"Babeş-Bolyai" University Cluj-Napoca

Faculty of Biology and Geology

Doctoral School of Integrative Biology

Increasing the therapeutic potential of Febuxostat by developing new formulations with improved solubility and bioavailability

SUMMARY OF THE DOCTORAL THESIS

Scientific Supervisor:

Prof. Dr. Manuela BANCIU

PhD Student:

Doriana-Timeea UNGUR

Scientific co-supervisor:

Prof. Dr. Alice MIJA

Côte d'Azur University

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Outline of the thesis

The present doctoral thesis is organized into five core chapters, preceded by the definition of the research aims and objectives and followed by final reflections, supplementary information, and documentation of scientific dissemination. The work focuses on the rational design, preparation and characterization of innovative solid-state forms of febuxostat, aimed at enhancing its solubility, bioavailability and overall therapeutic potential. The structure of the thesis is described below:

Chapter I – provides an overview of febuxostat, highlighting its physicochemical and pharmacological properties, therapeutic uses, and formulation challenges related to poor aqueous solubility and bioavailability. It reviews strategies such as salt formation, co-crystallization, and amorphous solid dispersions, alongside crystal engineering principles and multi-component systems. The chapter also outlines febuxostat's mechanism of action and clinical profile in comparison with allopurinol, establishing the scientific context for the research.

Chapter II – reports the first structural elucidation of febuxostat Form A, the marketed polymorph, using 3D electron diffraction and single-crystal X-ray diffraction. It compares the crystallographic results from both techniques and includes thermal and lattice energy analyses, offering critical insight into the solid-state behavior of febuxostat and establishing a reference for developing new forms.

Chapter III – describes the synthesis and solid-state characterization of a febuxostat–*p*-toluenesulfonic acid multi-component form (Tos1), using techniques such as XRPD, DSC, TGA, FT-IR and SC-XRD. The study clarifies the salt *versus* co-crystal nature of the system and highlights the Tos1 form's improved aqueous solubility and dissolution, supporting its potential as a superior alternative to crystalline febuxostat.

Chapter IV – explored the development and characterization of novel febuxostat formulations, including salts, a co-crystal with acridine (Acd1) and amorphous solid dispersions with PEG6000. These systems were evaluated using solid-state analytical techniques and tested *in vitro* on HepG2 liver cells for cytotoxicity and on RAW 264.7 murine macrophages for anti-inflammatory effects under oxidative stress conditions.

Chapter V – summarises the main findings and contributions of this thesis, offering an integrated perspective on the formulation strategies developed to enhance the solubility and therapeutic performance of febuxostat. This research provides structural and pharmaceutical insights into new salt, co-crystal and amorphous forms of the drug, demonstrating the potential of crystal engineering and solid dispersion approaches in overcoming solubility-related limitations. Notably, the structural elucidation of the marketed polymorph (Form A), the development of a febuxostat–acridine co-crystal with extended-release properties, and the generation of amorphous PEG6000-based systems represent key original outcomes of this work. These systems were evaluated using solid-state analytical techniques and tested in vitro on HepG2 liver cells for cytotoxicity and on RAW 264.7 murine macrophages for anti-inflammatory effects under oxidative stress conditions. A national patent has been granted for the co-crystal, highlighting its innovation potential.

Looking forward, the findings of this thesis pave the way for broader application of these formulation strategies to other poorly soluble compounds and support future efforts toward preclinical evaluation and clinical translation of the most promising candidates.

CHAPTER I: Introduction

Chapter I serves as the foundation for the doctoral thesis, addressing the scientific context and the motivation behind the development of new formulations of febuxostat. The chapter begins by highlighting the general characteristics of febuxostat, a potent drug used in the treatment of gout, and the challenges posed by its poor solubility and bioavailability. This serves as the primary reason for the need to explore and develop novel formulation strategies to enhance its therapeutic potential.

The chapter explores various methods aimed at improving drug solubility and bioavailability, such as salt formation, co-crystallization and amorphous solid dispersions. These strategies are essential for overcoming the barriers of poor aqueous solubility and variable absorption, which limit febuxostat's clinical efficacy. Special emphasis is placed on crystal engineering techniques, which are critical in enhancing the drug's physical properties and optimizing its bioavailability.

The mechanism of action of febuxostat is also thoroughly reviewed, including a detailed comparison with allopurinol, the standard treatment for gout. The clinical efficacy of febuxostat is discussed, focusing on its ability to inhibit xanthine oxidase and reduce uric acid levels in patients with hyperuricemia, which is the underlying cause of gout. The pharmacokinetic profile of febuxostat, its recommended dosage, and its route of administration are all factors contributing to its therapeutic use.

Overall, **Chapter I** establishes the scientific context for the thesis, emphasizing the need for new formulation strategies to improve febuxostat's solubility and bioavailability, which in turn could increase its therapeutic efficacy for treating gout. The research set forth in this thesis aimed to address these challenges, providing innovative solutions for the future clinical application of febuxostat.

CHAPTER II: Crystal structure of febuxostat marketed polymorph determined by Electron Diffraction and reinforced by X-ray crystallography

Chapter II focuses on the crystallographic study of febuxostat, specifically its marketed polymorph (Form A), which had not been characterized in detail until this work. The chapter highlights the use of electron diffraction (ED) and single-crystal X-ray diffraction (SC-XRD) as complementary techniques to solve the crystal structure of this polymorph. The need for such studies stems from the complexity of the solid forms of febuxostat, a drug used in the treatment of gout, which exhibits multiple polymorphic forms. Despite its commercial availability, the structure of the marketed polymorph (Form A) remained unknown, posing challenges for further optimization in the drug development process.

The chapter discusses the successful determination of the crystal structure of Form A, achieved through a combination of ED on nanocrystals and SC-XRD on larger crystals obtained by chance during an experiment aimed at co-crystallizing febuxostat with benzoic acid. The results demonstrate that ED is a reliable and efficient tool for solving complex structures of active pharmaceutical ingredients (APIs), providing an alternative to traditional X-ray crystallography, particularly for small crystals. This work highlights the importance of ED in pharmaceutical research, offering a quicker and more resource-efficient approach to structural analysis.

Moreover, the study emphasizes the relevance of understanding polymorphic systems in drug development, as the crystal structure plays a crucial role in determining the drug's stability, solubility and bioavailability. The chapter concludes by reinforcing the potential of ED in pharmaceutical research and development, positioning it as a valuable tool to overcome the challenges of characterizing solid forms and contributing to more efficient and cost-effective drug development processes.

CHAPTER III: Febuxostat–*p*-toluenesulfonic acid multi-component crystal: characterization, crystal growth and elucidation of the salt/ co-crystal nature

Chapter III presents the synthesis and characterization of a multi-component solid form of febuxostat (FEB) with *p*-toluenesulfonic acid (Tos1). This new formulation was developed using solvent-drop grinding and cooling-evaporative crystallization techniques, and its properties were thoroughly evaluated through a range of solid-state analytical techniques, including powder X-ray diffraction (XRPD), thermogravimetry (TGA), differential scanning calorimetry (DSC), and infrared spectroscopy (FT-IR). The new multi-component form showed a significant improvement in aqueous solubility, with a five-fold increase in solubility compared to pure febuxostat, making it a promising candidate for enhanced therapeutic efficacy.

The chapter highlights the stability of this multi-component form under conditions of elevated temperature and humidity, suggesting its potential for use in pharmaceutical formulations. In addition, the dissolution studies indicated a potential for improved drug absorption in the lower stomach, owing to a decrease in pH during dissolution, which could facilitate the bioavailability of febuxostat.

A key focus of the chapter was the elucidation of the salt/co-crystal nature of the formulation. The study combined crystallization process development with single-crystal X-ray diffraction and FT-IR analysis to confirm the salt nature of the compound. Despite febuxostat's weak basicity, the proton from p-toluenesulfonic acid was transferred to the drug's 1,3-thiazole nitrogen atom, forming a tosylate salt. This structural insight is crucial for understanding the interactions between the components in the multi-component crystal, as well as meeting the regulatory requirements for drug development.

The chapter concludes by emphasizing the significance of crystal structure knowledge in understanding the interactions within multi-component systems and highlights the potential of the Tos1 salt for improving the solubility and bioavailability of febuxostat, which is vital for enhancing its therapeutic potential in the treatment of gout.

CHAPTER IV: Integrating crystal engineering with PEG6000 conjugation to formulate advanced solid dispersions of febuxostat for gout management

Chapter IV explored the development of new formulations of febuxostat to enhance its solubility, bioavailability, and therapeutic efficacy, with a particular focus on biological evaluation. FEB, a selective xanthine oxidase inhibitor used for treating gout and hyperuricemia, suffers from limited solubility, which hinders its effectiveness in achieving therapeutic plasma concentrations.

The study investigated several novel forms of FEB, including three salts (Tos1, Tro1, Nmg1), a co-crystal (Acd1) and amorphous solid dispersions with PEG6000. These formulations were tested for their solubility, dissolution behavior and stability in both aqueous and biorelevant media.

In addition to physicochemical evaluations, the biological efficacy of these formulations was assessed through cytotoxicity assays on HepG2 liver cells to evaluate hepatotoxicity and macrophage-based studies to assess anti-inflammatory effects, particularly in the context of gout pathophysiology. The results revealed that the Tos1 salt, along with its amorphous solid dispersion using PEG6000, significantly improved solubility in aqueous and Fasted State Simulated Intestinal Fluid (FaSSIF). These formulations did not increase cytotoxicity, confirming their safety profile.

Furthermore, the novel formulations exhibited enhanced antioxidant activity, notably reducing oxidative stress markers like malondialdehyde. The Tos1 + PEG6000 formulation also lowered intracellular uric acid levels, demonstrating its potential to improve gout management. This study indicates that the combination of crystal engineering and polymer-based dispersions can synergistically enhance FEB's solubility, biological activity, and therapeutic efficacy without compromising safety, providing promising new treatment options for patients with gout and hyperuricemia.

The findings suggest that such novel formulations, particularly those incorporating PEG6000, offer an effective strategy to optimize febuxostat’s pharmacological response and could have significant clinical implications.

CHAPTER V: General Conclusions, Originality of the Work and Future Perspectives

General Conclusions

This doctoral thesis focused on enhancing the therapeutic potential of febuxostat by developing new formulations with improved solubility and bioavailability, aiming to optimize its treatment of gout and hyperuricemia. The study addressed the limitations of febuxostat's solubility and bioavailability and explored several innovative formulation strategies. The research was structured into three main components (**Chapters II**, **III** and **IV**).

Objective 1:

The crystal structure of febuxostat in its marketed polymorph (Form A) was successfully determined using electron diffraction and single-crystal X-ray diffraction. For the first time, the crystal structure of Form A was elucidated through ED on nanocrystals obtained from a controlled crystallization process, with confirmation from SC-XRD on larger crystals grown incidentally during a co-crystallization experiment. These results highlight the reliability and efficiency of ED as a technique for solving complex crystal structures, particularly for small crystals unsuitable for conventional X-ray diffraction. The accuracy of ED, comparable to SC-XRD, underscores its potential in pharmaceutical research, especially in polymorph characterization. The determination of Form A's crystal structure significantly enhances the understanding of febuxostat's polymorphic behavior, which is crucial for optimizing its formulations.

Objective 2:

Novel febuxostat forms and formulations, including salts with *p*-toluenesulfonic acid, tromethamine, and N-methyl-D-glucamine, a co-crystal with acridine, and amorphous solid dispersions with PEG6000, were successfully developed and characterized. The multi-component crystal of febuxostat with *p*-toluenesulfonic acid, synthesized in **Chapter III**, demonstrated a five-fold increase in aqueous solubility compared to pure febuxostat, highlighting its potential to improve bioavailability. Further work in **Chapter IV** focused on the development of additional febuxostat salts and co-crystals, and their incorporation into amorphous solid dispersions. These formulations showed significant improvements in solubility, stability and dissolution rate, with the Tos1 salt combined with PEG6000 exhibiting enhanced solubility in both aqueous and FaSSIF

media. These findings underscore the successful application of advanced crystal engineering techniques to create febuxostat forms with improved physicochemical properties, offering promising alternatives for optimizing febuxostat formulations to overcome its solubility limitations and enhance therapeutic efficacy.

Objective 3:

The evaluation of the cytotoxicity, biological safety and therapeutic efficacy of novel febuxostat formulations confirmed the favorable safety and efficacy profiles of these formulations. Cytotoxicity assays conducted on HepG2 liver cells revealed that the formulations, particularly the Tos1 salt and its solid dispersion with PEG6000 (Tos1+PEG6000), did not significantly increase cytotoxicity, indicating an acceptable safety profile. This is critical for ensuring that the formulations do not present liver-related risks. In macrophage-based models, the Tos1+PEG6000 formulation exhibited enhanced antioxidant activity and significantly reduced oxidative stress markers, such as malondialdehyde, compared to pure febuxostat. It also demonstrated a reduction in intracellular uric acid levels, suggesting its potential therapeutic benefit in managing gout and hyperuricemia. These findings demonstrate that the Tos1+PEG6000 formulation improves solubility, bioavailability, and therapeutic efficacy while maintaining a favorable safety profile. The novel formulations present promising alternatives to conventional febuxostat, with potential benefits in the treatment of gout and hyperuricemia, aiming to enhance patient outcomes while minimizing side effects.

Originality and Novelty of the Studies

The originality and novelty of this research lie in the innovative approaches to overcoming the long-standing challenges associated with the solubility and bioavailability of febuxostat, a compound that has shown significant therapeutic potential in the treatment of gout and hyperuricemia. This work introduces groundbreaking formulations and strategies that offer promising solutions to optimize the drug's pharmacological profile, focusing on enhancing its solubility, dissolution rate and therapeutic efficacy.

Key innovations in this research include:

- ✓ **Discovery and characterization of the crystal structure of febuxostat's marketed polymorph (Form A):** This study presents the first comprehensive analysis of the crystal structure

of febuxostat's marketed polymorph (Form A), a crucial milestone as this polymorph had not been previously reported (**Chapter II**). This discovery not only adds valuable structural data to the pharmaceutical field but also provides the foundational understanding required for further modifications to the molecule's crystalline forms. Understanding the properties of this polymorph aids in optimizing formulation strategies to enhance the drug's bioavailability and stability, ensuring that it is more effective when administered.

- ✓ **Development of novel salts, co-crystal and amorphous solid dispersions:** This research marks the successful design and synthesis of several novel solid forms of febuxostat, including salts (with *p*-toluenesulfonic acid, tromethamine and N-methyl-D-glucamine), co-crystals (with acridine) and amorphous solid dispersions with PEG6000. These formulations were designed using advanced crystal engineering techniques to improve the physicochemical properties of febuxostat. The novel salts and co-crystals demonstrated a significant improvement in solubility, which is a major hurdle for febuxostat's oral bioavailability. Furthermore, the amorphous solid dispersions created with PEG6000 offered a unique advantage in preventing recrystallization and maintaining stability under accelerated environmental conditions, thus enhancing the solubility and dissolution rate (**Chapters III** and **IV**).

- ✓ **First-time synthesis and characterization of the febuxostat-acridine co-crystal with extended release properties:** The synthesis of the febuxostat-acridine co-crystal represents a pioneering achievement in the field. This co-crystal demonstrated extended release properties, a novel and promising approach for improving the bioavailability of poorly soluble drugs like febuxostat. The extended release profile of this co-crystal formulation offers several advantages, including prolonged therapeutic effects, reduced dosing frequency and improved patient compliance. This formulation not only represents a new method for enhancing febuxostat's therapeutic potential but also provides a new paradigm for improving the pharmacokinetics of other drugs with similar solubility challenges (**Chapter IV**).The innovative nature of this research is further underscored by the granting of a patent for the febuxostat-acridine co-crystal with extended release properties. The patent, titled *"Co-cristal Febuxostat-Acridină cu eliberare prelungită pentru tratamentul gutei și procedee de obținere a acestuia"* (A2023/00737), marks a significant milestone in pharmaceutical development. This patent covers both the co-crystal itself and the novel methods used for its preparation, offering a new avenue for the treatment of gout and hyperuricemia. The unique combination of febuxostat with acridine opens up new possibilities for

the optimization of its pharmacological performance, providing a more effective and safer treatment option for patients suffering from these conditions.

✓ **Innovative approach to drug formulation:** In addition to the novel formulations and their characterization, this research also introduces an innovative approach to improving the therapeutic potential of febuxostat. The combination of advanced crystallization techniques (including solvent-drop grinding and cooling-evaporative crystallization) with polymer-based systems (like PEG6000) creates a synergistic effect that not only enhances solubility but also optimizes the dissolution rate and stability of the drug. The exploration of synergistic effects between salts and co-crystals, especially in combination with extended release properties, presents a new strategy for addressing the complex challenges of drug delivery and bioavailability (**Chapter IV**).

✓ **Implications for future pharmaceutical development:** This work paves the way for future studies in pharmaceutical development, particularly in the design of multi-component crystals and solid dispersions that can enhance the solubility and bioavailability of poorly soluble drugs. The strategies developed in this research offer new perspectives for the optimization of existing drugs, as well as the development of novel therapeutics in various therapeutic areas. The combination of advanced formulation strategies with comprehensive biological evaluations ensures that these new formulations hold significant potential for improving patient outcomes, particularly in the treatment of gout and hyperuricemia.

✓ **Comprehensive evaluation of cytotoxicity, biological safety and therapeutic efficacy of novel febuxostat formulations.** The originality and novelty of this research are underscored by the comprehensive evaluation of the cytotoxicity, biological safety and therapeutic efficacy of novel febuxostat formulations, as detailed in **Chapter IV.** This study introduces an innovative *in vitro* evaluation system, employing HepG2 liver cell models to assess potential hepatotoxicity, which is critical for understanding the liver's role in drug metabolism. Furthermore, the use of macrophage-based models to evaluate the anti-inflammatory effects provides a novel approach to examining febuxostat's therapeutic potential in the context of gout and hyperuricemia. By evaluating the formulations' impact on uric acid levels, solubility and dissolution rate, this research addresses both safety concerns and the need for enhanced therapeutic efficacy, contributing significantly to the development of safer and more effective treatments for these conditions.

In conclusion, this research represents a paradigm shift in febuxostat formulation development, marked by significant advances in pharmaceutical technology. By creating novel

salts, co-crystals and amorphous solid dispersions, as well as pioneering the synthesis of the febuxostat-acridine co-crystal with extended release properties, this work demonstrates an innovative approach to enhancing febuxostat's solubility, bioavailability and therapeutic efficacy. Additionally, the comprehensive evaluation of both the therapeutic potential and biological safety of these formulations, combining advanced crystallization strategies with thorough biological testing, ensures that these new approaches not only improve solubility and bioavailability but also provide safer, more effective treatment options for gout and hyperuricemia.

List of publications included in the thesis

Chapter II

Ungur, D. T., Lanza, A., Stam, D., Guguta, C., Iordache, C., Fruth, V., ... & Pop, M. M. (2024). Crystal structure of Febuxostat marketed polymorph determined by electron diffraction and reinforced by X-ray crystallography. *CrystEngComm*, *26*(32), 4295-4304.
https://doi.org/10.1039/D4CE00518J

Chapter III

Ungur, D. T., Santiso-Quinones, G., Pop, M. M., Tămaș, T. L., Guguta, C., Stam, D., ... & Iordache, C. A. (2023). Febuxostat–*p*-Toluenesulfonic Acid Multi-Component Crystal: Characterization, Crystal Growth and Elucidation of the Salt/Co-Crystal Nature. *Crystals*, *13*(5), 836.
https://doi.org/10.3390/cryst13050836

Chapter IV

Ungur, D. T., Pătraș L., Pop, M. M., Iordache, C. A., Tămaș, T. L., Mija, A., Banciu, M. (2025). Integrating crystal engineering with PEG6000 conjugation to formulate advanced solid dispersions of febuxostat for gout management.
→ Manuscript under preparation

List of publications not included in the thesis

1. Ungur, D. T., Iordache, C. A., Brăilă, C. A., Pop, D. A., David, M. C., Pandele-Cuşu, J., ... & Pop, M. M. (2025). Structural Insights into the Resveratrol-Piperazine Cocrystal Forms Enabling the Cocrystallization Process Development from Solution. *Crystal Growth & Design*, *25*(5), 1330–1343. https://doi.org/10.1021/acs.cgd.4c01113

2. Ungur, D. T., Codrea C. I., Mitran R.-A., Pandele-Cusu J., Fruth Oprisan V., Pop M.M., Iordache C.A. (2023): Co-cristal Febuxostat-Acridină cu eliberare prelungită pentru tratamentul gutei și procedee de obținere a acestuia (patent application A2023/00737), Romania.

Attendances at international conferences

Throughout my doctoral studies, I have actively participated in four international conferences, presenting posters on the following occasions:

1. Presented a poster at *International Conference Processes in Isotopes and Molecules (PIM)*, 13th edition, 21st to 23rd September 2021, Cluj, Romania. "D. T. Ungur, C. A. Brăilă, M. C. David, D. A. Pop, T. L. Tămaș, I. Kacso, X. Filip, M. M. Pop (2021). Crystallization process development of Febuxostat most stable polymorph and of a soluble salt thereof. PIM book of abstracts Poster T2-35, pp 55" – BEST POSTER AWARD.
2. Attended the *International Conference on Crystal Growth of Organic Materials (CGOM14)*, 14th edition, 11th to 14th September 2022, Brussels, Belgium.
3. Presented a poster at *12th Bologna's convention on crystal forms*, 10th to 12th September 2023 (Bologna, Italy). "D. T. Ungur, M. M. Pop, T. L. Tămaș, M. Banciu, A. Mija (2023). Boosting the solubility of febuxostat salt and co-crystal forms by polymeric solid dispersions. Book of abstracts, Poster 20, pp 13".

4. Presented a poster at *From Molecules to Materials: 1st Workshop on Benchmarking Solid State Properties, COST Action BEST-CSP*, 9th to 11th September 2024, Warsaw (Poland). "D. T. Ungur, D. A. Pop, C. A. Brăilă, M. C. David, A. Lanza, C. Iordache, V. Fruth, D. Stam, G. Santiso-Quinones, M. M. Pop (2024). Crystallographic insights into Febuxostat solid forms based on single-crystal and electron diffraction analysis. BEST-CSP book of abstracts, pp 19."

Scientific training and international mobility during the PhD

1. Erasmus+ Traineeship Program

Type of program: Erasmus+ Mobility for Traineeship

Period: 3rd March – 30th June 2023

Host Institution: Côte d'Azur University, Nice, France

Supervisor: Prof. Dr. Alice Mija

Traineeship Title: *Increasing the therapeutic potential of Febuxostat by developing new formulations with improved solubility and bioavailability.*

2. European Project COST, BEST-CSP

Since November 2023, I have been involved in the European Project COST (European Cooperation in Science and Technology) CA22107 (COST OC-2022-1-25740) - *Bringing Experiment and Simulation Together in Crystal Structure Prediction (BEST-CSP)*. I am the *tracker* for the compound **Febuxostat**, which is being studied within the project at an international level.

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