Primjena ciklodekstrina u poboljšanju in vitro brzine otapanja flufenaminske kiseline

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Application of cyclodextrins to enhance *in vitro* dissolution rate of flufenamic acid

DIPLOMA THESIS

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1 INTRODUCTION

1.1 PAIN

Task Force on Taxonomy of the International Association for the Study of Pain (IASP) back in 1979 defined the pain as:

"An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage."

Definition formed like this suggests that:

- Pain is always unpleasant and therefore an emotional experience (IASP, 1986).
- The pathophysiological mechanism of pain is immensely complex. Pain coming from the periphery (organs, tissues, nervous system) is just a rough impulse that is further modulated before it becomes a conscious perception in the cortical centers of the brain. Modulation plays an important role in the perception of pain (IASP, 2018; Francetić and Vitezović, 2014).
- Pain is always subjective and individuals learn the usage of that word experiencing the pain during their lifespan (IASP, 1986).
- Actual or potential tissue damage explains that the experience of the pain does not have to relate to the stimuli and there is usually no way to differentiate the experience of the pain caused by the tissue damage from that with the lack of tissue damage (IASP, 1986).

The definition was revised and updated by the IASP Task Force two more times (in 1986 and 2011) and was being commented on by other independent authors (Cohen et al., 2018) few more times, but the essence of the definition stayed the same until today. Numerous revisions of the definition were meant to further deepen the "matured" definition.

Pain is one of the most common reasons why patients come to their family physician (30% of the patients) or why they report to the emergency service (50% of the patients). Therefore, pain treatment belongs to the fundamental rights of the patient (Francetić and Vitezović, 2014).

1.1.1 Classifications of pain

According to the International Association for the Study of Pain (IASP) as well as the USA National Academies of Sciences, Engineering, and Medicine, pain can be classified based on pain's location (e.g. low back pain, orofacial pain, joint disorder, migraine) and/or type (nociceptive – somatic or visceral and neuropathic), as well as duration (acute/subacute/chronic).

According to Woolf et al., the above-mentioned types of classification have few limitations and should be disregarded in favor of mechanism. Based on that, pain is usually classified into three major classes — nociceptive pain, neuropathic pain, and inflammatory pain (Woolf et al., 1998).

On the other hand, because of the difference in managing acute and chronic (persistent, long-lasting) pain, classification of pain based on the duration of pain sensation is also important in clinical practice (Goucke, 2013).

Most of the time it is not unambiguous which type of pain we are dealing with and since they tend to coexist, it is of most importance to take up a detailed medical history and current status for determination of proper treatment (Francetić and Vitezović, 2014; IOM, 2011).

1.1.2 Pain management

Depending on the mechanism and the duration of the painful sensation, we are talking about different management approaches (Francetić and Vitezović, 2014).

Institute of Medicine (IOM) in their 2011 Relieving Pain in America report concluded that pain treatment must be custom-made for each person since there is a variation in people's tolerance of pain and therefore their need for pain management. Appropriate and on-time detection and management of the underlying cause of the pain has become a crucial part of pain treatment.

Clinical evaluation of pain should include the patient's medical record, comorbidities, past medication history, as well as the cause, site, severity, and impact of the pain. Inappropriate assessment of the pain is the usual cause of inappropriate treatment which leads to slower recovery, increased morbidity and increased cost of care, and an increased risk of progression of pain (National Academy of Science, 2019).

Pharmacotherapy is the most important therapeutic measure for pain management, even though sometimes non-pharmacological measures are a possible solution to achieve improvement. Non-pharmacological measures should be delegated early to yield the best possible outcome (Goucke, 2003). When properly appointed, they can reduce the need and/or dose of pharmacotherapy in use (Francetić and Vitezović, 2014).

Pharmacological pain management is based on the cause of the pain, intensity, and severity of the pain as well as the patient's history. Important notice is that before considering that NSAIDs are not effective, one must verify that the dose was high enough and that the drug was taken as soon as the headache appeared (Pardutz, 2010).

The International Association for the Study of Pain advocates that the relief of pain should be recognized as a human right and that chronic pain should be considered a disease in its own right (www.iasp-pain.org).

1.1.2.1 Classification of pain based on duration and appropriate treatment

Regarding its duration, pain can be classified as acute (lasting less than 6 weeks), subacute (6–12 weeks), or chronic (more than 12 weeks) (NICE, 2021; National Academy of Science, 2019; Dowell et al., 2016; Goucke 2003; Woolf, 1998)

Acute pain is usually linked to a specific event, injury, or illness. It may also be recurrent with pain-free periods and such pain typically lasts up to 7 days but can be prolonged to 30 days (National Academy of Science, 2019).

When pain lasts longer than 3 months or more than the time expected for an injury to heal, we are talking about chronic pain (IOM, 2011). Due to its longer-lasting, the patient's experience of pain is more complex, with more psychological features, and seeks more complex treatment (National Academy of Science, 2019).

The goals for patients with acute pain are to manage the pain and prevent chronic pain and long-term treatment (National Academy of Science, 2019). Acute pain is usually managed with medications such as analgesics and anesthetics. Initial treatment steps include non-pharmaceutical measures, non-opioid analgesics, or a combination of them and opioids (Brinar et al., 2009).

Chronic or persistent pain can be considered a chronic disease and therefore its management is complex and more difficult (Francetić and Vitezović, 2014). It is considered that patients with chronic pain have lower levels of serotonin in the upper parts of the CNS

which is why antidepressants (amitriptyline, citalopram, duloxetine, fluoxetine, paroxetine, sertraline) may help with the quality of life, pain, sleep, and psychological distress, even in the absence of a diagnosis of depression (NICE, 2011; Brinar et al., 2009; Goucke, 2003).

1.1.2.2 Classification of pain based on mechanism and appropriate treatment

1.1.2.2.1 Nociceptive pain

Nociceptive pain represents the body's protective system and is early activated as a warning and is essential for minimizing contact with potentially damaging or noxious stimuli (Julius, 2001). It arises as a result of actual or threatening mechanical, chemical, or thermal stimulation of peripheral sensory nerves – nociceptors (Francetić and Vitezović, 2014; IASP, 2011; Woolf, 2010).

We can distinguish somatic (superficial and deep) and visceral nociceptive pain and each of them responds to different kinds of treatment (Kyung-Hoon et al., 2020).

Nociceptive pain is usually described as well localized and sharp (Goucke, 2003).

Somatic nociceptive pain responds to non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen (paracetamol), acetylsalicylic acid, and steroids (Kyung-Hoon et al., 2020). On the other hand, visceral nociceptive pain usually responds to opioids (Brinar et al., 2009).

1.1.2.2.2 Neuropathic pain

Neuropathic pain is caused by a lesion or disease of the somatosensory nervous system and can be supported by several different mechanisms. It can develop spontaneously or as a response to usually non-painful stimuli (IASP, 2011; Nicholson, 2006; Goucke, 2003).

Neuropathic pain is characterized as burning, shooting (Goucke, 2003), different from nociceptive sensations, like sinking, piercing, shining, discomfort (Francetić and Vitezović, 2014).

In general, neuropathic pain is characterized by poor response to conventional treatment, especially peripheral analgesics as nonsteroidal anti-inflammatory drugs (NSAIDs) (Francetić and Vitezović, 2014), while the tricyclic antidepressants, antiepileptics (carbamazepine, phenytoin, gabapentin, and lamotrigine), corticosteroids, antiarrhythmics (lidocaine), neuroleptics and opioids have an effect in the treatment, and in general all of the drugs with an effect on CNS (NICE, 2021; Brinar et al., 2009).

Because of the complex background of neuropathic pain, the diagnose can be inconclusive or inconsistent (IASP, 2011). In such instances, clinical judgment is of the highest importance (Nicholson, 2006; Goucke, 2003).

1.2 NSAID

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed therapeutics that have well-documented analgesic, antipyretic and, at higher doses, anti-inflammatory properties (Hill and Zawia, 2021; Kyung-Hoon, 2020; Liu et al., 2019; Jong-Suep et al., 2017; Musa 2017; Barker et al., 2012; Pardutz, 2010; Pfaffenrath et al., 1995).

The mechanism behind NSAIDs was not completely understood until the early 1990s (Hill and Zawia, 2021). Today we know that these effects are achieved by inhibiting cyclooxygenase (COX), an enzyme responsible for prostaglandin synthesis. NSAIDs prevent the conversion of arachidonic acid to eicosanoids. The result is a reduction of proinflammatory prostaglandin synthesis (Hill and Zawia, 2021; Barker et al., 2012; Pardutz, 2010; Pérez Ruiz et al., 1998; Pfaffenrath et al., 1995; Sanger and Bennett, 1979; Flower et al., 1972).

NSAIDs are approved as an analgesic in a wide range of conditions: headache, toothache, migraines, musculoskeletal pain (rheumatoid arthritis, osteoarthritis, ankylosing spondylitis), dysmenorrhea, postoperative pain, renal colic, gout (but not salicylate), allergic conjunctivitis (Hill and Zawia, 2021; Kyung-Hoon, 2020; Katzung et al., 2012).

1.2.1 Chemistry

When we are talking about the chemical structure, NSAIDs are a heterogeneous therapeutic group. We can distinguish several chemical classes (Liu et al., 2019; Katzung et al., 2012; Pfaffenrath et al., 1995):

- salicylic acid - acetylsalicylic acid
- pyrazolones - propyphenazone, metamizole
- phenylacetic acids - diclofenac
- indoles - indomethacin
- propionic acids - ibuprofen, ketoprofen, naproxen
- anthranilic acids – fenamates
- enolic acids - oxicams - piroxicam
- coxibs - etoricoxib, celecoxib

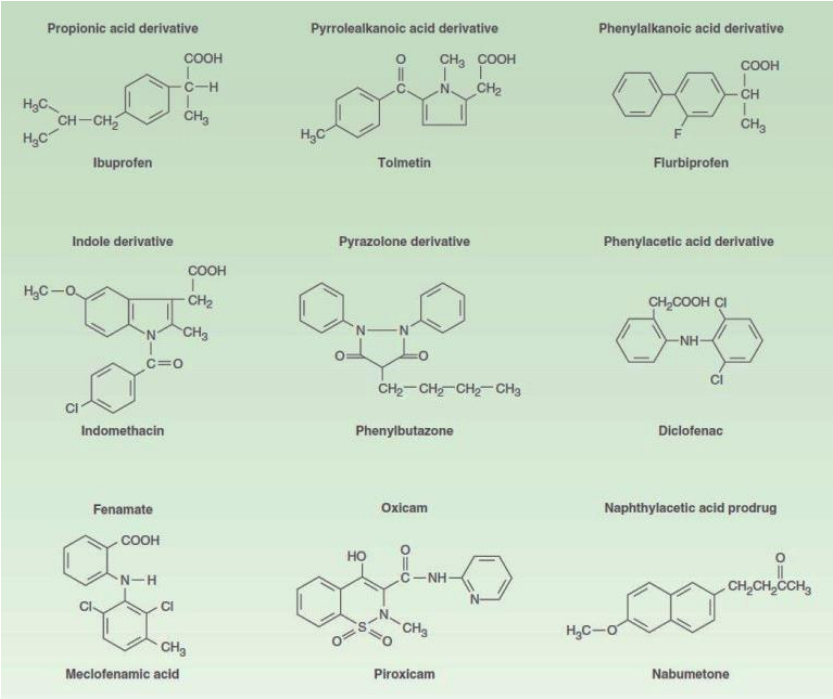


Figure 1. Chemical classes of NSAIDs (Katzung et al, 2012).

This chemical diversity is one of the reasons for a different range of pharmacokinetic characteristics as well as other properties that are causing differences in action among the drugs in this group (Liu et al., 2019; Katzung et al., 2012).

1.2.2 Pharmacokinetics

1.2.2.1 Absorption

All NSAIDs are quickly absorbed after oral administration with a time peak plasma concentration (t_{max}) of less than 2 hours (e.g. aspirin t_{max} < 0.5 h vs. naproxen t_{max} = 2 h) (Pardutz, 2010; Pfaffenrath et al., 1995). Half-lives in plasma are in the range of 2 hours for most NSAIDs besides naproxen, it gets to up to 14 hours (Pfaffenrath et al., 1995).

Most NSAIDs are, therefore, well absorbed, and food does not substantially change their bioavailability (Katzung et al., 2012).

1.2.2.2 Metabolism

NSAIDs are mainly highly metabolized. Some are substrates for phase I metabolism followed by phase II mechanisms and others only by direct glucuronidation (phase II metabolism) alone (Katzung et al., 2012).

NSAIDs are further metabolized mostly by CYP3A or CYP2C families of P450 enzymes in the liver (Katzung et al., 2012).

1.2.2.3 Excretion

Renal excretion presents the most important path of elimination. However, most NSAIDS go through various degrees of enterohepatic circulation which includes biliary excretion and reabsorption. With that in mind, the extent of lower gastrointestinal irritation correlates with the extent of NSAIDs enterohepatic circulation (Katzung et al., 2012).

1.2.2.4 Distribution

All NSAIDs can be found in synovial fluid after repeated dosing. Even the drugs with shorter half-lives remain longer than it would be predicted from their half-lives (Katzung et al., 2012).

1.2.3 Pharmacodynamics

The pharmacodynamics of NSAIDs can, as previously mentioned, be explained by cyclooxygenase (COX) inhibition (Kyung-Hoon, 2020; Musa 2017; Rouzer et al., 2009). Cyclooxygenase or prostaglandin G/H synthase catalyzes the first two steps in the biosynthesis of prostaglandins (PGs) (Rouzer et al., 2009). The COX is traditionally classified in the constitutive COX-1 and the inducible COX-2 isoform (Kyung-Hoon, 2020). The two COX isoforms COX-1 and COX-2 are the targets of NSAIDs which indicate their role in pain, fever, inflammation, and tumorigenesis (Rouzer et al., 2009).

COX-1 is widely distributed and constitutively expressed in most tissues (Hill and Zawia, 2021; Rouzer et al., 2009). It produces prostaglandins that are involved in homeostatic mechanisms including gastrointestinal protection and hemodynamics. COX-1 is present in the majority of cells including endothelial cells, gastrointestinal epithelium, and platelets, and functions continuously (Hill and Zawia, 2021; Kyung-Hoon, 2020; Jong-Suep, 2017; Musa 2017; Pardutz, 2010; Rouzer et al., 2009).

On the other hand, the gene for COX-2 is activated by a wide variety of inflammatory and proliferative stimuli and therefore COX-2 is expressed in inflammation and tumorigenesis. Constitutive COX-2 expression is well recognized in the brain, kidney, and female reproductive tract and is inducible in cell types such as macrophages and colorectal cancer cells. COX-2 inhibitors were originally thought to be safer (Hill, Zawia, 2021; Kyung-Hoon, 2020; Jong-Suep, 2017; Musa 2017; Pardutz, 2010; Rouzer et al., 2009).

The constitutive expression of COX-1 and inducible expression of COX-2 have led to the belief that COX-1 produces only homeostatic PGs, while COX-2 produces primarily pathophysiological PGs. Discoveries in the last two decades put this thesis to question and

reveal an underappreciated function of both enzymes (Kyung-Hoon, 2020; Rouzer et al., 2009; Pfaffenrath et al., 1995).

An additional property of NSAIDs is the blocking of platelet cyclooxygenase. NSAIDs block the formation of thromboxane A2, a potent aggregating agent. All, except the COX-2-selective agents and the non-acetylated salicylate, inhibit platelet aggregation.

More additional possible mechanisms of action include inhibition of chemotaxis, down-regulation of interleukin-1 production, decreased production of free radicals and superoxide, and interference with calcium-mediated intracellular events (Katzung et al. 2012; Pardutz, 2010).

1.2.4 Adverse effects of NSAIDs

NSAIDs mode of action – cyclooxygenase inhibition and blockage of PG synthesis – is responsible for side effects. Traditionally, it was thought that effects coming from COX-1 inhibition are connected with adverse effects while inhibition of COX-2 arises desired effects.

The most affected area is the gastrointestinal tract. COX-1 inhibition causes decreased production of cytoprotective prostaglandins. Due to it, the mucosa is damaged and gastrointestinal problems arise such as dyspepsia, gastric pain, nausea, vomiting, constipation, diarrhea, and gastric erosion, ulceration, perforation, and bleeding. Even though traditionally COX-1 is the cause for gastrointestinal problems, researchers have discovered that the combination of COX-1 and COX-2 inhibition and with that the reduction in the total levels of PGs, is more important than inhibition of PGs generated just from the COX-1 enzyme (Hill and Zawia, 2021; Kyung-Hoon, 2020; Musa 2017; Rouzer et al., 2009; Pfaffenrath et al., 1995).

Less common are renal problems (renal insufficiency, renal failure, hyperkalemia, and proteinuria), cardiovascular and platelet aggregation problems (stroke, fluid retention, hypertension, edema, myocardial infarction), skin (rashes, all types, pruritus, allergic reactions), and CNS intolerabilities (headache, tiredness, dizziness, confusion, tinnitus) (Hill and Zawia, 2021; Kyung-Hoon, 2020; Katzung et al., 2012; Pfaffenrath et al., 1995).

Despite all of that, NSAIDs are effective and generally recognized as safe drugs.

1.3 FENAMATES

As previously mentioned, NSAIDs can be divided by their chemical structure and one of the classes are drugs derived from anthranilic acids called fenamates.

Fenamates are more specifically derivatives of N-aryl-substituted anthranilic acid - all but niflumic acid. By belonging to NSAIDs, they work as potent analgesics, antipyretics, and anti-inflammatory drugs (Hill and Zawia, 2021; Jiang et al., 2020; Young-Jin et al., 2020; Klose et al., 2011; Pérez Ruiz et al., 1998; Dhanaraj et al., 1988).

Fenamates include mefenamic acid (MFA), tolfenamic acid (TA), meclofenamic acid (MCFA), niflumic acid (NFA), and flufenamic acid (FFA) (Hill and Zawia, 2021; Mantas and Mihranyan, 2020; Klose et al., 2011; Dhanaraj et al., 1988).

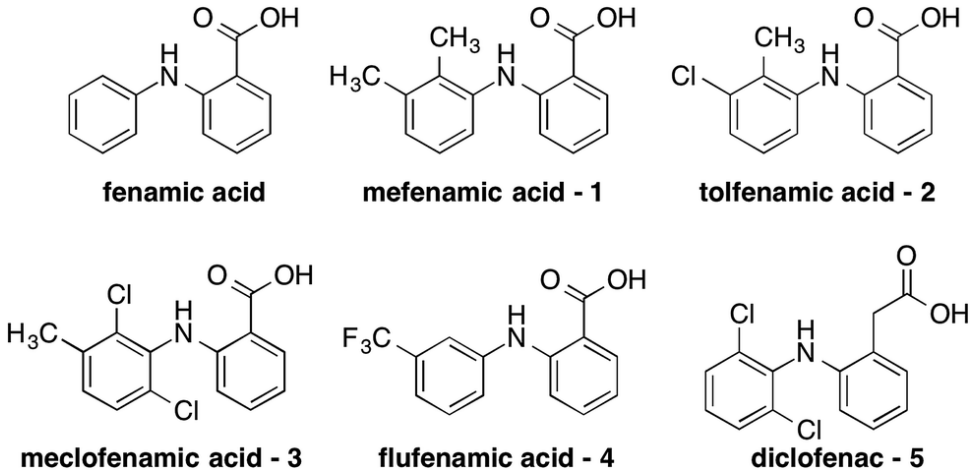


Figure 2 Chemical structures of fenamates (Davis et al., 2017).

Fenamates have been developed in the 1960s, in the 1990s they have been among the most prescribed NSAIDs but in the last decade, their rate of prescription has been declining (Mantas and Mihranyan 2020; Cimolai, 2013).

Today they are recognized as chemical reagents with some of them, mentioned in **Figure 3,** having their monographies. Still, not all of them have found their place in treatment. (Cimolai, 2013).

Table 1. Registered fenamates (Hill and Zawia, 2021; JP16; USP2020.; BP2020.; Ph. Eu. 10;).

Drug	Monography	Registered forms	Single-dose
Mefenamic acid	**USP 2020, Ph. Eu. 10, BP 2020, JP 17**	***Ponstel* (USA) for mild to moderate pain**	**500 mg**
Etofenamate	**Ph. Eu. 10 BP 2020**		
Tolfenamic acid	**Ph. Eu. 10 BP 2020**	***Clotam Rapid* (EU) for migraine headaches**	**200 mg**
Flufenamic acid	**Not registered**	*Assan, Combec 5%, Fenazol 5%, Flufemin, Kanyen, Mobilisin and others for rheumatic disorders, pain, and inflammation in Japan, Switzerland, and Taiwan*	**200 mg**

Pharmacodynamically, all fenamates exhibit their activity through COX inhibition in animal models and humans (Hill and Zawia, 2021; Bindu et al., 2020).

Chemical similarity entails similarity in the mechanism. Fenamates show acting mechanisms as well as an adverse effect similar to other NSAIDs. In the latest research, it has been noticed that certain *in vitro* discovered effects are unique for fenamates showing the true potential of fenamates that could distinguish them from other NSAIDs, giving them a new purpose (Cimolai, 2013).

Fenamates are recognized as low potency modulators of various enzymes and ion channels (Sun et al., 2019; Yau et al. 2010; Habjan and Vandenberg, 2009; Dvorzhak, 2008;

Guinamard et al., 2004; Lee et al., 2003; Greenwood and Large, 1995; Kankaanranta and Moilanen, 1995; Farrugia et al., 1993; Gögelein et al., 1990; White and Aylwin, 1990).

There is an obvious and vast potential for expanding the application of fenamates (Cimolai, 2013).

It should be noted that fenamates show a higher incidence of gastrointestinal side effects (30–60%) compared to over-the-counter available NSAIDs, like ibuprofen (Mantas and Mihranyan, 2020).

1.3.1 Polymorphism of fenamates

Polymorphism is the ability of a solid-state compound to exist in more than one crystalline form. Crystalline forms differ from each other by the arrangement of the molecules in a unit cell within a crystal grid and it may involve conformational changes. This property of a matter is responsible for numerous chemical and physical properties of molecules including stability, solubility, rate of reaction, as well as dissolution rate, and with that, bioavailability. Therefore, acknowledging the polymorphism of the matter is one of the fundamentals when researching matter (Jabeen, 2012).

One of the reasons why fenamates are not represented in greater presence as NSAIDs is due to the problem with their low solubility in the water as well as the other common organic solvents. This property is influenced by different polymorphic forms in which fenamates are known to exist (Gilpin and Zhou, 2005).

Most of the polymorphs of fenamates were discovered and characterized by X-ray or differential scanning calorimetry (DSC) (Bhargavi et al, 2018; Gilpin and Zhou, 2004).

1.3.2 Uses of fenamates and their potential

As previously mentioned, traditional uses of fenamates include analgesic treatment of various conditions, as well as anti-inflammatory and antipyretic effects. Because of the ubiquitous presence of COX enzyme throughout the whole body as well as the fact that fenamates are known modulating agents in several different pathways, studies have suggested that fenamates may be great candidates for repurposing outside pain management (Hill and Zawia, 2021; de Anda-Jauregui et al., 2018).

Altogether, studies have presented evidence for several potential repurposed usages of fenamates:

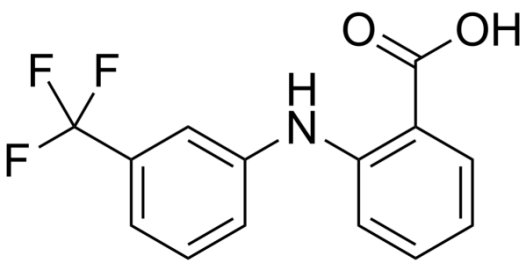
- Primary dysmenorrhea and menorrhagia (Mantas and Mihranyan, 2020)
- Alzheimer's disease (Hill and Zawia, 2021)
- Huntington's disease (Hill and Zawia, 2021)
- Epilepsy (Hill and Zawia, 2021)
- Stroke (Hill and Zawia, 2021; Jian-Fang, 2020)
- Cancer (Hill and Zawia, 2021; Rouzer et al., 2009)
- Potent anti-gonococcal activity (Young Jin et al., 2020).

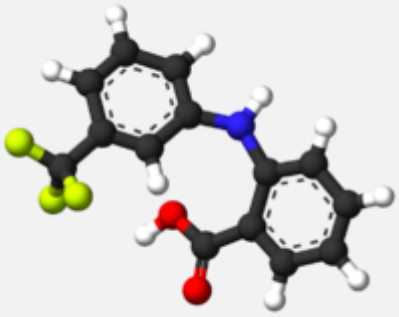
1.4 FLUFENAMIC ACID

Flufenamic acid belongs to the fenamates or anthranilic acid family of NSAIDs and therefore carries all the chemical and mechanical similarities mentioned before. Flufenamic acid is an aromatic amino acid with an organofluorine compound. It consists of anthranilic acid with N-(trifluoromethyl)-phenyl substituent which is the reason for its IUPAC name. Before that, the name was N-(α, α, α-trifluoro-m-tolyl) anthranilic acid (ChEBI; Badran, 2014; Abignente and Caprariis, 1982).

Flufenamic acid is a non-selective cyclooxygenase inhibitor and has an important role in rheumatic, musculoskeletal, and joint disorders and it can be administered orally and topically, even though it is rarely used in oral solid dosage forms and is more often used in topical formulations (Drug Bank; Alshehri, 2020; Alshehri and Shakeel, 2017; Mura et al., 2010; Perlovich et al., 2007; Warner et al., 1999; www.ebi.ac.uk; www.go.drugbank.com).

Table 2. Some pharmaceutically significant characteristics of flufenamic acid (Mantas and Mihranyan, 2020; Warner et al., 1999; Akbuga et al., 1983; Kapadia and Elder, 1978; www.guidetopharmacology.org; www.echa.europa.eu).

Name	Flufenamic acid
IUPAC's name	2-[[3-(trifluoromethyl) phenyl] amino] benzoic acid
Molecule	

3D structure	
Anatomical Therapeutic Chemical (ATC) Classification	M01AG03
Mechanism of action	Non-selective cyclooxygenase inhibitor
Legislative	Registered under REACH Regulation *(substance for use at industrial sites and in manufacturing and can be manufactured in and/or imported to the European Economic Area, for intermediate use only)*
	No monographies in newest pharmacopeias
	Used in Japan, Taiwan, and Switzerland, in Italy registered for topical application *(Mobilisin)*
Dose	Daily dose 400 – 600 mg; single dose 200 mg per 8 hours
The median lethal dose (LD_{50})	249 mg/kg after the oral administration in rats

The main reason why flufenamic acid is not more present in the market is its poor water solubility and poor wettability together with well-known NSAIDs gastrointestinal irritation (as high as 60% when using FFA). This arises problems with bioavailability, design of pharmaceutical formulation leading to reduced therapeutic efficacy (Alshehri, 2020; Belhocine et al., 2017; Mura et al., 2010; Akbuga et al., 1983; Abignente and Caprariis, 1982).

1.4.1 Chemical and physical characteristics

Selected chemical and physical properties of flufenamic acid are given in **Table 3**. The high value of the octanol/water partition coefficient indicates that it is a drug that is practically insoluble water and that, as previously mentioned, represents the main obstacle for drug formulation (Hill and Zawia, 2021; Alshehri and Shakeel 2017; Badran, 2014; Shazly et al., 2012).

Table 3. Chemical and physical characteristics of flufenamic acid (Hill and Zawia, 2021; Alshehri, 2020; Alshehri and Shakeel 2017; Badran, 2014; Shazly et al., 2012; Moffat, 1986; Abignente and Caprariis, 1982; go.drugbank.com; www.sigmaaldrich.com).

Appearance, color	Pale yellow needles
Melting point	132 - 135˚C; 133.5 ˚C
pKa	3.9
	Weak carboxylic acids, >99% ionized at physiological pH levels
Octanol/water partition (LogP)	4.88
Solubility in water, atm, 22˚C	0.0067 mg/ml
Solubility of water, mole fraction, atm, 22˚C	4.29×10^{-7}
BCS categorization	Class II - poor solubility and high permeability
Synthesis	Metz, 1984., U.S. Patent US4980498

Flufenamic acid is insoluble in the water but soluble in an organic solvent. It has been proposed as being soluble in light mineral oil, PEG-400, hexane, methylbenzene, ethanol. More so, it has been freely soluble in 2-butanol, 1-butanol, isopropanol, ethanol, and methanol, soluble in PEG-400, propylene glycol, and ethylene glycol with the highest solubility being in dimethyl sulfoxide (DMSO) and practically insoluble in water (Alshehri and Shakeel, 2017; Badran, 2014; Lee et al., 2012; Rytting et al., 2005; Wenkers and Lippold, 1999).

Since the most common use of flufenamic acid is topical, the problem arises because most of these solubilizers could damage the skin, which may worsen inflammations and therefore it is important that the vehicle potentiates and not diminishes the drug effect (Rubio et al., 2013; Rytting et al., 2005).

Flufenamic acid shows polymorphism, as described in more detail in section **1.4.4. Polymorphism of flufenamic acid**.

1.4.2 Pharmacokinetics

1.4.2.1 Absorption

Abignente and Caprariis as well as Perez Ruiz in their studies reported that flufenamic acid is readily absorbed after oral administration, at almost 100%. On the other hand, Lentjes and van Ginneken's research showed the absorption rate of flufenamic acid being irregular and the absorption rate is up to 80%. In addition, plasma concentrations varied between 6 and 20 μg/mL, which was reached between 1.5 and 5 hours. The plasma elimination half-life was found to be approximately 3 hours (Abignente and Caprariis, 1982).

The difference in bioavailability arises from the polymorphic nature of the flufenamic acid which will be discussed later. Having highly variable bioavailability makes flufenamic acid a problematic drug for clinical application.

Food affects drug bioavailability if taken accompanied, with variation in absorption being up to 30% (Mantas and Mihranyan, 2020).

1.4.2.2 Metabolism and elimination

Flufenamic acid is excreted mainly in the form of its metabolites with the two major and at least six minor metabolites. Most of the drug absorbed is metabolized by enzymes from both the I and II phase.

The main phase I metabolism reaction is oxidation by CYP enzymes on the 3'-, 4'- and 5- methyl groups. 4'-hydroxy- and 5-hydroxy-metabolites continue their metabolism with phase II enzymes, while active 3'-hydroxy metabolite undergoes another oxidation and forms inactive 3’-carboxy metabolite. To a lesser extent, monodehalogenation and ring hydroxylation can occur or drugs can be excreted unchanged (Hill and Zawia, 2021; Pérez Ruiz et al., 1998; Abignente and Caprariis, 1982).

Main phase II reaction is glucuronidation (Hill and Zawia, 2021).

Both the 4'-hydroxy- and 5-hydroxy-metabolites and flufenamic acid are eliminated in urine chiefly in conjugated form and the 4',5-dihydroxy-derivative in non-conjugated form. 4'-hydroxy-, 5-hydroxy-, 4',5-dihydroxy-metabolites, and unchanged flufenamic acid have been found in stool but in an unconjugated form (Hill and Zawia, 2021; Pérez Ruiz et al., 1998; Abignente and Caprariis, 1982).

The renal excretion of the drug and its conjugated metabolites was 51%, out of which only 2,6% was an unaltered drug (Hill and Zawia, 2021; Pérez Ruiz et al., 1998; Abignente and Caprariis, 1982).

1.4.3 Pharmacodynamics

Flufenamic acid has been known since the 1960s and since then its anti-inflammatory properties attributable to inhibition of prostaglandin synthesis have been known. Studies in the 90s showed that flufenamic acid appeared to be an ion channel modulator. Due to its low water solubility as well as extensive gastrointestinal side effects, it is difficult for it to be used in therapy and therefore its role in medicine diminished over the years. With the discovery of its role as a channel modulator, flufenamic acids' role in studies expanded and it turned out to be a useful and important tool in ion channel research (Mantas and Mihranyan, 2020; Guinamard et al., 2013).

Flufenamic acid, as part of NSAIDs, exerts its effects thanks to the action it has on the COX enzymes. Adding to previously discussed possible targets of fenamates, flufenamic acid has been assumed to possess effects on non-selective chloride, potassium, calcium, and sodium channels. To be more precise, flufenamic acid is:

- The blocker of a broad range of transient receptor potential (TRP) channels (Baek et al., 2017; Mohammed and Matijevic, 2012; Klose et al., 2011; Nazıroğlu et al., 2011; Guilbert et al., 2009; Albert et al., 2006; Nazıroğlu et al., 2006; Guinamard et al., 2004; Hill et al., 2004; Lee et al., 2003; Tesfai et al., 2001).
    - First described as TRPM2 blocker (Hill and Benham, 2004).
    - In addition to TRPM2 activity, studies showed FFA’s influences both canonical (TRPC) and melastatin (TRPM) subfamilies, with emphasis on TRPC6, TRPM3, TRPV4 activity (Klose et al., 2011).
- Inhibits Cl-sensitive and PGE_2-triggered Ca^{2+} entry into erythrocytes, thus providing clearance of human erythrocytes (Kasinathan et al., 2007).
- The blocker of calcium-activated cationic currents (Wang et al., 2006; Takahira et al., 2005; Peña et al., 2004; Ghamari-Langroudi and Bourque, 2002).

- Modulatory effect on neuronal sodium channels.
    - Decreasing sodium current availability, recovery from inactivation leading to decreased cellular excitability. Therefore, potentially complementary to the existing anti-epileptic treatment strategy (Yau et al., 2010).
- The blocker of gap junctions (Yau et al., 2010; Srinivas and Spray, 2003).
- Interfering with cellular volume regulation (Klose et al., 2011; Numata et al., 2007; Lambert and Oberwinkler, 2005).
- Inhibitors of aldo-keto reductase AKR1C3 (Skarydova et al., 2009; Matsuura et al., 1998).

1.4.4 Polymorphism of flufenamic acid

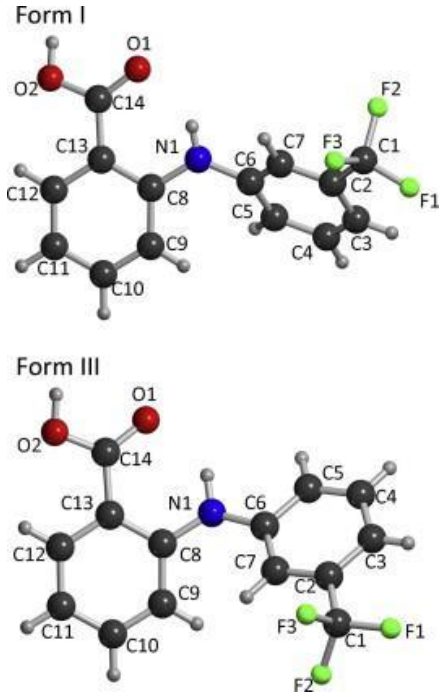


Figure 3. Models of two flufenamic acid polymorphs, the form I and III (Delaney et al., 2014).

From the very discovery and first studies conducted with flufenamic acid, it was a known fact that it exhibits polymorphism in the crystalline state. During that time, spectroscopic, conformational, and thermodynamic studies have been made about the relationship of proposed flufenamic acid polymorphs. Discovery and explanation of the first crystal form – Form III - occurred in 1973 by McConnell and nearly ten years later the structure of the second form – Form I - was reported by Kirshna et al.

As early as 1974 Kuhnert-Brandstatter has described five different polymorphs but reported melting points and infrared spectra and DSC thermograms for the first four forms.

In 1977 Krc reported the existence of at least seven crystalline modifications with different melting points. Polymorphs I, II, and III were described with crystal morphology, optical properties, X-ray diffraction powder data, and infrared spectra.

Abignente and Caprariis in 1982 reported eight crystalline modifications of flufenamic acid obtained by quantitative DSC, qualitative solubility determination, and IR spectroscopy.

Since then, numerous studies have elucidated flufenamic acid polymorphs reporting and characterizing seven (Jabeen, 2012; Gilpin and Zhou, 2005), eight (Lopez-Mejias, 2012), nine or more (Mantas and Mihranyan, 2020; Zhang et al., 2020) but all of the studies emphasize the existence of several polymorphs.

Studies showed that only two polymorphs – forms I and II – are stable under typical conditions at room temperature and can be obtained by recrystallization from either xylene or methanol. Studies establish that form III is the thermodynamically most stable form by at least 0.3 kcal/mol among all forms examined (Bhargavi N. et al., 2018; Jabeen, 2012; López-Mejías et al., 2012; Eun Hee Lee et al., 2010).

The difference between the two polymorphs of flufenamic acid – form I and form II – arises from a geometrical positioning of the [3-(trifluoromethyl) phenyl] amino moiety regarding the primary benzoic acid moiety. X-ray crystallography showed that the carboxyl group, the ring containing it, and the bridging amino group are all coplanar due to resonance interactions and internal hydrogen bonding between the NH and the carboxyl group on the ring. Various polymorphic states have been suggested as the result of out-of-plane rotational differences between the central NH group and ring 2 containing the different substituents (i.e., rotations about the N-C bond).

We can mention as well that the carboxyl group and the imino N atom are connected through an intramolecular hydrogen bond (Jabeen, 2012; Gilpin and Zhou, 2005; Murthy and Vijayan, 1982).

Knowing all of that, flufenamic acid is a problematic drug because it is difficult to provide solubilization conditions because more than eight polymorphs with different chemical and physical characteristics can coexist (Mantas and Mihranyan, 2020).

1.4.5 Applications

In addition to all the established and potential clinical use of fenamate's group of NSAIDs, some specific indications of flufenamic acid are going to be highlighted.

1.4.5.1 Rheumatoid arthritis

Back in 1967, Rajan et al in a double-blind clinical trial compared flufenamic acid, aspirin, and phenylbutazone in patients with rheumatoid arthritis. The research did not reveal any difference between these drugs when it comes to the ability to relieve symptoms. That proved that FFA may be used as a satisfactory substitution for aspirin and phenylbutazone but it showed no particular superiority.

Today flufenamic acid is a non-steroid drug approved for clinical use to relieve inflammation and pain in rheumatoid arthritis patients (Zhang et al., 2020).

1.4.5.2 Osteoporosis

Flufenamic acid could be used in preventing postmenopausal osteoporosis. By inhibition of the NF-κB signaling pathway, it promotes osteogenic differentiation in low concentration. The best concentration for osteogenesis promotion being 50 μM (Zhang et al., 2020; Liu et al., 2019).

1.4.5.3 Antibacterial effect

Newer studies showed that flufenamic acid has potential as an antibacterial drug. Preethi suggested in his research that flufenamic acid may be potential drug candidates against *Salmonella enterica Typhimurium*, bacteria responsible for different gastrointestinal diseases.

In separate studies, Lee et al. in 2018 discovered and Hung in 2019 confirmed that a triple combination of flufenamic acid-zanamivir-clarithromycin improves the inflammatory markers and survival of severe influenza A(H1N1) infection in mice. (Hung, 2019; Lee et al., 2018).

Knowing that flufenamic acid inhibits different ion channels, among them being Cl^- and Ca^{2+} Kasinathan et al. in 2007 connected this mechanism with Cl^- sensitive and PGE_2-triggered Ca^{2+} channels that can be found on erythrocytes. There is a possibility that due to this mode of action, FFA can interfere with intraerythrocytic growth of the malaria parasite *Plasmodium falciparum in vitro*, thus influencing the course of malaria *in vivo*.

In 2020 in research carried on by Zhang et al, flufenamic acid showed potential to be a potent therapeutic compound against MRSA infections as well as a promising candidate for antimicrobial coating of implants and surgical devices.

1.4.5.4 Antiepileptic add-on therapy

As mentioned before, among ion channels that flufenamic acid modulates, sodium channels are one of them. Flufenamic acid's modulation of sodium current in neuronal cells, as well as cellular excitability, raises the potential role of flufenamic acid in anti-epileptic therapy. Having various effects on sodium as well as calcium-activated cationic current and gap junctions, flufenamic acid shows promise as an add-on therapy that could work synergistically with the existing epilepsy therapy and gap junctions (Hau-Jie et al., 2010; Srinivas and Spray, 2003; Ghamari-Langroudi and Bourque, 2002).

1.4.5.5 Gout

In 1970 research by Douglas and Thompson, the efficacy of flufenamic acid was compared with phenylbutazone in acute gout management. Phenylbutazone was by far more effective, but flufenamic acid showed a potential to be included in the acute gout control in case phenylbutazone is not tolerated or contraindicated.

This usage of flufenamic acid never came to life and the studies on this topic were not repeated.

1.4.5.6 Unknown but possible effects on neuronal cells

Because of the lack of potent yet selective drugs that could serve as pharmacological tools to disrupt TRP channel activity, flufenamic acid may show potential here, greater than other fenamates (Klose et al., 2011; Hill and Benham, 2004).

Being a known TRPM2 channel blocker, and knowing the fact that TRPM2 is widely expressed and involved in the health and disease of neurological cells in dorsal root ganglion, its importance may be of physiological and clinical relevance that has yet to be discovered (Guinamard et al., 2013; Nazıroğlu et al., 2011; Clapham, 2007).

1.5 CYCLODEXTRINS

Cyclodextrins (CDs) or α-1,4-D-glucopyranoside are a group of naturally occurring cyclic oligosaccharides obtained by the enzymatic degradation of starch by the enzyme glucosyltransferase. They consist of multiple glucose subunits linked by α-1,4 glycosidic bonds (Crini, 2020; Shepelytskyi et al., 2020; Saokham et al., 2018; Erdogar et al., 2017; Davis and Brewster, 2004; Szejtli, 1981).

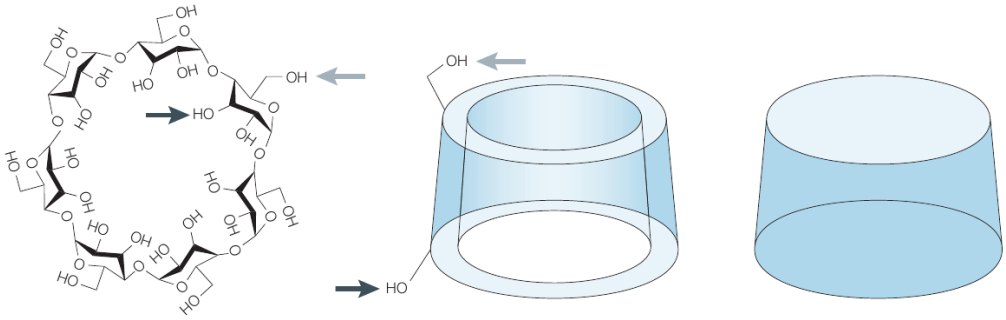


Figure 5. Schematic representations of β-cyclodextrin (Davis and Brewster, 2004).

Because of the chair conformation of glucopyranose units, CDs are shaped like a truncated cone with a central cavity and hydroxyl groups extending from both edges. A large number of hydroxyl groups can be found on the external surface of CDs. Because of that, the entire CD molecule is water-soluble. On the other hand, the interior of the cavity is hydrophobic due to the C-C and C-O-C bonds. Therefore, the CD has outer surface hydrophilic and central cavity hydrophobic. Due to the hydrophobic cavity, they can form a stable complex with a variety of molecules in aqueous media, a property that proved very useful for pharmaceutical applications (Crinia, 2021; Bingren, 2020; Crini, 2020; Patel and Hirlekar, 2019; Loftsson et al., 2005; Davis and Brewster, 2004).

1.5.1 Cyclodextrins' nomenclature and derivatives

Nomenclature system of CDs derived from the number of glucopyranose units in the structure and the most common natural CDs consist of six (α-cyclodextrin), seven (β-cyclodextrin), and eight (γ-cyclodextrin) glucopyranose units (Loftsson et al., 2005; Davis and Brewster, 2004).

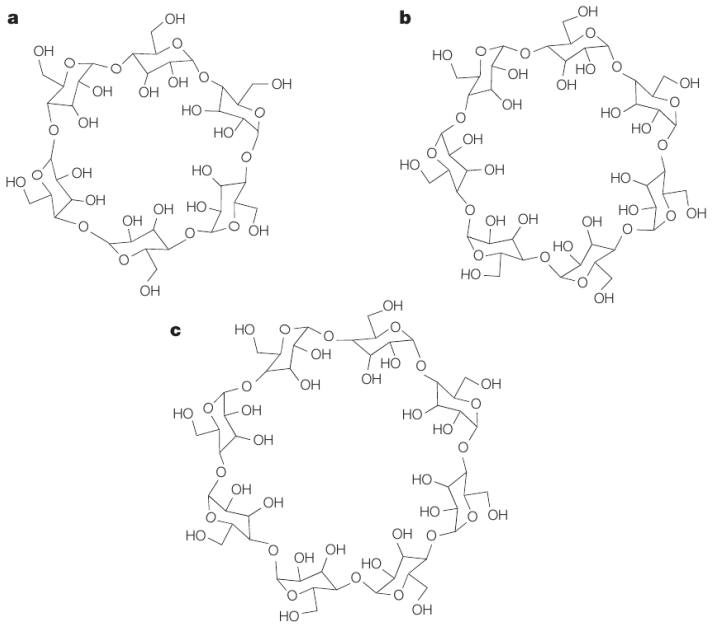


Figure 6 Schematic representations of cyclodextrins. a) α-CD b) β-CD c) γ-CD (Davis and Brewster, 2004).

The water solubility of α-, β- and γ-CD at ambient conditions are different and are shown in Table 4, as well as some other properties of CD (Davis and Brewster, 2004).

Table 4. Selected properties of natural cyclodextrins (Davis and Brewster, 2004).

	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin
Number of glucose units	6	7	8
Molar mass (g/mol)	973	1135	1297
Diameter of the cavity (nm)	0.47…0.53	0.6…0.65	0.75…0.83
Water solubility at 25℃ (g/100mL)	14.5	1.85	23.2
Crystal-water –content (mass %)	10.2	13.2…14.5	8.13…17.7
Hydrolysis by α-amylase	negligible	negligible	rapid

The lower solubility of β-CD compared with α-CD, even though β-CD contains a higher number of hydroxyl groups, is due to the formation of strong inter- and intramolecular hydrogen-bond which limits molecules water solubility at 1.85 g/100mL at 25°C (Jug et al., 2014).

Due to the tendency of natural cyclodextrins to self-assemble and strongly bind by aggregates formation, their complexes show limited aqueous solubility. Modification of hydroxyl group, depending on the type of substituent and the degree of substitution, will enhance solubility. The reason is that the random modification changes crystalline CDs into amorphous (Saokham et al., 2018; Loftsson et al., 2005).

βCD is used the most often. Its properties of low solubility and nephrotoxicity are avoided with chemical modifications and due to βCD low cost, its chemical derivatives are widely spread in the industry (Patel and Hirlekar, 2019).

Most simple modifications of CDs were obtained by methylation of parent CD species. The degree of methylation/substitution can vary, but CD always shows improved aqueous solubility (Szente and Szejtli, 1999).

CDs derivatives result from the introduction of functional groups at the 2-, 3- and 6-hydroxyl groups of the glucose residues. These changes improve solubility through two mechanisms: by breaking the hydrogen bonds, and by preventing crystallization, which gives rise to an amorphous product (Davis and Brewster, 2004).

CD derivatives of pharmaceutical interest include the hydroxypropyl derivatives of β- and γ-cyclodextrin (HPβCD and HPγCD), the randomly methylated β-cyclodextrin (RMβCD or RAMEB), sulfobutylether β-cyclodextrin (SBEβCD) – the newest CD derivative to be approved (JP and USP), and branched cyclodextrins such as glucosyl- and maltosyl-β-cyclodextrin (Patel and Hirlekar, 2019; Davis and Brewster, 2004; Szente and Szejtli, 1999).

The presence of CDs in the newest available editions of pharmacopeias (Ph. Eu., JP, USP, and BP) can be reported:

Table 5. Cyclodextrins as chemical reagents in pharmacopeias (JP 16, BP 2020, Ph. Eu.10, BP 2020).

CDs registered as chemical reagents
α-Cyclodextrin
β-Cyclodextrin
β-Cyclodextrin for chiral chromatography, modified
β-Cyclodextrin for chiral chromatography, modified R1
Dimethyl-β-cyclodextrin
2-Hydroxypropylbetadex for chromatography
Hydroxypropyl-β-cyclodextrin
Silica gel BC for chiral chromatography *(very finely divided silica gel for chromatography (5 µm) coated with β-cyclodextrin, used for chiral purity of caraway oil, coriander oil, lavender oil, neroli oil)*

As chemical reagents, they perform as additives for chiral separation in chiral chromatography, capillary zone electrophoresis (e.g. enantiomeric purity in galantamine hydrobromide and ropivacaine hydrochloride monohydrate), micellar electrokinetic chromatography (MEKC) as well as additives to modify selectivity.

Table 6. Compendial monographs of cyclodextrins' (JP 16, BP 2020, Ph. Eu. 10, BP 2020)**.**

MONOGRAPHIES	**PHARMACOPOEIA**			
	Ph. Eu.10	**BP 2020**	**JP 16**	**USP2020**
α-Cyclodextrin ALFADEX	+	+	+	+
β-Cyclodextrin BETADEX	+	+	+	+
2-Hydroxypropyl-β-Cyclodextrin HYDROXYPROPYLBETADEX	+	+	+	+
Sulfobutyl-ether-β-cyclodextrin SULFOBUTYL BETADEX SODIUM	+	+	+	+
γ-Cyclodextrin GAMMADEX	+	+	+	+
Alprostadil Alfadex (Prostaglandin E1 α-Cyclodextrin Clathrate Compound 400)	-	-	+	-
Limaprost Alfadex (α-cyclodextrin clathrate compound of limaprost)	-	-	+	-

1.5.2 Cyclodextrin inclusion complexes

Years ago it has been discovered that CDs may form the supramolecular inclusion complexes with lipophilic molecules. The inclusion complex is formed, with drug molecules of sufficient size, geometry, and appropriate properties, by substituting the enthalpy-rich water molecules from the cavity of the CD cone with the molecule or part of a molecule. Covalent bonds do not form or break during the formation of the inclusion complex. This means that drug molecules in the complex and free molecules in solution are in the rapid equilibrium ($t_{1/2}$ of the only 1μs). The connection between CD and drug is based solely on electrostatic, van der Waals' interactions, hydrophobic interaction, and hydrogen bonding (Jug et al., 2014; Loftsson et al., 2005; Davis and Brewster, 2004).

The important factor of inclusion complex formation is whether the molecule possesses the certain parameters which make a molecule suitable to form an inclusion complex with CDs (Jug et al., 2014):

- More than 5 atoms (C, P, S, N) in their structure
- Solubility lower than 10 mg/mL
- Melting point lower than 250°C; otherwise the cohesive bonds are too strong
- Mw in the range from 100 to 400; for the lower Mw ratio of the drug in the complex is too low while for molecules with higher Mw is too high
- Less than 5 condensed rings in the structure

Complex formation kinetically excites behavior predicted by Higuchi and Connors's theory of phase solubility. Formed complexes have been classified based on their effect on substrate solubility (Loftsson et al., 2005). Details can be found in section 3.2.3. Solubility studies.

The most common type of cyclodextrin complex is 1:1 in which one drug molecule forms a complex with one CDs molecule. In case that one more CD molecule forms a complex, we are talking about consecutive complexation and the ratio 1:2. Stability constant $K_{1:2}$ is much lower than $K_{1:1}$ (Saokham et al., 2018; Loftsson et al., 2005; Szejtli, 1981).

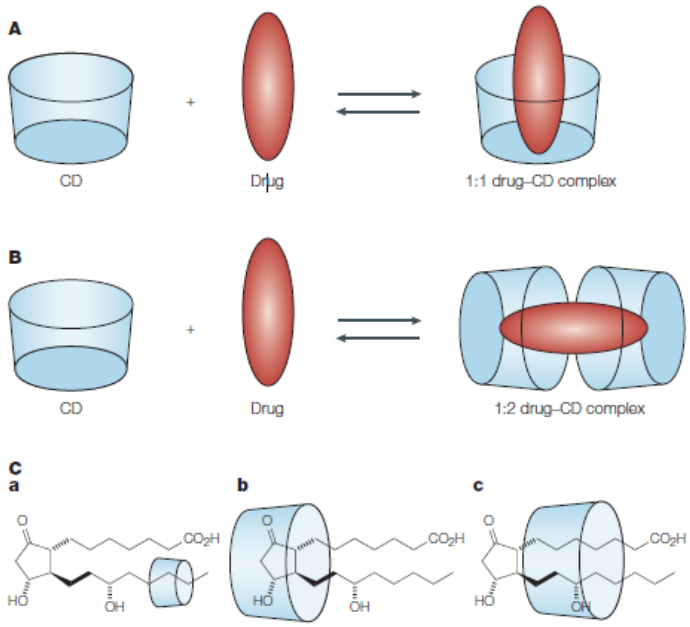


Figure 7. Dynamic equilibrium for 1:1 and 1:2 drug–CD complexes. C) PGE2 with α-, β- or γ-CD inclusion complexes (Davis and Brewster, 2004)

Cyclodextrins are known for forming not only inclusion complexes but also non-inclusive complexes as well as complex aggregates (they form micellar-like structures and are capable to act as solubilizers) but neither their structure nor their function in drug delivery is known (Loftsson et al., 2005).

Complexation with CDs makes hydrophobic drugs molecular dispersed in hydrophilic matrix making the poorly soluble drug more soluble in water, with consequently improved dissolution rate. In many cases, the same dose of drug results in higher biological effects. The addition of surfactants, water-soluble polymers, and preservatives can influence the solubilization provided with CDs (Saokham et al., 2018; Szejtli, 1981).

1.5.3 Absorption, elimination, toxicity

Cyclodextrin molecules are poorly absorbed through biological membranes. Lipophilic derivatives are absorbed to some insignificant extent but natural CDs are not susceptible to absorption in the gut, acting only as drug carriers (Loftsson et al., 2005; Szejtli, 1981).CDs are resistant to degradation by human enzymes, only γ-cyclodextrin can be hydrolyzed by salivary and pancreatic amylases. All CDs are fermented by bacterial and fungal amylases of the intestinal flora in the colon, before the excretion (Loftsson et al., 2005; Davis and Brewster, 2004).

What is important when talking about CDs as excipients is that they are biocompatible, do not produce an immune response, and are generally proven of low toxicities in both animals and humans (Davis and Brewster, 2004).

The toxicities of CDs are dependent on their route of administration. The research was described by Davis and Brewster in 2004. showed toxicity, expressed as the dose that causes 50% death (LD_{50}), of natural CDs after intravenous administration in mice. β-cyclodextrin proved to be the least toxic.

Table 7. LD_{50} of CDs after intravenous administration in mice (Davis, Brewster, 2004).

Natural CD	LD_{50} after i.v., in mice (g/kg)
α-cyclodextrin	1.0
β-cyclodextrin	0.79
γ-cyclodextrin	>4.0

1.5.3.1 Absorption and toxicological profile of CD regarding the delivery path

1.5.3.1.1 Oral delivery

After oral application, an insignificant amount of cyclodextrin (< 10%) is absorbed in the gut, while the rest of the applied dose is metabolized by microflora in the colon. That is the reason why CD is completely non-toxic (LD_{50} is impossible to determine) after oral administration (Loftsson et al., 2005; Szejtli, 1981).

Presently, only the oral administration of methylated β-cyclodextrin is limited by its potential toxicity (Loftsson et al., 2005; Szejtli, 1981).

1.5.3.1.2 Topical delivery

Cyclodextrins can act as penetration enhancers for drug delivery through aqueous diffusion layers, but not through lipophilic barriers such as the stratum corneum. CD can only increase the amount of drugs at the surface. CDs could be combined with conventional penetration enhancers due to different modes of action (Loftsson et al., 2005).

Toxicology-wise, it has been found that hydrophilic derivatives of CD are safe for skin and mucosa application because they do not cause local irritation or contact dermatitis (Loftsson et al., 2005).

1.5.3.1.3 Parenteral delivery

After parenteral administration, CD complex release drug rapidly due to dilution and binding of the drug molecule to plasma proteins and tissues (Loftsson et al., 2005).

Hydrophilic β-cyclodextrin derivatives, 2-hydroxypropyl-β-cyclodextrin (HPβCD) and sulfobutylether β-cyclodextrin (SBEβCD), hydrophilic γ-cyclodextrin, and natural α-cyclodextrin are toxicologically safe for being a parenteral drug carrier.

Natural γ-Cyclodextrin forms visible aggregates in aqueous solutions and because of that is not suitable for parenteral use.

Natural β-cyclodextrin cannot be administered parenterally due to its poor solubility that then leads to forming microcrystalline precipitations in the kidney, thus manifesting nephrotoxicity (Brewster and Loftsson, 2007; Loftsson et al., 2005; Davis and Brewster, 2004).

Dimethyl-β–CD (DMβCD) reacts with components of the cell membrane of the erythrocytes thus causing hemodialysis.

HPβCD and SBEβCD are, when compared with organic solvents, less toxic and they have a minimal effect on the drugs pharmacokinetic which is the reason why CDs are becoming more and more favorable excipients (Loftsson et al., 2005; Szente and Szejtli, 1999).

1.5.4 Applications in the pharmaceutical field

There are thousands of possible variations of CDs obtained by varying the size of the ring and site of functionalized modification. This fact is what makes CDs' structure unique. They possess promising ability for complex formation with significant practical impacts in chemistry, biology, biochemistry, health science and agrochemistry, textile industry, and environmental domains (Crini, 2020; Saokham et al., 2018; Erdogar et al., 2017; Davis and Brewster, 2004).

What makes them interesting to us is CDs role in the improvement of physicochemical properties - solubility and bioavailability - of the hydrophobic drug molecules without molecular modifications, presenting an enormous potential as a drug delivery system (Shepelytskyi et al., 2020; Patel and Hirlekar, 2019; Kfoury et al., 2018; Saokham et al., 2018; Erdogar et al., 2017; Davis and Brewster, 2004).

In 1999 Szente and Szejtli reported that the total number of studies published and dedicated to cyclodextrins was 16 300. While writing this paper, the Pubmed site, which was visited early in July of 2021, had 23 600 publications regarding CDs (https://pubmed.ncbi.nlm.nih.gov).

Some of the applications of CD are summoned below:

- The main purpose of CDs is to increase the solubility in water as well as the dissolution rate of poorly water-soluble drugs. CDs increase $_{Cmax}$ and AUC while reducing T_{max} thereby enhancing drugs' oral bioavailability (Davis and Brewster, 2004; Szejtli, 1981).
- Improvement of oral, rectal, transdermal, nasal, ocular, sublingual bioavailability (Hedges, 1998; Szejtli, 1981).
- CDs inclusion complex increases the drugs chemical stability against unfavorable environmental factors by slowing down the reaction (oxidation, hydrolysis, racemization, isomerization, polymerization, and enzymatic degradation of drug) (Davis and Brewster, 2004; Hedges, 1998; Perez-Ruiz et al., 1998; Szejtli, 1981).
- CDs can catalyze drug degradation if the functional group is situated close to external –OH groups (Cai Bai et al., 2017; Hedges, 1998).
- CDs in the solid-state can stabilize the amorphous form of the drug (Hedges, 1998).
- CDs can reduce local gastrointestinal drug irritation or damage (Loftsson et al., 2005; Davis and Brewster, 2004; Hedges, 1998; Szejtli, 1981).
- CDs transform liquid drugs into microcrystalline or amorphous powder (Loftsson et al., 2005; Davis and Brewster, 2004; Hedges, 1998; Szejtli, 1981).
- CDs provide stabilization of volatile compounds by reducing the evaporation (Kfoury et al., 2018; Pereva et al., 2015; Davis and Brewster, 2004; Szejtli, 1981).
- CD complexation enables the elimination of incompatibility between drugs or drugs and excipients (Loftsson et al., 2005; Davis and Brewster, 2004; Hedges, 1998; Szejtli, 1981).
- Catalysis of stereospecific reactions can be obtained by CDs (Cai Bai et al., 2017; Hedges, 1998) and they are registered as additives for chiral separations in pharmacopeias (JP 16; Ph. Eu.10; USP 2020; BP 2020).
- CDs stabilize flavors, mask unpleasant odors and tastes (Davis and Brewster, 2004; Hedges, 1998; Szejtli, 1981).

- Amphiphilic CDs, CDs surface modified with long aliphatic chains, have applications in targeted drug delivery (Erdogar et al., 2017).
- CDs can complex DNA, RNA improving their delivery and targeting as well as reduction of immunogenicity (Zhang et al., 2019; Davis and Brewster, 2004), showing the potential for application in gene therapy (Loftsson et al., 2005).
- CDs can stabilize protein conformation, prevent aggregation, reduce denaturation and improve drug delivery of protein and peptides when in complex (e.g. interleukin-2, insulin, human growth hormone) (Loftsson et al., 2005; Davis and Brewster, 2004).
- CD nano-systems act as drug-specific and target-specific therapeutics, having the potential for enhancement of cancer therapy (Bingren, 2020; Sivakumar et al., 2020; Adeoye and Cabral-Marques, 2017).
- Recently, CDs might find their purpose in the field of medical imaging as a potential scaffold for the contrast agent development suitable for magnetic resonance imaging, ultrasound imaging, etc. (Shepelytskyi et al., 2020).
- A mini-review was published by Fatmi et al. in early 2021. finding a potential purpose of CDs in drug treatment of Severe Acute Respiratory Syndrome Coronavirus 2. Cholesterol-rich parts of the cell membranes of SARS-COV-2 serve as docking places of host cells for the viruses. *In vitro* cell models have shown that depletion of cholesterol by cyclodextrin, and particularly methyl-β-cyclodextrin disturb the host cell membrane lipid composition this way, reducing the attachment of the virus to the protein receptors (Fatmi et al., 2021).

1.6 BIOPHARMACEUTICAL CLASSIFICATION SYSTEM - BCS CLASSIFICATION

Due to the significant evolution in the drug development process by extended and routine usage of screening techniques, current pharmaceutical lead compounds have higher molecular mass, lipophilicity, and consequently lower water solubility. Therefore, most of the compounds in pharmaceutical studies are drugs that are challenging to work with. The nature of the drugs that are being developed is important since, in the end, we will need a product that can be orally and parenterally applicable. For example, for a drug to be orally available, the compound must dissolve and be absorbed through the gastrointestinal tract and assure adequate drug levels at the pharmacologically active site.

This has had a significant impact on what is required from drug delivery formulators (Davis and Brewster, 2004).

To sum up and emphasize these factors, EMA and other drug regulatory organizations have defined a Biopharmaceutical Classification System (BCS) in which drugs are categorized according to their solubility in water and permeability through the intestinal mucosa (Loftsson et al., 2005; Davis and Brewster, 2004).

For a drug to be defined as highly soluble, EMA said that the drugs' highest single therapeutic dose must be completely soluble in ≤ 250 ml of aqueous media over the pH range of 1.2–6.8 at 37±1°C. For a drug to be highly permeable, according to EMA, it must have absolute bioavailability of ≥90% (EMA, 2020).

For an immediate-release tablet, ≥ 85% of the labeled amount of drug substance must dissolve within 30 min (Loftsson et al., 2005).

According to these definitions, BSC drugs have been classified into four categories (Loftsson et al., 2005; Davis and Brewster, 2004).

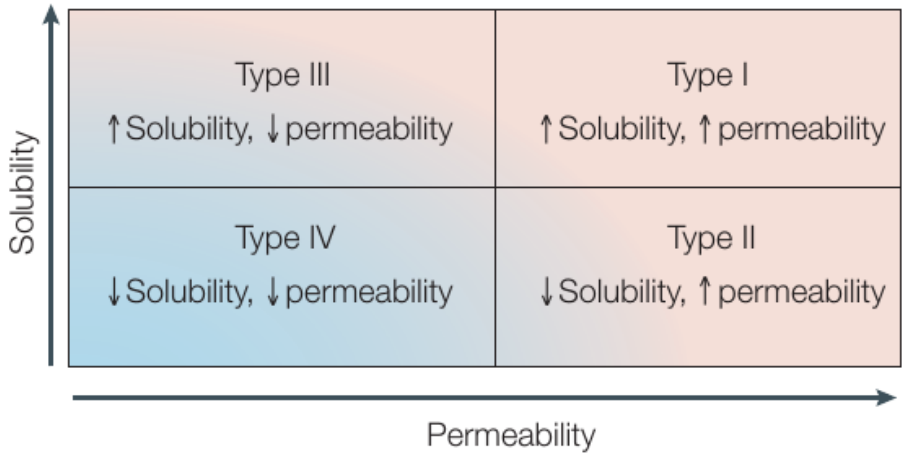


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Figure 8. Table of Biopharmaceutical Classification System (Davis and Brewster, 2004)

Class I drugs include relatively water-soluble drugs and their absolute bioavailability is ≥ 90%.

Class II drugs possess limited aqueous solubility, but permeate biological membranes rather easily, reaching ≥ 90% absolute bioavailability. In this case, dissolution is a rate-limited step for oral absorption.

Class III drugs are water-soluble but do not easily permeate biological membranes due to, for example, their size and/or extent of hydration.

Class IV drugs are water-insoluble and do not readily permeate lipophilic biological membranes.

As mentioned before, high-throughput screening approaches to drug development have led to an increasing number of drugs classified as Class II (poorly soluble/highly permeable) or Class IV (poorly soluble/poorly permeable) drugs according to the Biopharmaceutics Classification System (Loftsson et al., 2005).

In general, formulation techniques that increase the apparent aqueous solubility of Class II and Class IV drugs without decreasing their lipophilicity, since drugs need to be lipophilic to be able to permeate biological membranes, will enhance their absorption through biological membranes. These techniques include particle size reduction, salt formation, solid dispersion, melt extrusion, spray drying, and complexation, as well as drug formulation into microemulsions, liposomes, and non-aqueous solvents (Loftsson et al., 2005).

2 AIM OF THE PROJECT

Advancements in drug discovery and development have evolved over the years resulting in drugs with increased molecular mass and lipophilicity, drugs that are categorized by biopharmaceutical classification system (BCS) as Type II and Type IV. Both classes of drugs are difficult to work with due to their limited solubility and/or permeability. More and more active compounds on the market are classified as difficult Type II or Type IV. Therefore, in the latest years, the formulation of said substances have become of interest in numerous studies. One of the possible solutions was found in the complexation with cyclodextrins. Cyclodextrins, being a long-present and therefore well-researched as well as biocompatible and rather safe excipient, have come to attention and became more present in different formulations.

Pain is an omnipresent condition that is experienced by almost every person throughout their lifetime. As it is mentioned in the IASP definition, pain is an unpleasant experience and most of the people experiencing it want the pain to last as short as possible. Therefore, analgesic drugs are one of the most prescribed and most sold medicines in the world. Being present on the market for almost 60 years, as well as their all-around usage makes analgesic a well-studied group of medicines, with non-steroidal anti-inflammatory drugs (NSAID) on the main position. Therefore, NSAIDs' positive effects, as well as their side effects, are well investigated and well known. It is a known fact that the long-lasting use of NSAIDs raises special attention to the gastrointestinal system since the most common side effect are connected to it. For them to be safer for the GI system and therefore to avoid severe side effects, the specific formulation may be of help. And this is where, again, cyclodextrins' role is important since the formulations with CDs have proven to be safer for the GI system, thus reducing side effects.

The NSAID of our interest was flufenamic acid. Due to its remarkably low solubility, flufenamic acid is classified as a Class II drug, with poor solubility, but high permeability. Therefore, an improvement of aqueous solubility, the limiting factor of flufenamic acid absorption, would lead to an increase in absorption and an improvement in bioavailability, accompanied by enhanced therapeutic activity. Flufenamic acid is currently not registered for oral application in Europe or the USA, and in Croatia, it is not registered at all (as mentioned, for example, in Italy it is registered for topical use). On the other hand, studies performed in

the last 20 years have shown great potential for this drug. Flufenamic acid, known as an inhibitor/activator of numerous ionic channels, hides a great potential for its repurposing. The only problem is its low solubility which can be resolved with a specific formulation. And here again, studies suggest that the problem might be solved by the application of cyclodextrins.

Saying all of this, this project aimed to consider all of the problems mentioned above and propose a potential solution. Flufenamic acid, a medicine of great potential but scarce usage, is known for its low solubility. That solubility was tried to be improved with complexation with cyclodextrins in this research. By complexing it with four different cyclodextrins and by preparing each of the mixtures with the different methods we tried to show the possible solubility improvement. Furthermore, it was examined how different methods of complex preparation and different types of cyclodextrins influence flufenamic acid's solubility and *in vitro* dissolution, the main property we were observing during the experiment.

This work will be concentrated only on *in vitro* dissolution studies of the drug-cyclodextrin binary mixtures, investigating the improvement of the drug solubility and dissolution rate after preparing the inclusion complexes with different cyclodextrins.

The contribution of this research is rather modest but sufficient to draw attention to the importance of considering the repurposing of pre-existing drugs as well as the adaptation of formulations of some intolerable drugs.

3 MATERIALS AND METHODS

3.1 MATERIALS

The materials used to conduct this experiment:

Active Pharmaceutical Ingredient – Flufenamic acid
Natural β-cyclodextrin
β-cyclodextrins derivatives (HP-β-CD, RAMEB, SBE β-CD, HMS)
Solvents

- Flufenamic acid (FFA)
  - IUPAC name: 2-{[3-(Trifluoromethyl) phenyl] amino} benzoic acid
  - $C_{14}H_{10}F_3NO_2$
  - Practically insoluble in water, soluble in ethanol, diethyl ether, and chloroform
  - Molecular weight: 281.234 g/mol
  - Melting point: 124 to 125˚C
  - Λ_{max} = 290 nm
  - FFA used from two sources
    - white crystalline powder (TCI, an American company, freshly purchased)
    - pale yellow powder (SIMS, an Italian company, in the laboratory since 2006)

- Natural β-cyclodextrin
  - Molecular weight: 1135 g/mol
  - Solubility: 16 mg/mL in water at 25 ° C.

→ produced by pharmaceutical company "Roquette"

- Derivatives of β-cyclodextrin

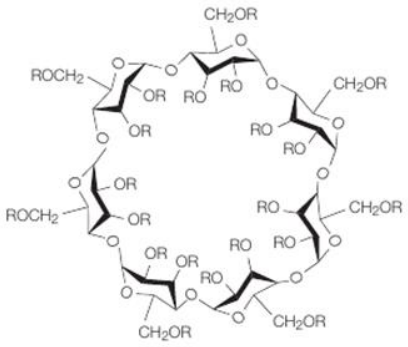

Figure 9. β-Cyclodextrin and its chemical derivatives (Davis and Brewster, 2004.)

- HP-β-CD - hydroxypropyl-β-cyclodextrin
  - R = $-CH_2CHOHCH_3$
  - Degree of molar substitution 0.65
  - Molecular weight = 1399 g/mol
  - Solubility: 200 mg/mL in water at 25 ° C

  → produced by pharmaceutical company Roquette

- RAMEB (**RA**ndomly **ME**thylated **β**-CD)
  - R = $-CH_3$
  - M.W. = 1310 g/mol
  - Solubility: 800 mg/mL in water at 25 ° C.

  → produced by Wacker-Chemie GmbH

- SBE β-CD - sulfobutylether sodium β-cyclodextrin
  - R = $-O(CH_2)_4SO_3\text{-}Na^+$
  - Molecular weight = 2163 g/mol
  - Solubility: >500 mg/mL in water at 25 ° C.

  → produced by Cyclolab

- Solvents
  - Ethanol, EtOH (SIGMA-ALDRICH)
  - Phosphate buffer: KH_2PO_4, Na_2HPO_4 (SIGMA-ALDRICH)
    - We are preparing two solutions:
      - The first solution is prepared by dissolving 27.2 g of KH_2PO_4 in 1 L flask

- Second solution is prepared by dissolving 71.6 g of Na_2HPO_4 dodecahydrate (MW = 358.14 g / mol) or 32.2 g of the monohydrate (MW = 160.9 g / mol) in the 1 L flask.

- Buffer is prepared by mixing 510 mL of the first solution with 490 ml of the second solution.
- The pH was always checked using a pH meter and adjusted to 6.8

3.2 INSTRUMENTS

- UV-vis spectrophotometer 1600 Shimadzu, Japan
- High Energy Grinding Mill MM 200 (MixerMill), Retsch, Germany
- Rotary evaporator Heidolph, Laborota 4000, Sigma Aldrich, USA
- Cryostat Lauda Bio Class E00

3.2.1 Methods

3.2.2 Spectrophotometric determination of active compound

The technique used for the determination of the concentration of the active substance in examined samples throughout our experiment was spectrophotometry.

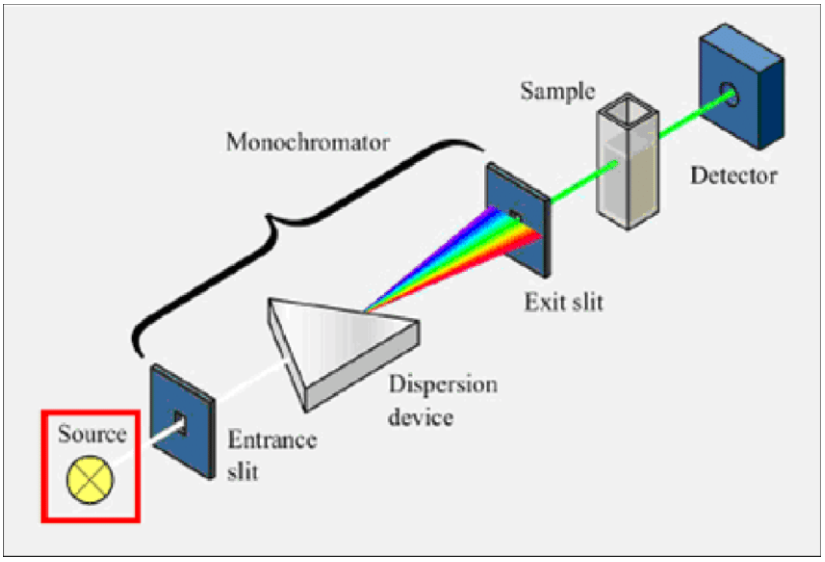

Figure 10. Schematic representation of UV-Vis spectrophotometer (Gohain and Neelakshi, 2009)

This technique is based on the interaction between electromagnetic radiation and matter: electromagnetic radiation of intensity I_0 is passing through a solution of the drug which results in lesser intensity in output, $I < I_0$. This means that the portion of radiation has been absorbed by the solution. To absorb electromagnetic radiation a molecule must have chemical groups called chromophores, which possess easily excitable electrons, in their structure.

Transmittance (T) represents the ratio of the intensity of radiation after the ray has passed through the sample, I, and initial intensity of radiation, I_0, and can be shown as:

$$T = \frac{I}{Io}$$

More commonly used quantification is absorbance that tells us how much light is absorbed by our sample. It is connected with transmittance:

$$A = -\log\log T = -\log\log \frac{I}{Io}$$

$$A = log\ log\ \frac{Io}{I}$$

It has been shown that the concentration of a sample influences the absorbance; as the concentration of the sample is higher, more radiation is absorbed by the sample and with that, the measure of absorbed light, absorbance, gets higher values. Therefore, the absorbance is directly proportional to the sample concentration.

Beer-Lambert's law defines this linear relationship between the absorbance and the concentration, as well as molar absorption and path length with the equation:

$$A = \varepsilon \cdot C \cdot l$$

ε – molar absorptivity, the measure of how well the sample absorbs the specific wavelength (M^{-1} cm^{-1})

l – optical pathway length (cm)

If and when a substance absorbs electromagnetic radiation of wavelengths between about 200 and 700 nm, or in the UV-visible region, it can be studied by UV-visible spectroscopy

The wavelength of the input ray was chosen at the wavelength at which most of the light is absorbed by the sample/flufenamic acid, i.e. on the peak of maximum UV absorption of the species. Measurements were performed at λ_{max} because the higher the absorbed light, the higher are the absorbance and molar absorptivity. With them being higher, the lower concentrations of the sample can be measured which then provides us with the lowest detection limits.

In the case of flufenamic acid, λ_{max} is set out at 290 nm.

Before measuring the concentrations of unknown samples (which in our case were the samples taken during the dissolution studies of various combinations of CD with FFA as well as different preparation methods), we have first obtained a standard curve or calibration plot.

The standard curve or calibration plot was obtained by preparing a series of solutions (usually 3-5) with known concentrations of our analyte. To zero the spectrophotometer before measuring the absorbance of the standard and unknown solutions, was set using a blank – in our case, it was a buffer pH = 6.8. The absorbance of each standard sample is measured at

λ_{max} and the obtained absorptions were plotted against the concentration of our samples. The linearity of this plot arises from the Beer-Lambert law.

The calibration plot for our experiment was taken from a previous study conducted in the same laboratory (Saturno, 2016).

3.2.3 Phase solubility studies

The first thing we needed to clarify before starting the preparation of flufenamic acid and CDs binary mixtures, was the stoichiometric ratio of these two substances in the complex formed. Phase solubility method, developed by Higuchi and Connors in 1965 can help us out with that.

Higuchi and Connors have classified complexes based on the effect of increasing ligand concentration, in our case the increasing concentration of CD, on substrate solubility. Different types of possibly-made complexes are represented with the phase solubility profiles (isotherms of solubility). Isotherms also indicate the stoichiometry of the complex and possible additional solubilization mechanisms.

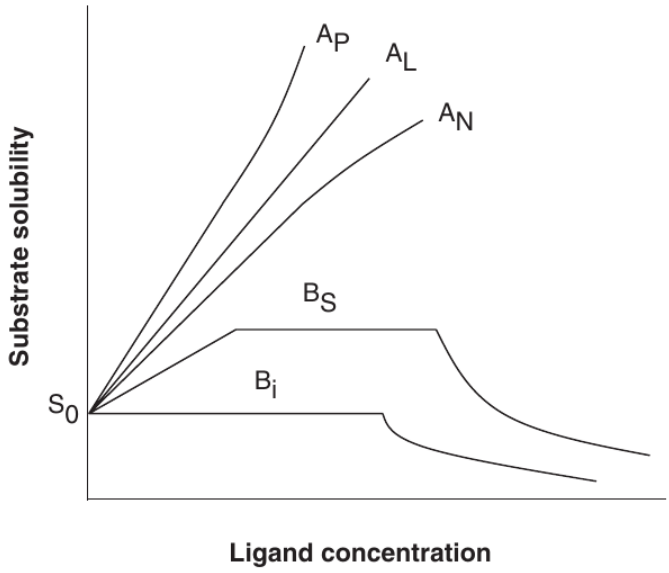

Figure 11. Phase-solubility profiles and classification of complexes according to Higuchi and Connors (Loftsson et al., 2005).

S_0 – intrinsic solubility of the substrate (drug) in the aqueous medium when no ligand (CD) is present

A-type profile diagram – drug-ligand complex is soluble and the solubility of the drug is increased with increasing ligand concentration (usually associated with the water-soluble CD derivatives)

A_L - linear diagram; the complex is the first order concerning substrate and ligand

A_P - positive deviation in solubility from linearity;

A_N - negative deviation in solubility from linearity;

B-type profile diagram – indicates that a formation of complexes with limited solubility occurred (typical for natural CDs)

B_s – complex has some but limited solubility

B_i – complex is insoluble in water

The phase solubility profiles do not verify the formation of inclusion complexes. They only describe how the increasing concentration of the ligand influences drug stability. To distinguish between inclusion and non-inclusion complexes, experimental results from phase-solubility studies must be compared with other experimental results from UV/Vis, fluorescence, NMR, etc.

In 2010 Mura et al performed a study in the same laboratory in the frame of the project aimed to investigate the effects of CD complexation of flufenamic acid on the performance of mucoadhesive films intended for buccal administration. For that research, the first step was to evaluate the efficacy of CD (in their case it was only HPβCD) towards FFA by phase solubility studies (Figure 12.).

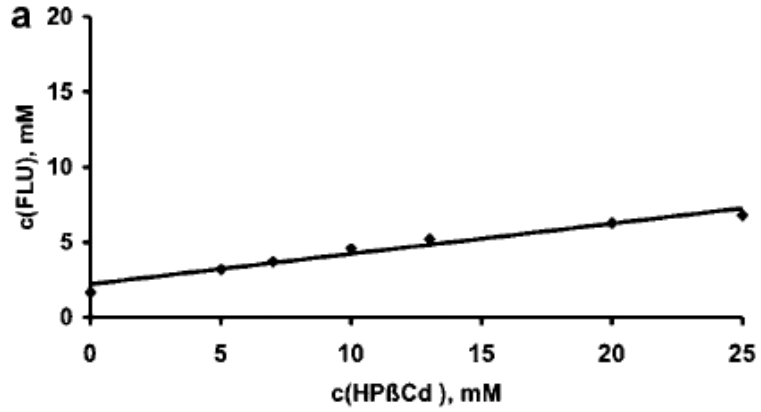

Figure 12. Phase-solubility diagram of FFA in pH 6.75 simulated salivary fluid at 25˚C in the presence of the increasing concentration of HPβCD (Mura et al., 2010).

This study showed that FFA solubility increased linearly with the concentration of HPβCD and the A_L type phase-solubility diagram was obtained, indicating the formation of the complex of probable 1:1 (mol/mol) stoichiometry. Because of that, complexes prepared within this study were prepared at 1:1 (mol/mol) stoichiometry.

3.2.4 Preparation of drug-cyclodextrin binary systems

The binary systems were prepared with four different cyclodextrins (βCD, HPβCD, SBEβCD, RAMEB). The main purpose was to determine both the best preparation method and the most suitable cyclodextrin derivative to enhance the performance/solubility of the drug.

As discussed earlier, based on the results from the phase-solubility studies, that indicated that FLU: CD complexes are formed in a 1:1 ratio, in our experiment Drug-CD solid systems were prepared in the equimolar ratio (Mura et al., 2010).

The methods we used to form a drug-cyclodextrin complex are classified into:

- solid-state techniques: physical mixing, co-grinding
- semisolid-state techniques: kneading
- solution techniques: co-evaporation

3.2.4.1 Solid-phase methods

3.2.4.1.1 Physical mixture (PM)

This is the basic preparation, also used as a reference and starting point for all other preparations. It was obtained simply by weighing the quantities of drug and CD and mixing them in a porcelain mortar with the geometric dilution method (when combining fine powders of unequal amounts, we used doubling-up technique – after adding weighted ingredient present in the lowest (FFA) into the mortar, we add a second ingredient (CD) in approximately the same amount that is present in the mortar, therefore doubling-up the bulk already in the mortar and then we mix it).

The physical mixture was prepared by weighing exactly the quantities of flufenamic acid and CD that satisfy the 1: 1 molar ratio. It is mixed with a bone spatula for a time of 10-15 minutes sufficient to obtain a homogeneous system (Alshehri et al., 2020; Alshehri and Sarim, 2020; Patel and Hirlekar, 2019; Saturno, 2016; Mura et al., 2010).

White-colored powders are obtained.

3.2.4.1.2 Co-Grinding (GR)

The co-ground system (GR) was prepared first by making the physical mixture. That physical mixture was then inserted into a high-energy mill together with stainless steel balls. The grinding process was carried out for 30 minutes at 24 Hz until obtaining fine white powders. The drug-CD interaction obtained by this technique is greater than in the simple physical mixture, due to the mechanical forces applied and the generation of heat during grinding in high-energy mills (Saturno, 2016; Mura et al., 2010).

White-colored powders are obtained.

3.2.4.2 Methods in semi-solid phase

3.2.4.2.1 Kneading (KN)

Components were weighed and prepared as a physical mixture. Then this mixture was placed into a mortar followed by the addition of a few drops of ethanol-water 1:1 (v/v) solution. The solvent was added in quantity needed to maintain the suitable consistency required for kneading. In this way, we obtain a semi-solid paste which we worked (kneaded) thoroughly with the pestle for about 45 minutes, until the added solvent completely evaporated. To ensure the complete evaporation of the solvent, the system was placed in a vacuum desiccator for 24 hours at 35-40 °C up until constant weight (Patel and Hirlekar, 2019; Saturno, 2016; Mura et al., 2001).

The final product obtained is a white powder

3.2.4.3 Methods in liquid phase

3.2.4.3.1 Co-evaporation (CoE)

Co-evaporated products (CoE) were obtained by co-evaporation of equimolar FFA/CD mixture dissolved in ethanol-water 1:1 (v/v) solutions. The FFA was solubilized in a small volume of ethanol in a beaker, whilst the CD was solubilized in a small (depending on the CD) volume of water in another beaker. The two clear solutions were combined in a 250 mL or 500 mL flask on a magnetic plate very slowly, to avoid massive precipitation of the drug, since FFA is poorly soluble in water. It was necessary to add ethanol to the final mixture to solubilize any precipitated drug. The solution thus obtained was brought dry by the rotary evaporator, Rotavapor (T = about 78 ° C, 120 rpm) within 3 to 5 hours. The co-evaporated product obtained was then kept in an oven or dryer for 24 hours before use to remove traces of residual solvents (Patel and Hirlekar, 2019; Saturno, 2016; Mura et al., 2010).

The powders prepared by co-evaporation appeared as a yellow crystalline solid.

3.2.5 *In vitro* dissolution and solubility studies

Drug dissolution is a multistage kinetic process in which particles of the solid phase are passing into the liquid phase. It is the process in which molecules from the solid phase separate and mixes with molecules of the liquid phase. Dissolution is the process by which a solute interacts with the solvent and becomes dissolved.

Solubility, on the other hand, is a thermodynamic property of a matter. A solute can dissolve in a pure solvent. More specifically, it is the maximum amount of solute that the pure solvent can hold (saturated solution) at specific environmental conditions (temperature, pressure, pH) and it depends on the physical and chemical properties of both solute and solvent. Solubility represents the quantity of the dissolved drug, thus representing only the endpoint, without information on how long it takes to get there. Solubility is the intrinsic property of the matter which can be changed only by performing a chemical modification of the matter and it represents one of the most critical physical-chemical properties in drug production.

Solubility can be expressed precisely, as a concentration with units such as g of solute per kg of solvent, g per 100mL of solvent, etc., or it can be expressed descriptively. Figure 21, taken from Ph. Eu. 10, shows those descriptors.

Table 8. Descriptive terms for solubility (Ph. Eu. 10).

Descriptive term	Approximate volume of solvent in millilitres per gram of solute			
Very soluble	less than	1		
Freely soluble	from	1	to	10
Soluble	from	10	to	30
Sparingly soluble	from	30	to	100
Slightly soluble	from	100	to	1000
Very slightly soluble	from	1000	to	10 000
Practically insoluble	more than			10 000

Once the capacity of a solvent to dissolve any further solute is reached, further addition of solute will simply result in settling of solute to the bottom of the container.

A solute may have poor solubility in a solvent, but the dissolution rate may be rapid.

3.2.5.1 Kinetic of dissolution

Dissolution represents a heterogeneous process that takes place on the border of two phases, liquid and solid.

The total speed of this way performed experiment is controlled by the speed of the slowest process happening during the dissolution and that, in the case of solid-phase dissolution, is diffusion: it consists of the passage of solute molecules from the solid surface to the dissolution medium, spreading through the diffusion layer (film of liquid that surrounds each particle of solid that goes into dissolution).

The Noyes-Whitney equation relates the dissolution rate with the concentration:

$$\frac{dm}{dt} = A\frac{D}{d}(Cs - Ct)$$

A = surface of the solid particle exposed to the solvent (m^2)

D = diffusion coefficient of dissolved substance in solvent (ms^{-1}), related to the viscosity of solvent;

h = diffusion layer thickness (stationary layer of solvents around the solid) (m);

C_s = saturation concentration of a solid at certain temperature (maximum solubility) (kg or mol/L);

C_t = concentration in the bulk solvent/solution; concentration at time t (kg or mol/L)

The Noyes-Whitney equation provides practical information relevant to the dissolution process. For example, the dissolution rate (*dm/dt*) will be faster with smaller particles because surface area *A* increases as the particle size decreases, some type of stirring or agitation during dissolution will decrease diffusion gradient by removing solute molecules more quickly from the particle surface to increase dissolution rate.

Table 9. Parameters which we can vary and the effect on dissolution rate (Shekunov and Montgomery, 2016)

Parameter	What affects it?
D	Concentration, temperature, pressure, solvent properties (viscosity)
A	Size, porosity, and dispensability of the particles
H	Stirring
Cs	Temperature, solvent properties, molecular and crystal properties
C	Solvent volume, removal of dissolved matter

The kinetics describing the dissolution rate is of order 1.

The equation can be transformed by substituting *dm* with the *dc*, and that is possible knowing that *m=c*V:*

$$\frac{dm}{dt} = A\frac{D}{d}(Cs - Ct) \quad \Longrightarrow \quad \frac{dc}{dt} = \frac{AD}{Vd}(Cs - Ct)$$

When the concentration measured at time *t* is much lower than the solubility of the drug (for example if *Ct* is 10-15% of *Cs*), *Ct* can be neglected compared to *Cs* and it is said that the system is in "sink conditions". Solvent here never becomes saturated since we have a greater volume of it, so the dissolution takes place continuously. In case of the sink conditions, when *Cs>>C*, the dissolution rate is proportionate to the solubility of the substance. These conditions are similar to the physiological present in the gut. *In vivo*, as the drug passes into solution in the blood, there is no increase in the concentration of the drug in the surrounding medium since the systemic circulation takes away the drug as it passes in solution and the blood arriving at the absorption site is always "fresh", at a concentration of 0 of the drug.

Since we want to simulate the physiological conditions, we had to make sure to keep Ct always very low, so that we can neglect it:

$$Ct \ll Cs; \qquad \frac{dm}{dt} = \frac{A \cdot D \cdot Cs}{d} \quad or \quad \frac{dC}{dt} = \frac{A \cdot D \cdot Cs}{d \cdot V}$$

In sink conditions, the kinetics describing the dissolution rate is of order 0 (Straight).

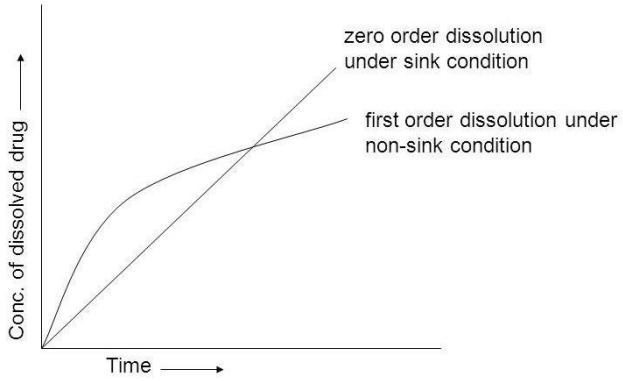

Figure 13. Dissolution rate under non-sink and sink conditions; the slope of the curve: kACs/V (USP 2020).

In traditional *in vitro* dissolution methods, sink conditions are recommended. However, this makes the dissolution rate very fast and experiments' conduction becomes problematic, especially in the case of nanoparticles. The Federation Internationale Pharmaceutique (FIP)/American Association of Pharmaceutical Scientists (AAPS) dissolution testing guidelines recommend the use of the non-sink conditions to decrease the dissolution rate and to get discriminating dissolution results. In non-sink conditions, the solvent becomes saturated with the drug and after some time the concentration of bulk drug and dissolved drug are equal, and after that point, no further dissolution will take place. Therefore, such an experimental setup will provide the possibility to separate the samples with enhanced solubility and *in vitro* dissolution rate (Shekunov and Montgomery 2016**,** Cirri et al., 2008).

3.2.6 Preparation of the dissolution media for the *in vitro* dissolution studies

Any dilutions of the samples, necessary to work in the concentration range of the calibration curve, in which a linear concentration-absorbance relationship has been verified, were carried out using a phosphate buffer at pH = 6.8 prepared by dissolving 27.2 g of KH_2PO_4 in a 1 L flask and 71.6 g of Na_2HPO_4 dodecahydrate (MW = 358.14 g / mol) or 32.2 g of the monohydrate (MW = 160.9 g / mol) in a 1 L flask and subsequently mixing 510 mL of the first solution with 490 mL of the second one.

The pH was always checked using a pH meter.

3.2.7 Performing the *in vitro* dissolution studies

Dissolution studies were performed on pure FFA from two producers as well as all prepared binary mixtures of FFA and CDs. All of them were performed in the same way.

The apparatus used in this study was a beaker method according to Levy, providing a non-sink condition, a technique already used in our laboratory in previous studies (Mura, 2010; Saturno, 2016).

Adequate amounts of samples (pure FFA or binary complex) to achieve non-sink conditions, were added to 75 mL of phosphate buffer at pH = 6.8 in a 150 mL beaker immersed in a bath thermostated at 25 ° C. The powders were inserted from above and in such quantities that the drug remains undissolved. A three-blade stirrer (both glass and metal were tested) was immersed in the beaker always at the same height and rotated at 100 rpm. At time specified intervals (5, 10, 15, 20, 30, and 60 minutes) 3 ml samples were withdrawn by the syringe, immediately filtered by Millipore nitrocellulose filters with 0.45 μm porosity, diluted and measured in the spectrophotometer. Each time the 3 mL withdrawn were replaced with 3 mL of fresh buffer which was kept the whole time in the bath at the same temperature as our studied solution: therefore, correction in the calculation of the dilution was performed.

Each withdrawal was diluted with pH = 6.8 phosphate buffer in 1:100 to 1:200 ratio and the concentration for each was measured spectrophotometrically. Each experiment was performed in duplicate, the average of the values was calculated and the values obtained were used to build a drug concentration graph (mg/L) as a function of time (min), to evaluate the effectiveness of the dissolution process.

The standard deviation for each time-point was calculated and was always <5% (Saturno, 2016; Mura et al. 2010).

4 RESULTS AND DISCUSSION

4.1 SPECTROPHOTOMETRIC DETERMINATION OF ACTIVE COMPOUND

As mentioned, before measuring the concentrations of unknown samples, we first have to generate a standard curve or calibration plot. Also as mentioned, data for our calibration curve was borrowed from the experiment that was previously conducted in the same laboratory by colleague Saturno in 2016.

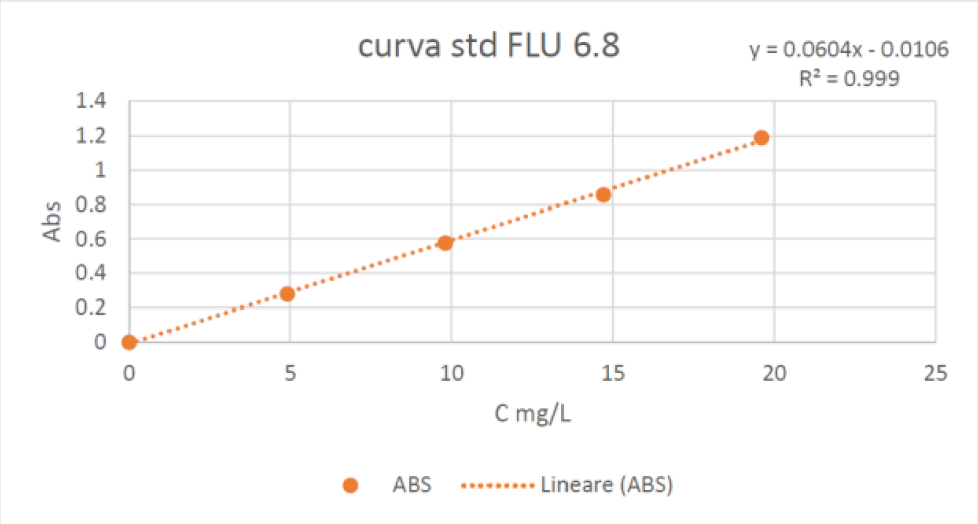

Figure 14. Calibration curve for flufenamic acid (Saturno, 2016).

The linear equation enabled us to calculate the concentration of each sample analyzed, considering the measured absorbance. By locating the absorbance of the unknown sample on the vertical axis, concentration can be found on the horizontal axis. This may be more accurately done using the linear equation presented in Figure 14.

The equation for Beer's law is a straight line with the general form:

$$y = mx + b$$

If we compare this form with Beer-Lambert's equation mentioned above, we can see that the slope, *m*, is equal to $\varepsilon \cdot l$ and it is kept a constant during our whole experiment. This fact allows us to determine the concentration, *c*, from the measured absorbance of unknown samples by extrapolation from the calibration plot.

The equation that colleague Saturno got in her measurements is:

$$y = 0{,}0604 \cdot x - 0.0106$$

$$R^2 = 0{,}999$$

Here *y* represents measured absorbance and x presents the concentration of the measured sample. Unit of the concentration is mg/l.

Another important parameter in the spectrophotometric quantification of the drug is the working range, i.e. the range in which the linear correlation between sample concentration and absorbance is established. In general, the highest concentration that can be precise should not exceed the absorbance of 1.2, as at higher values, a deviation of the linearity occurs, resulting in experimental errors. Therefore, each of the analyzed samples must be suitably diluted with the phosphate buffer at pH = 6.8 to avoid high absorbance values. In our experiment, the maximum measured absorbance was 1.191 that corresponded to the sample concentration of 19.6 mg/L.

4.2 *IN VITRO* DISSOLUTION STUDIES

4.2.1 *In vitro* dissolution rate of pure flufenamic acid

The first step in the experiment was to determine *in vitro* dissolution kinetic behavior of FFA. As mentioned before, in our experiments we used FFA produced by two different companies (SIMS and TCI) so we first analyzed their *in vitro* dissolution behavior. The obtained results are presented in Figure 15. Tested samples presented somewhat different *in vitro* dissolution profiles. The FFA sample obtained by TCI presented a somewhat faster *in vitro* dissolution rate and relatively higher equilibrium solubility in phosphate buffer pH 6.8 (200.74 vs. 156.10 mg/L, respectively). However, in both cases the observed *in vitro* dissolution behavior was typical of a poorly soluble compound, as neither of the tested samples reached the dissolution of more than 50% of the initially added drug dose.

The observed difference in solubility and *in vitro* dissolution rate between tested samples may be attributed to the different particle sizes and/or different polymorphic forms of the drug, as discussed earlier in the Introduction. However, the elucidation of exact reasons behind the observed solubility and *in vitro* dissolution behavior was outside the scope of this research.

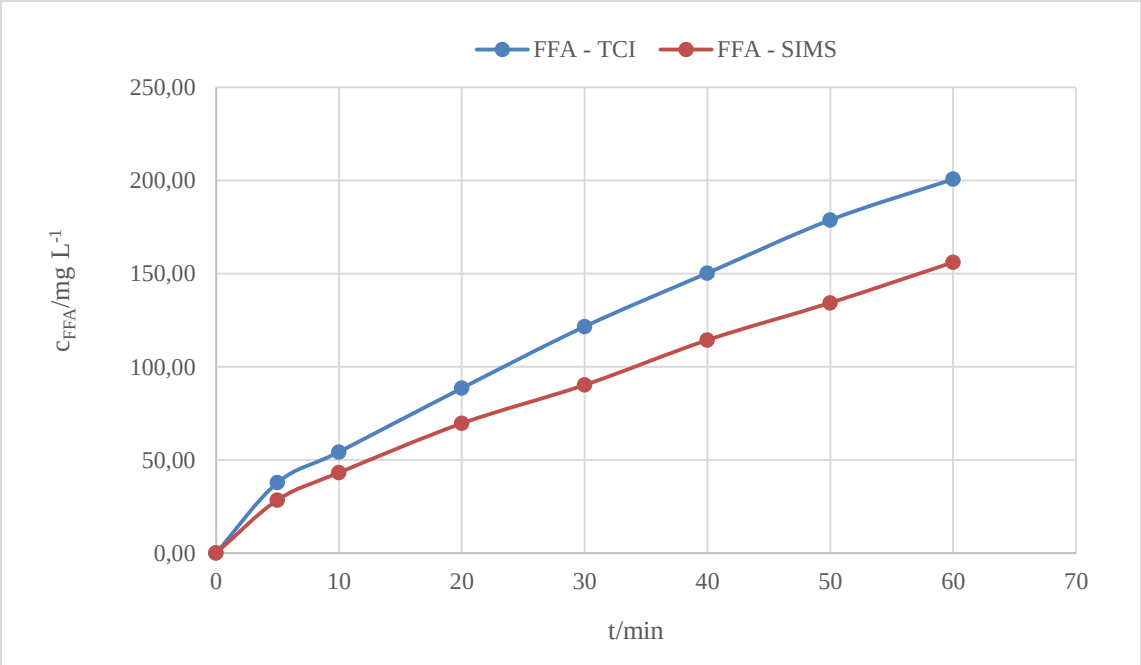

Figure 15. *In vitro* dissolution profile of FFA TCI and SIMS in phosphate buffer pH 6.8 at 25°C.

Based on the results presented in Figure 15, we decided to continue this study using the FFA sample produced by TCI, as it presented a more favorable solubility and *in vitro* dissolution rate in phosphate buffer. However, according to the definition from Ph. Eur. 10, the selected sample of FFA should be considered very slightly soluble in phosphate buffer at pH=6.8, requiring 3392.82 mL of the buffer to dissolve 1 gram of selected FFA.

4.2.2 Binary systems of FFA and selected CDs

We prepared and tested several binary systems containing FFA and β-CD, HP-β-CD, RAMEB, and SBE-β-CD at equimolar ratio 1:1 (mol/mol). Binary systems were prepared by physical mixing of the components (PM), grinding in high energy vibrational mills (GR), kneading (KN), and co-evaporation technique (CoE), as elaborated in detail in section 3.2.4. For each binary system obtained, the *in vitro* dissolution test was performed taking the amount of powdered sample equivalent to 120 - 200 mg of FFA, to provide non-sink conditions, as discussed in detail in section 3.2.5.

4.2.2.1 In vitro dissolution profiles of FFA binary mixtures with β-CD

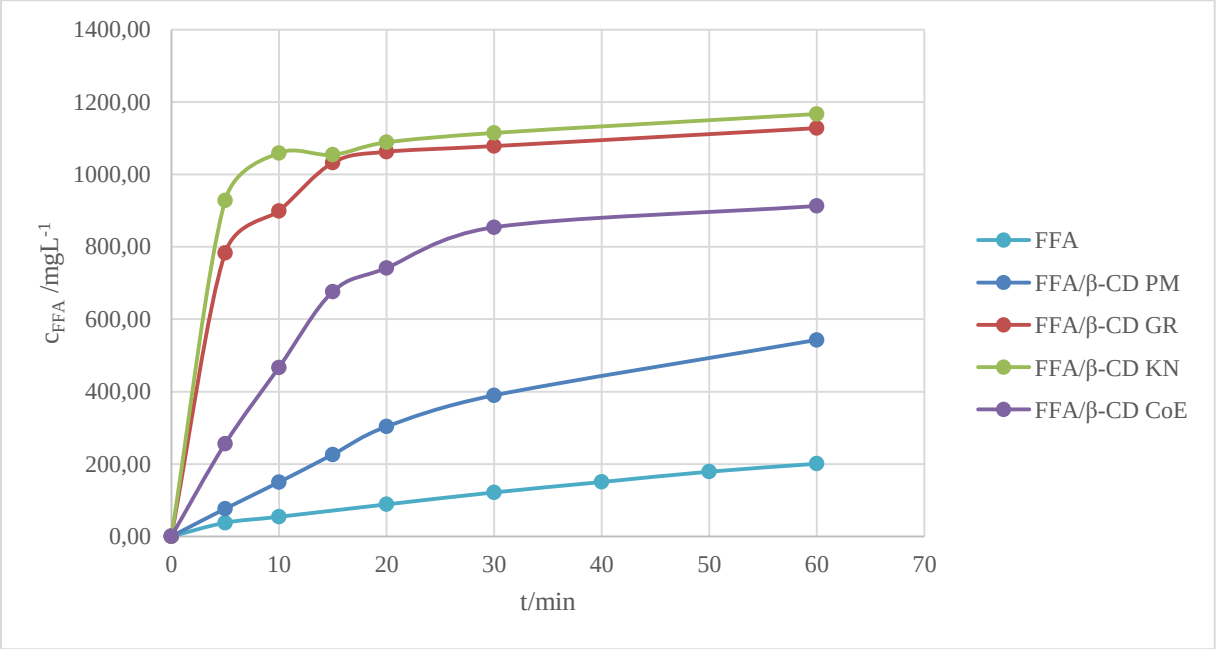

Figure 16. *In vitro* dissolution profiles of binary mixtures of FFA and β-CD in phosphate buffer pH 6.8 at 25˚C.

The *in vitro* dissolution profiles of FFA/β-CD equimolar binary systems prepared by different techniques are shown in Figure 16. β-CD significantly enhanced the *in vitro* dissolution rate of FFA, depending on the preparation method employed. Plain physical mixing of the FFA and β-CD resulted in a sample with the lowest increase of the dissolution rate while grinding and kneading produced samples with the most prominent *in vitro* dissolution properties, characterized by "burst" release. Co-evaporation appears to be less efficient in establishing the solid-state interactions between FFA and β-CD compared to kneading and grinding, resulting in samples with a somewhat worse *in vitro* dissolution rate.

Nevertheless, the FFA dissolution rate was significantly improved by β-CD, regardless of the method employed. Even with the FFA/β-CD prepared as a physical mixture, which showed the lowest improvement of dissolution, the concentration of dissolved FFA was almost three times higher (200 mg/L vs 500 mg/L after 60 minutes) when compared to the dissolution of pure FFA.

The increase of *in vitro* dissolution rate by grinding and kneading might be correlated with the reduction of the particle size, as well as a different degree of amorphization of FFA in those samples that still needs to be examined in the future studies. The use of the grinding

technique was shown to provide the FFA/ β-CD binary sample with the best *in vitro* dissolution characteristics.

4.2.2.2 In vitro dissolution profiles of FFA binary mixtures with HP-β-CD

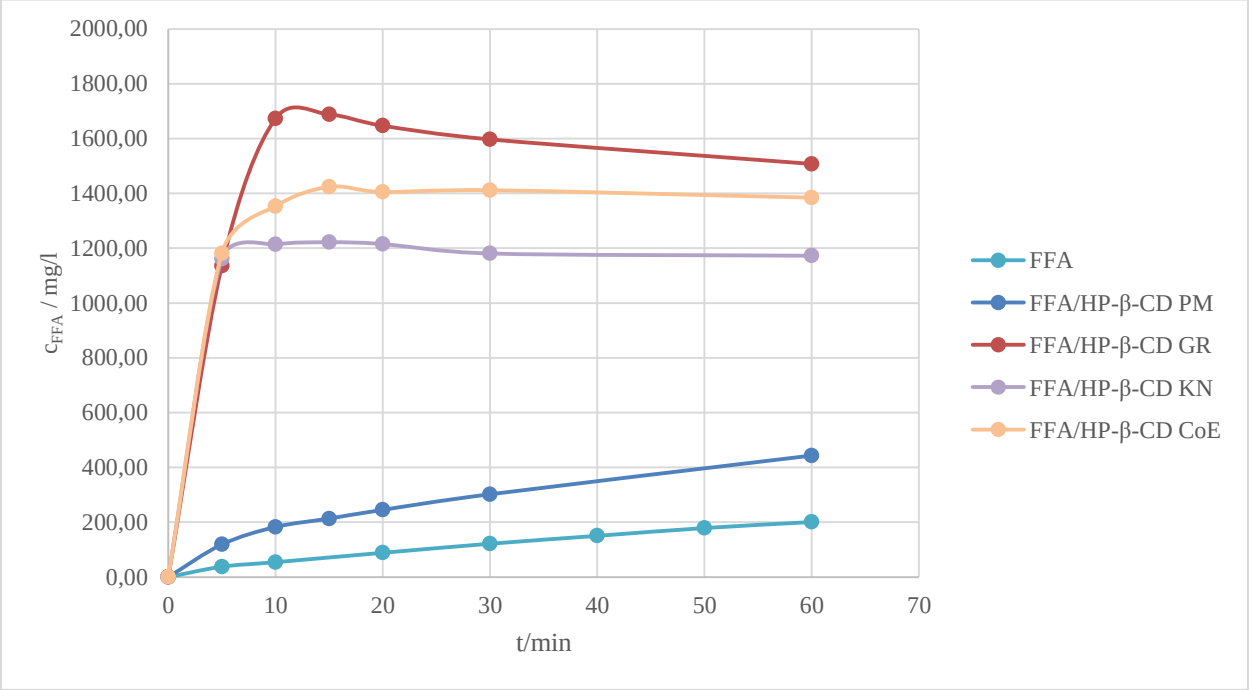

Figure 17. *In vitro* dissolution profiles of binary mixtures of FFA and HP-β-CD in phosphate buffer pH 6.8 at 25˚C.

The *in vitro* dissolution profiles of FFA/HP-β-CD equimolar binary systems prepared by different techniques are shown in Figure 17. HP-β-CD was more efficient than β-CD in enhancing the *in vitro* dissolution behavior of FFA. Here also the employed preparation technique had a significant effect on the characteristics of the products obtained.

In binary complexes prepared with three (out of four) techniques, we can again see the similarity in the release of FFA over time. The most pronounced *in vitro* dissolution was observed for the FFA/HP-β-CD binary systems prepared with grinding, co-evaporation, and kneading, presenting a very steep incline of the dissolution curve during the first 10 minutes of the experiment (Figure 17). For the co-ground sample, the highest concentrations of the dissolved drug were reached after 15 minutes, followed by a slight decline over the remaining time of the experiment. Such behavior may be attributed to the supersaturation phenomena, where initially fast dissolution of this sample leads to the formation of the supersaturated

solution. Such a system is unstable, leading to partial drug precipitation until the system reaches the dynamic equilibrium with the non-dissolved complex (Jug et al., 2014).

Nevertheless, the *in vitro* dissolution of FFA was significantly improved by HP-β-CD. In the case of a physical mixture, the most modest increase of 2 times was obtained after 60 minutes of the dissolution time (200 mg/L vs 443 mg/L), while other preparation methods lead to the samples for which concentration of the dissolved drug after 60 minutes ranged between 1172 and 1507 mg/L, resulting in 6 to 7,5 improvement in FFA *in vitro* dissolution.

Once again, the system obtained with the grinding technique showed the best dissolution characteristics, followed by that prepared by co-evaporation and that obtained with the kneading method.

4.2.2.3 In vitro dissolution profiles of FFA binary mixtures with RAMEB

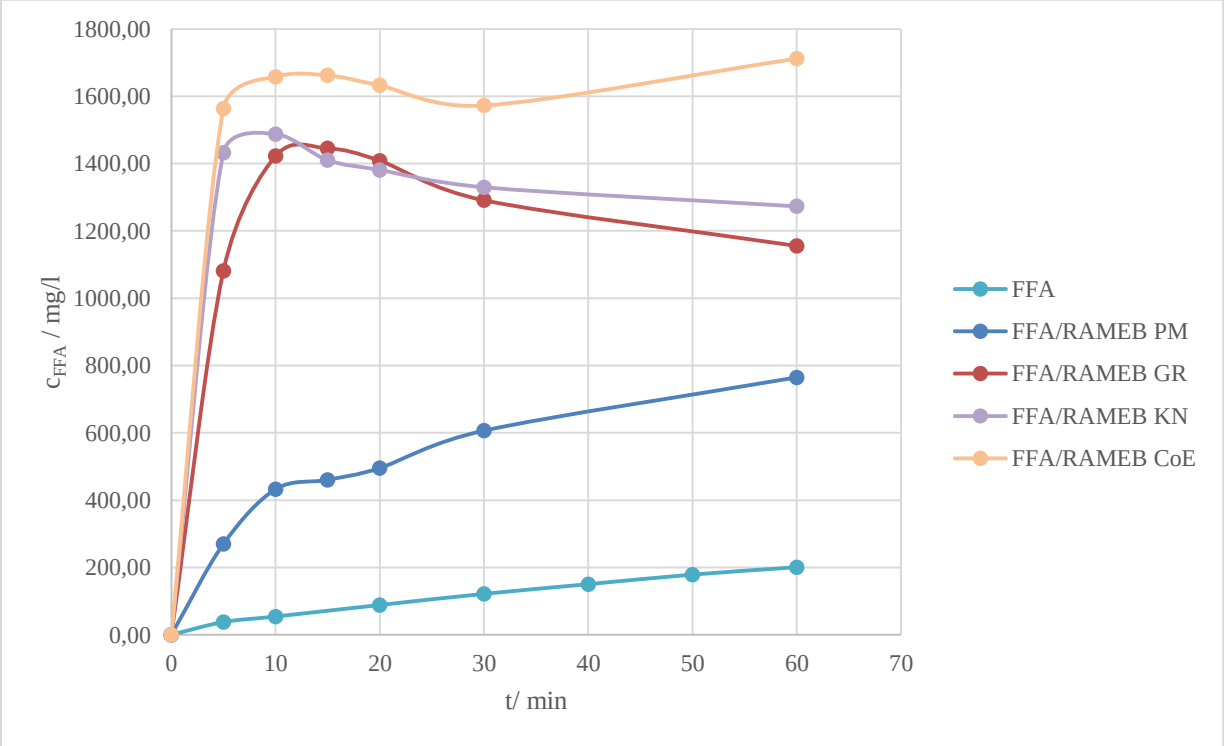

Figure 18. *In vitro* dissolution profiles of binary mixtures of FFA and RAMEB in phosphate buffer pH 6.8 at 25°C.

The *in vitro* dissolution profiles of FFA/RAMEB equimolar binary systems prepared by different techniques are shown in Figure 18. In this case, we can also observe a trend somewhat different than mentioned before for FFA/HP-β-CD and FFA/β-CD binary systems. *In vitro* dissolution of FFA/RAMEB samples prepared by the kneading, co-evaporation and grinding methods showed a sudden burst release, leading to the rapid incline of dissolved drug

concentration in the first 10 to 15 minutes, accompanied by a slow decrease in dissolved drug concentration for the rest of the experiment. The only exception of such behavior is the mixture prepared by the co-evaporation technique, showing an additional increase in the concentration of dissolved FFA after 30 minutes of the experiment. It seems that, in the case of RAMEB, the co-evaporation technique is exceptionally favorable for the FFA complexation, resulting in highly soluble products, forming stable solutions. The concentration of the dissolved drug after 60 minutes in case of FFA/RAMEB binary system obtained by co-evaporation method with the values of concentration being 1711 mg/L, showing 8.5-time dissolution enhancement with respect to pure FFA.

4.2.2.4 In vitro dissolution profiles of FFA binary mixtures with SBE-β-CD

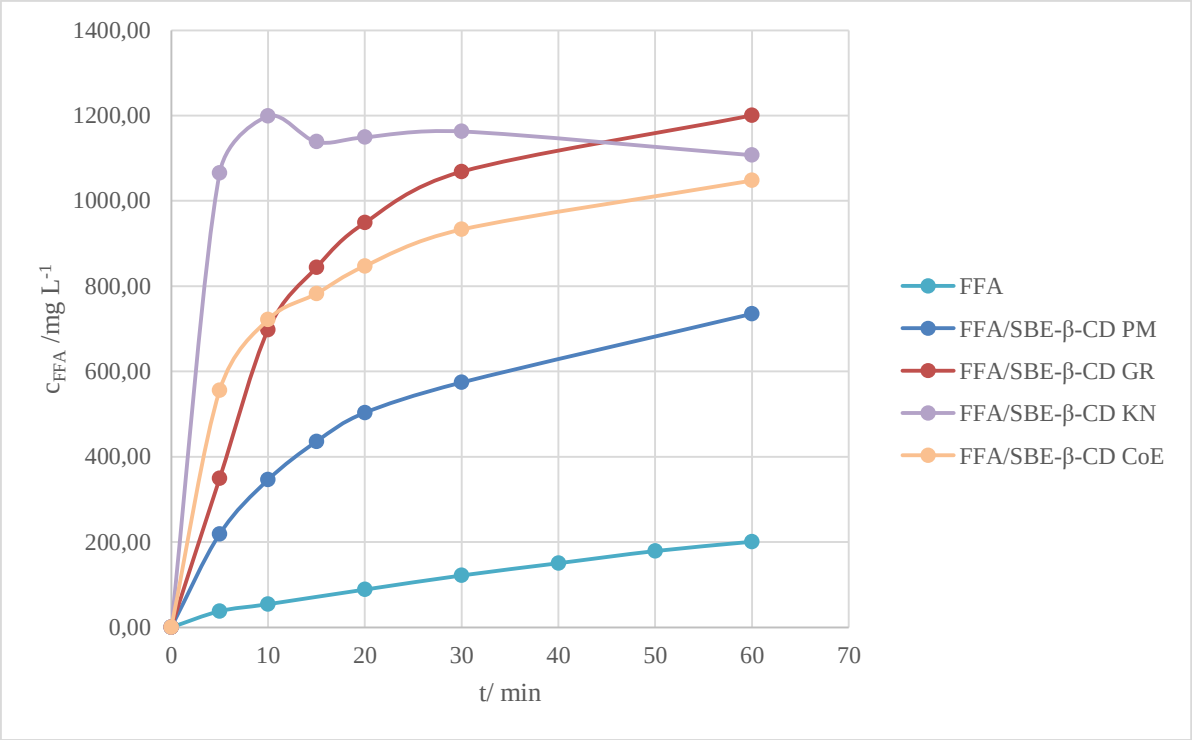

Figure 19. *In vitro* dissolution profiles of binary mixtures of FFA and SBE-β-CD in phosphate buffer pH 6.8 at 25°C.

The *in vitro* dissolution profiles of FFA/ SBE-β-CD equimolar binary systems prepared by different techniques are shown in Figure 19. Binary samples prepared with that CD derivative showed completely different *in vitro* dissolution behavior with respect to that observed in previous cases. Here the fast initial dissolution resulting in the formation of the supersaturated system was observed in the case of FFA/SBE-β-CD prepared with kneading. Other samples presented a significantly slower initial dissolution rate. Even though the kneaded sample presented initially faster dissolution, at the end of the *in vitro* dissolution

experiment FFA/SBE-β-CD system prepared by grinding resulted in the highest concentration of the dissolved drug, thus presenting the best dissolution profile.

Among CD derivatives studied, the plain physical mixture with SBE-β-CD showed the highest drug dissolution enhancement, indicating that this ionic CD derivative has the highest potential for *in situ* interaction with the drug, thus enhancing its solubility. Such behavior might be related to the high wettability of this ionic CD, enabling better contact of the drug with the dissolution medium. However, such an assumption must be confirmed through additional research that is above the scope of this diploma thesis.

4.2.3 Selection of the optimal CD derivative and complex preparation method to enhance FFA *in vitro* dissolution rate

To select the optimal CD derivative and the most suitable complex preparation method the best *in vitro* dissolution profiles for each of examined CD derivatives were compared. Such comparison is presented in Figure 20.

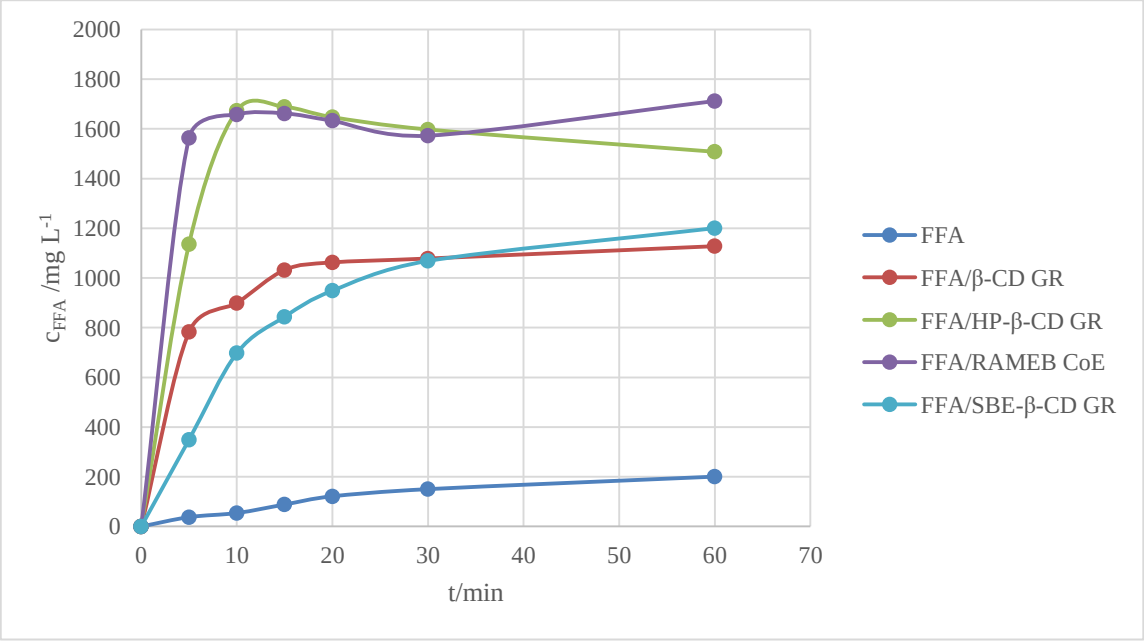

Figure 20. Comparison of the best *in vitro* dissolution profiles of FFA binary mixtures prepared with β-CD, HP-β-CD, RAMEB, and SBE-β-CD.

The comparison of the dissolution curves obtained demonstrates that both the co-grinding with HP-β-CD and co-evaporation with RAMEB result in products with comparable *in vitro* dissolution efficiency. Their efficiency should be further investigated using *the Gastrointestinal Simulator* device proposed by Hens et al. (2020), which can simulate more

accurately the conditions present *in vivo*. However, this is again above the scope of this diploma thesis. But even with the best result provided by this study where the equilibrium concentration of dissolved FFA after 60 minutes reached 1711.86 and 1507,66 mg/L, respectively, it appears that to dissolve 1g of FFA prepared as co-ground HP-β-CD and co-evaporated RAMEB system, we need 584.45 and 663,3 ml of phosphate buffer pH=6.8, respectively. This indicates that RAMEB and HP-β-CD only provided us *slightly soluble* FFA products, according to the definition of Ph. Eur. 10, clearly highlighting the need for further investigation in this field.

5 CONCLUSION

This study was aimed to investigate whether the combination of FFA with CDs will increase the limited aqueous solubility and *in vitro* dissolution rate of this drug, both recognized as critical properties presenting a limiting factor for its wider therapeutic use.

Complexes of FFA were prepared with four different, natural β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP- β-CD), randomly methylated-β-cyclodextrin (RAMEB), and sulfobutyl ether-β-cyclodextrin (SBE- β-CD), using four different preparation methods (physical mixture, grinding, kneading, and co-evaporation). Obtained products were subjected to *in vitro* dissolution study, comparing their dissolution performance to that of pure FFA.

CDs enhanced the *in vitro* dissolution properties of FFA, depending on the CD type and complex preparation method used. By that, the dissolution enhancement in the range from 2.5 (FFA + β-CD PM) to almost 8 times (FFA+RAMEB CoE) was obtained.

In general, physical mixtures showed consistently the lowest dissolution enhancement for each of the CD tested. Even so, it is the least demanding and the quickest method which still showed a certain improvement of drug dissolution, probably having some minor potential for further application.

Both the co-grinding with HP-β-CD and co-evaporation with RAMEB resulted in products with the most enhanced *in vitro* dissolution efficiency. But when revising results obtained with other CDs, co-evaporation was not found to be the most successful method and grinding appeared to be more effective. Considering the complexity and price of co-evaporation, grinding appears as the method of choice, being fast, reproducible, and solvent-free, thus environmentally friendly. β-CD and SBE-β-CD appeared less efficient in the enhancement of FFA *in vitro* dissolution rate and solubility.

However, both co-ground products with HP-β-CD and co-evaporated ones with RAMEB are considered as slightly soluble, highlighting the need for further investigation and development through the addition of ternary components that would enhance the solubilizing efficiency of HP-β-CD and RAMEB.

6 REFERENCES

Abignente E, De Caprariis P. Research on heterocyclic compounds XII - Preparation and antiinflammatory activity of 2-methylimidazo [1,2-α] pyridine-3-carboxylic acids. *Farmaco Ed Sci,* 1982, 37(1), 22-35.

Adeoye O, Cabral-Marques H. Cyclodextrin nanosystem in oral drug delivery: A mini review. *Int J Pharm*, 2017, 531(2), 521-531.

Aina A, Hargreaves MD, Matousek P, Burley JC. Transmission Raman spectroscopy as a tool for quantifying polymorphic content of pharmaceutical formulations. *The Analyst*, 2010, 35(9), 2328-2333.

Akbuga J, Gulhan S, Bayrakta R, Alpmen G. Studies on flufenamic acid capsules and tablets. *Pharmazie,* 1983, 38(7), 478-480.

Albert AP, Large WA. Signal transduction pathways and gating mechanisms of native TRP-like cation channels in vascular myocytes. *J Physiol*, 2006, 570(1), 45-51.

Alshehri S, Sarim Imam S, Altamimi MA, Hussain A, Shakeel F, Alshehri A. Stimulatory effects of soluplus on flufenamic acid β-cyclodextrin supramolecular complex. *AAPS PharmSciTech,* 2020, 21, 145.

Badran M. Formulation and in vitro evaluation of flufenamic acid loaded deformable for improved skin delivery. *Dig J Nanomater Bios,* 2014, 9(1), 83-91.

Baek JS., Yeo EW, Lee YH, Tan NS, Loo SCJ. Controlled-release nanoencapsulating microcapsules to combat inflammatory diseases. *Drug Des Devel Ther*, 2017, 11, 1707–1717.

Bal R, Ustundag Y, Bulut F, Demir C F, Bal A. Flufenamic acid prevents behavioral manifestations of salicylate-induced tinnitus in the rat. *Arch Med Sci*, 2016, 12(1), 208-215.

Barker RA, Cicchetti F, Neal MJ. Neuroanatomy and Neuroscience at a Glance. UK, John Wiley & Sons, 2012, pp 250-254.

Bates D, Schultheis BC, Hanes MC, Jolly SM, Chakravarthy KV, Deer TR, Levy RM, Hunter CW. A Comprehensive Algorithm for Management of Neuropathic Pain. *Pain Medicine*, 2019, 20, 2–12.

Belhocine Y, Bouhadiba A, Rahim M, Nouar L, Djilani I, Khatmi DE. Inclusion complex formation of β-cyclodextrin with the nonsteroidal anti-inflammatory drug flufenamic acid: Computational study. *Macroheterocycles*, 2017, 11(2), 150-155.

Bindu S, Mazumder S, Bandyopadhyay U. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and Organ Damage: A Current Perspective. *Biochem Pharmacol*, 2020, 180, 114-147.

Bingren T, Shiyao H, Jiayue L. Cyclodextrin-based delivery systems for chemotherapeutic anticancer drugs: A review. *Carbohydr Polym*, 2020, 232.

Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. *Adv Drug Deliv Rev*, 2007, 59, 645 – 666.

Brinar V. et al. Neurology for Medical Students. In: Pain and Pain Syndroms. Zagreb, Medicinska naknada, 2009, 193-214.

British pharmacopoeia. London: Medicines and Healthcare Products Regulatory Agency, 2020.

Cai Bai C, Tian BR, Zhao T, Huang Q, Wang ZZ. Cyclodextrin-catalyzed organic synthesis: Reactions, mechanisms and applications. *Molecules*, 2017, 22(9), 1475.

Chemical Entities of Biological Interest (ChEBI), https://www.ebi.ac.uk/chebi/, accessed May 15th, 2021.

Cimolai N. The potential and promise of mefenamic acid. *Expert Rev Clin Pharmacol*, 2013, 6(3), 289-305.

Cirri M, Maestrelli F, Corti G, Furlanetto S, Mura P. Simultaneous effect of cyclodextrin complexation, pH and hydrophilic polymers on naproxen solubilization. *J Pharm Biomed Anal*, 2006, 42, 126–213.

Clapham DE. SnapShot: mammalian TRP channels. *Cell*, 2007, 129(1), 220.

Cohen M, Quintnerb J, van Rysewyk S. Reconsidering the international association for the study of pain definition of pain. *Pain rep*, 2018, 3(2), 634.

Crini G. The contribution of Franz Schardinger to cyclodextrins. *J Incl Phenom Macrocycl Chem*, 2020, 97, 19–28.

Daniels MJ, Rivers-Auty J, Schilling T et al. Fenamate NSAIDs inhibit the NLRP3 inflammasome and protect against Alzheimer's disease in rodent models. *Nat Comm*, 2016, 7, article number: 12504.

Davis CA, Erickson P, McNeill K, Elisabeth M, Janssen EM. Environmental photochemistry of fenamate NSAIDs and their radical intermediates. *Environ Sci Processes Impacts*, 2017, 19, 656-665.

Davis ME, Brewster ME. Cyclodextrin-based Pharmaceutics: Past, Present and Future. *Nat Rev Drug Discov*, 2004, 3, 1023-1035.

De Anda-Jáuregui G, Guo K, McGregor BA, Hur J. Exploration of the Anti-Inflammatory Drug Space Through Network Pharmacology: Applications for Drug Repurposing. *Front Physiol*, 2018, 9, 151.

Delaney SP., Smith TM., Korter TM. Conformational origins of polymorphism in two forms of flufenamic acid. *Journal of Molecular Structure*, 2014, 1078, 83-89.

Dhanaraj V, Vijayan M. Structure and interactions of anti-inflammatory fenamates. A concerted crystallographic and theoretical conformational study. *Acta Crystallogr B*, 1988, 44 (Pt 4), 406-412.

Drug Bank, https://go.drugbank.com/, accessed June 5th, 2021.

Dvorzhak AY. Effects of fenamate on inhibitory postsynaptic currents in Purkinje's cells. *Bull Exp Biol Med*, 2008, 145(5), 564-568.

Domańska U, Pelczarska A, Pobudkowska A. Effect of 2-hydroxypropyl-β-cyclodextrin on solubility of sparingly soluble drug derivatives of anthranilic acid. *Int J Mol Sci*, 2011, 12, 2383-2394.

Douglas G, Thompson M. A comparison of phenylbutazone and flufenamic acid in the treatment of acute gout. *Ann Phys Med*, 1970, 10, 275-280.

Dowell D, Haegerich TM, Chou R. CDC Guideline for Prescribing Opioids for Chronic Pain. *JAMA*, 2016, 315(15), 1624-1645.

EMA. ICH M9 guideline on biopharmaceutics classification system-based biowaivers, EMA/CHMP/ICH/493213/. *Committee for Medicinal Products for Human Use*, 2020.

Erdogar N, Varan G, Bilensoy E. Amphiphilic cyclodextrin derivatives for targeted drug delivery to tumors. *Curr Top Med Chem*, 2017, 17(13), 1521-1528.

European Chemical Agency, ECHA EU, https://echa.europa.eu/hr/home, accessed June 15th 2021.

European Pharmacopoeia. Council of Europe. 10th ed. Strasbourg: Council of Europe, 2020.

Fatmi S, Taouzinet L, Skiba M, Iguer-Ouada M. The use of cyclodextrin or its complexes as a potential treatment against the 2019 novel coronavirus: A mini-review. *Current Drug Delivery*, 2021, 18(4), 382-386.

Flower RJ, Gryglewski R, Herbaczńska-Cedro K et al. Effects of anti-inflammatory drugs on prostaglandin biosynthesis. *Nature New Biology*, 1972, 238, 104–106.

Flower RJ. Drugs which inhibit prostaglandin biosynthesis. *Pharmacol Rev*, 1974, 26(1), 33-67.

Flower RJ. The development of COX2 inhibitors. *Nat Rev Drug Discov*, 2003, 3, 179-191.

Francetić I, Vitezović D. Klinička farmakologija, Smjernice u liječenju bola i primjeni analgetika. Bilušić M, editor, Zagreb, Medicinska naklada, 2014, pp. 470-476.

Ghamari-Langroudi M, Bourque CW. Flufenamic acid blocks depolarizing after potentials and phasic firing in rat supraoptic neurons. *J Physiol*, 2002, 545, 537–542.

Gilpin RK, Zhou W. Infrared studies of the polymorphic states of the fenamates. *J Pharm Biomed Anal*, 2005, 37, 509–515.

Gohain N. Studies on the structure and function of phenazine modifying enzymes PhzM and PhzS involved in the biosynthesis of pyocyanin. *Max-Planck-institut für molekulare physiologie*, 2009, 148.

Gögelein H, Dahlem D, Englert HC, Lang HJ. Flufenamic acid, mefenamic acid and niflumic acid inhibit single nonselective cation channels in the rat exocrine pancreas. *FEBS Lett*, 1990, 268(1), 79-82.

Guide to pharmacology, https://www.guidetopharmacology.org/, accessed June 15th 2021.

Guinamard R, Chatelier A, Demion M, Potreau D, Patri S, Rahmati M, Bois P. Functional characterization of a Ca^{2+}-activated non-selective cation channel in human atrial cardiomyocytes. *J Physiol*, 2004, 558(Pt 1), 75-83.

Guinamard R, Simard C, Del Negro C. Flufenamic acid as an ion channel modulator. *Pharmacol Ther*, 2013, 138(2), 272-284.

Goucke CR. Clinical update: The management of persistent pain. *Med J Aust*, 2003, 178, 444–447.

Habjan S, Vandenberg RJ. Modulation of glutamate and glycine transporters by niflumic, flufenamic and mefenamic acids. *Neurochem Res*, 2009, 34(10), 1738-1747.

Hall JE, Guyton AC. Textbook of Medical Physiology. Saunders, Elsevier, 2016, 49, 621-628.

Hedges AR. Industrial Applications of Cyclodextrins. *Chem Rev*, 1998, 98(5), 2035-2044.

Hee Lee E, Boerrigter SXM, Byrn SR. Epitaxy of a Structurally Related Compound on the (100) Faces of Flufenamic Acid Form I and III Single Crystals. *Cryst Growth Des*, 2010, 10, 2, 518–527.

Hens B, Kataoka M, Ueda K, Gao P, Tsume Y, Augustijns P, Kawakami K, Yamashita S. Biopredictive *in vitro* testing methods to assess intestinal drug absorption from supersaturating dosage forms. *J Drug Del Sci Technol*, 2020, 56, 101275.

Higuchi T, Connors KA. Phase-solubility techniques. *Adv Anal Inst*, 1965, 4, 117-210.

Hill K, Benham CD, McNulty S, Randall AD. Flufenamic acid is a pH-dependent antagonist of TRPM2 channels. *Neuropharmacology*, 2004, 47, 450–460.

Hill J, Zawia NH. Fenamates as Potential Therapeutics for Neurodegenerative Disorders. *Cells*, 2021, 10, 702.

IASP, Declaration of Montréal, 2010. http://www.iasp-pain.org/, accessed May 2nd 2021.

Jabeen R, Kaplan MH. The symphony of the ninth: the development and function of Th9 cells. *Curr Opin Immunol*, 2012, 4(3), 303-307.

Jian-Fang S., Ming-Yi Z., Yi-Jia X., Yang S., Xiao-Hua K., Zhan-You W., Fenamates inhibit human sodium channel Nav1.2 and protect glutamate-induced injury in SH-SY5Y cells, *Cell Mol Neurobiol*, 2020, 40, 1405–1416.

Jong-Suep B, Eng Wan Y, Yin Hao L, Nguan Soon T, Say Chye JL. Controlled-release nanoencapsulating microcapsules to combat inflammatory diseases. *Drug Des Devel Ther*, 2017, 11, 1707–1717.

Jug M, Mennini N, Kövér KE, Mura P. Comparative analysis of binary and ternary cyclodextrin complexes with econazole nitrate in solution and in solid state. *J Pharm Biomed Anal*, 2014, 91, 81-91.

Julius D, Basbaum AI. Molecular mechanisms of nociception. *Nature*, 2001, 413, 203-210.

Kankaanranta H, Moilanen E. Flufenamic and tolfenamic acids inhibit calcium influx in human polymorphonuclear leukocytes. *Mol Pharmacol* 1995, 47(5), 1006-1013.

Kapadia L, Elder MG. Flufenamic acid in treatment of primary spasmodic dysmenorrhea, *Lancet*, 1978, 18(1), 348-350.

Kasinathan RS, Föller M, Koka S, Huber SM, Lang F. Inhibition of eryptosis and intraerythrocytic growth of *Plasmosium falciparum* by flufenamic acid. *Naunyn Schmiedebergs Arch Pharmacol*, 2007, 374, 255–264.

Katzung BG, Masters SB, Trevor AJ. Basic and Clinical Pharmacology. In: Nonsteroidal anti-inflammatory drugs, disease-modifying antirheumatic drugs, nonopioid analgesics, & drugs used in gout. Borazan HN, Furst DE, editors. New York, The McGraw-Hill Companies, Inc, 2012, 635.-659.

Kauppila A, Vapaatalo H, Taskinen PJ. A double-blind clinical study on long-term use of oxyphenbutazone and tolfenamic acid in connection with telecobalt therapy. *Arzneimittelforschung*, 1975, 25(7), 1082-1085.

Kfoury M, Landy D, Fourmentin S. Characterization of cyclodextrin/volatile inclusion complexes: A review. *Molecules*, 2018, 23(5), 1204.

Klose C, Straub I, Riehle M, Ranta F, Krautwurst D, Ullrich S, Meyerhof W, Harteneck C. Fenamates as TRP channel blockers: mefenamic acid selectively blocks TRPM3. *Br J Pharmacol*, 2011, 162, 1757–1769.

Krc J. Crystallographic properties of flufenamic acid. *Microscope,* 1977, 25, 31.

Krishna Murthy HM, Bhat TN, Vijayan M. Structure of a new crystal form of 2-{[3-(trifluoromethyl) phenyl] amino} benzoic acid (flufenamic acid). *Acta Cryst B* 38, 1982, 315-317.

Nakanishi K, Masukawa T, Nadai T, Yoshii K, Okada S, Miyajima K. Sustained release of flufenamic acid from a drug-triacetyl-beta-cyclodextrin complex. *Biol Pharm Bull*, 1997, 20(1), 66-70.

Kuhnert-Brandstätter M, Borka L, Friedrich-Sander G. Zur Polymorphie von Arzneimitteln: Flufenaminsäure und BL 191 [On polymorphism of drugs: flufenamic acid and BL 191 author's transl]. *Arch Pharm (Weinheim),* 1974, 307(11), 845-853.

Kyung-Hoon K, Hyo-Jung S, Salahadin A, Billy H. All about pain pharmacology: what pain physicians should know. *Korean J Pain*, 2020, 33(2), 108–120.

Lambert S, Oberwinkler J. Characterization of a proton-activated, outwardly rectifying anion channel. *J Physiol* 2005, 567(Pt 1), 191–213.

Lee ACY, To KKW, Yhang AJX, Zhu H, Li C, Zhang RR, Hung IFN, Kao RYT. Triple combination of FDA-approved drugs including flufenamic acid, clarithromycin and zanamivir improves survival of severe influenza in mice. *Arch Virol*, 2018, 163 (9), 2349-2358.

Lee EH, Byrn SR, Pinal R. The solution properties of mefenamic acid and a closely related analogue are indistinguishable in polar solvents but significantly different in nonpolar environments. *J Pharm Sci*, 2012, 101, 4529–4539.

Lee YM, Kim BJ, Kim HJ, Yang DK, Zhu MH, Lee KP, et al. TRPC5 as a candidate for the nonselective cation channel activated by muscarinic stimulation in the murine stomach. *Am J Physiol Gastrointest Liver Physiol*, 2003, 284, 604–616.

Lentjes EG, van Ginneken CA. Pharmacokinetics of flufenamic acid in man. *Int J Clin Pharmacol Ther Toxicol,* 1987, 25(4), 185-187.

Liu X, Li Z, Liu H, Zhu Y, Xia D, Wang S, Gu R, Wu W, Zhang P, Liu Y, Zhou Y. Low concentration flufenamic acid enhances osteogenic differentiation of mesenchymal stem cells and suppresses bone loss by inhibition of the NF-κB signaling pathway. *Stem Cell Res Ther*, 2019, 10, 213.

Loftsson T, Jarho P, Másson M, Järvinen T. Cyclodextrins in delivery (review). *Expert Opin Drug Deliv*, 2005, 2(2), 335-351.

López-Mejías V, Kampf JW, Matzger AJ. Nonamorphism in Flufenamic Acid and a New Record for a Polymorphic Compound with Solved Structures. *J Am Chem Soc*, 2012, 9872–9875.

Mantas A, Mihranyan A. Dissolution behavior of flufenamic acid in heated mixtures with nanocellulose. *Molecules*, 2020, 25(6), 1277.

Matsuura K, Shiraishi H, Hara A, Sato K, Deyashiki Y, Ninomiya M, Sakai S. Identification of a principal mRNA species for human 3alpha-hydroxysteroid dehydrogenase isoform (AKR1C3) that exhibits high prostaglandin D2 11-ketoreductase activity. *J Biochem*, 1998, 124 (5), 940-946.

McConnell JB, Glasgow JFT, McNair R. Effect on neonatal jaundice of oestrogens and progestogens taken before and after conception. *Br Med J*, 1973, 3, 5881, 605-607.

Merskey H, Albe Fessard D, Bonica JJ, Carmon A, Dubner R, Kerr FWL, Lindblom U, Mumford JM, Nathan PW, Noordenbos W, Pagni CA, Renaer MJ, Sternbach RA, Sunderland S. Pain terms II: a list with definitions and notes on usage. IASP press, 1986.

Merskey H, Albe Fessard D, Bonica JJ, Carmon A, Dubner R, Kerr FWL, Lindblom U, Mumford JM, Nathan PW, Noordenbos W, Pagni CA, Renaer MJ, Sternbach RA, Sunderland S. Part III: Pain Terms, A Current List with Definitions and Notes on Usage, IASP press, 2011, pp 209-214.

Merskey H, Bogduk N. and members of the IASP Subcommittee on Taxonomy. Classification of chronic pain. *IASP press*, 2002.

Metz G. Method of producing 2-(2-hydroxyethoxy)-ethanol ester of flufenamic acid, 1984., U.S. Patent US4980498

Moffat AC. Clarke's Isolation and Identification of Drugs. *Pharmaceutical Press*, 1986, 1248.

Mohamed AA, Matijevic E. Preparation and characterization of uniform particles of flufenamic acid and its calcium and barium salts. *J Colloid Interface Sci*, 2012, 381(1), 198–201.

Mura P, Faucci MT, Parrini PL. Effects of grinding with microcrystalline cellulose and cyclodextrins on the Ketoprofen physicochemical properties. *Drug Dev Ind Pharm*, 2001, 27(2), 119-128.

Mura P, Bettinetti GP, Cirri M, Maestrelli F, Sorrenti M, Catenacci L. Solid-state characterization and dissolution properties of naproxen-arginine-hydroxypropyl-beta-cyclodextrin ternary system. *Eur J Pharm Biopharm*, 2005, 59(1), 99-106, 2005.

Mura P, Corti G, Cirri M, Maestrelli F, Mennini N, Bragagni M. Development of Mucoadhesive Films for Buccal Administration of Flufenamic Acid: Effect of Cyclodextrin Complexation. *J Pharm Sci*, 2010, 99(7), 3019-3029.

Mura P, Faucci T, Bettinetti GP. The influence of polyvinylpyrrolidone on naproxen complexation with hydroxypropyl-β-cyclodextrin. *Eur J Pharm Sci*, 2001, 187–194.

Musa D. Peptic ulcer disease and non-steroidal anti-inflammatory drugs. *Aust Prescr*, 2017, 40(3), 91–93.

National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Board on Health Care Services; Committee on Evidence-Based Clinical Practice Guidelines for Prescribing Opioids for Acute Pain. Framing Opioid Prescribing Guidelines for Acute Pain: Developing the Evidence, Managing Acute Pain. *National Academies Press (US)*, 2019.

Nazıroğlu M, Özgül C, Çelik Ö, Çiğ B, Sözbir E. APB and flufenamic acid inhibit Ca^{2+} influx through TRPM2 channels. *J Membr Biol*, 2011, 241, 69–75.

NICE guidelines, Chronic pain (primary and secondary) in over 16s: assessment of all chronic pain and management of chronic primary pain, 2021., https://www.nice.org.uk/, accessed 15th July 2021.

Nicholson B. Differential Diagnosis: Nociceptive and Neuropathic Pain. *Am J Manag Care*, 2006, 256-262.

Numata T, Shimizu T, Okada Y. TRPM7 is a stretch- and swelling-activated cation channel involved in volume regulation in human epithelial cells. *Am J Physiol Cell Physiol*, 2007, 292(1), 460-467.

Pardutz A, Schoenen J. NSAIDs in the acute treatment of migraine: A review of clinical and experimental data. *Pharmaceuticals*, 2010, 3, 1966-1987.

Patel M, Hirlekar R. Multicomponent cyclodextrin system for improvement of solubility and dissolution rate of poorly water soluble drugs. *Asian J Pharm Sci*, 2019, 14(1), 104–115, 2019.

Pongkorpsakol P, Yimnual C, Chatsudthipong V, Rukachaisirikul V, Muanprasat C. Cellular mechanisms underlying the inhibitory effect of flufenamic acid on chloride secretion in human intestinal epithelial cells. *J Pharmacol Sci*, 2017, 134(2), 93-100.

Pereva S, Himitliiska T, Spassov T, Stoyanov SD, Arnaudov LN, Dudev T. Cyclodextrin-based solid-gas clathrates. *J Agric Food Chem*, 2015, 63(29), 6603-6613.

Pérez Ruiz T, Martínez Lozano C, Tomás V, Carpena J. Analysis of binary mixtures of flufenamic, meclofenamic and mefenamic acids by derivative synchronous fluorescence spectrometry. *Talanta*, 1998, 47, 537 – 545.

Perlovich GL, Surov AO, Bauer-Brandl A. Thermodynamic properties of flufenamic and niflumic acids—specific and non-specific interactions in solution and in crystal lattices, mechanism of solvation, partitioning and distribution. *J Pharm Biomed Anal*, 2007, 45, 679-687.

Peña F, Parkis MA, Tryba AK, Ramirez JM. Differential contribution of pacemaker properties to the generation of respiratory rhythms during normoxia and hypoxia. *Neuron*, 2004, 43, 105–117.

Pfaffenrath V, Scherzer S. Analgesics and NSAIDs in the treatment of the acute migraine attack. *Cephalalgia*, 1995, 15, 14-20.

The Pharmacopoeia of Japan. Tokyo, Japan, Society of Japanese Pharmacopoeia, 2011.

Pobudkowska A, Domanska U. Study of pH-dependent drugs solubility in water. *Chem Ind & Chem Eng Q*, 2014, 20 (1), 115−126.

Preethi B, Shanthi V, Ramanathan K. Identification of Potential Therapeutics to Conquer Drug Resistance in *Salmonella typhimurium*: Drug Repurposing Strategy. *BioDrugs*, 2016, 30, 593–605.

PubMed, https://pubmed.ncbi.nlm.nih.gov/, accessed on 12th July 2021.

Rae JL, Farrugia G. Whole-cell potassium current in rabbit corneal epithelium activated by fenamates. *J Membr Biol*, 1992, 129, 81–97.

Rajan KT, Hill AGS, Barr A, Whitwell E. Flufenamic acid in rheumatoid arthritis. *Ann Rheum Dis*, 1967, 26, 43-46.

Roberts MS, Walters KA. Dermal absorption and toxicity assessment. Boca Raton, CRC press, 2008, 253-265.

Romero S, Escalera B, Bustamante P. Solubility behavior of polymorphs I and II of mefenamic acid in solvent mixtures. *Int J Pharm*, 1999, 178, 193-202.

Rubio C, Bellver J, Rodrigo L, Bosch E, Mercader A, Vidal C, De los Santos MJ, Giles J, Labarta E, Domingo J, Crespo J, Remohí J, Pellicer A, Simón C. Preimplantation genetic screening using fluorescence in situ hybridization in patients with repetitive implantation failure and advanced maternal age: two randomized trials. *Fertil Steril*, 2013, 99(5), 1400-1407.

Rytting E, Lentz KA, Chen XQ, Qian F, Venkatesh S. Aqueous and co-solvent solubility data for drug-like organic compounds. *AAPS J*, 2005, 7, 10.

Sanford-Driscoll M, Knodel LC. Induction of hemolytic anemia by nonsteroidal antiinflammatory drugs. *Drug Intell Clin Pharm*, 1986, 20(12), 925-34.

Saokham P, Muankaew C, Jansook P, Loftsson T. Solubility of cyclodextrins and drug/cyclodextrin complexes. *Molecules*, 2018, 23(5), 1161.

Saturno A. Tesi di laurea: Sviluppo e caratterizzazione di complessi Acido Flufenamico- Ciclodestrina. 2016-2017.

Shazly GA, Ibrahim MA, Badran MM, Zuheir KMA. Utilizing Pluronic F-127 and Gelucire 50/13 solid dispersions for enhanced skin delivery of flufenamic acid. *Drug Dev Res*, 2012, 73, 299–307.

Shekunov B, Montgomery ER, Theoretical Analysis of Drug Dissolution: I. Solubility and Intrinsic Dissolution Rate, *J Pharm Sci*, 2016, 105, 2685-2697.

Shepelytskyi Y, Newman CJ, Grynko V, Seveney LE, DeBoef B, Hane FT, Albert MS. Cyclodextrin-based contrast agents for medical imaging. *Molecules*, 2020, 25(23), 5576.

Sivakumar MP, Peimanfard S, Zarrabi A, Khosravi A, Islami M. Cyclodextrin-based nanosystems as drug-carriers for cancer treatments. *Anticancer Agents Med Chem*, 2020, 20(11), 1327-1339.

Sigma-Aldrich, https://www.sigmaaldrich.com/HR/, accessed 20th July 2021.

Skarydová L, Zivná L, Xiong G, Maser E, Wsól V. AKR1C3 as a potential target for the inhibitory effect of dietary flavonoids. *Chem Biol Interact*, 2009, 178 (1-3), 138-144.

Smolková R, Zeleňák V, Smolko L, Sabolová D, Kuchár J, Gyepes R. Novel Zn(II) complexes with non-steroidal anti-inflammatory ligand, flufenamic acid: Characterization, topoisomerase I inhibition activity, DNA and HSA binding studies. *J Inorg Biochem*, 2017, 177, 143-158.

Srinivas M, Spray DC. Closure of gap junction channels by arylaminobenzoates. *Mol Pharmacol*, 2003, 63(6):1389-1397.

Sun JF, Xu YJ, Kong XH, Su Y, Wang ZY. Fenamates inhibit human sodium channels Nav1.7 and Nav1.8. *Neurosci Lett*, 2019, 23;696, 67-73.

Suzuki R, Fujita T, Mizuta K, Kumamoto E. Inhibition by non-steroidal anti-inflammatory drugs of compound action potentials in frog sciatic nerve fibers. *Biomed Pharmacother*, 2018, 103, 326-335.

Szejtli J. Enhancement of Drug Bioavailability by Cyclodextrins. *Starch/Starke*, 1981, 33, 387-390.

Szente L, Szejtli J. Highly soluble cyclodextrin derivatives: chemistry, properties and trends in development. *Adv Drug Deliv Rev*, 1999, 36, 17–28.

Takahira M, Sakurai M, Sakurada N, Sugiyama K. Fenamates and diltiazem modulate lipid-sensitive mechano-gated 2P domain K^+ channels. *Pflugers Arch*, 2005, 451, 474–478.

Tesfai Y, Brereton HM, Barritt GJ. A diacylglycerol-activated Ca^{2+} channel in PC12 cells (an adrenal chromaffin cell line) correlates with expression of the TRP-6 (transient receptor potential) protein. *Biochem J*, 2001, 358, 717–726.

Tomita K, Takano T. Interaction of flufenamic acid on ethanol metabolism in rat. *Ind Health*, 1992, 30(2), 85-95.

Venkatachalam K, Montell C. TRP Channels. *Annu Rev Biochem*, 2007, 76, 387–417.

The United States pharmacopeia. National formulary. Vol. 1. Rockville (MD): United States Pharmacopeial Convention. 2020,

Wang D, Grillner S, Wallen P. Effects of flufenamic acid on fictive locomotion, plateau potentials, calcium channels and NMDA receptors in the lamprey spinal cord. *Neuropharmacology*, 2006, 51, 1038–1046.

Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Non-steroid drug selectivities for cyclooxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full in vitro analysis. *Proc Natl Acad Sci U S A*, 1999, 96, 7563–7568.

Wenkers BP, Lippold BC. Skin penetration of nonsteroidal anti-inflammatory drugs out of a lipophilic vehicle: influence of the viable epidermis. *J Pharm Sci*, 1999, 88, 1326–1331.

White MM, Aylwin M. Niflumic and flufenamic acids are potent reversible blockers of Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes. *Mol Pharmacol*, 1990, 37(5), 720-724.

Woolf CJ. What is this thing called pain. *J Clin Investig*, 2010, 120(11), 3742–3744.

Woolf CJ, Bennett GJ, Doherty M, Dubner R, Kidd B, Koltzenburg M, Lipton R, Loeser JD, Payne R, Torebjork E. Towards a mechanism-based classification of pain. *Pain*, 1998, 77(3), 227-229.

Yau HJ, Baranauskas G, Martina M. Flufenamic acid decreases neuronal excitability through modulation of voltage-gated sodium channel gating. *J Physiol*, 2010, 588(20), 3869–3882.

Young Jin S, Alhashimi M, Mayhoub A, Mohammad H, Seleem MN. Repurposing fenamic acid drugs to combat multidrug-resistant *Neisseria gonorrhoeae. Antimicrob Agents Chemother*, 2020, 64(7), 2206-2219.

Zhang K, Fellah N, López-Mejías V, Ward MD. Polymorphic phase transformation pathways under nano-confinement: Flufenamic acid. *Cryst Growth Des*, 2020, 11, 7098–7103.

Zhang S, Tang H, Wang Y, Nie B, Yang H, Yuan W, Qu X, Yue B. Antibacterial and antibiofilm effects of flufenamic acid against methicillin-resistant *Staphylococcus aureus*. *Pharmacol Res*, 2020, 160.

7 SAŽETAK/SUMMARY

7.2 SAŽETAK

Fenamati su zanimljiva skupina nesteroidnih protuupalnih lijekova s obzirom da djeluju kao modulatori širokog spektra ionskih kanala i enzima. Zbog toga potencijalno predstavljaju zanimljive analgetike s drugačijim mehanizmom djelovanja. Unutar ove skupine, ističe se flufenaminska kiselina, koja se ubraja u II. skupinu lijekova prema BCS klasifikaciji, s obzirom da je niske topljivosti i visoke permeabilnosti. Zbog niske topljivosti u vodi je njena oralna bioraspoloživost niska što posljedično ograničava kliničku primjenu ovog lijeka. Na tržištu Europske unije dostupna je samo u Italiji kao topikalni pripravak za primjenu na kožu. Cilj ovoga rada je poboljšanje biofarmaceutskih karakteristika flufenaminske kiseline primjenom različitih ciklodekstrina. Istraživanje obuhvaća prirodni β-ciklodekstrin (β-CD) te njegove hidrofilne derivate hidroksipropil-β-ciklodekstrin (HP-β-CD), nasumično metilirani β-ciklodekstrin (RAMEB) te sulfobutileter-β-ciklodekstrin (SBE-β-CD). Binarni sustavi flufenaminske kiseline s ispitivanim ciklodekstrinima pripremljeni su primjenom različitih tehnologija kojima je praćena brzina otapanja u *in vitro* uvjetima. Uzorci pripremljeni mljevenjem lijeka s HP-β-CD te koevaporacijom s RAMEB pokazuju najviše potencijala u prevladavanja biofarmaceutski nepovoljnih karakteristika flufenaminske kiseline, u prvom redu ograničenog *in vitro* oslobađanja, koji ograničavaju širu terapijsku uporabu flufenaminske kiseline.

7.3 SUMMARY

Fenamates are an interesting group of nonsteroidal anti-inflammatory drugs acting as modulators of a wide range of ion channels and enzymes. Therefore, they potentially represent interesting analgesics with a different mechanism of action. Within this group of drugs, flufenamic acid stands out, which belongs to the II. group of drugs according to the BCS classification, given its low solubility and high permeability. Due to its low aqueous solubility, its oral bioavailability is low and variable, which consequently limits the clinical use of this drug. It is only available on the European Union market in Italy as a topical preparation for application to the skin. This work aims to improve the biopharmaceutical properties of flufenamic acid by applying various cyclodextrins. The study includes natural β-cyclodextrin (β-CD) and its hydrophilic derivatives hydroxypropyl-β-cyclodextrin (HP-β-CD), randomly methylated β-cyclodextrin (RAMEB), and sulfobutylether-β-cyclodextrin (SBE-β-CD). Binary systems of flufenamic acid with tested cyclodextrins were prepared using various technologies that monitor the dissolution rate *in vitro*. Samples prepared by milling the drug with HP-β-CD and co-evaporating with RAMEB show the greatest potential in overcoming the biopharmaceutical unfavorable characteristics of flufenamic acid, primarily limited in vitro dissolution rate, which restricts the wider therapeutic use of that drug.

Temeljna dokumentacijska kartica

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Diplomski rad

PRIMJENA CIKLODEKSTRINA U POBOLJŠANJU *IN VITRO* BRZINE OTAPANJA FLUFENAMINSKE KISELINE

Anamaria Maričić

SAŽETAK

Fenamati su zanimljiva skupina nesteroidnih protuupalnih lijekova s obzirom da djeluju kao modulatori širokog spektra ionskih kanala i enzima. Zbog toga potencijalno predstavljaju zanimljive analgetike s drugačijim mehanizmom djelovanja. Unutar ove skupine, ističe se flufenaminska kiselina, koja se ubraja u II. skupinu lijekova prema BCS klasifikaciji, s obzirom da je niske topljivosti i visoke permeabilnosti. Zbog niske topljivosti u vodi je njena oralna bioraspoloživost niska što posljedično ograničava kliničku primjenu ovog lijeka. Na tržištu Europske unije dostupna je samo u Italiji kao topikalni pripravak za primjenu na kožu. Cilj ovoga rada je poboljšanje biofarmaceutskih karakteristika flufenaminske kiseline primjenom različitih ciklodekstrin. Istraživanje obuhvaća prirodni β-ciklodekstrin (β-CD) te njegove hidrofilne derivate hidroksipropil-β-ciklodekstrin (HP-β-CD), nasumično metilirani β-ciklodekstrin (RAMEB) te sulfobutileter-β-ciklodekstrin (SBE-β-CD). Binarni sustavi flufenaminske kiseline s ispitivanim ciklodekstinima pripremljeni su primjenom različitih tehnologija kojima je praćena brzina otapanja u *in vitro* uvjetima. Uzorci pripremljeni mljevenjem lijeka s HP-β-CD te koevaporacijom s RAMEB pokazuju najviše potencijala u prevladavanja biofarmaceutski nepovoljnih karakteristika flufenaminske kiseline, u prvom redu ograničenog *in vitro* oslobađanja, koji ograničavaju širu terapijsku uporabu flufenaminske kiseline.

Rad je pohranjen u Središnjoj knjižnici Sveučilišta u Zagrebu Farmaceutsko-biokemijskog fakulteta.

Rad sadrži:	72 stranica, 20 grafičkih prikaza, 9 tablica i 148 literaturnih navoda. Izvornik je na engleskom jeziku.
Ključne riječi:	Flufenaminska kiselina, ciklodekstrini, mljevenje, gnječenje, koevaporacija, *in vitro* ispitivanje brzine otapanja
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Basic documentation card

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Diploma thesis

APPLICATION OF CYCLODEXTRINS TO ENHANCE *IN VITRO* DISSOLUTION RATE OF FLUFENAMIC ACID

Anamaria Maričić

SUMMARY

Fenamates are an interesting group of nonsteroidal anti-inflammatory drugs acting as modulators of a wide range of ion channels and enzymes. Therefore, they potentially represent interesting analgesics with a different mechanism of action. Within this group of drugs, flufenamic acid stands out, which belongs to the II. group of drugs according to the BCS classification, given its low solubility and high permeability. Due to its low aqueous solubility, its oral bioavailability is low and variable, which consequently limits the clinical use of this drug. It is only available on the European Union market in Italy as a topical preparation for application to the skin. This work aims to improve the biopharmaceutical properties of flufenamic acid by applying various cyclodextrins. The study includes natural β-cyclodextrin (β-CD) and its hydrophilic derivatives hydroxypropyl-β-cyclodextrin (HP-β-CD), randomly methylated β-cyclodextrin (RAMEB) and sulfobutylether-β-cyclodextrin (SBE-β-CD). Binary systems of flufenamic acid with tested cyclodextrins were prepared using various technologies that monitor the dissolution rate in vitro. Samples prepared by milling the drug with HP-β-CD and co-evaporating with RAMEB show the greatest potential in overcoming the biopharmaceutical unfavorable characteristics of flufenamic acid, primarily limited *in vitro* dissolution rate, which restricts the wider therapeutic use of that drug.

The thesis is deposited in the Central Library of the University of Zagreb Faculty of Pharmacy and Biochemistry.

Thesis includes: 72 pages, 20 figures, 9 tables and 148 references. Original is in English.

Keywords: Flufenamic acid, cyclodextrins, grinding, kneading, co-evaporation, *in vitro* dissolution studies

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