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Studies on the application of eutectic systems in the formulation of pharmaceuticals

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In the development of modern pharmaceutical forms, one of the biggest challenges is represented by the low solubility of active substances. This factor directly influences their bioavailability and therapeutic efficacy. About 40% of existing drugs on the market and almost 90% of new chemical entities developed in the pharmaceutical industry show limited solubility in water, which significantly reduces the ability of these substances to adequately reach the therapeutic target. This solubility limitation, which can lead to low absorption and consequently to diminished therapeutic efficacy, is a challenge, especially in the case of non-steroidal anti-inflammatory drugs (NSAIDs), a class of drugs commonly used in the treatment of pain and inflammation.

To overcome these limitations, natural eutectic solvents (NADES) represent a promising solution, offering the possibility to significantly improve the solubility and bioavailability of some active pharmaceutical substances. NADES are mixtures of natural compounds, such as organic acids, alcohols, amino acids, and sugars, which, through molecular interactions, lower the melting point of the mixture and form an effective solubilizing medium for hydrophobic substances. These systems are recognized not only for their ability to solubilize and stabilize active pharmaceutical ingredients (APIs) but also for their biodegradable, non-toxic, and environmentally friendly nature, thus offering a viable alternative to conventional solvents used in pharmaceutical formulations.

This work consists of two parts: the general-theoretical part and the part of personal contributions.

The general-theoretical part is structured in three chapters, the first two chapters offering a detailed approach to the critical aspects of the solubilization of nonsteroidal anti-inflammatory drugs (NSAIDs) using natural eutectic solvents (NADES), their formulation in eutectogels, as an innovative solution for their topical administration, along with a presentation of the main physicochemical and pharmacological characteristics of the NSAIDs selected as drug models.

Chapter 1 introduces the field of eutectic mixtures, focusing on ionic liquids (ILs) and deep eutectic solvents (DES). These systems are formed by combining components that generate a eutectic mixture in a certain molar ratio with a reduced melting point compared to the values of the individual components. DES and IL are widely used in various fields, including pharmacy and chemistry, due to their versatility and ability to solubilize poorly soluble substances. Moreover, NADES are a superior alternative by using natural compounds and low production costs, making them attractive for pharmaceutical applications. Also, this chapter focuses on how eutectic solvents, especially NADES, can improve the solubility and

bioavailability of poorly soluble drugs, thus providing an innovative solution for pharmaceutical formulations. These systems offer a superior alternative to traditional solvents due to their biocompatibility, reduced toxicity, and favorable environmental profile. The compositional flexibility of NADES allows its modulation to significantly improve the solubility of hydrophobic drugs, leading to superior absorption and therapeutic efficacy.

Chapter 2 explores the concept of eutectogels, hybrid materials that combine the properties of eutectic solvents with those of gels, thus providing a three-dimensional structure with improved rheological and textural characteristics. Eutectogels show significant potential in the pharmaceutical field due to their ability to ensure the controlled release of active substances and improve their stability. These colloidal systems are biocompatible and biodegradable, which makes them ideal for topical administration and mucosal application, thus optimizing the absorption of active substances and prolonging the therapeutic effect. The synergistic interaction between NADES and the gel matrix maximizes the therapeutic effect. NADES, having a high capacity to solubilize hydrophobic substances, ensures the uniform dispersion of the active substance, which leads to a more efficient absorption at the level of mucous membranes. Encapsulation in a gel provides a prolonged and controlled release, reducing the frequency of administrations. Eutectogels, applied to mucous membranes, such as the oral one, facilitate rapid absorption and avoid hepatic metabolism.

Chapter 3 presents the physicochemical properties and challenges associated with NSAIDs, with a focus on ibuprofen and mefenamic acid, two NSAIDs with wide therapeutic use. Although therapeutically effective, these active substances present significant difficulties related to their low solubility, which limits absorption and bioavailability.

The research in this PhD thesis aims to explore how NADES and eutectogels can be used to improve the physicochemical characteristics of these NSAIDs, thus contributing to the development of innovative pharmaceutical formulations capable of optimizing both the release and therapeutic efficacy of active substances. Thus, the part of personal contributions is structured in chapters 4-6, representing the core of the experimental research. Chapter 4 presents the working hypotheses and general objectives of the thesis, which establish the fundamental directions of the research.

Chapter 5 details the experimental steps that include formulating and characterizing natural eutectic solvent-based systems (NADES) and innovative excipients to optimize the solubility and bioavailability of selected NSAIDs. In this chapter, we performed a series of experiments to evaluate the performance of DESs in terms of drug solubilization and stability. The eutectic systems were formulated by heating and controlled stirring, and the tested

compositions included organic acid-based DES and menthol-based hydrophobic formulations. Each of these systems was prepared by considering the choice of components to assess a wide range of intermolecular interactions, including hydrogen bonding and van der Waals forces, which contribute to increased solubility. The experiments performed included the determination of the equilibrium solubility of ibuprofen and mefenamic acid in DESs, followed by quantification by the HPLC method. We also investigated the rheological properties of the eutectic systems to evaluate the flow behavior and performed thermal analysis, namely differential scanning calorimetry (DSC), to characterize the glass transitions and melting of the eutectics. In addition, we performed surface tension measurements. We evaluated the impact of water on the physicochemical properties of DESs, demonstrating that adding water can modulate viscosity and influence drug solubility. These experiments provided valuable information on the interactions between drugs and DESs, revealing the potential of these solvents to significantly improve the bioavailability of low-solubility active substances.

In Chapter 6, research focused on optimizing gel formulations by combining NADES (choline:sorbitol:glycerol in a 2:1:1 ratio) with natural polymers such as xanthan gum and hyaluronic acid. These polymers gave the gels appropriate rheological and textural properties, ensuring an efficient and comfortable application on the oral mucosa. The resulting formulations were evaluated to determine their effectiveness.

The development of hybrid eutectogels, by combining natural eutectic solvents (NADES) with gels based on xanthan gum (XTG) and hyaluronic acid (HA), brings a significant innovation in pharmaceutical formulations intended for mucosal administration. Xanthan gum provides mechanical stability and thixotropic properties, facilitating uniform application and prolonged retention at the administration site, while hyaluronic acid improves adhesion and hydration, extending residence time and optimizing the controlled release of the active substance. These eutectogels allow a prolonged and controlled release of the drug, increasing the therapeutic efficiency and stability of the formulation, being ideal for oral application and on other mucosal surfaces. The tests included rheological characterization to analyze the flow behavior and viscosity of eutectogels, which are important for administration on sensitive surfaces, such as oral mucosa. Evaluation of thixotropy was important to determine the ability of the gels to return to their initial state after applying a mechanical force, an important parameter for gels that must provide controlled drug release. In addition, in vitro release tests of ibuprofen were performed using a Sotax apparatus to assess the release kinetics of the active substance, ensuring that the formulations provide prolonged and controlled release, thus reducing the need for frequent applications. The textural characterization of the

eutectogels provided data on the firmness, adhesion, and cohesion of the gels, important parameters for their stability and applicability on mucous membranes. SEM tests provided detailed information on the microstructure of the formulations, and DSC analysis allowed the evaluation of thermal stability and interactions between components. Tests were also performed to evaluate the antimicrobial and anti-inflammatory activity and validate the formulations' therapeutic potential in combating local infections and inflammations. These experiments demonstrated that the developed eutectogels not only improve drug solubility and release, but also provide a beneficial therapeutic profile.

This PhD thesis makes a significant contribution to the field of pharmaceutical formulations by developing innovative hybrid eutectogels based on natural eutectic solvents and biopolymers - xanthan gum and hyaluronic acid. By improving the solubility, bioavailability and controlled release of drugs, especially NSAIDs, and integrating antimicrobial properties alongside testing anti-inflammatory properties, the PhD thesis opens new therapeutic perspectives for oral mucosal administration and topical application, positioning eutectogels as promising solutions in disease management inflammatory and infectious.

The subject of this doctoral thesis allowed the completion of the complex in silico, in vitro, and in vivo studies that led to the development of pharmaceutical formulations with medicinal substances from the class of nonsteroidal anti-inflammatory drugs with low water solubility (ibuprofen and mefenamic acid), which have proven antimicrobial and anti-inflammatory therapeutic efficacy.

The research has exploited the potential of natural eutectic solvents (NADES) as solubilization vectors for biopharmaceutical class II model active substances with low aqueous solubility (ibuprofen and mefenamic acid). In this sense, the use of menthol in the hydrophobic systems of NADES significantly increased the solubility of the medicinal substances used compared to the classical solubilization methods.

The association of menthol with hydrogen bond donors led to a significant increase in the solubility of the tested substances, creating the premise for the development of new strategies to increase the bioavailability of poorly water-soluble NSAIDs.

Studies to optimize the balance between viscosity, thermal stability, and solubilization capacity in NADES-based systems allowed the introduction of water as a strategic modifier in the development of the studied systems, based on the elucidation of structure-physico-chemical property interactions. These results led to the premise of developing personalized therapeutic

systems, applicable in the case of active substances with low solubility, capable of overcoming their biopharmaceutical limitations, to increase the therapeutic benefits.

Hybrid eutectogels based on xanthan (XTG) and hyaluronic acid (HA) were developed and characterized, incorporating a NADES system (ChCl: sorbitol: glycerol, in molar ratio 2:1:1) and ibuprofen. They were optimized based on critical formulation parameters (water content, percentage of HA and XTG).

The experimental results demonstrated that the optimized eutectogels exhibit remarkable characteristics such as high viscosity, shear thinning behavior and thixotropic recovery, essential properties for easy application, prolonged retention at the site of administration and controlled release of the active substance. The NADES system also contributed significantly to improving the consistency and adhesion of eutectogels, generating superior mechanical properties compared to traditional gels.

The amount of bound, non-freezing water in these systems was a key parameter leading to pharmaceutical formulations with mechanical integrity and optimal adhesive properties to allow local administration in the oral cavity.

The pharmaceutical formulations were stable under salivary exposure, ensuring their resilience in the dynamic environment of the oral cavity.

In vitro release studies of the active substance showed that the developed eutectogels release ibuprofen in a controlled and sustained manner, combining a rapid initial release with a prolonged profile.

The anti-inflammatory activity of eutectogels was demonstrated in two experimental models of inflammation, and the potential of these formulations in the localized treatment of inflammatory conditions was confirmed.

XTG-HA-NADES-IBU gel containing 2.5% ibuprofen demonstrated comparable efficacy to commercial 5% ibuprofen gel in reducing induced plantar edema in rats.

Eutectogels showed significant antimicrobial activity against strains of *Bacillus cereus*, *Enterococcus faecium* and *Klebsiella pneumoniae*, and highlighted the potential of these formulations in the treatment of oral cavity infections.

Antimicrobial activity combined with anti-inflammatory effects position eutectogels as promising multifunctional therapeutics for the management of inflammatory conditions with a bacterial component.

Ibuprofen eutectogels have demonstrated a significant increase in the solubility of the active substance, a controlled release over 24 hours, anti-inflammatory effects, and antimicrobial activity. These results highlight the significant therapeutic potential of

eutectogels in treating inflammatory conditions complicated by bacterial infections, opening new perspectives for their clinical application.

The research resulted in the obtaining of high-performance pharmaceutical forms that are easy to apply, have prolonged retention at the application site, and have a therapeutic effect.

The PhD thesis makes a significant contribution to pharmaceutical formulations by investigating the use of natural eutectic solvents and eutectogels to improve the solubility and bioavailability of low-solubility active substances.

The research carried out in this work allowed the development of innovative pharmaceutical formulations, which showed remarkable performance in both in vitro and in vivo tests, proving their therapeutic potential. These results make it possible to continue research with a view to translating it into effective and safe clinical use.

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