PREPARATION OF STARCH-BASED PRODRUGS OF FENAMATES: YIELD OPTIMATION AND RELEASE PROFILE STUDY

A dissertation submitted in the partial fulfilment of
the requirement for the degree of
Master of Science
in
Chemistry

Submitted by:
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Under the supervision of
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May, 2024

DECLARATION

I declare that the thesis entitled **"PREPARATION OF STARCH-BASED PRODRUGS OF FENAMATES, YIELD OPTIMATION AND RELEASE PROFILE STUDY"** has been prepared by me under the supervision of **Dr Parteek Prasher and Dr Shefali Arora** from **the Department of Chemistry, School of Engineering, University of Petroleum & Energy Studies, Dehradun, India.**

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CERTIFICATE

I certify that, **Garima Chandrasen** has prepared his project entitled **"PREPARATION OF STARCH-BASED PRODRUGS OF FENAMATES, YIELD OPTIMATION AND RELEASE PROFILE STUDY"** for the award of **M.Sc. Chemistry,** under our guidance. She has carried out work at the **Department of Chemistry, School of Engineering, University of Petroleum & Energy Studies, Dehradun, India.**

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I, **Garima Chandrasen**, hereby certify that the research dissertation titled "**PREPARATION OF STARCH-BASED PRODRUGS OF FENAMATES, YIELD OPTIMATION AND RELEASE PROFILE STUDY**" submitted for the partial fulfilment of a B.Sc. degree from University of Petroleum Energy & Studies, Dehradun, India is an original idea and has not been copied/taken verbatim from anyone or from any other sources.

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Abstract

In this study, our objective was to enhance the therapeutic potential of fenamic acid, a non-steroidal anti-inflammatory drug (NSAID), by developing new derivatives using starch as a carrier with an ester boost linker. The synthesis process involved the use of dicyclohexylcarbodiimide (DCC) and 1,1'-carbonyldiimidazole (CDI) in combination with thionyl chloride, along with various tertiary amine catalysts such as pyridine, 4-dimethylaminopyridine (DMAP), and triethylamine (TEA).

To assess the effectiveness and feasibility of the synthesized compounds. We carefully monitored the reactions to ensure successful coupling between fenamic acid and starch, facilitated by the ester bond. The impact of different tertiary amine catalysts on the reaction kinetics and product yield was thoroughly evaluated, with pyridine, DMAP, and TEA serving as catalysts to optimize the synthesis process. Different characterization was carried out (FTIR, NMR, UV-visible spectroscopy). In FTIR analysis ester bond was observed at 1628cm^{-1}. The IR spectra of the ester-linked starch-flufenamic acid prodrug showed a sharp peak at 1628cm^{-1}, assigned to the C=O (ester bond) stretching. Due to preincubation of the prodrug with pancreatin, 94% of the drug was released in esterase-containing SIM.

The obtained results demonstrate advancements in the development of fenamic acid derivatives and the successful incorporation of starch as a carrier. The use of ester boost linkers provides a controlled release mechanism, potentially enhancing the drug's bioavailability and therapeutic efficacy. This research provides valuable insights into the design and synthesis of innovative NSAID derivatives, paving the way for further investigation of targeted drug delivery systems with improved pharmacological profiles.

Keywords: flufenamic acid, prodrugs, resistant starch, colon-targeting delivery, enzyme-responsive linker

ACKNOWLEDGEMENT

As a master's student, I extend my heartfelt gratitude to all those who have contributed to the successful completion of this research endeavour. Firstly, I express my sincerest appreciation to my supervisor, Dr Parteek Prasher, and co-supervisor, Dr Shefali Arora for their unwavering guidance, invaluable insights, and unwavering support throughout this journey, which has enriched the quality of this study.

I extend my thanks to the laboratory staff and technicians for their assistance during experimental procedures and data collection. Their expertise and dedication have been instrumental in the progress of this research.

Special thanks to Research scholar, Shraddha Chugh, Pankaj Bhandari and peers for their camaraderie, encouragement, and intellectual exchange, which have fostered a stimulating academic environment.

Lastly, I am profoundly thankful to my family for their unending love, encouragement, and understanding, which have been my source of strength throughout this academic pursuit.

This research would not have been possible without the support and encouragement of all the members of the chemistry department. Thank you for being part of this enriching journey.

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CHAPTER 1
INTRODUCTION

The focus of this thesis is to develop a prodrug of fenamic acids using resistant starch as a carrier, with an ester bond serving as an enzyme-responsive linker. Through characterization techniques such as NMR and FTIR spectroscopy, the presence of ester peaks in the prodrug was confirmed, validating the successful synthesis of the desired compound. To evaluate its pharmacokinetic behavior, release profile studies were conducted using UV-visible spectroscopy. These studies show that the prodrug exhibited resistance to metabolism in extracellular interstitial fluids, stomach, and small intestine. However, the prodrug was found to undergo fermentation by gut microbiota specifically in the colon.

This observation led to the conclusion that the prodrug possesses colon-targeting properties, as it remains intact in the upper gastrointestinal tract but undergoes enzymatic cleavage in the colon. This targeted delivery system enhances efficacy and reduces the systemic side effects of fenamic acids. Overall, this research represents a significant advancement in the field of drug delivery, offering a novel approach to achieving colon-specific drug release. The findings underscore the potential of enzyme-responsive prodrugs in optimizing therapeutic outcomes by precisely targeting the site of action within the colon.

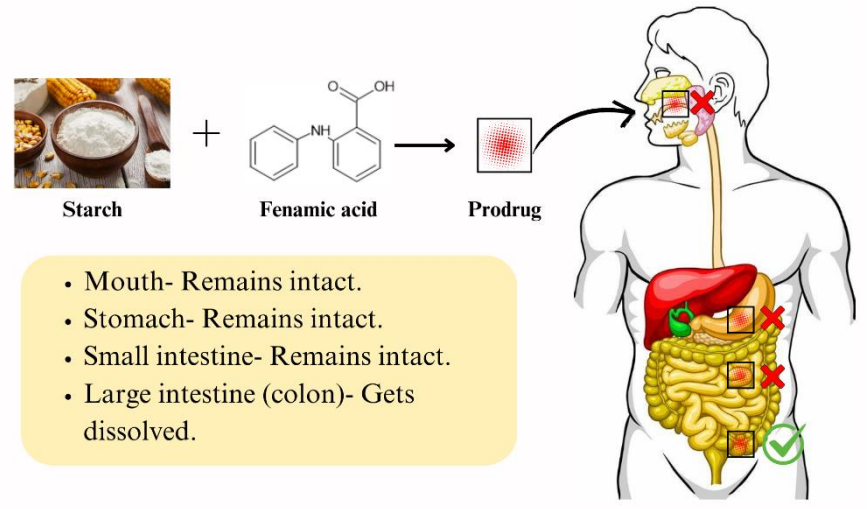


Figure 1 Diagram showing digestion of prodrug in Gastrointestinal Tract

1. NSAIDs:

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to reduce pain, inflammation, and fever. These drugs work by inhibiting the activity of enzymes COX-1 and COX-2, which are responsible for the production of prostaglandins, that play a crucial role in mediating inflammation, pain, and fever. However, while NSAIDs are effective in reducing inflammation and pain, they also cause adverse effects, especially in the gastrointestinal tract,

where they can disrupt the protective mucus lining of the stomach, maintained by COX enzymes. This disruption can cause various complications, including gastritis, peptic ulcers, and gastrointestinal bleeding. Examples of a few classes of NSAIDs are- Salicylates, Anthranilic Acid Derivatives (fenamates), Propionic Acid Derivatives, etc. Herein, fenamates are used for the research.

2. **Fenamates:** Fenamates are a class of non-steroidal anti-inflammatory drugs (NSAIDs). These are derived from anthranilic acid and have anti-inflammatory, antipyretic, and analgesic properties. Fenamates are classified into four types: mefenamic acid (MFA), tolfenamic acid (TA), meclofenamic acid (MCFA), and flufenamic acid (FFA).

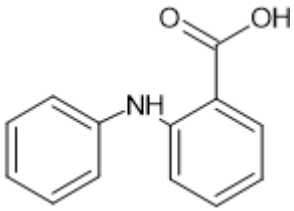


Figure 2. Structure of Fenamic Acid

2.1 Mefenamic Acid: It is the most commonly used fenamates. It is widely used for its analgesic and antipyretic properties. This is used to treat mild to moderate pain and also used to treat menstruation pain.

2.2 Tolfenamic acid (TA): Tolfenamic acid is prescribed to treat musculoskeletal pain and inflammation.

2.3 Meclofenamic acid (MCFA): Meclofenamic acid is used to treat pain and inflammation. It works by inhibiting the activity of the COX enzyme which reduces the secretion of prostaglandins responsible for pain and inflammation.

2.4 flufenamic acid (FFA): It is used to treat pain inflammation and gastrointestinal problems

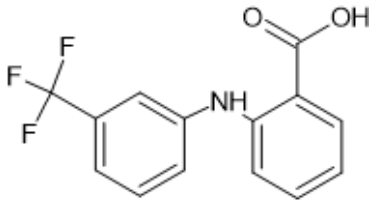


Figure 3 Structure of Flufenamic Acid

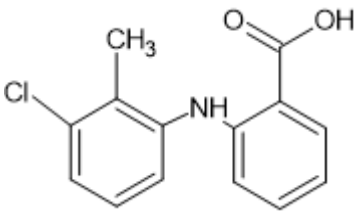


Figure 4 Structure of Tolfenamic acid

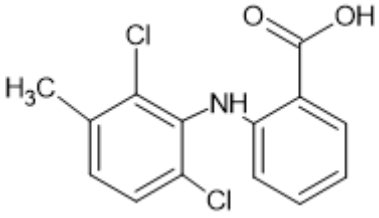


Figure 5 Structure of Meclofenamic Acid

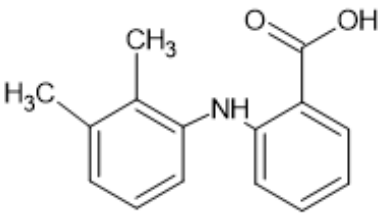


Figure 6 Structure of Mefenamic Acid

3. **Resistant Starch:** Resistant starch is a type of starch that resists digestion in the upper gastrointestinal tract (mouth, stomach, small intestine) rather get digested in the large intestine. It gets fermented by the gut microbiota in the large intestine. We used resistant starch in our prodrug because it also has a large size structure as compared to flufenamic acid, which helps by shielding the drug from pH and enzymes, inside the body.

3.1 Resistant starch is of five types: (RS1, RS2, RS3, RS4, RS5)

15.1.1. **RS1**: This resistant starch resists digestion due to its natural packaging within the plant cell wall and is found in whole grains, seeds, and legumes.

15.1.2. **RS2**: It consists of native starch granules that resist digestion due to their crystalline structure and low water content and are found in raw potatoes, green bananas, and high-amylose corn.

15.1.3. **RS3**: This starch is formed when starchy foods like potatoes, rice, or pasta are cooked and then cooled. The cooling process causes the starch molecules to realign into a more resistant structure. It is also known as Retrograded starch.

15.1.4. **RS4**: This type of resistant starch is formed by the physical and chemical processes to increase its resistance to digestion. This is used in processed

Figure 7 Diagram showing types of Resistant Starch with their examples

foods as a functional ingredient to improve texture, stability, and fibre content.

15.1.5. **RS5**: It is Amylose-lipid complex. It is found in food that contains more amount of amylose.

15.2. Structure of resistant starch: Resistant starch has 4 types of structures Depending on how the dextran chain double helix accumulates in the starch granules: Type A, Type B, Type C, and Type V.

15.2.1. Type A: It has a monoclinic structure.
15.2.2. Type B: It has hexagonal cells.
15.2.3. Type C: It has a crystal structure which is a mixture of Type A and Type B.
15.2.4. Type V: It has a helical structure (left-handed single amylose).

4. Esterification: Esterification is a type of reaction that leads to the formation of an ester (RCOOR) by combining organic acid (RCOOH) and alcohol (ROH). In this prodrug formation, esterification has been carried out between carboxylic acid (Fenamic acid) and alcohol (starch) in the presence of different solvents ($SOCl_2$, DCC, CDI) and catalysts (DMAP, Pyridine, TEA), to form enzyme-responsive ester linkage.

5. Research gaps:

5.1 **Limited investigation-** The use of starch-based carriers and pH/enzyme-responsive ester linkers for prodrug synthesis of fenamates remains largely unexplored. While fenamates like mefenamic acid are widely used NSAIDs, their gastrointestinal side effects necessitate colon-targeted delivery systems. Starch, resistant to digestion in the upper GI tract but degradable by colonic gut microbiota, combined with responsive ester linkers, offers a promising approach for creating prodrugs that selectively release the drug in the colon. However, limited research has been conducted to investigate the synthesis, characterization, and performance of such prodrug systems for fenamates. Unveiling the potential of this approach could enhance drug stability, and bioavailability, and minimize systemic side effects while paving the way for optimized colon-targeted delivery strategies.

5.2 **Optimization challenges**- Despite the potential benefits of starch-based prodrug synthesis for fenamates, the lack of established protocols and optimization strategies poses a significant challenge. Maximizing yields and efficiency requires systematic optimization of reaction conditions, solvents, catalysts, and purification methods. Critical aspects include conjugating the fenamate to the starch carrier via the responsive ester linker, and balancing reactant stoichiometry, time, and temperature for high coupling efficiency.

6. Motivation: -

6.1 **Enhanced Drug Delivery**: Fenamates, while effective anti-inflammatory drugs, often suffer from poor bioavailability and gastrointestinal side effects due to their physicochemical properties and non-targeted release. STARCH-based prodrugs with pH/enzyme-responsive linkers present a promising solution to enhance the delivery of fenamates. These prodrug systems can protect the drug from premature degradation, improve its solubility and absorption, and selectively release the active compound in the colon, thereby minimizing systemic exposure and associated side effects. By leveraging the unique properties of starch carriers and responsive linkers, this approach offers the potential to optimize the bioavailability, targeting, and safety profiles of fenamates, ultimately improving patient outcomes and medication adherence.

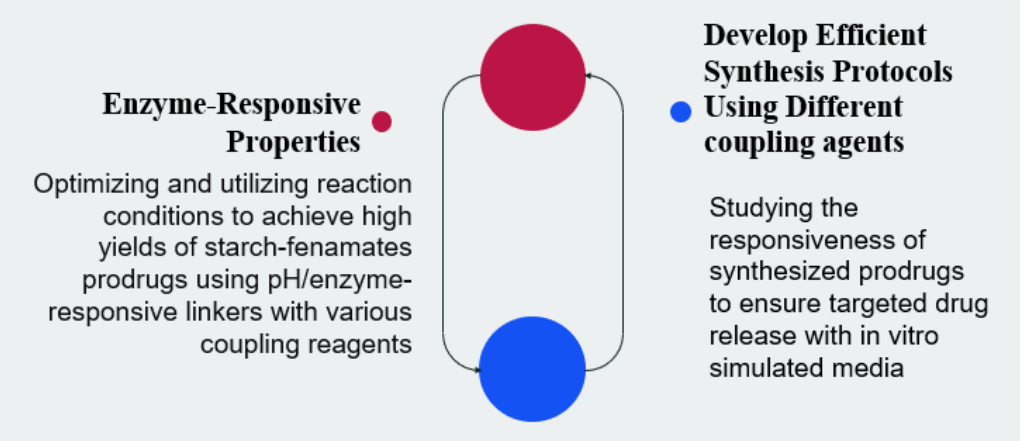
6.2 **Therapeutic Advancements**: By transcending existing limitations, research endeavours aim to usher in a new era of medical interventions that prioritize efficacy and safety. This pursuit underscores a commitment to refining treatment modalities, ultimately revolutionizing patient care paradigms. Through rigorous investigation and innovation, the aspiration is to forge breakthroughs that not only alleviate current shortcomings but also redefine the standards of healthcare excellence. This motivation propels researchers towards transformative discoveries poised to reshape the landscape of medical science.

6.3 **Mechanistic Insights:** By delving into these intricate processes, we gain insights essential for devising efficient and feasible synthetic routes. Understanding the intricate dance of reactions and their rates not only facilitates the creation of these prodrugs but also ensures their scalability, paving the way for their widespread application. In essence, unraveling the mysteries of reaction mechanisms and kinetics serves as the cornerstone for the strategic design of synthesis protocols, heralding a new era of innovation in pharmaceutical development.

7. Objective:

7.1 **Develop Efficient Synthesis Protocols Using Different coupling agents**: - fenamate prodrugs employing pH/enzyme-responsive linkers. This involves systematically investigating and optimizing reaction conditions to maximize the coupling efficiency between the starch carrier and fenamate drug using different coupling agents. Rigorous exploration of factors such as reactant stoichiometry, solvents, catalysts, temperature, and reaction times will be undertaken to achieve high product yields. Additionally, the integration of various coupling reagents presents an opportunity to evaluate their impact on the conjugation process and the resulting prodrug's properties. Establishing robust synthesis protocols through meticulous optimization will pave the way for reliable and scalable production of these innovative colon-targeted delivery systems.

7.2 **Enzyme-Responsive Properties**: Through rigorous in vitro studies employing simulated gastrointestinal media, the responsiveness of the prodrugs to specific enzymatic triggers will be evaluated. This involves assessing the stability and integrity of the prodrug in conditions mimicking the upper gastrointestinal tract, followed by monitoring the drug release kinetics upon exposure to enzymes present in the colonic environment. By systematically examining the influence of factors such as pH, and enzyme concentrations, valuable insights will be gained into the prodrugs' ability to selectively release the fenamate payload at the intended site of action. These studies will play a pivotal role in optimizing the prodrug design and ensuring targeted drug delivery to the colon.



References:

[1] Hill, Jaunetta, and Nasser H. Zawia. "Fenamates as potential therapeutics for neurodegenerative disorders." *Cells* 10.3 (2021): 702. MDPI

[2] Jiali, L., Wu, Z., Liu, L., Yang, J., Wang, L., Li, Z., & Liu, L. (2023). The research advance of resistant starch: structural characteristics, modification method, immunomodulatory function, and its delivery systems application. *Critical Reviews in Food Science and Nutrition*.

[3] Hassner, Alfred, and Vazken Alexanian. "Direct room temperature esterification of carboxylic acids." *Tetrahedron Letters* 19.46 (1978): 4475-4478.

[4] Chugh, Shraddha, et al. "Synthesis and evaluation of amylose-mefenamic acid conjugates as colon-targeting prodrugs." *Therapeutic Delivery* 0 (2024)

CHAPTER 2
MATERIALS AND METHODS

1. **Materials:** Corn starch, native soluble starch, dicyclohexylcarbodiimide (DCC), cyclohexyl diimidazole (CDI), thionyl chloride ($SOCl_2$), Triethyl amine (TEA), pyridine, dimethyl aminopyridine (DMAP), $NaCO_3$, Potassium Bromide Powder, Pestle and Mortar, pencil spatula, Pellet Die and Holder, Pressing Machine, Desiccator and moisture-free sample.

2. Methods of preparation for ester prodrug:

2.1 By using Thionyl Chloride:

- In a beaker of 100 mL, 9.25 mmol of Corn starch and 14 mmol of fenamic acid were dissolved in DMSO with continuous stirring.
- In ice-cold conditions, 8 drops of $SOCl_2$ were added dropwise after 10 minutes.
- The above mixture was kept in ice-cold condition for 35 minutes. After 35 minutes fumes were observed.
- Then the reaction mixture was stirred at room temperature at 450 rpm for 3 hours.
- $SOCl_2$ impurity was observed, and separation of $SOCl_2$ impurity was carried out with $NaCO_3$.
- Polar impurity was separated by adding H_2O.
- Keep the solution still for 15 minutes.
- The DMSO product was heated after adding ethanol and recrystallized by cold H_2O.
- The product was kept overnight in dark conditions at room temperature.
- The product was again reprecipitated under ethanol and cold H_2O and subjected to filtration.

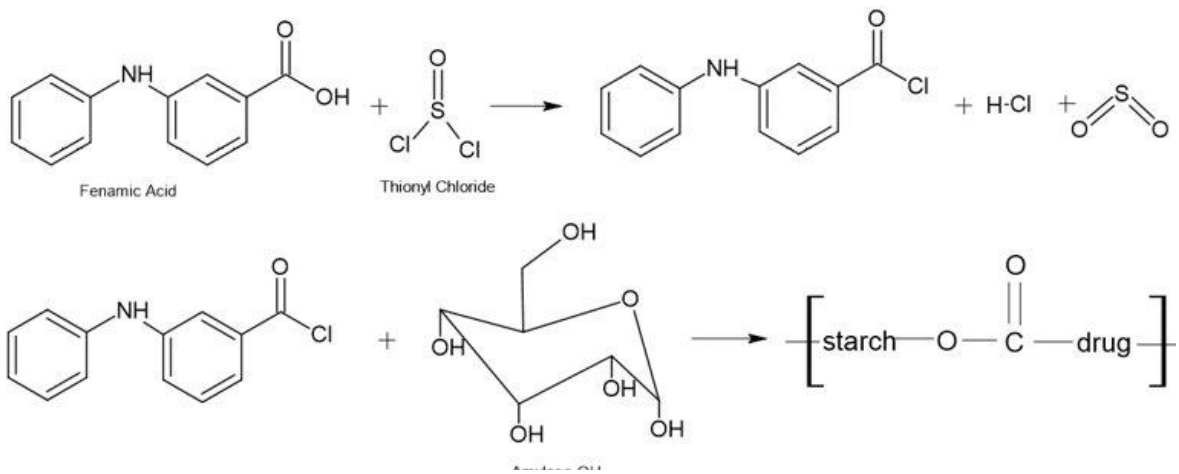


Figure 8 Reaction between Fenamic acid and Thionyl Chloride

2.1. By using DCC (N, N′-Dicyclohexylcarbodiimide)

- In a beaker 5.33 mmol of flufenamic acid and 5.57 mmol of DCC were added in 8 ml of DMF.
- This reaction mixture was kept for stirring at 600 rpm for 1 hour.
- In another beaker 18.50 mmol of starch was dissolved in 15 ml DMF along with catalyst (DMAP/TEA/DMAP) and then kept for stirring at 550 rpm for 1 hour.
- Starch mixture was added to the flufenamic acid mixture and kept for 3 hours at 550 rpm.
- The reaction was kept overnight in dark conditions at room temperature.
- Precipitates were filtered using a vacuum filter.
- Unreacted substances are removed by adding HCl and Na2CO3.
- Filtrate was crystallized using ethanol.
- Crystals were purified by washing them with water under a vacuum.

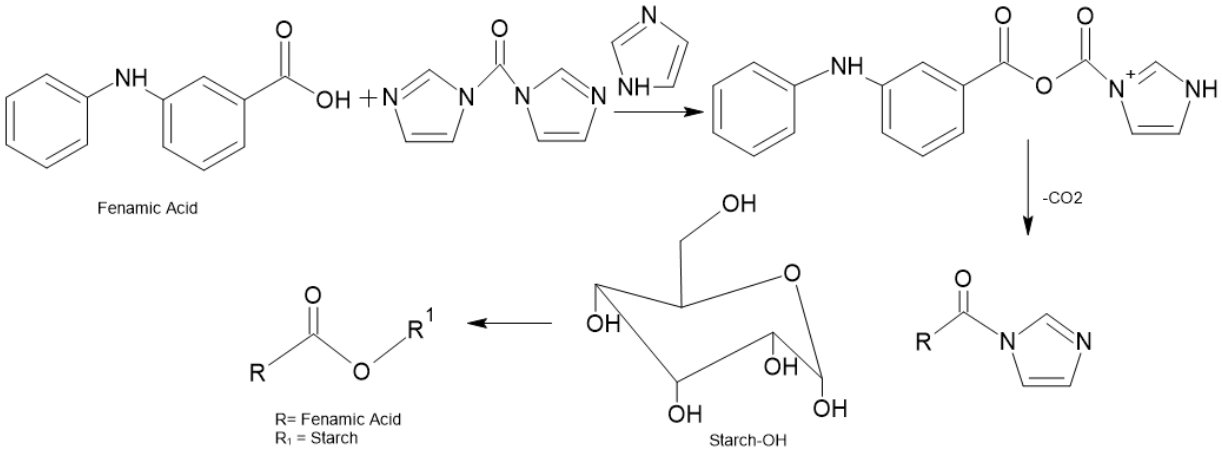


Figure 9 Reaction between fenamic acid and CDI

2.2. By using DCC (N, N′-Dicyclohexylcarbodiimide)

- In a beaker 5.33 mmol of flufenamic acid and 5.57 mmol of CDI were added in 8 ml of DMF.
- This reaction mixture was kept for stirring at 600 rpm for 1 hour.
- In another beaker 18.50 mmol of starch was dissolved in 15 ml DMF along with catalyst (DMAP/TEA/DMAP) and then kept for stirring at 550 rpm for 1 hour.
- Starch mixture was added to the flufenamic acid mixture and kept for 3 hours at 550 rpm.
- The reaction was kept overnight in dark conditions at room temperature.
- Precipitates were filtered using a vacuum filter.
- Unreacted substances are removed by adding HCl and Na2CO3.
- Filtrate was crystallized using ethanol.
- Crystals were purified by washing them with water under vacuum.

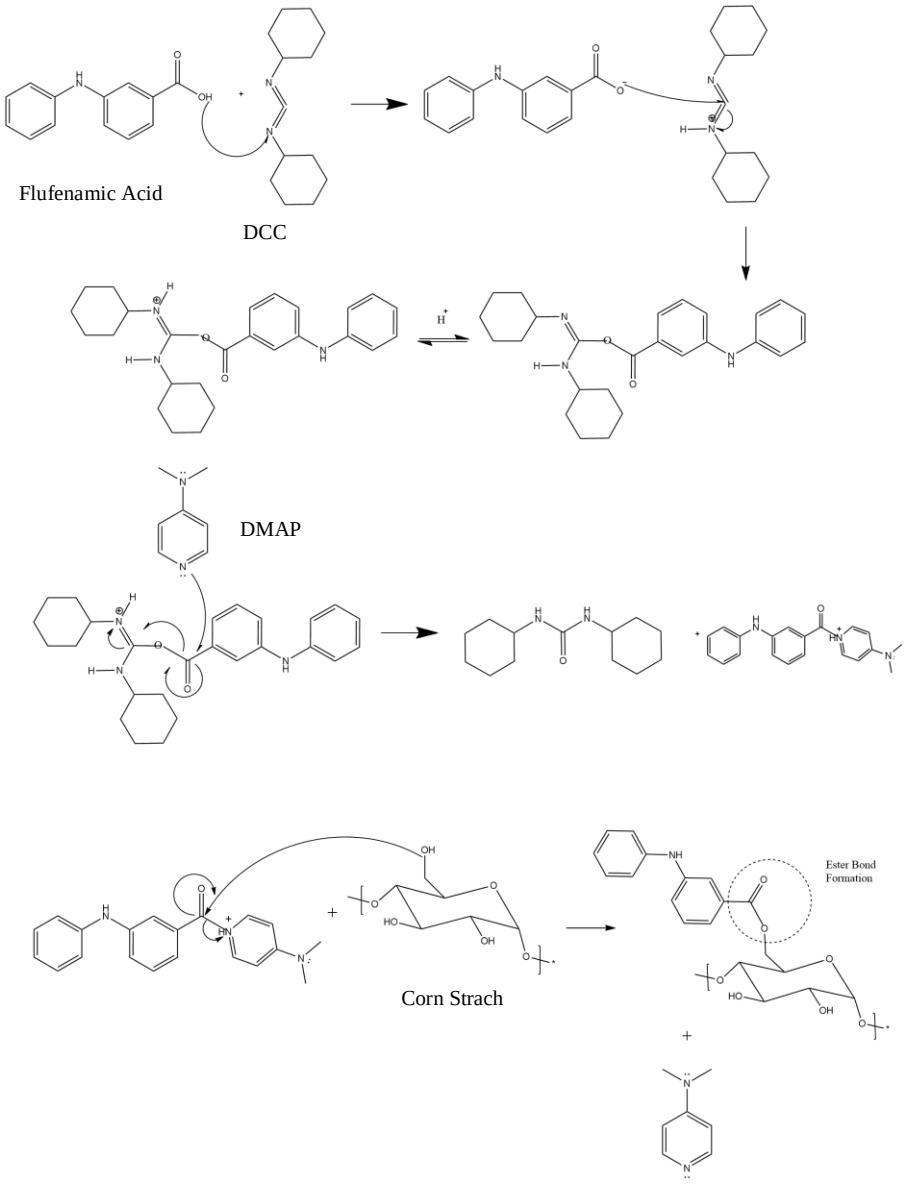


Figure 10 Reaction between fenamic acid and DCC

3. FTIR pallet Preparation:

- To make a KBr pellet, all the equipment was cleaned with ethanol to get rid of any dirt and impurities.
- 200 mg of KBr and 0.9 mg of sample were measured and transferred in the mortar.
- With the help of a pestle the sample was crushed until a homogeneous mixture was observed.
- The pallet press was fixed together and the die was inserted in the cavity.
- This homogeneous mixture was placed inside the cavity.
- To make the pallet even, bolt press was inserted and rotated.
- The whole die set was placed under a hydraulic pellet press.
- After setting the pressure to 5 tons the die set and remained under the hydraulic press for 3 minutes.
- The pellet was removed from the die after applying a small amount of pressure.
- The pellet was collected and in butter paper.

4. **UV-visible sample preparation:** Different media were prepared for the UV-visible analysis.
    - **Gastric Media:** 0.1M HCl, pH 1.2.
    - **Intestinal media:** enzymes (esterase aminopeptidase and pancreatin), pH 7.2.
    - **Colon media:** Gut microbiota, electrolytes, pH6.

References:

[1] Mulet-Cabero, Ana-Isabel, et al. "A standardised semi-dynamic in vitro digestion method suitable for food–an international consensus." *Food & function* 11.2 (2020): 1702-1720.

[2] Freire C, Podczeck F, Veiga F, Sousa J. Starch-based coatings for colon-specific delivery. Part II: physicochemical properties and in vitro drug release from high amylose maize starch films. Eur J Pharm Biopharm. 2009 Aug;72(3):587-94. doi: 10.1016/j.ejpb.2009.02.010. Epub 2009 Mar 3. PMID: 19264128.

[3] Wallace PM, Senter PD. Selective activation of anticancer prodrugs by monoclonal antibody-enzyme conjugates. Methods Find Exp Clin Pharmacol. 1994 Sep;16(7):505-12. PMID: 7885077

CHAPTER 3
CHARACTERIZATION AND RELEASE STUDY

1. Characterization:

1.1 FTIR:

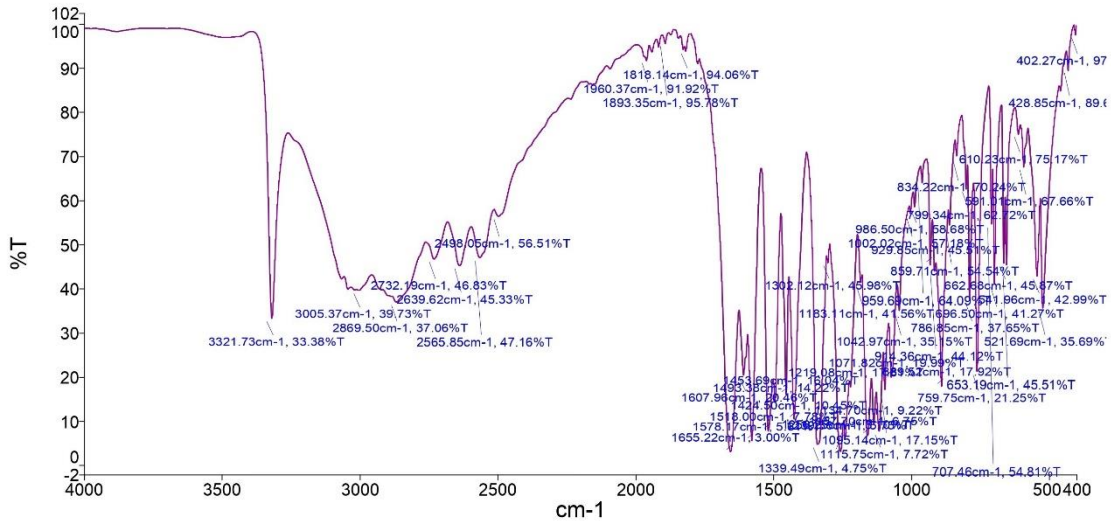


Figure 11 FTIR report of Starch-flufenamic acid prodrug

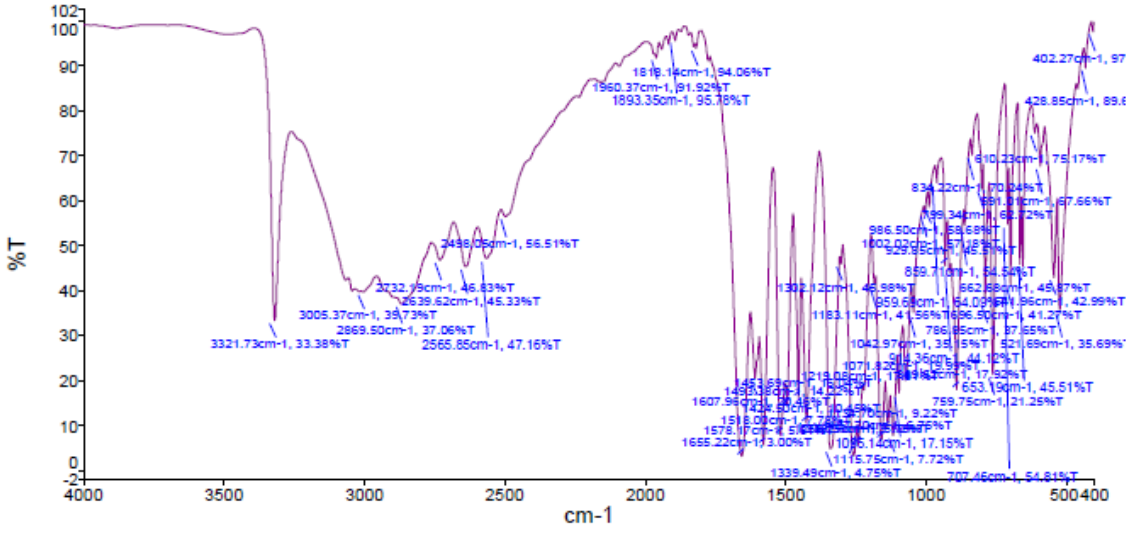


Figure 12 FTIR report of Flufenamic Acid

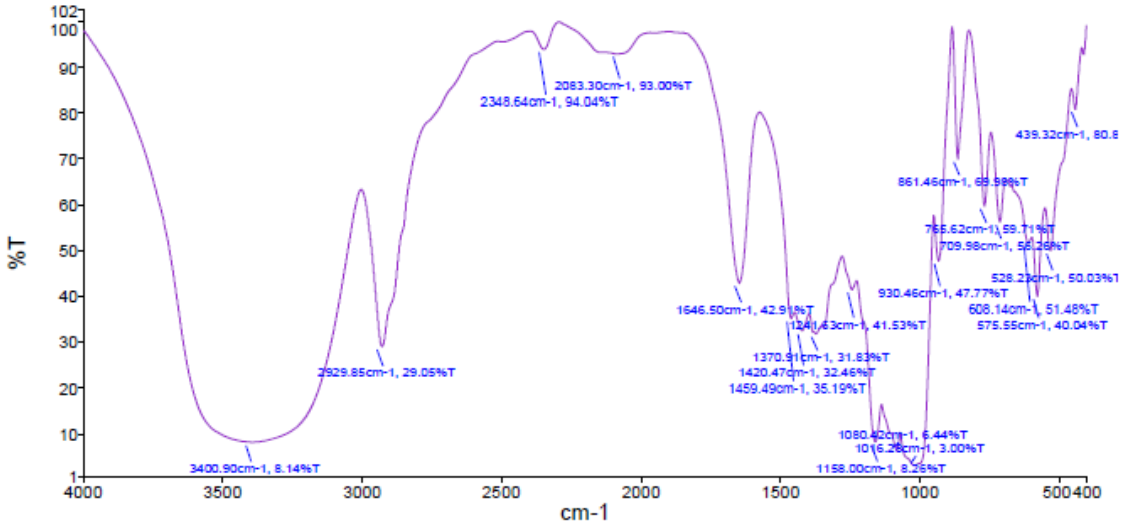


Figure 13 FTIR report of Corn Starch

1.2. NMR:

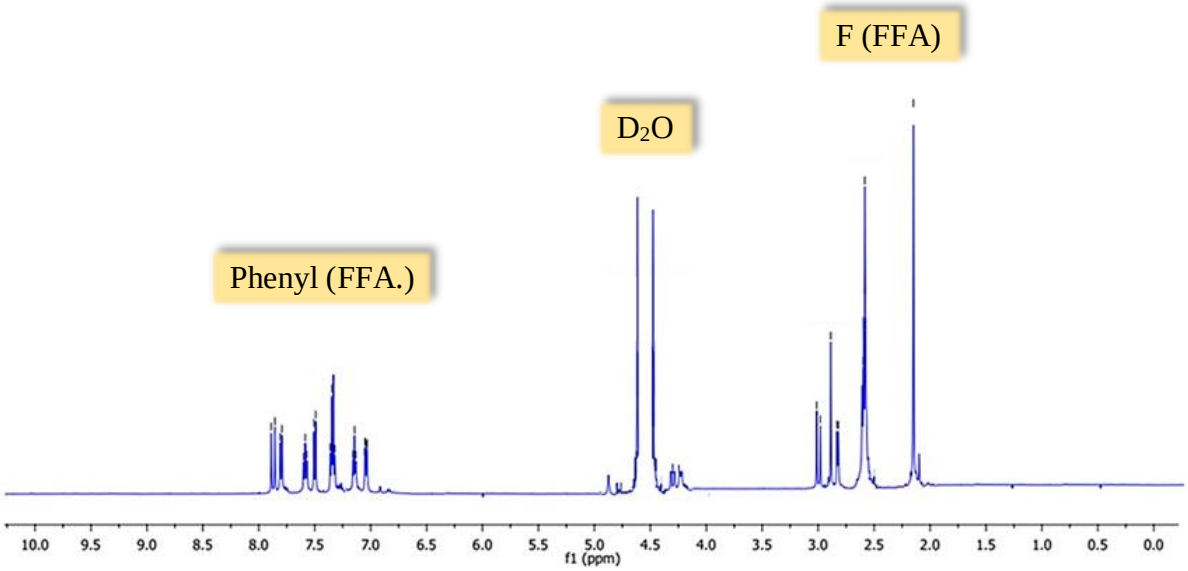


Figure 141H NMR spectra of amylose-flufenamic acid prodrug

1.3. Release study by UV-Visible spectroscopy:

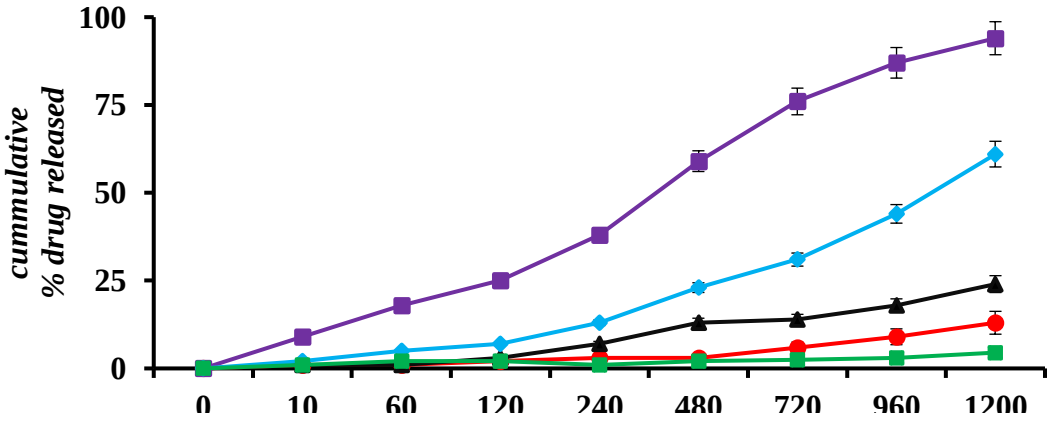


Figure 15 Release profile of amylose-flufenamic prodrug in buffer solution (green trail), SGM (red trail), SIM (black trail), SIM containing esterase (blue trail), and SIM containing aminopeptidase + pancreatin preincubated prodrug conjugate (purple trail)

- **Buffer Media :**

2% drug release at 2 hours, Max 4.5% drug release at 20 hours

- **Simulated Gastric Fluid:**

2% drug release at 2 hours, Max 13% drug release at 20 hours

- **Simulated Intestinal Medium:**

3% drug release at 2 hours, Max 24% drug release at 20 hours

- **SIM with Esterase Enzyme:**

7% drug release at 2 hours, Max 61% drug release at 20 hours

- **SIM with Pancreatin and Esterase:**

results in a maximum 94% drug release.

CHAPTER 4
RESULTS AND CONCLUSIONS

1. Results:

1.1 From prodrug yield:

- The reported yields for the coupling agents were as follows:
- CDI: 76% with pyridine, 72% with TEA, and 48% with DMAP.
- DCC: 73% with pyridine, 67% with TEA, and 45% with DMAP.
- Among the coupling agents, CDI exhibited superior yields compared to DCC.
- The reported yield for SOCl2 was 57%.
- Regarding carboxylic acid activating agents/catalysts:
- Pyridine yielded the highest reported yield, followed by TEA and then DMAP.
- In summary, CDI showed better yields compared to DCC as a coupling agent.
- Pyridine proved to be the most effective carboxylic acid activating agent, followed by TEA and then DMAP.
- These findings suggest that utilizing CDI as the coupling agent and pyridine as the carboxylic acid activating agent can optimize yields in the synthesis process.

1.2 FROM FTIR and NMR:

FTIR CHARACTERISTIC PEAKS-

For prodrug:

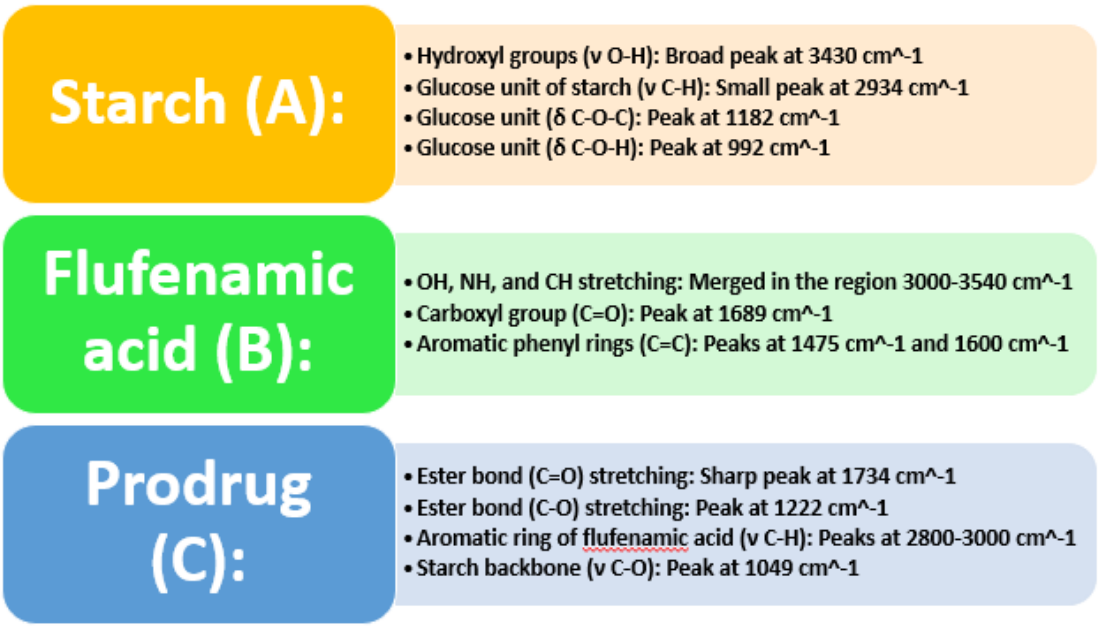
- 1628 cm^-1: Represents the C=O stretching characteristic of the ester bond.
- 1243 cm^-1: Indicates the C-O stretching typical of an ester linkage.
- 1017 cm^-1: Reveals a new peak corresponding to C-O stretching, suggesting the formation of an ester or hydroxyl group.

For Flufenamic Acid:

- 1707 cm^-1: Demonstrates the C=O stretching indicative of a carboxylic acid group, which may be absent or reduced in the product**.**

For Corn Starch:

- 2929 cm^-1: Indicates the presence of C-H stretching in the amylose backbone.
- 1459 cm^-1: Represents C-H bending within the amylose backbone.
- 1243 cm^-1: Displays C-O stretching characteristic of the amylose backbone, possibly overlapping with the ester C-O stretch observed in the product.

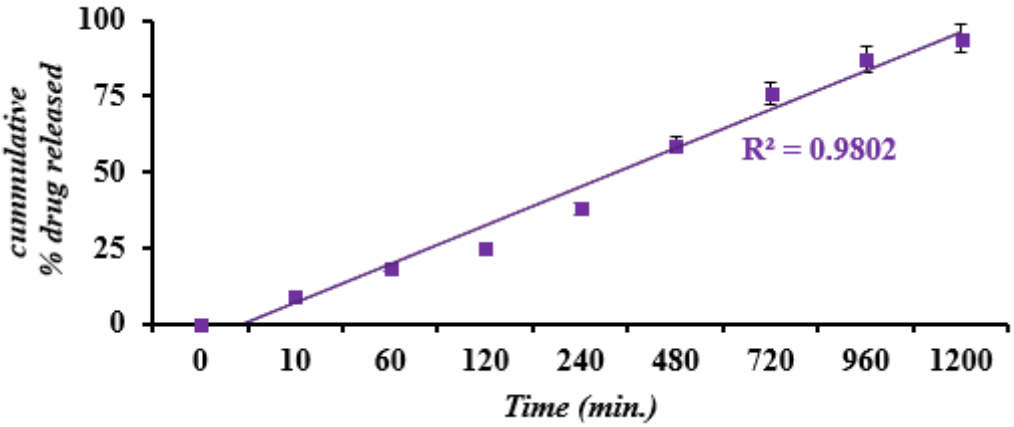


1.3. NMR PEAKS-

- Ester-linked starch-fenamic acid conjugated peaks were observed at δ = 2.15 ppm and 2.65 ppm.
- The -CH- protons of starch appear at δ = 2.80-3.10 ppm.
- The aromatic -CH- protons of fenamic acid appear between δ = 7.0-7.90 ppm

1.4. From UV-Visible (release study):

- **In buffer media:** In 2 hours only 2% of the drug was released with increasing of 4.5% in 20 hours.
- **In simulated gastric media (SGM):** In 2 hours only 2% of the drug was released with increasing of 13% in 20 hours.
- **Simulated intestinal media (SIM):** In 2 hours 3% of the drug was released with increasing of 24% in 20 hours. This can be due to the enzymatic hydrolysis of the bond between fenamic acid and starch
- Due to preincubation of the prodrug with pancreatin 94% of the drug was released in esterase-containing SIM.
- The glycosidic bond of the starch backbone is likely to be hydrolysed with pancreatin which reduces the shielding effect and enhances the enzymatic degradation of ester linkages.



The drug release kinetics were evaluated from the provided plot by constructing a trendline along the data points obtained for the release of flufenamic acid in esterase-containing simulated intestinal media (SIM) after preincubation of the prodrug with pancreatin. The data points fitted for **zero-order kinetics** along the trendline showed excellent correlation (R^2 = 0.9802), indicating a controlled release of the drug in the parent media.

2. **Conclusion:** The development of a starch-based fenamic acid prodrug represents a significant advancement in drug delivery systems, particularly for addressing pain and inflammation in the body with targeted precision and controlled release. This innovative formulation offers promising potential for colon-specific delivery, enhancing the efficacy of treatment while minimizing systemic side effects. By utilizing starch as a carrier, the prodrug ensures biocompatibility and stability during transit through the gastrointestinal tract until it reaches the colon, where it can exert its therapeutic effects directly at the site of inflammation. Furthermore, the controlled release mechanism of the prodrug prolongs drug action, optimizing therapeutic outcomes and patient compliance. This breakthrough holds considerable promise for improving the management of conditions such as colitis, inflammatory bowel disease, and other gastrointestinal disorders, offering a more tailored and effective approach to pain and inflammation management. Through targeted delivery and controlled release, the starch-based fenamic acid prodrug represents a valuable addition to the arsenal of treatments for colon-related ailments, potentially enhancing patient quality of life and clinical outcomes.