages by which the excipient significantly enhances the solubility of the active principles.

Furthermore, pK_a values were used to identify the pH range in which the active principles predominated in their non-ionized form. With this data, pH was frequently adjusted in solubility studies to ensure the compounds were non-ionized throughout the experiment. This adjustment effectively evaluated the excipients' impact on solubility in an aqueous medium at 25°C.

2.5.3 Preparation of standards for HPLC

To prepare standards for Ibuprofen (IBU) and Fluconazole (FLU) in HPLC, high-purity samples of each compound are selected and precisely weighed or measured. These standards were obtained from stock solutions (5.1 mg of IBU in 25 mL of methanol and 26.4 mg of FLU in 5 mL of methanol, resulting in concentrations of 204 ppm and 5280 ppm, respectively). Subsequently, several dilutions were performed to create sets of 5-6 standards for each API, with concentrations presented in Table 1.

Table 1. Standards concentrations of Ibuprofen (IBU) and Fluconazole (FLU)

Standards Concentrations	
Ibuprofen (IBU)	**Fluconazole (FLU)**
204 PPM	5280 ppm
40.8 PPM	2640 ppm
20.4 PPM	1320 ppm
8.16 PPM	660 ppm
4.08 PPM	280 ppm
2.04 PPM	

It is important to mention that these standards represent indispensable assets in guaranteeing the precision and consistency of the HPLC measurements, thereby bolstering the credibility and reliability of analytical findings.

2.5.4 Shake-Flask Determinations

In the initial phase of sample preparation for Shake Flask Determinations, IBU and FLU were mixed with a selection of excipients, namely BCD, HPMC, PVP, CBER, and OCAS. This process, which involved no prior treatment except the necessary grinding using a mortar to obtain a homogeneous solid mixture, yielded solid mixtures primed for solubility assays. These mixtures were prepared in approximate proportions of 95±0.3% and 5±0.3%, 85±0.2% and 15±0.2%, 75±0.2% and 25±0.2%, 55±0.2% and 45±0.2%, and 25±0.2% and 75±0.2% of excipient and API, respectively. Thus, Tables A.1 and B.1 in the Appendix show the specific proportions used for each API and excipient.

In this process, the protocol suggested by Avdeef et al. [11] was implemented. Around 150 mg of mixtures were prepared and placed in 5 mL amber vials. Each one of mixtures were divided into three equal parts, using weights from 30±2 mg, 55±5 mg, 80±5 mg, and 150±2 mg depending of the sample, and distributed in 3 mL test tubes (refer to exact weights in the tables in the Appendix A.2 and B.2). Then, 2.5 mL of aqueous medium was added to each test tube for the subsequent solubility study. For IBU, the Ac/P buffer was used as the aqueous medium, while for FLU, the BPP buffer was used. It is essential to mention that the specific choice of these buffers is based on the need to keep the drugs in

their neutral forms during the solubility study. By maintaining a pH that favors the non-ionized form, acid-base interactions that could affect the solubility and chemical stability of the drugs are minimized. This balance is essential to ensure that the results accurately reflect the solubility properties of the drugs under pharmacologically relevant conditions.

After preparing the samples, they were agitated using orbital shakers for 24 hours. During this process, the pH was frequently monitored and adjusted to keep it stable, close to the initial pH at which the compound is completely neutral. 0.5 M HCl or NaOH solutions were used to correct the pH to the value closest to the initial.

After agitation, the samples rested for an additional 24 hours, and at the end of this period, the pH was measured before centrifugation to obtain the final pH. It is important to note that the samples were maintained at 25 ± 3 °C during the agitation and resting periods. Centrifugation was conducted at 25 °C for a duration of 30 minutes at 4500 rpm. Subsequently, the supernatant was collected with Pasteur pipettes to fill 1.5 mL glass HPLC vials, avoiding the collection of solids. The collected supernatants were injected into the liquid chromatograph (HPLC) under the following experimental conditions: a mobile phase composed of a mixture of chromatographic-grade methanol and BPP buffer in a ratio of 70:30 v/v. The BPP buffer was adjusted to a pH of 6.01 for samples with Ibuprofen (IBU) and 7.06 for samples with Fluconazole (FLU).

The liquid flow operated at a 1 mL/min rate, and a 5 µL injection volume was utilized. A UV detector with a wavelength of 265 nm for Fluconazole (FLU) and 220 nm for Ibuprofen (IBU) was employed. The data quantified by HPLC was processed with Microsoft Excel, generating a calibration curve from standards to calculate unknown concentrations and, hence, obtaining the solubility values of the APIs with respect to the added excipients. With these solubility data obtained, the respective graphs, calculations, plots, and statistical analyses (correlation analysis with R^2, ANOVA and Tukey Tests) necessary to achieve the established objectives in the research were carried out.

In the case of correlation analysis, scatter plots were first created to visualize possible patterns and trends. Subsequently, the determination coefficient (R^2) was computed for each sample to analyze and

assess the relationship between each excipient and the enhancement in compound solubility. This involved identifying whether the correlation between API: Excipient is positive, negative, or null, and determining the type of relationship that best aligns with the data, be it linear, logarithmic, or another form.

On the other hand, the construction of the Henderson-Hasselbalch model was based on pK_a and logS_0 values obtained through a comprehensive analysis of scientific literature. Once the solubility curve as a function of pH was obtained, the solubilities corresponding to each percentage of excipients were incorporated, thus determining the optimal percentage of excipient that maximizes the solubility of each active principle with respect to each excipient.

The introduction of the solubilities of the active principles into the pH-dependent solubility reference curve allowed for accurately identifying the point or points corresponding to the excipient percentages at which the solubility of the API is improved. To statistically validate these results, ANOVA and Tukey tests were conducted, providing a solid and reliable assessment of the significance of the excipient on the solubility of the APIs.

Thus, this comprehensive approach, combining literature data, Henderson-Hasselbalch modeling, and rigorous statistical analysis, ensures an accurate and reliable interpretation of how excipients impact the solubility of active principles, significantly contributing to understanding the results obtained in the study.

CHAPTER III: Results and Discussion

3. RESULTS AND DISCUSSION

3.1 Reference pK_a and logS_0 values for Ibuprofen (IBU) and Fluconazole (FLU)

The carefully selected pK_a and logS_0 values for Ibuprofen (IBU) and Fluconazole (FLU) are critical in assessing excipients' impact on these APIs' solubility. This choice is supported by the need for values to be comparable to the specific conditions of the study, considering that the experiments took place in a water-based medium at room temperature (25°C).

In relation to IBU a monoprotic compound with a single pK_a in acidic conditions, literature reports multiple pK_a values at different temperatures (see Table 2). However, this study solely focused on values obtained at room temperature, finding two values in this context. To address variability, the decision was made to average these two values, resulting in an average pK_a of 4.48. Similarly, for the logS_0 of IBU, two values were reported at room temperature (see Table 2), which averaged, is a value of logS_0 of -3.805.

Table 2. Reference values of pK_a and logS_0 for Ibuprofen (IBU) at different temperatures.

IBUPROFEN (IBU)					
pK_a	**T °C**	**Ref. (pK_a)**	**logS_0**	**T °C**	**Ref. (logS_0)**
4.45	25±0.5; 37	[59, 60]	-3.17	37	[39, 61, 62]
4.51	25±0.1	[63]	-3.48	37	[64]
4.57	37	[64]	-3.62	24.9	[65]
4.91	-	[66, 67]	-3.99	25	[68]
5.2	-	[69]	-4.31	-	[70, 71]
			-4.47	37	[71]

In contrast, FLU generally has two pK_as. However, this study focuses explicitly on the pK_a of the base, as the other pK_a is not relevant to the investigation, given that in this study, it is working in a zone where

the basic API is completely neutral. Thus, like IBU, the pK_a at room temperature (25°C) was selected, resulting in a value of 1.76. Similarly, investigating the LogS_0 of FLU at 25°C revealed a value of -1.79. It is worth to notice that, in the case of FLU, there was no need to average the values, as the literature provided only these values for logS_0 and pK_a at room temperature, as seen in Table 3. This work was developed under 25°C, thus the reference values were also selected at this temperature.

Table 3. Reference values of pK_a and logS_0 for Fluconazole (FLU) at different temperatures.

FLUCONAZOLE (FLU)					
p*Ka*	**T °C**	**Ref. (pK_a)**	**logS_0**	**T °C**	**Ref. (logS_0)**
1.5	-	[72, 73]	-1.79	23	[39, 74–77]
1.76±0,1	23; 24	[39, 75, 77]	-1.80	-	[76, 78]
2.03	37	[79–82]	-1.90	-	[76, 83]

From this perspective, using pK_a and logS_0 values was crucial for constructing the Henderson-Hasselbalch (HH) model. In the present study, this model facilitated a practical comparison between experimental solubilities in the presence of excipients and under neutral conditions of the APIs, considering an aqueous medium at room temperature (25°C).

For IBU, an acid with a pK_a of 4.48, a pH of 2.05±0.15 was stablished during the procedure because according to its HH model at this pH value, this acidic compound will be in its neutral form [84, 85]. In contrast, for FLU with a pK_a of 1.76, a pH higher than the pK_a was chosen, with a value of 4.5±0.5. This is because, at this pH, the deprotonated form predominates, ensuring that the base is in its neutral state [84, 85]. Hence, these values are the reference for the present work.

3.2 Molecular interactions during the procedure

Figures 11 and 12 show the variability between initial and final pH of Fluconazole (FLU) and Ibuprofen (IBU). It is essential to highlight that, during stirring, the pH variations for IBU were not significant. However, in the case of FLU, considerable changes in pH were recorded. These changes were corrected

during the procedure using pH adjusters such as 0.5 M NaOH and 0.5 M HCl, achieving a final pH similar to the initial values.

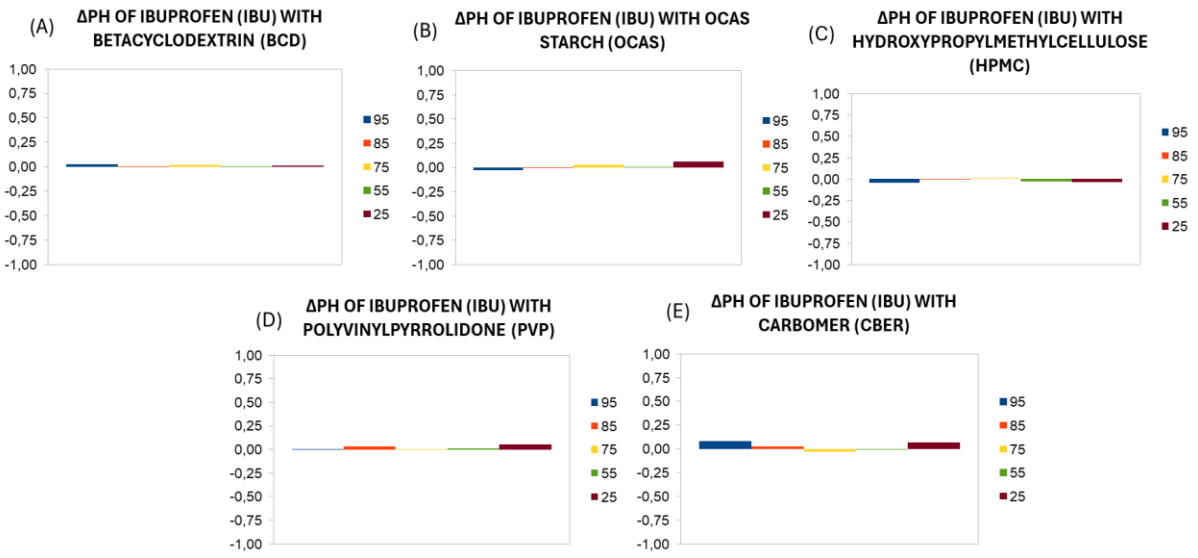

Figure 11. *pH variation in the mixture of Ibuprofen (IBU) with different excipients, which are BCD (A), OCAS (B), HPMC (C), PVP (D) and CBER (E); evaluated in different proportions of excipients, which are 95% (blue), 85% (orange), 75% (yellow), 55% (green) and 25% (brown).*

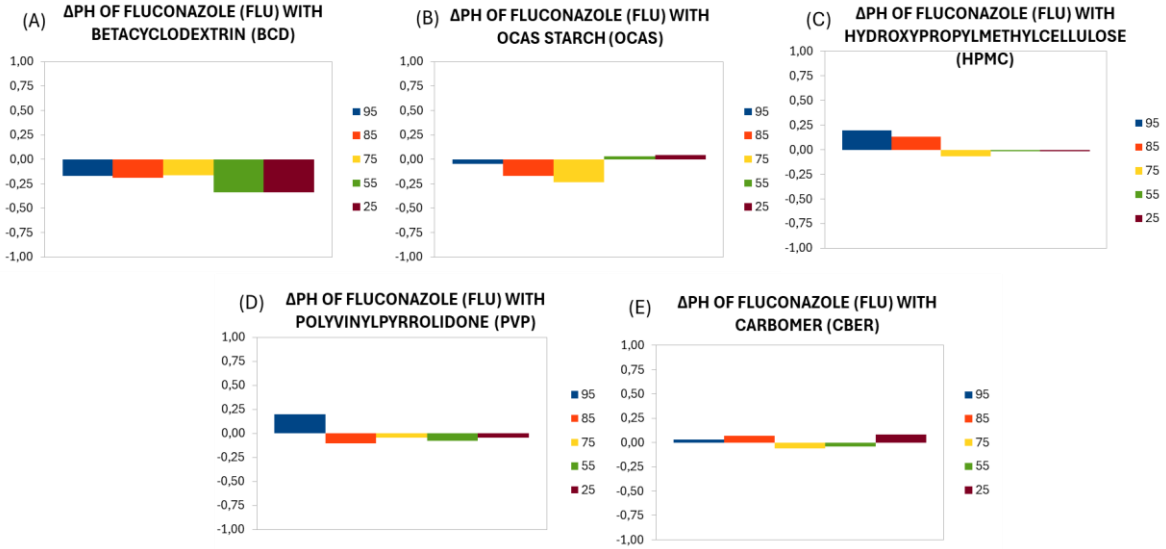

Figure 12. *pH variation in the mixture of Fluconazole (FLU) with different excipients, which are BCD (A), OCAS (B), HPMC (C), PVP (D) and CBER (E); evaluated in different proportions of excipients, which are 95% (blue), 85% (orange), 75% (yellow), 55% (green) and 25% (brown).*

To understand the observed variations in pH, it is crucial to delve into the study of molecular interactions in the system. Molecules can engage in various interactions, ranging from electrostatic forces and van der Waals dispersion to hydrophobic interactions and covalent and ionic bonds [86, 87]. However, it is essential to note that the manifestation of these interactions is conditioned by the

surrounding environment, ion concentration, and the possible pK_a. In situations where the pK_a is particularly weak, extreme pH conditions outside the standard range of 0 to 14 may be necessary for the molecule to participate in specific interactions. Nonetheless, in the present study conducted in an aqueous medium, interactions are explained considering the standard pH range. These interactions arise from functional groups acting as hydrogen donors and acceptors in the molecule. In the aqueous medium, some functional groups that could act as donors or acceptors may not meet the appropriate conditions to exhibit such behavior. Although in other solvents, they might behave as acids or bases, releasing or accepting protons, but not always this activity takes place in water. However, this does not exclude the possibility that they can form hydrogen bonds through weaker electrostatic interactions with either excipients, buffer components or API itself. These interactions could also influence the concentration of hydrogen ions in the surrounding environment; therefore, this is manifested in the pH change. Thus, the higher the H^+ concentration the pH decreases and vice-versa [88].

From this perspective, discrepancies in pH variation of Fluconazole (FLU) and Ibuprofen (IBU) find their explanation in the intrinsic ability of both compounds to form hydrogen bonds. The polarity of a molecule is determined by the distribution of its electrons and the presence of functional groups with the ability to accept or donate protons. In this case, FLU exhibits a more pronounced polarity than IBU, supported by its higher number of hydrogen acceptors and donors (seven acceptors and one donor for FLU) [89], compared to IBU, which has only two acceptors and one donor [90].

To better understand the influence of this polarity, it is crucial to consider how hydrogen bonds, a specific form of interaction between molecules, affect chemical properties. In the case of FLU, its structure allows for forming more hydrogen bonds, intensifying its interactions with other molecules in an aqueous environment. These strong interactions can directly impact the acid-base balance and, consequently, the observed pH variation during the procedure.

On the other hand, IBU, with fewer available sites for hydrogen bond formation, may not manifest as intense interactions compared to FLU. This could explain why pH variations are more pronounced in

the presence of FLU, as its molecular interactions have a more significant impact in the aqueous medium.

In the case of Fluconazole (FLU), it was observed that even upon adding minimal amounts of NaOH and HCl, there were drastic changes in the pH, indicating a high sensitivity of the medium containing FLU to pH adjusters. Therefore, to determine the necessity of adjusting FLU and excipient mixtures, a pH range of approximately ±0.5 units from the initial value of these mixtures was established. pH adjustment was conducted if the variation was outside this range, while no adjustments were made if the variation fell within the range. The primary objective of this decision was to avoid excessive use of pH adjusters, as they could lead to unfavorable interactions with the molecules present in the solution, thereby affecting the solubility of the compound. Based on this, it was decided not to adjust the pH of the mixtures with BCD, as they maintained pH values close to the initial one within the established range. However, it was necessary to adjust the pH for mixtures with PVP, HPMC, CBER, and OCAS, as their pH variations were outside of that range. Nevertheless, upon analyzing the final results, it was observed that in all proportions of FLU: BCD mixtures, there was a greater pH variation (final pH - initial pH) than other mixtures containing FLU with the other excipients. This highlights the importance of using adjusters in appropriate quantities to prevent interactions that may affect the pH in the case of Fluconazole (FLU), emphasizing the utility of these measures in manipulating acid-base interactions in the study context.

On the other hand, our study methodology focused on obtaining a saturated solution after the procedure, aiming to examine the impact of excipients on the solubility of API precisely. It is crucial to highlight that mixtures of FLU and IBU differed in the total mass, aiming to achieve a balance between the aqueous and solid phases. This equilibrium constitutes the distinctive feature of a saturated solution. Thus, in the specific case of IBU with excipients, 30 mg was initially weighed in all mixtures, and no additional mass was necessary. This indicates that the intrinsic solubility of IBU was achieved, suggesting that the initial conditions were suitable to reach saturation of the solution with this compound.

In contrast, to achieve saturation of the solutions with mixtures with FLU, it was decided to increase the total mass in combinations containing excipients such as PVP and HPMC up to 150 mg. On the contrary, other excipients such as BCD, CBER, and OCAS achieved saturation with only 30 mg. These results suggest the possibility of specific interactions between the drug and the mentioned excipients, thereby improving the solubility of FLU. Therefore, in the following sections, a more precise analysis of the impact of excipients on the solubility of both FLU and IBU will be conducted.

3.3 Correlation Analysis

In the context of this research, an exhaustive correlation analysis has been conducted to explore the interrelation between excipient percentages and concentrations of active pharmaceutical ingredients (APIs), with a particular emphasis on comparing the behavior of Fluconazole (FLU) as a basic API and Ibuprofen (IBU) as an acidic API, both in their neutral forms, dissolved in an aqueous medium at room temperature. It is crucial to highlight that the concentrations obtained in this analysis directly reflect the intrinsic solubility of the APIs. It becomes especially relevant when exploring how different excipient concentrations affect the concentrations of both APIs, carefully considering their basicity and acidity properties.

During this research, various correlation models have been applied, such as linear, polynomial, logarithmic, potential, and exponential. The choice of the most appropriate model has been based on the coefficient of determination (R^2), establishing that it must exceed a value of 0.9 to assert that the data adequately fit the behavior associated with a specific type of correlation. Furthermore, it is fundamental to highlight that this approach focuses on understanding the dynamics and general trends of the relationship, concentrating on comprehending the behavior of the data without determining a specific mathematical model. Thus, for this analysis, determination coefficients were determined using excipient percentages of 95%, 85%, 75%, 55%, and 25%, and also, new determination coefficients were determined using only 85%, 75%, 55%, and 25%, where it can be mentioned that the 95% was excluded, given that an excess concentration of excipient could reach the solubility limit of the solution and negatively affect the solubilization of the API.

Thus, in the case of ibuprofen (IBU), which is an acid, Table 4 presents the coefficient of determination in the relationship between IBU concentration and various excipients at different percentages, including 95%. The data predominantly suggest a behavior associated with polynomial correlation only in the relationship between BCD percentages and IBU concentration. However, this pattern could be attributed to an abnormal result related to the inhibition of solubilization due to excess excipient.

Table 4. Coefficients of determination (R^2) in the different types of correlations for Ibuprofen (IBU) with percentages of excipients ≈ (95%, 85%,75%,55%, 25%)

	LINEAR	LOGARITHMIC	EXPONENTIAL	CUBIC POLYNOMIAL	POTENTIAL
BCD	0.1495	0.076	0.113	0.9278	0.0604
OCAS	0.104	0.0481	0.1055	0.4766	0.0487
PVP	0.0989	0.1348	0.0911	0.6143	0.129
CBER	0.3882	0.3312	0.3882	0.6133	0.3324
HPMC	0.7887	0.7743	0.7883	0.8223	0.7789

IBUPROFEN (IBU) WITH PERCENTAGES OF EXCIPIENTS ≈ (95%, 85%,75%,55%, 25%)

On the other hand, in Table 5, by excluding the 95%, it is observed that, like in Table 4, with BCD all the determination coefficients are higher than 0.9. In this sense, although the points fit all correlations in BCD, linear correlation might be the most suitable due to its simplicity. This is because determining if this relationship follows a more complex model would require more experimental points, while linear correlation only demands a few and, R^2 for linear correlation is good enough, as it not only simplifies the interpretation of results but also proves helpfulness in situations where the amount of available data could be limited, providing a valuable understanding of how variables relate in general, even beyond the specific data collected up to this point [96].

Table 5. Coefficients of determination (R^2) in the different types of correlations for Ibuprofen (IBU) with percentages of excipients ≈ (85%, 75%, 55%, 25%)

	LINEAR	LOGARITHMIC	EXPONENTIAL	QUADRATIC POLYNOMIAL	POTENTIAL
BCD	**0.9782**	0.9966	0.9717	0.9983	0.9969
OCAS	0.0146	0.0013	0.0146	0.4091	0.0013
PVP	0.7629	0.6623	0.8108	0.8909	0.7081
CBER	0.51	0.3827	0.5137	0.8805	0.3852
HPMC	0.7254	0.7154	0.7264	0.7262	0.7181

IBUPROFEN (IBU) WITH PERCENTAGES OF EXCIPIENTS ≈ (85%, 75%, 55%, 25%)

It is important to mention that the deduction to exclude the 95% because an excess of excipient could affect the produced solubility of IBU is reinforced by observing Figure 13, where percentages up to 85% show an upward trend, unlike the 95%, which experiences an abrupt decrease. Additionally, it can be observed that the interaction of IBU with BCD results in a higher concentration of API compared to interactions with other excipients; that is, BCD could be providing greater solubility to the API, aligning with the literature as BCD, unlike other excipients, is primarily used as a solubilizing agent [91–94]. Therefore, in the next sections, a statistical evaluation will be conducted to clarify the impact of BCD and other excipients on the solubility of IBU.

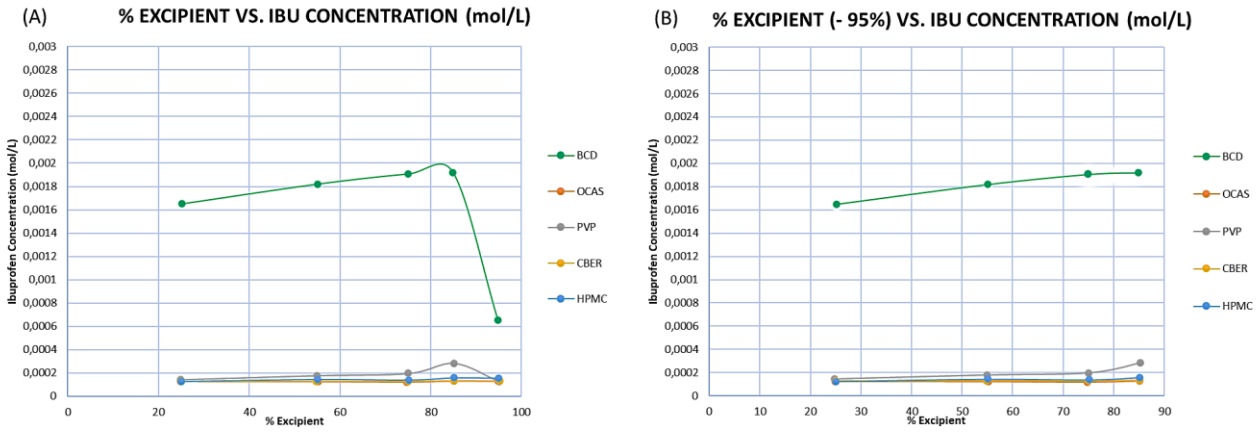

Figure 13. *Linear relationship between various excipients and Ibuprofen (IBU) concentration measured in mol/L. This graph illustrates the distinctive impact of each excipient on Ibuprofen (IBU) concentration, with color-coded curves (BCD in green, OCAS in orange, PVP in lead gray, CBER in yellow, and HPMC in light blue) that provide a clear visual representation of their respective influences. For graphs A, excipients include approximate percentages of ≈ (95%, 85%, 75%, 55%, and 25%); and for B, excipients include approximate percentages of ≈ (85%, 75%, 55%, and 25%).*

On the other hand, in the case of Fluconazole (FLU), which is a base, Table 6 reveals that by including the excipient percentage equal to 95%, the relationship of FLU with all excipients seems to follow a polynomial correlation. However, although polynomial correlations exhibit coefficients higher than 0,9, the validity of these results cannot be asserted due to the complexity of the model, necessitating the collection of more data to obtain more accurate confirmation.

Table 6. Coefficients of determination (R^2) in the different types of correlations for Fluconazole (FLU) with percentages of excipients ≈ (95%, 85%,75%,55%, 25%).

	LINEAR	LOGARITHMIC	EXPONENTIAL	CUBIC POLYNOMIAL	POTENTIAL
BCD	0.8942	0.7751	0.7286	0.988	0.6279
OCAS	0.9338	0.8278	0.787	0.9997	0.6897
PVP	0.117	0.088	0.1076	0.9384	0.0842
CBER	0.5567	0.3862	0.4038	0.9999	0.2865
HPMC	0.8417	0.7026	0.651	0.9837	0.545

FLUCONAZOLE (FLU) WITH PERCENTAGES OF EXCIPIENTS ≈ (95%, 85%, 75%, 55%, 25%)

Furthermore, for this basic API, the excess of excipient could also negatively influence solubility, so new determination coefficients (R^2) were determined, excluding the data from 95%. In this context, Table 7 shows the relationship between excipient percentages and Fluconazole (FLU) concentrations, where it can be observed that BCD and OCAS apparently follow linear and polynomial correlations. However, in this case, it could be argued that linear correlation is more appropriate due to the number of available experimental points. On the other hand, HPMC and CBER, in their relationship with Fluconazole (FLU) concentration, exhibit a coefficient of determination greater than 0.9 in polynomial correlation. However, as mentioned earlier, more data is needed to allow the confirmation of this correlation, highlighting the need for more comprehensive data collection to validate these behavior patterns.

Table 7. Coefficients of determination (R^2) in the different types of correlations for Fluconazole (FLU) with percentages of excipients ≈ (85%,75%,55%, 25%).

	LINEAR	LOGARITHMIC	EXPONENTIAL	QUADRATIC POLYNOMIAL	POTENTIAL
BCD	**0.9448**	0.8608	0.8922	0.987	0.8002
OCAS	**0.9574**	0.8801	0.8989	0.9961	0.816
PVP	0.0278	0.0103	0.0287	0.1296	0.0107
CBER	0.3791	0.2396	0.3309	0.9991	0.2113
HPMC	0.7923	0.669	0.6919	0.9734	0.5876

FLUCONAZOLE (FLU) WITH PERCENTAGES OF EXCIPIENTS ≈ (85%, 75%, 55%, 25%)

This exclusion of the 95% for fluconazole can be justified by analyzing figure 14, where a decrease in concentration is evidenced when reaching this percentage, suggesting a possible negative impact on solubilization. Additionally, it can be observed that its concentration, i.e., its solubility in relation to excipients such as BCD, OCAS, and HPMC, tends to decrease as the excipient percentage increases. CBER, on the other hand, follows a similar trend, with a slight elevation between 25% and 55%, followed by a decrease. On the other hand, PVP shows fluctuations, exhibiting a different behavior compared to other excipients. In this context, it is crucial to emphasize that the graphical representation does not provide a definitive conclusion about which excipient impacts FLU solubility better, as the concentrations of this API are similar at certain percentages of various excipients. Therefore, the next sections will address the reliable determination of the influence of excipients on FLU solubility.

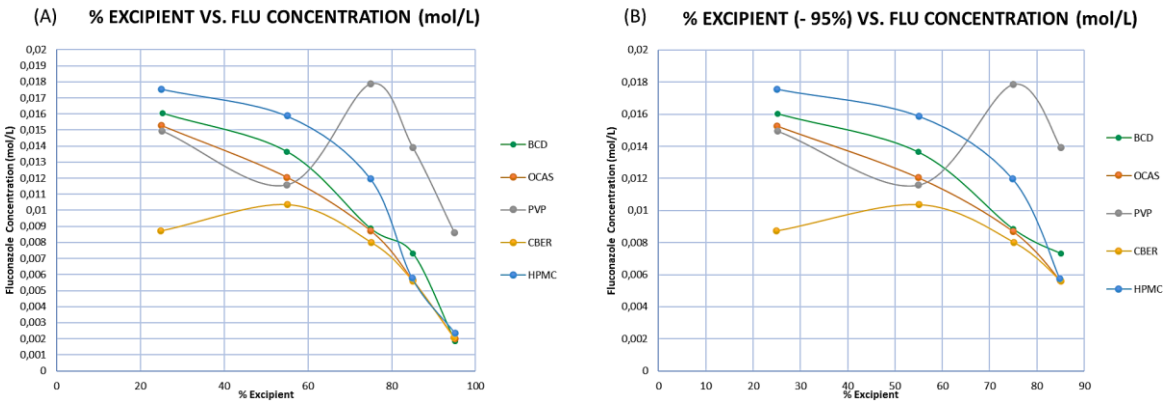

Figure 14. *Linear relationship between various excipients and Fluconazole (FLU) concentration measured in mol/L. This graph illustrates the distinctive impact of each excipient on Fluconazole (FLU) concentration, with color-coded curves (BCD in green, OCAS in orange, PVP in lead gray, CBER in yellow, and HPMC in light blue) that provide a clear visual representation of their respective influences. For graphs A, excipients include approximate percentages of ≈ (95%, 85%, 75%, 55%, and 25%); and for B, excipients include approximate percentages of ≈ (85%, 75%, 55%, and 25%).*

It is also relevant to note that the relationships between PVP with FLU, PVP with IBU, OCAS with IBU, HPMC with IBU, and CBER with IBU do not exhibit any of the studied correlation behaviors. Therefore, in the absence of observable patterns that align with the analyzed correlation types, a more detailed analysis and potentially exploration of alternative models are required to comprehend the nature of these relationships. The lack of clear trends could suggest complexity in the interaction between excipients and active ingredients that extends beyond the linear, exponential, potential, logarithmic, or polynomial correlation forms considered thus far.

3.4 Statistical Analysis of solubility in presence of excipients

Applying the Henderson Hasselbalch Model in this study provides a theoretical and structured approach to examine how variations in excipient and API proportions influence the solubility of these compounds. In this way, this model indicates whether they are acidic or basic, when they are in neutral state in aqueous solutions at room temperature.

In this context, implementing ANOVA and Tukey statistical tests will allow to determine significant differences between reference and experimental determined solubility values, and also, to determine statistical differences between the results with each excipient.

Due to the nature of our experimental design, one-way ANOVA was employed in the initial statistical analysis stage. This design focused on comparing the means of three or more independent groups. In

this case, these groups represent the average experimental solubilities of the API (IBU or FLU) associated with different percentages of a specific excipient (BCD, OCAS, PVP, CBER, or HPMC), as seen in Table 8.

Table 8. P-values obtained from the ANOVA tests for Fluconazole (FLU) and Ibuprofen (IBU) indicate whether significant differences exist between the experimental solubilities obtained from the 5 percentages of each excipient.

Analysis of Variance (ANOVA)			
Ibuprofen (IBU)		**Fluconazole (FLU)**	
Excipient	**P-VALUE**	**Excipient**	**P-VALUE**
BCD	2.4615E-12	**BCD**	8.83673E-11
OCAS	0.05679255	**OCAS**	9.1941E-12
PVP	6.3665E-09	**PVP**	0.012749506
CBER	0.24682615	**CBER**	0.002089037
HPMC	3.9108E-05	**HPMC**	2.20499E-07

Each group represented an excipient percentage (95%, 85%, 75%, 55%, 25%), where three measurements of API experimental solubility were conducted to ensure result reliability. The mean of these three measurements was calculated for each group, and the variability of the mean experimental solubilities among the five groups, represented by each excipient percentage, was assessed using the ANOVA test, when the p-value was less than 0.05, the null hypothesis was rejected, which means that that at least one mean experimental solubility in a specific excipient percentage differed from the rest of the mean solubilities.

So, Table 8 presents the p-values obtained from ANOVA tests for Fluconazole (FLU) and Ibuprofen (IBU) on solubilities in presence of 5 levels of excipients, comparing the mean solubilities of the API between the five percentages of each excipient. In the case of IBU, it was observed that among the percentages of BCD, PVP, and HPMC, at least one group exhibited a mean solubility different from the others. At the same time, OCAS and CBER showed no significant differences in their means when varying the percentages. In contrast, regarding FLU, all excipients revealed at least one mean solubility, which differs among their respective groups.

These results lay the basis for the next step in the statistical analysis: identifying groups of excipient percentages that differ in API solubility, which the Tukey test addresses. It is essential to mention that the disparity between the means of solubilities for each excipient percentage compared to the reference solubility value (logS_0) has yet to be evaluated.

Table 9. Tukey test for Ibuprofen (IBU) showed a p-value, indicating whether or not there was a significant difference between its intrinsic solubility reference value ($\log S_0 = -3.805$) and the solubility corresponding to a specific percentage of excipient.

TUKEY TEST FOR IBUPROFEN (IBU) WITH ASSUMED MEAN=-3.805					
EXCIPIENT	**P-VALUE**				
	95%	**85%**	**75%**	**55%**	**25%**
BCD	2.101E-11	5.3968E-13	5.4168E-13	5.5589E-13	6.0718E-13
OCAS	0.00671885	0.01119144	0.00049919	0.00160199	0.00266753
PVP	0.02366509	3.396E-06	0.00924205	0.18409582	0.531545
CBER	0.00031287	0.00104472	0.0002279	0.0001643	0.00017551
HPMC	0.99999972	0.99967525	0.07368129	0.34732182	0.00187171

Therefore, in this case, the Tukey test also was conducted. So, the p-value again played a crucial role for this test. The highlighted values in the previous tables, indicate significant differences, either between a specific excipient percentage and the intrinsic reference solubility (logS_0 = -3,805 for IBU) or among the specific percentage groups evaluated.

In case of FLU, which referential log$S0$ = -1.79, there are less differences according to results showed in Table 10.

Table 10. Tukey test for Fluconazole (FLU) showed a p-value, indicating whether or not there was a significant difference between its intrinsic solubility reference value ($logS_0$ = -1.79) and the solubility corresponding to a specific percentage of excipient.

TUKEY TEST FOR FLUCONAZOLE (FLU) WITH ASSUMED MEAN=-1.79					
EXCIPIENT	**P-VALUE**				
	95%	**85%**	**75%**	**55%**	**25%**
BCD	3.8853E-09	5.0135E-05	0.00050457	0.43865149	0.99999303
OCAS	1.6523E-10	2.3955E-07	3.3124E-05	0.0106252	0.93479317
PVP	0.15321489	0.98919716	0.99876634	0.76473642	0.99941242
CBER	0.00486068	0.17995973	0.52744048	0.86611275	0.4492623
HPMC	2.0097E-05	0.00295279	0.61128297	0.99999691	0.99826952

In this case, PVP practically matches the experimental solubility of Fluconazole (FLU) with its intrinsic reference solubility despite the inclusion of different percentages of this excipient. Adding 25% of OCAS shows no significant differences compared to the intrinsic reference solubility, whereas other percentages exhibit discrepancies. In the case of BDC, no differences are detected at 55% and 25%, but they do occur at other percentages. Similarly, CBER shows significant differences only at 95%, while solubility remains similar to the reference at other percentages. Lastly, HPMC displays differences at 95% and 85% but not at other percentages. It is crucial to highlight that employing 25% of any of the studied excipients results in an alignment of the experimental solubility of Fluconazole (FLU) with the intrinsic reference solubility.

The following table shows now existing differences among the applied levels of excipients, identifying at which percentage of each excipient the solubility of IBU is already affected (see Table 11).

Table 11. Tukey test for Ibuprofen (IBU) shows the p-value, indicating whether or not there was a significant difference between the experimental solubilities represented by specific percentages of each excipient.

TUKEY TEST FOR IBUPROFEN (IBU)					
CONTRAST	**P-VALUE**				
	BCD	**OCAS**	**PVP**	**CBER**	**HPMC**
95% vs. 85%	1.42E-11	0.99517988	8.39E-09	0.72781863	0.99526515
95% vs. 75%	1.49E-11	0.14139389	2.93E-06	0.99844716	0.0114152
95% vs. 55%	1.96E-11	0.68704337	3.37E-05	0.96047082	0.11711868
95% vs. 25%	3.36E-11	0.92843592	0.08404628	0.97504664	1.21E-04
85% vs. 75%	0.99941895	0.0673692	1.25E-05	0.5121954	0.0055799
85% vs. 55%	0.28806897	0.4189298	1.32E-06	0.31518579	0.05562831
85% vs. 25%	0.00085264	0.71042557	3.40E-08	0.3506023	7.20E-05
75% vs. 55%	0.42691797	0.77530215	0.13145934	0.99795243	0.64634027
75% vs. 25%	0.00127878	0.48110765	4.52E-05	0.9993115	0.04308163
55% vs. 25%	0.0199255	0.9929733	0.00109818	0.99999917	0.00440138

Interestingly, the results show significant differences in the solubility of IBU observed between certain groups of percentages used for BCD, PVP and HPMC, while no significant differences are detected in any group of percentages for OCAS and CBER. Specifically, in the cases of BCD, PVP, and HPMC, significant differences were found between the percentages of 75% vs. 25%, 55% vs. 25%, 95% vs. 75%, and 85% vs. 25%. However, in other percentage groups of these same excipients, variations are observed: specific percentages show significant differences only for PVP but not for BCD or HPMC, while some other percentage groups have significant differences for BCD and PVP but not for HPMC, and vice versa (for example, in HPMC and PVP, but not in BCD; in HPMC and BCD, but not in PVP). Additionally, all the excipients used show a lack of differences in the produced solubility of IBU between 75% and 55%.

About FLU, due to its basic nature, the results are different as can be seen in Table 12.

Table 12. Tukey test for Fluconazole (FLU) shows the p-value, indicating whether or not there was a significant difference between the experimental solubilities represented by specific percentages of each excipient.

TUKEY TEST FOR FLUCONAZOLE (FLU)					
CONTRAST	**P-VALUE**				
	BCD	**OCAS**	**PVP**	**CBER**	**HPMC**
95% vs. 85%	1.17E-08	1.02E-08	0.09986719	0.05251355	0.0001979
95% vs. 75%	3.17E-09	1.76E-10	0.01275172	0.00860219	2.97E-07
95% vs. 55%	1.53E-10	3.74E-11	0.41575119	0.00237547	4.47E-08
95% vs. 25%	7.22E-11	1.90E-11	0.05554634	0.01169721	2.49E-08
85% vs. 75%	0.1068195	3.90E-05	0.74610653	0.82430247	0.00081437
85% vs. 55%	2.02E-05	2.18E-07	0.8938008	0.34754429	3.3501E-05
85% vs. 25%	2.41E-06	1.60E-08	0.99847495	0.90871483	1.30E-05
75% vs. 55%	0.00045917	0.00050082	0.24106909	0.93326145	0.25732806
75% vs. 25%	3.01E-05	3.56E-06	0.91575854	0.99992315	0.0756313
55% vs. 25%	0.19805931	0.00463815	0.71159249	0.85968304	0.9643751

The results reveal that the solubilities obtained from FLU in the presence of OCAS differ across all compared percentage groups. Similarly, the situation occurs with BCD, where solubilities differ in most groups, except in 85% vs 75% and 55 vs 25%. On the other hand, for PVP, CBER, and HPMC, no significant differences are observed between 75% vs. 55%, 75% vs. 25%, and 55 vs 25%. However, in the case of PVP, it is evident that significant differences exist only in 95% vs. 75%, and furthermore, this percentage group significantly differs across all excipients.

3.5 Effects of the excipients on the intrinsic solubility of Ibuprofen (IBU) and Fluconazole (FLU)

- **Ibuprofen (IBU) in addition with BCD**

The presence of BCD enhances the experimental solubilities of ibuprofen (IBU) across all studied percentages, as shown in Figure 15, and as revealed in Table 9, where significant differences are

evidenced when comparing each percentage of BCD with the intrinsic reference solubility of ibuprofen. However, when comparing the experimental solubilities among themselves, represented by the percentages of excipients, according to Table 11, it was found that certain groups exhibit significant differences while others do not, such as 85% vs. 75%, 85% vs. 55%, and 75% vs. 55%, which show the same solubility among them. This suggests that, from the perspective of enhancing the solubility of ibuprofen (IBU), there would be no difference if 85%, 75%, or 55% of BCD is employed, where it is important to mention that these percentages yield the highest solubility of IBU in the presence of this excipient.

Nevertheless, when comparing this solubility at 25% with the solubilities at 85%, 75%, and 55%, Table 11 reflects significant differences, indicating lower solubility at 25% of BCD compared to 85%, 75%, and 55%. On the other hand, a solubilization limit is observed concerning the 95% BCD percentage due to its excess, as inferred in previous analyses; furthermore, upon examining Figure 15, the previously deduced conclusion that BCD functions as a more effective solubilizing agent than other excipients in most proportions for IBU is confirmed. The only exception is found at 95%, where no improvement was observed in any of the analyzed excipients.

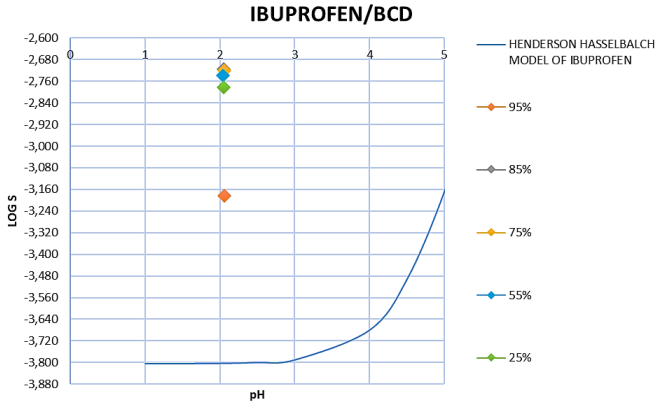

Figure 15. *Henderson-Hasselbalch (HH) Model with a curve plotted based on the intrinsic reference solubility for Ibuprofen (IBU). Experimental solubility values for different percentages of BCD are also shown for comparison. Components in the graph are color-coded, with the reference HH curve shown in blue, 95% in orange, 85% in lead, 75% in yellow, 55% in light blue, and 25% in green.*

To explain these previously mentioned facts, it is crucial to highlight that according to the literature [101–103], the enhancement in Ibuprofen (IBU) solubility with BCD is attributed to specific interactions between both molecules. These interactions occur when BCD acts as an encapsulating agent

due to its molecular cavity, a three-dimensional structure that serves as a "container" capable of harboring molecules like Ibuprofen (IBU) [95–97]. In this regard, a significant increase in solubility is observed at percentages of 85%, 75%, and 55% of BCD. This could suggest that at these percentages, a delicate balance is reached between the formation of inclusion complexes and solution saturation, in which the adequate availability of BCD (85%, 75%, and 55%) can effectively envelop Ibuprofen (IBU), forming stable inclusion complexes without reaching a saturation point that limits this beneficial interaction. However, it is important to highlight that the results of this study differ from what has been reported in the literature [107, 108], where it is suggested that stable inclusion complexes form when the BCD percentage is equal to 50%. This discrepancy could be attributed to differences in experimental conditions or specific characteristics of the materials used in the present study. Despite this discrepancy, observing an increase in the solubility of IBU in the presence of BCD does not dismiss the idea that these complexes can act as a protective "shield" around ibuprofen (IBU) at percentages of 55%, 75%, and 85%. This could protect it from adverse external forces and prevent the aggregation or crystallization of ibuprofen (IBU), a phenomenon that could affect its ability to dissolve properly and, therefore, its therapeutic effectiveness [109–111].

Additionally, upon analyzing the 25% BCD percentage, lower solubility is observed compared to the previously mentioned percentages, which aligns with the findings of the study [112], where it is demonstrated that the solubility of IBU increases as the amount of BCD increases. Also, it is essential to note that even with this lower solubility, the BCD percentage remains effective in improving Ibuprofen (IBU) solubility compared to the intrinsic reference solubility. This lower solubility at 25% of BCD suggests that the BCD proportion in this case may not be optimal for efficiently forming inclusion complexes with Ibuprofen (IBU). One possible reason for this lower solubility at 25% of BCD could be reduced encapsulation capacity at that specific proportion. With less BCD available in the solution, there may be fewer BCD molecules to efficiently encapsulate Ibuprofen (IBU) molecules, limiting the formation of stable inclusion complexes. As a result, Ibuprofen (IBU) may have less protection against aggregation or crystallization, leading to lower solubility compared to higher BCD percentages [112].

On the other hand, in the case of 95% BCD, a critical saturation point is reached due to BCD excess in the solution [113], leading to competition for interactions [114]. In this situation, BCD is not only devoted to encapsulating Ibuprofen (IBU) but may also interact with other BCD molecules. This competition results in inefficiencies in inclusion complex formation, decreasing the effectiveness of BCD and negatively affecting Ibuprofen (IBU) solubility, which is also observed in the study [112]. Thus, the excess of BCD does not provide additional benefits and could interfere with the beneficial interaction between BCD and the drug.

- **Ibuprofen (IBU) in addition with PVP**

Figure 16 demonstrates that the presence of different percentages of PVP variably affects the solubility of Ibuprofen (IBU), either enhancing or diminishing it when compared to the intrinsic reference solubility. However, it is crucial to note that the literature lacks similar previous studies specifically addressing the interaction between PVP and IBU concerning solubility, making it difficult to determine if the results obtained in this study align with previous research. In this regard, the study compares experimental solubilities with intrinsic solubility, providing a unique perspective on the influence of PVP on IBU solubilization. Thus, table 9 shows that PVP percentages of 55% and 25% do not show significant improvements compared to the intrinsic reference solubility. This could be because the specific proportion of PVP about Ibuprofen (IBU) does not favor the formation of beneficial complexes or micelles [115]. In these lower percentages, PVP molecules may not be present sufficiently to interact effectively with IBU, or the stoichiometric relationship between both components may not be optimal for favoring drug solubilization [116].

On the other hand, at the 95% PVP percentage, there is a sharp decrease in solubility, suggesting that excess PVP could lead to unfavorable molecular interactions [113]. This excess could promote competition between PVP molecules, aggregation, or even system saturation, thus limiting the ability of IBU to dissolve efficiently [114, 115]. These phenomena highlight the critical importance of maintaining an appropriate ratio in the formulation to avoid adverse interactions that affect solubility and, consequently, the effectiveness of the medication.

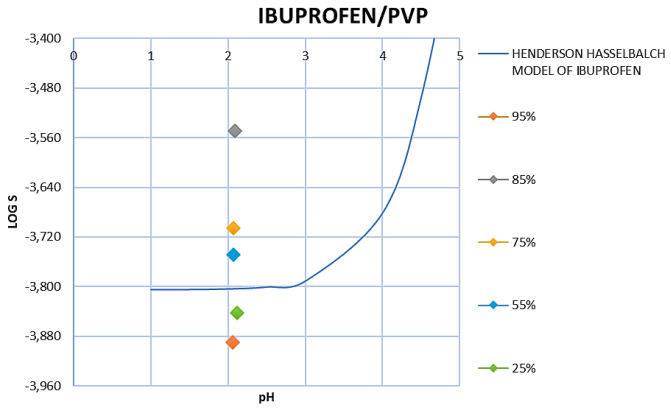

Figure 16. *Henderson-Hasselbalch (HH) Model with a curve plotted based on the intrinsic reference solubility for Ibuprofen (IBU). Experimental solubility values for different percentages of PVP are also shown for comparison. Components in the graph are color-coded, with the reference HH curve shown in blue, 95% in orange, 85% in lead, 75% in yellow, 55% in light blue, and 25% in green.*

Additionally, there is an increase in IBU solubility at the 75% and 85% PVP percentages, which exhibit different solubilities (higher in 85% than in 75%). It is likely that PVP, in these cases, facilitates the formation of complexes or micelles with the drug. According to the literature, this could be explained by the polymeric properties of PVP, such as its hydrophilic nature and the presence of polar groups [117–119], suggesting beneficial interactions with IBU in aqueous environments. These interactions could create stable structures that enhance the dispersion and solubility of IBU in the aqueous medium. In contrast, these interactions do not manifest significantly in other percentages, possibly due to suboptimal proportions of PVP about IBU. These results highlight the sensitivity of solubility to the specific proportion of PVP, emphasizing the importance of precise formulation for substantial improvements.

It is also relevant to note that all evaluated percentages used the same temperature and pH. However, there may still be differences in how these conditions affect molecular interactions between PVP and Ibuprofen (IBU). The formation of complexes or micelles between PVP and IBU may critically depend on temperature and pH [120]. These conditions influence the structure and charge of molecules, affecting PVP's ability to wrap IBU and improve its solubility efficiently. If, in specific percentages, the conditions are not conducive, molecular interactions may not positively contribute to improving solubility. For example, complex formation could be less effective at specific temperatures or pH levels, limiting PVP's ability to favor IBU solubilization. In contrast, in the 85% and 75% PVP percentages,

where an improvement in solubility is observed, optimal conditions may allow stable complexes or micelles to form. These optimal conditions enable molecular interactions between PVP and IBU to be more efficient, resulting in better drug dispersion and solubility in the aqueous medium.

- **Ibuprofen (IBU) in addition to OCAS, CBER, and HPMC**

Upon examining Figure 17 and Tables 9 and 11 for the percentages of OCAS and CBER, it can be observed that the solubility of Ibuprofen (IBU) remains unchanged at any percentage. In fact, these experimental solubilities are even lower than the intrinsic reference solubility.

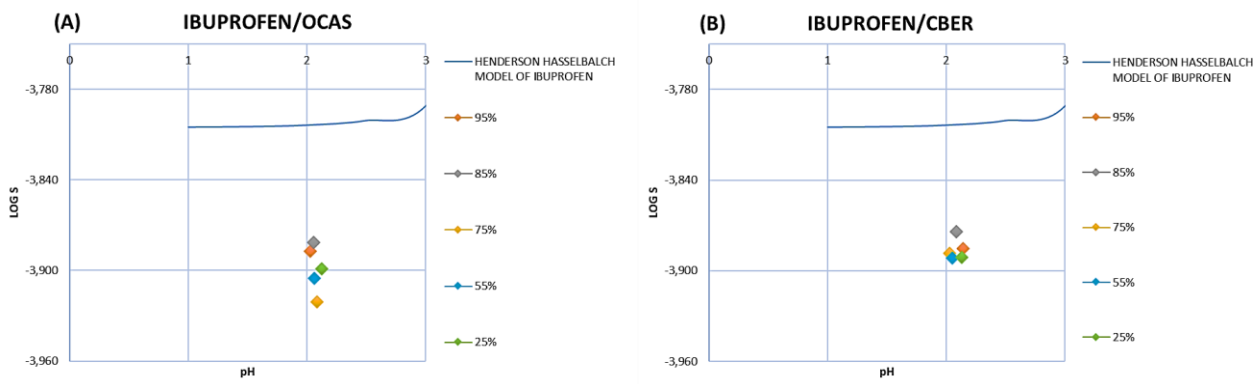

Figure 17*. Henderson-Hasselbalch (HH) Model with a curve plotted based on the intrinsic reference solubility for Ibuprofen (IBU). Experimental solubility values for different percentages of OCAS (A) y CBER(B) are also shown for comparison. Components in the graph are color-coded, with the reference HH curve shown in blue, 95% in orange, 85% in lead, 75% in yellow, 55% in light blue, and 25% in green.*

The same pattern is observed with HPMC, where no significant improvements in solubility are noted. However, when analyzing the percentages of 95%, 85%, 75%, and 55% of HPMC, we find that the experimental solubility shows no significant differences compared to the intrinsic solubility, as indicated in Table 9. The only exception occurs in the 25% HPMC percentage, where a significant decrease is observed in relation to the intrinsic reference solubility (see Figure 18).

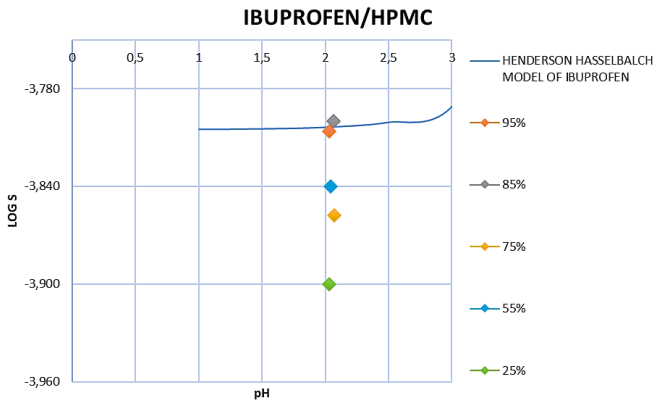

***Figure 18**. Henderson-Hasselbalch (HH) Model with a curve plotted based on the intrinsic reference solubility for Ibuprofen (IBU). Experimental solubility values for different percentages of HPMC are also shown for comparison. Components in the graph are color-coded, with the reference HH curve shown in blue, 95% in orange, 85% in lead, 75% in yellow, 55% in light blue, and 25% in green.*

Regarding the percentages of OCAS and CBER, the presence of experimental solubilities lower than the intrinsic reference solubility of IBU and the absence of significant differences between them suggest that these excipients do not significantly improve the solubility of IBU. This lack of improvement could be due to the absence of chemical or structural affinity between IBU and the OCAS and CBER excipients [121], preventing the formation of complexes or beneficial interactions that could enhance the drug's solubility, negatively affecting it. It's worth noting that there are no existing comparable studies to contrast these results, underscoring the novelty of these findings.

Similarly, the lack of substantial improvements and the absence of significant differences in the experimental solubilities of IBU at different percentages of HPMC compared to its intrinsic reference solubility, except in the case of the 25% percentage, suggest that the interaction between HPMC and the drug does not help improve solubility at the evaluated proportions. The stability in solubility at 95%, 85%, 75%, and 55% indicates that, at these specific concentrations, there are no notable changes in HPMC's ability to influence IBU solubility, either positively or negatively. Additionally, the relationship between HPMC and IBU at the 25% percentage may not be suitable for improving solubility, resulting in decreased observed solubility compared to other concentrations. It's worth noting that while previous studies have shown promising results, such as a notable increase in the solubility of IBU when co-milled with hydroxypropylmethylcellulose (HPMC) using different proportions [60], the present study, in contrast, does not demonstrate such improvements. The contrast in results could stem

from differences in experimental methodology, specific characteristics of the excipients employed, or the unique interaction between IBU and the excipients in this study. The approach of the present study may be valid for offering a novel or more detailed perspective on IBU solubility under various conditions, which could inform more precise formulation decisions or identify areas for future research.

So, all these results related to CBER, OCAS, and HPMC suggest exploring alternative excipients or adjusting proportions to improve IBU solubility effectively.

- **Effects of BCD, OCAS, HPMC, PVP, and CBER on the solubility of Fluconazole (FLU)**

When studying the interaction between Fluconazole (FLU) and various excipients such as BCD, OCAS, HPMC, PVP, and CBER, it is observed that despite some of these excipients being recognized for their solubilizing properties in the literature [91–94], there is no significant improvement in solubility when combined with this drug. These results can be examined in detail in Figures 19-23, where the experimental solubilities for each percentage considering a specific excipient are graphically represented compared to the intrinsic reference solubility. Similarly, Tables 10 and 12 offer a thorough description, presenting highlighted p-values indicating statistically significant differences between the compared groups. It is important to mention that there are no previous studies investigating the effect of the excipients used in this study on the solubility of FLU.

In this context, concerning PVP, significant differences are observed in the experimental solubilities of FLU for the various percentages compared to its intrinsic reference solubility. However, when comparing the solubilities obtained from FLU corresponding to the different percentages of PVP, it was demonstrated that they were equal, except in the case of 95% vs. 75%, despite an apparent separation between them observed in Figure 19.

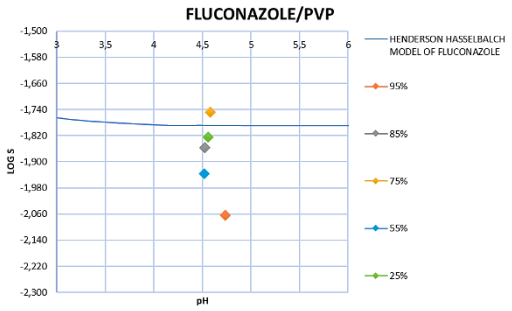

Figure 19. *Henderson-Hasselbalch (HH) Model with a curve plotted based on the intrinsic reference solubility for Fluconazole (FLU). Experimental solubility values for different percentages of PVP are also shown for comparison. Components in the graph are color-coded, with the reference HH curve shown in blue, 95% in orange, 85% in lead, 75% in yellow, 55% in light blue, and 25% in green.*

Similarly, the experimental solubilities of FLU associated with the percentages of 25%, 55%, 75%, and 85% of CBER are equal to the intrinsic reference solubility. However, the solubility at 95% does not match this intrinsic solubility, being lower at this percentage, as depicted in Figure 20.

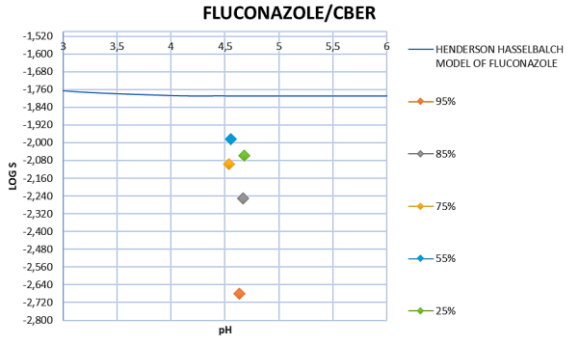

Figure 20. *Henderson-Hasselbalch (HH) Model with a curve plotted based on the intrinsic reference solubility for Fluconazole (FLU). Experimental solubility values for different percentages of CBER are also shown for comparison. Components in the graph are color-coded, with the reference HH curve shown in blue, 95% in orange, 85% in lead, 75% in yellow, 55% in light blue, and 25% in green.*

On the other hand, the solubility of FLU in the presence of 25% OCAS is the same when compared to the intrinsic reference solubility of this basic API. However, the solubilities obtained from 55%, 75%, 85%, and 95% of OCAS differ from the reference solubility. Additionally, all of these solubilities differ from each other, where it can be observed in Figure 21 that the solubility decreases as a higher percentage of OCAS is applied.

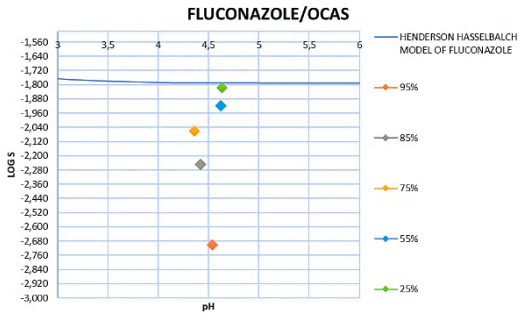

Figure 21. *Henderson-Hasselbalch (HH) Model with a curve plotted based on the intrinsic reference solubility for Fluconazole (FLU). Experimental solubility values for different percentages of OCAS are also shown for comparison. Components in the graph are color-coded, with the reference HH curve shown in blue, 95% in orange, 85% in lead, 75% in yellow, 55% in light blue, and 25% in green.*

The solubility of FLU in the presence of 55% and 25% BCD percentages coincides with its intrinsic reference solubility. However, significant differences were found in the 75%, 85%, and 95% BCD percentages compared to this reference solubility. Although the percentages of 85% vs. 75% and 55% vs. 25% showed statistical equivalence, it could be suggested, as they are consecutively studied proportions, that these comparison groups could represent intervals in which solubility remains constant. On the other hand, these compared percentages differed significantly from the 95% percentage. Therefore, when observing Figure 22 and considering the aforementioned, it can be inferred that as the BCD percentage increases, solubility varies and tends to increase.

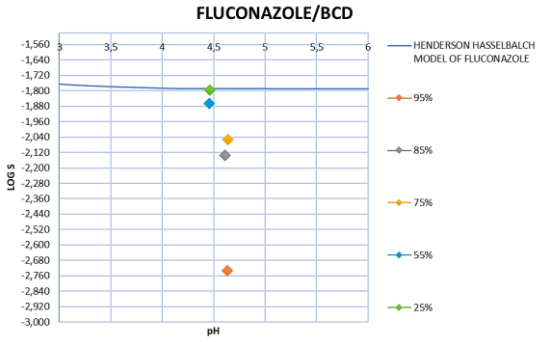

Figure 22. *Henderson-Hasselbalch (HH) Model with a curve plotted based on the intrinsic reference solubility for Fluconazole (FLU). Experimental solubility values for different percentages of BCD are also shown for comparison. Components in the graph are color-coded, with the reference HH curve shown in blue, 95% in orange, 85% in lead, 75% in yellow, 55% in light blue, and 25% in green.*

Additionally, concerning HPMC, significant differences were observed in the experimental solubilities of FLU at its higher percentages (85% and 95%) compared to the intrinsic reference solubility. Whereas, at lower percentages (75%, 55%, and 25%), there were no significant differences from the intrinsic reference solubility. Furthermore, as depicted in Figure 23, similar to BCD, OCAS, and CBER, there

exists an inverse relationship between the increase in the percentage of BCD and the solubility of the compound.

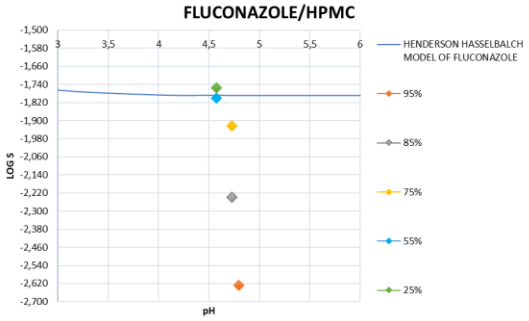

Figure 23. *Henderson-Hasselbalch (HH) Model with a curve plotted based on the intrinsic reference solubility for Fluconazole (FLU). Experimental solubility values for different percentages of HPMC are also shown for comparison. Components in the graph are color-coded, with the reference HH curve shown in blue, 95% in orange, 85% in lead, 75% in yellow, 55% in light blue, and 25% in green.*

All these results suggests that excess of excipients could negatively affect FLU solubility by saturating the system and generating unfavorable molecular interactions [113].

From this perspective, the lack of significant improvements in FLU solubility, despite the inclusion of excipients known for their solubilizing properties, raises the possibility that molecular interactions between FLU and the excipients are not effectively overcoming specific challenges associated with solubilization under the conditions and percentages employed. The unique nature of these connections lies in the importance of considering specific molecular interactions between FLU and excipients. It is not simply a generic interaction; its effectiveness depends mainly on the compatibility between the complex chemical structures of both elements [120, 122]. This level of molecular detail is crucial for understanding how excipients, known to improve the solubility of various compounds, can affect FLU specifically.

Each drug, including FLU, exhibits particular molecular characteristics that influence the ability of excipients to improve its solubility. Variability in these molecular characteristics among different drugs means that the effectiveness of excipients can fluctuate [123], even if they are recognized for their general ability to improve solubility.

Moreover, various molecular factors such as polarity, molecular geometry, and intermolecular forces form solubilization complexes between Fluconazole (FLU) and excipients. These complexes are

essential for facilitating drug solubility in the body. Thus, if the specific molecular interactions necessary for this process do not develop optimally, even in the presence of solubilizing excipients, the improvement in Fluconazole (FLU) solubility may be limited. The effectiveness of these excipients depends on how accurately they adapt to the particular molecular characteristics of Fluconazole (FLU), and any mismatch may compromise the desired improvement in drug solubility. Furthermore, medium conditions such as pH and temperature also play a crucial role. If these conditions are not optimal, there will be no significant molecular interaction to improve Fluconazole (FLU) solubility.

On the other hand, it is well known that during experimentation with mixtures of Fluconazole (FLU) and excipients PVP or HPMC, rapid dissolution of samples with high percentages (75%, 85%, 95%) was observed during the first hours of agitation, requiring the addition of more mass to balance the aqueous and liquid phases, suggesting that Fluconazole (FLU) in the presence of PVP and HPMC achieved an improvement in solubility. However, despite this rapid dissolution in the experimental process, when quantitatively evaluating the solubility of these percentages compared to the reference solubility of Fluconazole (FLU), lower solubility was evidenced in such percentages. The complex dynamics of molecular interactions between Fluconazole (FLU) and excipients could explain this apparent contradiction. The initial rapid dissolution could result from the formation of complexes or micelles, temporarily improving solubility during agitation. However, when obtaining quantitative solubility after performing the procedure, where low solubility was observed. So, according to the literature [124], this could indicate that these interactions were not stable enough to sustain a sustained improvement in solubility over time. Dissolution kinetics and interaction stability are crucial factors in this discrepancy. The initial rapid dissolution could be related to faster kinetics, but the lack of long-term stability could reverse these observed improvements. Therefore, a more detailed analysis of kinetics and molecular characterization of interactions could provide a more comprehensive understanding for future studies.

CHAPTER IV: Conclusions

4. CONCLUSIONS

After carrying out this thorough investigation into the effect of various excipients on the solubility of Ibuprofen (IBU) as an acid and Fluconazole (FLU) as a base in an aqueous medium at room temperature, it is essential to conclude that:

- Molecular properties play a crucial role in formulation design, particularly in solubility and stability, as highlighted by pH responses to excipients.
- Precise control of pH is essential for maintaining product stability and optimizing drug solubility in pharmaceutical formulations.
- Correlation analysis offers valuable insights into excipient effects on API solubility, aiding in understanding trends and patterns.
- While certain excipients significantly enhance Ibuprofen solubility, the need for deeper understanding of formulation interactions for Fluconazole is evident.
- Continued research is necessary to identify effective alternatives in pharmaceutical formulation design, addressing clinical needs accurately.
- Thorough understanding of molecular properties is crucial for tailoring formulation strategies to ensure optimal drug solubility and efficacy.
- Recognizing unique interactions between active ingredients and excipients is vital for precise formulation design and enhanced therapeutic efficacy.
- Innovative approaches leveraging molecular information hold promise for improved pharmaceutical outcomes.
- Understanding solubility's critical role at room temperature offers opportunities for enhancing drug release efficacy and improving patient safety.

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Appendix A

Experimental Findings: Data and Graphs Gathered Throughout the Ibuprofen (IBU) Research.

MIXTURES (IBUPROFEN (IBU)/EXCIPIENTS)			
Ibuprofen (IBU)/β-cyclodextrin (BCD)			
Ibuprofen (IBU)		BCD	
Percentage (%)	Mass (mg)	Mass (mg)	Percentage (%)
5,2	7,8	142,1	94,8
15,1	22,7	127,6	84,9
25,0	37,4	112,3	75,0
44,9	67,3	82,6	55,1
74,8	112,7	37,9	25,2
Ibuprofen (IBU)/OCAS			
Ibuprofen (IBU)		OCAS	
Percentage (%)	Mass (mg)	Mass (mg)	Percentage (%)
5,3	7,9	142,5	94,7
14,9	22,4	127,6	85,1
25,2	37,9	112,3	74,8
44,8	67,2	82,7	55,2
75,1	112,1	37,1	24,9
Ibuprofen (IBU)/Polyvinylpyrrolidone (PVP)			
Ibuprofen (IBU)		PVP	
Percentage (%)	Mass (mg)	Mass (mg)	Percentage (%)
4,9	7,4	142,7	95,1
14,8	22,1	127,2	85,2
24,9	37,3	112,4	75,1
45,0	67,7	82,6	55,0
75,2	112,6	37,2	24,8
Ibuprofen (IBU)/Carbomer (CBER)			
Ibuprofen (IBU)		HPMC	
Percentage (%)	Mass (mg)	Mass (mg)	Percentage (%)
4,8	7,2	142,2	95,2
14,8	22,2	127,6	85,2
25,0	37,6	112,6	75,0
45,2	67,8	82,2	54,8
74,9	112,2	37,5	25,1
Ibuprofen (IBU)/Hydroxypropylmethylcellulose (HPMC)			
Ibuprofen (IBU)		HPMC	
Percentage (%)	Mass (mg)	Mass (mg)	Percentage (%)
5,0	7,5	142,2	95,0
14,8	22,2	127,6	85,2
24,8	37,2	112,6	75,2
44,9	67,1	82,3	55,1
74,9	112,4	37,6	25,1

Table A.1: Mass of mixtures containing Ibuprofen (IBU) and its respective excipients in different proportions.

SUB-MIXTURES (IBUPROFEN (IBU) AND EXCIPIENTS)				
Blend of Ibuprofen (IBU) with β-cyclodextrin (BCD)				
% IBU: %BCD		Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
5,2	94,8	29,6	29,7	30,7
15,1	84,9	30,3	29,5	31
25,0	75,0	31,3	29,7	29,7
44,9	55,1	30,2	31	29,8
74,8	25,2	29,8	30	30,3
Blend of Ibuprofen (IBU) with OCAS				
% IBU: %OCAS		Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
5,3	94,7	29,2	31,1	29,6
14,9	85,1	29,6	31,6	30,8
25,2	74,8	29,4	29,5	30
44,8	55,2	30,3	31,4	30,3
75,1	24,9	31,6	30,9	30,9
Blend of Ibuprofen (IBU) with Polyvinylpyrrolidone (PVP)				
% IBU: %PVP		Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
4,9	95,1	31,3	29,5	30,1
14,8	85,2	30,5	30,1	29,4
24,9	75,1	29,5	29,4	29,3
45,0	55,0	30,2	30,9	29,8
75,2	24,8	31	29,8	29,6
Blend of Ibuprofen (IBU) with Carbomer (CBER)				
% IBU: %CBER		Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
4,8	95,2	29,1	30,5	30,3
14,8	85,2	29,7	29,4	30,5
25,0	75,0	29,3	30,4	29,5
45,2	54,8	29,6	29,6	29,4
74,9	25,1	30,8	30,3	31,2
Blend of Ibuprofen (IBU) with Hydroxypropylmethylcellulose (HPMC)				
% IBU: %HPMC		Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
5,0	95,0	29,6	29,9	31,3
14,8	85,2	29,9	30,9	30,2
24,8	75,2	29,5	29,6	31
44,9	55,1	29,3	29,6	30,8
74,9	25,1	31,5	31,7	29,7

Table A.2: Mass of sub-mixtures containing Ibuprofen (IBU) and its respective excipients in different proportions.

Ibuprofen (IBU)/BCD												
First day								Second day			Third day	Average Final pH
Samples	1st pH measurement				2nd pH measurement			3rd pH measurement (before resting)			4th pH measurement (after resting)	
	pH0 (12h 40)	pH (15h 00)	pHc (15h 00)	µl (NaOH/ HCl)	pH (16h 45)	pHc (16h 45)	µl (NaOH/ HCl)	pH (13h 00)	pHc (13h 00)	µl (NaOH/ HCl)	Final pH	
951	2,04	2,04	-	-	2,03	-	-	2	-	-	2,07	2,0633
952	2,04	2,02	-	-	2,04	-	-	2	-	-	2,06	
953	2,04	2,02	-	-	2,04	-	-	1,99	-	-	2,06	
851	2,04	2,05	-	-	2,04	-	-	1,98	-	-	2,04	2,0467
852	2,04	2,01	-	-	2,04	-	-	1,99	-	-	2,04	
853	2,04	2,02	-	-	2,05	-	-	2	-	-	2,06	
751	2,04	2,03	-	-	2,04	-	-	1,98	-	-	2,06	2,0567
752	2,04	2,02	-	-	2,03	-	-	2	-	-	2,06	
753	2,04	2,03	-	-	2,03	-	-	2,02	-	-	2,05	
551	2,04	2,05	-	-	2,04	-	-	2,04	-	-	2,04	2,0433
552	2,04	2,03	-	-	2,04	-	-	2,02	-	-	2,05	
553	2,04	2,02	-	-	2,05	-	-	2,03	-	-	2,04	
251	2,04	2,04	-	-	2,05	-	-	2,01	-	-	2,03	2,0533
252	2,04	2,03	-	-	2,05	-	-	2,03	-	-	2,07	
253	2,04	2,04	-	-	2,06	-	-	2,03	-	-	2,06	

Table A.3: pH variations in Ibuprofen (IBU) and BCD samples during experimentation: Each sample is identified by a three-digit code: the initial two digits indicate the percentage of BCD used in the respective mixture, and the last digit designates the submix number derived from that specific mixture. Three sub-mixtures are generated from every combination of Ibuprofen (IBU) and BCD with specified proportions. These sub-mixtures are evenly distributed, ensuring reproducibility in each mix with specific proportions of Ibuprofen (IBU) and BCD.

Ibuprofen (IBU)/OCAS									Average Final pH
Samples	First day				Second day			Third day	
	1st pH measurement				2nd pH measurement (before resting)			3rd pH measurement (after resting)	
	pH0 (14h30)	pH (17h00)	pHc (17h00)	µ (NaOH/HCl)	pH (10h30)	pHc (10h30)	µ (NaOH/HCl)	Final pH	
951	2,06	2	-	-	1,99	-	-	2	2,03
952	2,06	2	-	-	2,01	-	-	2,01	
953	2,06	1,97	-	-	1,97	2,03	10µl NaOH	2,08	
851	2,06	2,02	-	-	2	-	-	1,97	2,053333333
852	2,06	1,97	-	-	1,98	-	-	1,98	
853	2,06	2,01	-	-	1,92	2,15	30µl NaOH	2,21	
751	2,06	2,01	-	-	1,95	2,04	20µl NaOH	2,12	2,086666667
752	2,06	2,01	-	-	1,98	-	-	2,01	
753	2,06	1,97	-	-	1,96	2,09	20µl NaOH	2,13	
551	2,06	1,97	-	-	1,99	-	-	1,99	2,063333333
552	2,06	2,02	-	-	1,9	2,12	20µl NaOH	2,19	
553	2,06	1,99	-	-	1,98	-	-	2,01	
251	2,06	1,99	-	-	1,96	2,05	20µl NaOH	2,11	2,123333333
252	2,06	2,01	-	-	1,97	-	-	2,02	
253	2,06	2,01	-	-	1,93	2,14	30µl NaOH	2,24	

Table A.4: pH variations in Ibuprofen (IBU) and OCAS samples during experimentation: Each sample is identified by a three-digit code: the initial two digits indicate the percentage of OCAS used in the respective mixture, and the last digit designates the submix number derived from that specific mixture. Three sub-mixtures are generated from every combination of Ibuprofen (IBU) and OCAS with specified proportions. These sub-mixtures are evenly distributed, ensuring reproducibility in each mix with specific proportions of Ibuprofen (IBU) and OCAS.

Ibuprofen (IBU)/PVP									Average Final pH
Samples	First day				Second day			Third day	
	1st pH measurement				2nd pH measurement (before resting)			3rd pH measurement (after resting)	
	pH0 (14h30)	pH (17h00)	pHc (17h00)	µ (NaOH/HCl)	pH (10h30)	pHc (10h30)	µ (NaOH/HCl)	Final pH	
951	2,06	2,01	-	-	1,93	1,98	10µl NaOH	2,08	2,063333333
952	2,06	1,99	-	-	1,94	1,98	10µl NaOH	2,1	
953	2,06	2,03	-	-	1,92	1,97	10µl NaOH	2,01	
851	2,06	1,95	2,05	10µl NaOH	2,03	-	-	2,08	2,093333333
852	2,06	2,03	-	-	1,95	2	20µl NaOH	2,09	
853	2,06	2,02	-	-	1,95	2,04	20µl NaOH	2,11	
751	2,06	2,04	-	-	1,94	1,99	10µl NaOH	2,06	2,066666667
752	2,06	2	-	-	1,93	1,97	10µl NaOH	2,06	
753	2,06	2,02	-	-	1,95	2,04	20µl NaOH	2,08	
551	2,06	2	-	-	1,95	2,02	20µl NaOH	2,11	2,073333333
552	2,06	2,03	-	-	1,95	1,98	10µl NaOH	2,05	
553	2,06	2,02	-	-	1,96	1,98	10µl NaOH	2,06	
251	2,06	2,01	-	-	1,93	1,98	10µl NaOH	2,08	2,116666667
252	2,06	2,02	-	-	1,95	2,04	20µl NaOH	2,14	
253	2,06	1,96	-	-	1,94	2,07	20µl NaOH	2,13	

Table A.5: pH variations in Ibuprofen (IBU) and PVP samples during experimentation: Each sample is identified by a three-digit code: the initial two digits indicate the percentage of PVP used in the respective mixture, and the last digit designates the submix number derived from that specific mixture. Three sub-mixtures are generated from every combination of Ibuprofen (IBU) and PVP with specified proportions. These sub-mixtures are evenly distributed, ensuring reproducibility in each mix with specific proportions of Ibuprofen (IBU) and PVP.

Ibuprofen (IBU)/CBER									Average Final pH
Samples	First day				Second day			Third day	
	1st pH measurement				2nd pH measurement (before resting)			3rd pH measurement (after resting)	
	pH0 (14h30)	pH (17h00)	pHc (17h00)	µ (NaOH/HCl)	pH (10h30)	pHc (10h30)	µ (NaOH/HCl)	Final pH	
951	2,06	1,97	-	-	1,89	2,05	30µl NaOH	2,15	2,14
952	2,06	1,99	-	-	1,96	2,08	20µl NaOH	2,15	
953	2,06	1,99	-	-	1,93	2,03	20µl NaOH	2,12	
851	2,06	2	-	-	2	-	-	1,98	2,083333333
852	2,06	1,98	-	-	1,94	2,03	20µl NaOH	2,12	
853	2,06	1,99	-	-	1,94	2,08	30µl NaOH	2,15	
751	2,06	1,96	-	-	1,93	2,03	20µl NaOH	2,1	2,03
752	2,06	1,98	-	-	1,97	-	-	1,99	
753	2,06	1,98	-	-	2,02	-	20µl NaOH	2	
551	2,06	1,98	-	-	1,98	-	-	1,98	2,053333333
552	2,06	1,96	-	-	1,96	2,02	20µl NaOH	2,09	
553	2,06	1,99	-	-	1,95	2,05	20µl NaOH	2,09	
251	2,06	1,98	-	-	1,91	2,02	20µl NaOH	2,1	2,13
252	2,06	1,98	-	-	1,94	2,05	20µl NaOH	2,12	
253	2,06	1,97	-	-	1,91	2,05	30µl NaOH	2,17	

Table A.6: pH variations in Ibuprofen (IBU) and CBER samples during experimentation: Each sample is identified by a three-digit code: the initial two digits indicate the percentage of CBER used in the respective mixture, and the last digit designates the submix number derived from that specific mixture. Three sub-mixtures are generated from every combination of Ibuprofen (IBU) and CBER with specified proportions. These sub-mixtures are evenly distributed, ensuring reproducibility in each mix with specific proportions of Ibuprofen (IBU) and CBER.

Ibuprofen (IBU)/HPMC												Average Final pH
Samples	First day							Second day			Third day	
	1st pH measurement				2nd pH measurement			3rd pH measurement (before resting)			4th pH measurement (after resting)	
	pH0 (12h 30)	pH (13h 50)	pHc (13h 50)	µ (NaOH/ HCl)	pH (17h 30)	pHc (17h 30)	µ (NaOH/ HCl)	pH (14h 30)	pHc (14h 30)	µl (NaOH/ HCl)	Final pH	
951	2,07	2,01	-	-	2	-	-	1,95	2,01	10µl NaOH	2,07	2,03
952	2,07	2,02	-	-	2,03	-	-	2	-	-	1,99	
953	2,07	2,03	-	-	2,01	-	-	2	-	-	2,03	
851	2,07	2,03	-	-	2,01	-	-	1,94	2	10µl NaOH	2,07	2,067
852	2,07	2,01	-	-	2,01	-	-	1,96	2,03	10µl NaOH	2,08	
853	2,07	2,02	-	-	2,01	-	-	1,97	2,01	10µl NaOH	2,05	
751	2,07	2,02	-	-	2,02	-	-	1,98	2,01	10µl NaOH	2,08	2,073
752	2,07	2	-	-	2,02	-	-	1,96	2	10µl NaOH	2,07	
753	2,07	2	-	-	2,01	-	-	1,95	1,99	10µl NaOH	2,07	
551	2,07	2	-	-	2,01	-	-	1,99	-	-	2,02	2,043
552	2,07	2,01	-	-	2	-	-	1,95	2,01	10µl NaOH	2,08	
553	2,07	2,01	-	-	2,02	-	-	1,99	-	-	2,03	
251	2,07	2,02	-	-	2,03	-	-	1,99	-	-	2,02	2,033
252	2,07	2	-	-	2	-	-	1,95	2	10µl NaOH	2,07	
253	2,07	2,01	-	-	2,01	-	-	1,99	-	-	2,01	

Table A.7: pH variations in Ibuprofen (IBU) and HPMC samples during experimentation: Each sample is identified by a three-digit code: the initial two digits indicate the percentage of HPMC used in the respective mixture, and the last digit designates the submix number derived from that specific mixture. Three sub-mixtures are generated from every combination of Ibuprofen (IBU) and HPMC with specified proportions. These sub-mixtures are evenly distributed, ensuring reproducibility in each mix with specific proportions of Ibuprofen (IBU) and HPMC.

% Excipient (BCD)	Ibuprofen (IBU) Concentration (mol/L)	Ibuprofen (IBU) Concentration (log M)
94,79653102	0,000654446	-3,18412635
84,89687292	0,001921019	-2,716468323
75,01670007	0,001906656	-2,719727654
55,10340227	0,001820325	-2,739851138
25,16600266	0,001650551	-2,782371105
% Excipient (OCAS)	**Ibuprofen (IBU) Concentration (mol/L)**	**Ibuprofen (IBU) Concentration (log M)**
94,74734043	0,000129667	-3,887171723
85,06666667	0,000131405	-3,881389107
74,76697736	0,000120026	-3,920724345
55,17011341	0,000124452	-3,904996411
24,86595174	0,000126271	-3,898696356
% Excipient (PVP)	**Ibuprofen (IBU) Concentration (mol/L)**	**Ibuprofen (IBU) Concentration (log M)**
95,06995336	0,000128856	-3,889896854
85,19758875	0,000282688	-3,548693157
75,08350033	0,000196783	-3,706012825
54,95675316	0,000178242	-3,748989989
24,83311081	0,000143783	-3,842293404
% Excipient (CBER)	**Ibuprofen (IBU) Concentration (mol/L)**	**Ibuprofen (IBU) Concentration (log M)**
95,18072289	0,000130263	-3,885179276
85,18024032	0,000133624	-3,874115749
74,96671105	0,000129335	-3,888283501
54,8	0,000128347	-3,891614231
25,0501002	0,000128548	-3,890934077
% Excipient (HPMC)	**Ibuprofen (IBU) Concentration (mol/L)**	**Ibuprofen (IBU) Concentration (log M)**
94,98997996	0,000156277	-3,806106195
85,18024032	0,000158433	-3,800154303
75,16688919	0,000138733	-3,857818705
55,08701473	0,000144553	-3,839973669
25,06666667	0,0001259	-3,899974057

Table A.8: Experimental solubility values of Ibuprofen (IBU) in addition of different proportions of excipients

Appendix B

Experimental Findings: Data and Graphs Gathered Throughout the Fluconazole (FLU) Research.

MIXTURES (FLUCONAZOLE (FLU)/EXCIPIENTS)			
Fluconazole (FLU)/β-cyclodextrin (BCD)			
Fluconazole (FLU)		BCD	
Percentage (%)	Mass (mg)	Mass (mg)	Percentage (%)
4,8	7,2	142,4	95,2
14,9	22,4	127,5	85,1
25,1	37,5	112,2	74,9
45,0	67,6	82,5	55,0
74,8	112,2	37,8	25,2
Fluconazole (FLU)/OCAS			
Fluconazole (FLU)		OCAS	
Percentage (%)	Mass (mg)	Mass (mg)	Percentage (%)
4,9	7,3	142,6	95,1
15,0	22,5	127,6	85,0
25,0	37,5	112,5	75,0
45,1	67,4	82,1	54,9
75,0	112,3	37,4	25,0
Fluconazole (FLU)/Polyvinylpyrrolidone (PVP)			
Fluconazole (FLU)		PVP	
Percentage (%)	Mass (mg)	Mass (mg)	Percentage (%)
5,0	27,7	522,5	95,0
15,0	52,3	297,5	85,0
25,0	87,7	262,4	75,0
45,0	67,7	82,6	55,0
74,8	112,2	37,7	25,2
Fluconazole (FLU)/Carbomer (CBER)			
Fluconazole (FLU)		HPMC	
Percentage (%)	Mass (mg)	Mass (mg)	Percentage (%)
5,1	7,7	142,2	94,9
15,0	22,4	127,2	85,0
24,8	37,1	112,2	75,2
45,0	67,3	82,4	55,0
75,1	112,8	37,3	24,9
Fluconazole (FLU)/Hydroxypropylmethylcellulose (HPMC)			
Fluconazole (FLU)		HPMC	
Percentage (%)	Mass (mg)	Mass (mg)	Percentage (%)
4,9	14,6	284,7	95,1
15,1	45,4	255	84,9
25,1	50,3	150,1	74,9
45,0	67,3	82,3	55,0
75,0	112,2	37,4	25,0

Table B.1: Mass of mixtures containing Fluconazole (FLU) and its respective excipients in different proportions.

SUB-MIXTURES (FLUCONAZOLE (FLU) AND EXCIPIENTS)				
Blend of Fluconazole (FLU) with β-cyclodextrin (BCD)				
% FLU: %BCD		Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
75,9	95,2	30,3	29,9	30,1
73,1	85,1	31,3	30,1	30,4
70,9	74,9	30,8	31,3	29,9
65,1	55,0	29,5	30,4	30,5
44,8	25,2	31,1	30,9	30,4
Blend of Fluconazole (FLU) with OCAS				
% FLU: %OCAS		Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
4,9	95,1	31,1	29,8	30,5
15,0	85,0	30,7	31,1	30,1
25,0	75,0	29,7	31,2	29,9
45,1	54,9	31,9	29,6	30,1
75,0	25,0	30,7	31,2	30,6
Blend of Fluconazole (FLU) with Polyvinylpyrrolidone (PVP)				
% FLU: %PVP		Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
5,0	95,0	149,3	151,5	151
15,0	85,0	79,2	79,7	79,3
25,0	75,0	81,5	81,3	82,6
45,0	55,0	30,7	29,4	30,9
74,8	25,2	31,2	30,8	30,6
Blend of Fluconazole (FLU) with Carbomer (CBER)				
% FLU: %CBER		Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
5,1	94,9	30,2	29,4	30
15,0	85,0	30,1	29,6	30,2
24,8	75,2	29,8	29,8	30
45,0	55,0	29,4	30,1	29,4
75,1	24,9	29,2	30,4	29,8
Blend of Fluconazole (FLU) with Hydroxypropylmethylcellulose (HPMC)				
% FLU: %HPMC		Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
4,9	95,1	75,1	77,6	77,6
15,1	84,9	78,9	75	75,8
25,1	74,9	59,8	60	53,9
45,0	55,0	30,4	30,7	31,5
75,0	25,0	29,9	29,5	29,8

Table B.2: Mass of sub-mixtures containing Fluconazole (FLU) and its respective excipients in different proportions.

Fluconazole (FLU)/BCD												Average Final pH
Samples	First day				Second day						Third day	
	1st pH measurement				2nd pH measurement			3rd pH measurement (before resting)			4th pH measurement (after resting)	
	pH0 (13h 00)	pH (14h 30)	pHc (14h 30)	µ (NaOH/ HCl)	pH (8h30)	pHc (8h30)	µ (NaOH/ HCl)	pH (14h 00)	pHc (14h 00)	µl (NaOH/ HCl)	Final pH	
951	4,8	4,74	-	-	4,65	-	-	4,61	-	-	4,61	4,6333
952	4,8	4,75	-	-	4,6	-	-	4,65	-	-	4,63	
953	4,8	4,73	-	-	4,7	-	-	4,65	-	-	4,66	
851	4,8	4,66	-	-	4,63	-	-	4,63	-	-	4,63	4,61
852	4,8	4,68	-	-	4,6	-	-	4,59	-	-	4,61	
853	4,8	4,68	-	-	4,57	-	-	4,6	-	-	4,59	
751	4,8	4,66	-	-	4,57	-	-	4,56	-	-	4,57	4,6367
752	4,8	4,67	-	-	4,6	-	-	4,6	-	-	4,72	
753	4,8	4,65	-	-	4,56	-	-	4,56	-	-	4,62	
551	4,8	4,56	-	-	4,46	-	-	4,44	-	-	4,4	4,46
552	4,8	4,57	-	-	4,49	-	-	4,44	-	-	4,44	
553	4,8	4,6	-	-	4,52	-	-	4,53	-	-	4,54	
251	4,8	4,64	-	-	4,53	-	-	4,53	-	-	4,52	4,4633
252	4,8	4,46	-	-	4,41	-	-	4,43	-	-	4,44	
253	4,8	4,51	-	-	4,43	-	-	4,44	-	-	4,43	

Table B.3: pH variations in Fluconazole (FLU) and BCD samples during experimentation: Each sample is identified by a three-digit code: the initial two digits indicate the percentage of BCD used in the respective mixture, and the last digit designates the submix number derived from that specific mixture. Three sub-mixtures are generated from every combination of Fluconazole (FLU) and BCD with specified proportions. These sub-mixtures are evenly distributed, ensuring reproducibility in each mix with specific proportions of Fluconazole (FLU) and BCD.

Fluconazole (FLU)/OCAS												Average Final pH
Samples	First day				Second day						Third day	
	1st pH measurement				2nd pH measurement			3rd pH measurement (before resting)			4th pH measurement (after resting)	
	pH0 (12h 00)	pH (14h 00)	pHc (14h 00)	µl (NaOH/ HCl)	pH (9h00)	pHc (9h00)	µl (NaOH/ HCl)	pH (13h 30)	pHc (13h 30)	µl (NaOH/ HCl)	Final pH	
951	4,59	5,11	4,72	0,9µl HCl	4,65	-	-	4,58	-	-	4,53	4,54
952	4,59	5,05	4,69	0,9µl HCl	4,56	-	-	4,57	-	-	4,47	
953	4,59	5,11	4,81	0,9µl HCl	4,66	-	-	4,61	-	-	4,62	
851	4,59	5,04	4,4	0,9µl HCl	4,42	-	-	4,4	-	-	4,41	4,42
852	4,59	5,01	4,4	0,9µl HCl	4,44	-	-	4,39	-	-	4,36	
853	4,59	5,03	4,56	0,9µl HCl	4,5	-	-	4,51	-	-	4,49	
751	4,59	4,94	4,7	4µl HCl/3µl NaOH	4,55	-	-	4,54	-	-	4,47	4,3567
752	4,59	5,01	4,3	0,9µl HCl	4,37	-	-	4,39	-	-	4,33	
753	4,59	4,97	4,32	0,9µl HCl	4,41	-	-	4,39	-	-	4,27	
551	4,59	4,73	-	-	4,82	-	-	4,73	-	-	4,69	4,6233
552	4,59	4,82	-	-	4,76	-	-	4,8	-	-	4,6	
553	4,59	4,83	-	-	4,83	-	-	4,66	-	-	4,58	
251	4,59	4,61	-	-	4,51	-	-	4,51	-	-	4,63	4,6333
252	4,59	4,63	-	-	4,58	-	-	4,58	-	-	4,68	
253	4,59	4,61	-	-	4,59	-	-	4,58	-	-	4,59	

Table B.4: pH variations in Fluconazole (FLU) and OCAS samples during experimentation: Each sample is identified by a three-digit code: the initial two digits indicate the percentage of OCAS used in the respective mixture, and the last digit designates the submix number derived from that specific mixture. Three sub-mixtures are generated from every combination of Fluconazole (FLU) and OCAS with specified proportions. These sub-mixtures are evenly distributed, ensuring reproducibility in each mix with specific proportions of Fluconazole (FLU) and OCAS.

Fluconazole (FLU)/PVP (95%: 550 mg; 85% y 75%: 350 mg; 55% y 25%: 150 mg)											Average Final pH
Samples	First day				Second day					Third day	
					1st pH measurement (before resting)					2nd pH measurement (after resting)	
	pH0 (14h30)				pH (10h00)	pHc (10h00)	µl (NaOH/HCl)			Final pH	
951	4,54				4,05	4,79	1,8µl NaOH			4,78	4,7367
952	4,54				4,03	4,81	1,8µl NaOH			4,83	
953	4,54				4,06	4,64	1,8µl NaOH			4,6	
	First day				Second day					Third day	
	1st pH measurement				2nd pH measurement(before resting)					3rd pH measurement (after resting)	
	pH0 (12h40)	pH (14h30)	pHc (14h30)	µl (NaOH/HCl)	pH (10h00)	pHc (10h00)	µl (NaOH/HCl)			Final pH	
851	4,63	3,91	4,66	3,2µl HCl/3,9µl NaOH	4,54	-	-			4,55	4,5233
852	4,63	3,77	4,64	0,9µl HCl/5,2µl NaOH	4,53	-	-			4,52	
853	4,63	3,84	4,68	0,9µl HCl/3,9µl NaOH	4,53	-	-			4,5	
751	4,63	3,71	4,8	7µl NaOH	4,74	-	-			4,74	4,5833
752	4,63	3,89	4,58	3,9µl NaOH	4,48	-	-			4,48	
753	4,63	3,9	4,76	1,8µl HCl/3,9µl NaOH	4,59	-	-			4,53	
	First day				Second day					Third day	
	1st pH measurement				2nd pH measurement		3rd pH measurement (before resting)			4th pH measurement (after resting)	
	pH0 (12h30)	pH (13h50)	pHc (13h50)	µl (NaOH/HCl)	pH (10h00)	pHc (10h00)	µl (NaOH/HCl)	pH (14h30)	pHc (14h30)	µl (NaOH/HCl)	Final pH	
551	4,6	4,29	4,8	1,6µl HCl/1,6 µl NaOH	4,77	-	-	4,8	4,54	0,9µl HCl/0,9 µl NaOH	4,56	4,52
552	4,6	4,3	4,54	0,8µl HCl/0,8 µl NaOH	4,54	-	-	4,55	-	-	4,55	
553	4,6	4,29	4,52	0,8µl NaOH	4,45	-	-	4,45	-	-	4,45	
251	4,6	4,4	4,46	3,2µl HCl/3,2	4,4	4,76	1,8µl HCl/0,9	4,77	4,58	2,7µl HCl/2,7	4,61	4,5567

				µl NaOH			µl NaOH			µl NaOH		
252	4,6	4,31	4,79	1,8µl NaOH	4,73	-	-	4,76	4,59	0,9µl HCl	4,57	
253	4,6	4,41	-	-	4,38	4,45	0,9µl HCl/0,9 µl NaOH	4,46	4,49	0,9µl HCl/0,9 µl NaOH	4,49	

Table B.5: pH variations in Fluconazole (FLU) and PVP samples during experimentation: Each sample is identified by a three-digit code: the initial two digits indicate the percentage of PVP used in the respective mixture, and the last digit designates the submix number derived from that specific mixture. Three sub-mixtures are generated from every combination of Fluconazole (FLU) and PVP with specified proportions. These sub-mixtures are evenly distributed, ensuring reproducibility in each mix with specific proportions of Fluconazole (FLU) and PVP.

Fluconazole (FLU)/CBER												
Samples	First day				Second day						Third day	Average Final pH
	1st pH measurement				2nd pH measurement			3rd pH measurement (before resting)			4th pH measurement (after resting)	
	pH0 (12h 30)	pH (13h 50)	pHc (13h 50)	µl (NaOH/ HCl)	pH (10h 00)	pHc (10h 00)	µl (NaOH/ HCl)	pH (14h 30)	pHc (14h 30)	µl (NaOH/ HCl)	Final pH	
951	4.6	2,92	4,59	70µl NaOH	4,32	4,68	20µl NaOH	4,61	-	-	4,68	4,6333
952	4,6	3,15	4,41	70µl NaOH	4,66	-	-	4,7	-	-	4,71	
953	4,6	2,81	4,7	70µl NaOH	4,55	-	-	4,52	-	-	4,51	
851	4,6	2,92	4,6	0,9µl HCl/90 µl NaOH	4,49	4,55	3µl NaOH	4,7	-	-	4,71	4,6667
852	4.6	2,92	4,47	70µl NaOH	4.31	4,64	15µl NaOH	4,69	-	-	4,72	
853	4,6	3,09	4,48	75µl NaOH	4,55	-	-	4,57	-	-	4,57	
751	4,6	2,94	4,5	69µl NaOH	4,47	4,54	3µl NaOH	4,56	-	-	4,57	4,54
752	4,6	2,93	4,58	60µl NaOH	4,36	4,56	8µl NaOH	4,48	-	-	4,46	
753	4.6	3,04	4,6	74µl NaOH	4,58	-	-	4,56	-	-	4,59	

551	4,6	3,07	4,71	50µl NaOH	4,45	4,51	9µl NaOH	4,55	-	-	4,55	
552	4.6	3,23	4,51	35µl NaOH	4,56	-	-	4,58	-	-	4,58	4,5567
553	4.6	3,27	4,54	40µl NaOH	4,51	-	-	4,54	-	-	4,54	
251	4,6	3,35	4,68	0,9µl HCl/30 µl NaOH	4,63	-	-	4,65	-	-	4,64	
252	4,6	3,49	4,53	30µl NaOH	4,75	4,66	0,9µl HCl	4,72	-	-	4,7	4,68
253	4,6	3,48	4,68	0,9µl HCl/30 µl NaOH	4,67	-	-	4,69	-	-	4,7	

Table B.6: pH variations in Fluconazole (FLU) and CBER samples during experimentation: Each sample is identified by a three-digit code: the initial two digits indicate the percentage of CBER used in the respective mixture, and the last digit designates the submix number derived from that specific mixture. Three sub-mixtures are generated from every combination of Fluconazole (FLU) and CBER with specified proportions. These sub-mixtures are evenly distributed, ensuring reproducibility in each mix with specific proportions of Fluconazole (FLU) and CBER.

Fluconazole (FLU)/HPMC (95% y 85%: 300 mg; 75%: 200 mg; 55% y 25%: 150 mg)												Average Final pH
Samples	First day				Second day						Third day	
	1st pH measurement				2nd pH measurement			3rd pH measurement (before resting)			4th pH measurement (after resting)	
	pH0 (12h 30)	pH (13h 50)	pHc (13 h50)	µl (NaOH /HCl)	pH (10 h00)	pHc (10h0 0)	µl (NaO H/HCl)	pH (14h 30)	pHc (14h3 0)	µl (NaOH/ HCl)	Final pH	
951	4,6	4, 74	-	-	4,85	4,54	0,9µl HCl/0, 9µl NaOH	4,63	-	-	4,82	4,7967
952	4,6	4, 62	-	-	4,92	4,75	3,2µl HCl/3, 2µl NaOH	4,76	-	-	4,9	
953	4,6	4, 58	-	-	5,04	4,49	1,8µl HCl/1, 8µl NaOH	4,56	-	-	4,67	
851	4,6	4. 70	-	-	4,79	-	-	4,81	4,53	5,4µl HCl/5,4 µl NaOH	4,69	4,73
852	4,6	4, 57	-	-	4,74	-	-	4,75	-	-	4,75	
853	4,6	4, 73	-	-	4,82	4,59	1,8µl HCl/1, 8µl NaOH	4,7	-	-	4,75	
	First day				Second day						Third day	
	1st pH measurement				2nd pH measurement			3rd pH measurement (before resting)			4th pH measurement (after resting)	
	pH0 (13h 00)	pH (14h 30)	pHc (14 h30)	µl (NaOH /HCl)	pH (8h 30)	pHc (8h30)	µl (NaO H/HCl)	pH (14h 00)	pHc (14h0 0)	µl (NaOH/ HCl)	Final pH	
751	4,8	4, 81	-	-	4,7	-	-	4,74	-	-	4,73	4,73
752	4,8	4, 88	4,75	1,8µl HCl/0,9 µl NaOH	4,75	-	-	4,79	-	-	4,79	

753	4,8	4,85	-	-	4,69	-	-	4,67	-	-	4,67	
	First day	**Second day**									**Third day**	
		1st pH measurement			**2nd pH measurement(before resting)**						**3rd pH measurement (after resting)**	
	pH0 (11h30)	**pH (9h00)**	**pHc (9h00)**	**µl (NaOH/HCl)**	**pH (13h30)**	**pHc (13h30)**	**µ (NaOH/HCl)**				**Final pH**	
551	4,59	3,69	4,29	0,9µl HCl/2,7µl NaOH	4,42	-	-				4,42	4,5767
552	4,59	3,71	4,62	1,8µl NaOH	4,68	-	-				4,64	
553	4,59	3,77	4,63	0,9µl NaOH	4,7	-	-				4,67	
251	4,59	4,75	-	-	4,72	-	-				4,74	4,5733
252	4,59	4,87	4,86	0,9µl HCl/0,9µl NaOH	4,92	4,62	1,8µl HCl/0,9µl NaOH				4,62	
253	4,59	4,36	-	-	4,44	-	-				4,36	

Table B.7: pH variations in Fluconazole (FLU) and HPMC samples during experimentation: Each sample is identified by a three-digit code: the initial two digits indicate the percentage of HPMC used in the respective mixture, and the last digit designates the submix number derived from that specific mixture. Three sub-mixtures are generated from every combination of Fluconazole (FLU) and HPMC with specified proportions. These sub-mixtures are evenly distributed, ensuring reproducibility in each mix with specific proportions of Fluconazole (FLU) and HPMC.

% Excipient (BCD)	Fluconazole (FLU) Concentration (mol/L)	Fluconazole (FLU) Concentration (log M)
95,18716578	0,001841212	-2,734896184
85,05670447	0,007327682	-2,135033376
74,9498998	0,008864545	-2,052343558
54,96335776	0,013648071	-1,864928718
25,2	0,016032714	-1,794992968
% Excipient (OCAS)	**Fluconazole (FLU) Concentration (mol/L)**	**Fluconazole (FLU) Concentration (log M)**
95,13008672	0,00199355	-2,700372769
85,00999334	0,005651197	-2,24785954
75	0,008704958	-2,060233303
54,91638796	0,012046738	-1,919130528
24,98329993	0,015263392	-1,816348941
% Excipient (PVP)	**Fluconazole (FLU) Concentration (mol/L)**	**Fluconazole (FLU) Concentration (log M)**
94,9654671	0,008622813	-2,064351052
85,0485992	0,013910149	-1,856668211
74,95001428	0,017857976	-1,748167756
54,95675316	0,011591376	-1,935865
25,15010007	0,014951849	-1,825305108
% Excipient (CBER)	**Fluconazole (FLU) Concentration (mol/L)**	**Fluconazole (FLU) Concentration (log M)**
94,86324216	0,002091591	-2,679523183
85,02673797	0,005609616	-2,251066893
75,15070328	0,00800672	-2,096545379
55,04342017	0,010358809	-1,984690185
24,85009993	0,008721489	-2,059409352
% Excipient (HPMC)	**Fluconazole (FLU) Concentration (mol/L)**	**Fluconazole (FLU) Concentration (log M)**
95,12195122	0,002355704	-2,627879325
84,88681758	0,005769457	-2,238865096
74,9001996	0,011969679	-1,921917501
55,01336898	0,015874399	-1,799302719
25	0,017547323	-1,755789127

Table B.8: Experimental solubility values of Fluconazole (FLU) in addition of different proportions of excipients.