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Original Article

IN VITRO PREDICTION OF DRUG RELEASE PROFILE OF RIFAMPICIN IN CROHN'S DISEASE AND CHOLESTASIS DISEASE CONDITIONS USING BIOPHARMACEUTICAL TOOLS AND BIORELEVANT MEDIA

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ABSTRACT

Objective: Altered gastrointestinal physiology in Crohn's disease and cholestasis may affect the intraluminal environment and impact the dissolution of orally administered drugs. Rifampicin exhibits pH and bile salt–dependent solubility, making it susceptible to variations in gastrointestinal conditions. The present research investigates *in vitro* dissolution behaviour of rifampicin in biorelevant media that simulate both healthy and disease-specific gastrointestinal environments.

Methods: Biorelevant dissolution medium that mimic the fasted-state intestinal conditions for healthy people, Crohn's disease, and cholestasis were created by modifying pH, bile salt concentration, and the levels of mucin and inflammatory cytokines based on reported physiological values. Rifampicin fast-dissolving tablets (100 mg) were tested using United States Pharmacopeia (USP) Apparatus II (900 ml, 37 °C±0.5 °C) and spectrophotometric analysis. Dissolution profiles were analyzed descriptively.

Results: Rifampicin demonstrated slower drug release in Crohn's disease–simulated medium (FaSSIF-CD) compared with FaSSIF-NA (healthy condition). Drug release at 60 min exceeded 90% in FaSSIF control, whereas in FaSSIF-CD, it was limited to approximately 58–62% ($p<0.05$). Conversely, dissolution in the cholestasis medium exhibited no significant divergence from the FaSSIF-NA under the evaluated conditions. The data demonstrate significant diversity in dissolution behaviour among media that simulate both healthy and disease-altered gastrointestinal environments.

Conclusion: These findings support the idea that physiological characteristics of the gastrointestinal environment may affect how rifampicin dissolves, as well as highlight the importance of media composition in predicting oral bioavailability in pathological states. Additional research with adequate clinical data is needed to go deeper into these findings and examine the molecular impacts on specific components.

Keywords: Rifampicin, Crohn's disease, Cholestasis, Biorelevant media, *In vitro* release, Bioavailability, Altered gastric motility

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INTRODUCTION

Rifampicin is a primary antibiotic widely used for the treatment of tuberculosis, leprosy, and other bacterial infections. Rifampicin, classified as a Biopharmaceutics Classification System (BCS) Class II medication, exhibits high permeability but poor aqueous solubility, rendering its oral absorption highly contingent upon dissolution characteristics and gastrointestinal (GI) physiological conditions [1–3]. Upon oral administration, it is swiftly absorbed and extensively distributed across tissues and fluids within the body, encompassing macrophages and necrotic granulomatous areas necessary to achieve antitubercular activity. Rifampicin experiences significant hepatic metabolism and biliary excretion, with enterohepatic recirculation playing a considerable role in systemic absorption [4-6]. These attributes render its therapeutic efficacy particularly susceptible to fluctuations in gastrointestinal composition, biliary function, and intestinal motility.

Crohn's disease and cholestasis are important gastrointestinal and hepatobiliary illnesses that significantly modify the luminal environment, hence affecting the solubility, absorption, and bioavailability of orally administered medications. Crohn's disease is linked to diminished bile salt levels, elevated mucin synthesis, persistent inflammation, and modified intestinal permeability, all of which may hinder micellar solubilization and obstruct medication transport [7–9]. Cholestasis, conversely, entails disrupted bile flow or bile acid buildup, resulting in alterations in bile salt concentration and enterohepatic circulation that may affect medication solubilization and metabolism [10]. Disease-induced physiological alterations lead to significant interpatient heterogeneity in rifampicin systemic exposure, frequently requiring dosage adjustments or therapeutic drug monitoring [11].

Rifampicin's pronounced reliance on pH and bile salt-mediated micellar solubility suggests that changes in gastrointestinal circumstances associated with Crohn's disease and cholestasis may markedly affect its dissolution dynamics and *in vivo* pharmacokinetic efficacy [8–13]. *In vitro* biorelevant dissolution experimentation has developed into a mechanistic and predictive approach for elucidating drug behaviour in disease-simulated environments. The utilization of media that mimic abnormal physiological traits facilitates the systematic assessment of dissolution patterns and offers insights into possible clinical implications [14–18].

This work seeks to examine the *in vitro* dissolution characteristics of rifampicin utilizing biorelevant media that simulate Crohn's disease and cholestasis, and to compare these profiles with those of normal fasted-state intestinal fluid. This study aims to clarify disease-specific changes in dissolution behaviour to improve comprehension of rifampicin efficacy in impaired physiological conditions, thereby facilitating informed clinical decisions, formulation optimization, as well as potential dose individualization strategies to enhance therapeutic outcomes.

MATERIALS AND METHODS

Materials

Chemicals and reagents

Rifampicin was procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Phosphoric acid (H_3PO_4), phosphate buffer tablets, sodium hydroxide, sodium dihydrogen phosphate, and sodium chloride were purchased from Sigma Aldrich Chemicals Pvt. Ltd., Bengaluru, Karnataka, India. Hydrochloric acid (HCl), sodium taurocholate, lecithin, sodium glycocholate, phosphatidylcholine, oleic acid, and potassium phosphate were obtained from TCI Chemicals India Pvt. Ltd., Hyderabad, India. Sodium bicarbonate, cholesterol, polycarbonate membrane filters, ethanol, and dimethyl sulfoxide (DMSO) were purchased from Fisher Scientific Chemicals Pvt. Ltd., Delhi, India. All other chemicals, reagents, and solvents were of analytical grade.

Instruments

A UV–Visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) was used for drug analysis. Dissolution studies were performed using a dissolution tester (TDT-06P, Electrolab, Mumbai, India). pH measurements were conducted using a digital pH meter (Eutech Instruments pH 700, Singapore). Temperature control during dissolution was maintained using a thermostatic water bath (Julabo SW22, Germany). All experiments were carried out using calibrated laboratory equipment.

Methods

Preparation of stock solution of drug

The rifampicin fast-dissolving formulation consisted of rifampicin (100 mg), mannitol, microcrystalline cellulose, cross-linked PVP (crospovidone), magnesium stearate and talc. The tablets were prepared using direct compression and evaluated for standard physicochemical properties (hardness, friability, thickness, and weight variation) prior to dissolution testing.

Method A: A standard stock solution of Rifampicin (100 µg/ml) was prepared by dissolving its 10 mg in 100 ml 0.1 M HCl (method A). The dilution was scanned in–the UV-visible range. Further, different dilutions, i. e., 3.0, 6.0, 9.0, 12.0, 15.0, 18.0, 21.0, 24.0, 27.0 and 30 µg/ml of the standard solution, were prepared using 0.1 M HCl. The absorbances of each dilution were taken at absorption maxima of the drug and the graph was plotted between the concentration vs. absorbance to get the standard curve [19].

Method B: A standard stock solution of Rifampicin (100 µg/ml) was prepared by dissolving its 10 mg in 100 ml 0.1 M H_3PO_4. The dilution was scanned in–UV Visible range. Further, different dilutions, i. e., 3.0, 6.0, 9.0, 12.0, 15.0, 18.0, 21.0, 24.0, 27.0 and 30 µg/ml of the standard solution, were prepared using 0.1 M H_3PO_4. The absorbance's of each dilution were taken at absorption maxima, and the graph was plotted between the concentration vs absorbance to get the standard curve [19].

The standard curve of rifampicin in phosphate buffer (6.5 pH)

A standard stock solution of Rifampicin (100 µg/ml) was prepared by dissolving its 10 mg in 100 ml phosphate buffer pH 6.5. Buffer was prepared by adding about 1.74 g of Sodium hydroxide (NaOH), 19.77 gm of Sodium Dihydrogen Phosphate (NaH2PO4. H2O), and 30.93 gm of Sodium Chloride (NaCl) in dissolved in 5 l of purified water. The pH was adjusted to 6.5 using 1 N Sodium hydroxide (NaOH). Further a 20 µg/ml dilution was scanned in–UV Visible range. The absorption maxima were found to be 260 nm. Further, different dilutions, i. e., 9.0, 12.0, 15.0, 18.0, 21.0, 24.0, 27.0, 30.00, 33.00, 36.00 and 39.00 µg/ml of the standard solution, were prepared using 6.5 pH phosphate buffer. The absorbances of each dilution were taken at 260 nm, and the graph was plotted between the concentration vs absorbance to get the standard curve.

Selection of biorelevant media

Appropriate biorelevant media was chosen that mimic the physiological conditions of the gastrointestinal tract. Simulation of both the small intestine and colon conditions are required as Crohn's disease affects both areas. Various types of biorelevant media are used to simulate the fasting and fed states in gastrointestinal fluids. Rifampicin is mainly absorbed from intestine.

Fasted State Small Intestinal Conditions (FaSSIF) are created to reproduce the effects of fasting in the proximal small intestine. This medium includes bile salts and phospholipids (lecithin) in addition to a stable phosphate buffer system [20]. Sodium taurocholate is selected as a representative bile salt. Bile salt ought to be present in the medium at a concentration of 3-5 mmol. Bile salt and lecithin are found in a ratio of about 4:1 for adults (table 1).

Fed State Small Intestinal Conditions (FeSSIF) stimulate the drug dissolution in the proximal intestine. There are alterations in the hydrodynamics and intraluminal volume following a meal. Unique interactions between the medication and food components that are consumed may happen and an abrupt increase in bile salts can significantly impact the bioavailability of drugs [21]. To reflect the biliary response to meal intake, taurocholate, and lecithin are present in significantly higher amounts than in the fasting state medium.

Different levels of biorelevant media were introduced in order to consider the situations where less complex media can be appropriate or situations where additional factors need to be considered. Level 0 biorelevant media (pH) are considered for the approval of the immediate release of solid dosage forms comprising highly soluble drugs (BCS Class I and III drugs). On the other hand, Level I media (pH and buffer capacity) are considered for highly soluble drugs where the rate and extent of dissolution are governed by both pH and buffer capacity. Level II (pH, buffer capacity, and physiological solubilizing factors) is considered for poorly soluble drugs with a log P value of ≥ 2. In contrast, Level III media (special purpose) are in which the media is modified to respond to certain queries or unique formulations [22]. Examples include adding enzymes to the media for gelatin capsules that may exhibit crosslinking or when determining the amount of release from lipid-based dosage forms.

Therefore, level II media was selected for the investigation of Rifampicin release in Fasted state of the adult patients. The most appropriate media has been summarized in table 1.

Table 1: Types and composition of biorelevant media

Components	FaSSIF	FeSSIF	FaSSIF Adult
Sodium Taurocholate	3 mmol	15 Mm	3 mmol
Lecithin	0.75 mmol	3.75 mmol	0.75 mmol
Acetic acid	-	8.65 g	
Sodium chloride	-	11.874 g	125.5 mmol
Sodium hydroxide	qs ad pH 6.5	4.04 g pellets	qs ad pH 6.5
pH	6.5	5.0	6.5
Deionized water	1 l	1 l	1 l

Osmolality (mOsmol/kg)	~270	~ 670	~270
Buffer Capacity (mEq/pH/l)	~ 12	~ 72	~ 12
Surface tension (mN/m)	54	48	42

*FaSSIF-Fasted state Simulated Intestinal Fluid and FeSSIF-Fed state Simulated Intestinal Fluid

Following sections describes the most appropriate conditions for making biorelevant media specific to the Crohn's disease and cholestasis to ensure most appropriate simulation of these conditions and to ensure optimum correlation with the *in vivo* condition of the patients suffering from these diseases.

Simulation of disease condition and composition of biorelevant media for Crohn's disease

Biorelevant media for simulating Crohn's disease conditions should mimic the altered physiological environment of the gastrointestinal tract in individuals with this condition. While there is no single standardized biorelevant medium for Crohn's disease, one can create a custom medium that replicates key characteristics associated with the disease. The composition and preparation of such a medium involve considering factors like altered pH, inflammatory components, and microbiota changes.

Crohn's disease often results in shifts in intestinal pH. The pH in the small intestine typically ranges from 6 to 7, while the pH in the colon ranges from 5.5 to 7. Mimicking these conditions might use phosphate or acetate buffer adjusted pH of the medium accordingly. Crohn's disease is characterized by chronic inflammation. Inflammatory markers such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), or C-reactive protein (CRP) was added into the dissolution medium to simulate the inflammatory environment [23]. Commercially available inflammatory cytokines or laboratory-generated equivalents are recommended to be used in this case. Crohn's disease affects mucin production in the gastrointestinal tract. Adding mucin, obtained from animal or synthetic sources to the medium can replicate this aspect. Alterations in the gut microbiota are also associated with Crohn's disease. Specific bacterial strains isolated from the fecal microbiota transplant (FMT) of Crohn's disease patients was added to the medium [24]. Depending on the location of Crohn's disease (small intestine or colon), there is need to adjust the concentration of bile salts. Bile salts are crucial for digestion and absorption in the small intestine [25]. Consider the malabsorption issues associated with Crohn's disease and adjust the nutrient composition accordingly. This may involve reducing the concentration of certain nutrients like fats [26].

Preparation of Crohn's disease biorelevant Media

Mucin was used at a concentration of 2 mg·mL^{-1}(porcine gastric mucin, Sigma-Aldrich, St. Louis, MO, USA; Cat. No. M1778) and solubilized in the buffer solution in combination with bile salts, with the final bile salt concentration adjusted according to the targeted intestinal region. The pH of the medium was modified to simulate the physiological conditions of the small intestine or colon. Pro-inflammatory cytokines TNF-α, IL-1β, and C-reactive protein (CRP) were obtained from R and D Systems (Minneapolis, MN, USA; recombinant human proteins, TNF-α Cat. No. 210-TA, IL-1β Cat. No. 201-LB, CRP Cat. No. DY1707) and reconstituted in sterile phosphate-buffered saline (PBS) prior to administration at concentrations representative of the desired inflammation severity. The media's nutrient composition was altered to simulate malabsorption circumstances characteristic of Crohn's disease, particularly by decreasing fat and specific carbohydrate levels (table 2). Ultimately, chosen bacterial strains were injected, and the medium was cultured under anaerobic conditions for 24 h to replicate gastrointestinal microbial environments. The FaSSIF-CD medium served as experimental biorelevant system to qualitatively evaluate the possible impact of luminal disease-related and inflammatory variables on the behaviour of rifampicin dissolution. Mucin, certain pro-inflammatory cytokines, and microbiological elements were included in the medium composition to represent important pathological characteristics linked to Crohn's disease. This medium's goal was to offer a mechanistic foundation for analysing dissolution variability in inflammatory gastrointestinal circumstances rather than to create a fully verified physiological surrogate. However, It should be noted that the FaSSIF-CD medium is an exploratory mechanistic dissolution model rather than a validated physiological surrogate; the observed effects are a reflection of the combined system response, and the individual contributions of mucin, cytokines, and microbiological components were not isolated.

Simulation of disease condition and composition of biorelevant media for cholestasis

Biorelevant media for cholestasis aim to mimic the altered physiological conditions in the gastrointestinal tract of individuals with cholestasis. Cholestasis is characterized by impaired bile flow and changes in bile composition, which can affect drug solubility and absorption [27]. A basic formula for preparing a biorelevant media for cholestasis may include several things. Bile salts are a critical component of bile. Sodium glycocholate and sodium taurocholate are commonly used bile salts. A mixture of both can also be considered [28]. Phosphatidylcholine is a common phospholipid present in bile. Lecithin is used a source of phospholipids. Oleic acid is often used to mimic the fatty acid composition of bile [29]. To maintain a physiological pH, a buffer such as potassium phosphate or sodium bicarbonate is used. Sodium chloride (NaCl) is typically added for maintain ionic strength. Depending on the specific research objectives, adding cholesterol, cholesterol esters, and mucus may be considered to replicate the complexity of bile (table 2).

Preparation of cholestasis biorelevant media

The appropriate amounts of bile salts, phospholipids (lecithin), fatty acids (oleic acid), sodium chloride, and optional components were weighed based on the study requirements. A buffer solution was prepared to maintain the desired pH of the media like small intestine (around pH 6-7). The buffer was made separately and added to the media later. The dry ingredients were dissolved in an appropriate solvent (sufficient for complete dissolution) including 1:1 ethanol and dimethyl sulfoxide. The dissolved dry ingredients were gradually mixed with the prepared buffer solution while stirring continuously until a homogeneous mixture was obtained. If required, the pH of the media was adjusted to the desired level. The media was sterilized by filtration (0.45 μM) to remove any particulate matter and ensure its sterility. The prepared biorelevant media were stored to prevent from contamination until use. In order to enable mechanistic assessment of solubility-dependent drug dissolution, a higher bile salt concentration (10 mmol) was purposefully chosen to reflect a compensatory micellar environment scenario that is outlined in some cholestatic situations, where changed bile flow and composition may result in localized bile salt accumulation (table 2). In accordance with physiological conditions and commonly used formulations like FaSSIF and FeSSIF, biorelevant dissolution media were made with a bile-to-lecithin ratio of roughly 4:1 [30, 31]. The stable mixed micelles required for drug solubilization in the small intestine can be formed based on the ratio 1:1 ethanol–DMSO mixture was utilized solely to solubilize hydrophobic bile salts during stock preparation. After dilution into the final dissolution medium, the total organic solvent content was maintained at less than 1% v/v, a level deemed acceptable in dissolution testing that does not significantly affect micellar solubilization or polarity. Further, this facilitated rifampicin solubilization during preparation of cholestasis-simulated dissolution media. The solvent concentration was minimized to limit potential interference with dissolution behaviour and bile salt functionality. The produced medium was primarily watery and physiologically realistic.

Table 2: Composition of biorelevant media specific to Crohn's Disease and cholestasis

Components	FaSSIF Adult	FaSSIF-CD	FaSSIF-cholestasis
Sodium Taurocholate	3 mmol	1.2 mmol	10 mmol
Lecithin	0.75 mmol	0.3 mmol	2.5 mmol
Oleicacid	-	-	60.0 mmol
Sodium chloride	125.5 mmol	125.5 mmol	125.5 mmol
Sodium hydroxide	qs ad pH 6.5	qs ad pH 6	qs ad pH 6.5
pH	6.5	5.5-7	6-7
Deionized water	1 l	1 l	1 l
Osmolality (mOsmol/kg)	~270	~270	~270
Buffer Capacity (mEq/pH/l)	~ 12	~ 12	~ 12
Surface tension (mN/m)	42	53	43
Inflammatory markers	-	TNF-α, IL-1β, C-RP	-
Mucin	-	2 mg·mL^{-1}	-
Bacterial strains isolated from the fecal microbiota transplant (FMT) of Crohn's disease	-	Added	-

*FaSSIF-Fasted state Simulated Intestinal Fluid

The standard curve of rifampicin in biorelevant media

A standard stock solution of Rifampicin (100 µg/ml) was prepared by dissolving its 10 mg in 100 ml of each biorelevant media prepared in phosphate buffer pH 6.5. Further, a dilution of 20 µg/ml (prepared in the biorelevant media) was scanned in–UV Visible range. The absorption maxima were found to be 260 nm and 263 nm respectively in the case of biorelevant media of Crohn's disease and cholestasis, respectively. Subsequently, different dilutions were prepared using the same biorelevant mediums, i. e., 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.00, 18.00, 20.00 and 22.00 µg/ml of the standard solution. The absorbances of each dilution were taken at 260 nm and 263 nm respectively, and the graph was plotted between the concentration vs absorbance to get the standard curve. Rifampicin shows tautomeric and chemical shifts that depend on pH, which causes λmax to change in different dissolution media. Initial spectral scanning found λmax values of 260 nm in acidic medium (0.1 N HCl) and 263 nm in phosphate-based media (FaSSIF). These values gave the best peak definition and absorbance intensity. The chosen wavelengths correspond to experimentally determined maxima in each solvent system and were not given at random.

Release studies in biorelevant media

In vitro release study was conducted using dissolution tester in biorelevant media simulating Crohn's disease and cholestasis most appropriately. This was done using standard dissolution apparatuses, such as USP apparatus 2 (paddle) or apparatus 4 (flow-through cell), with modifications as needed. Effect of different diseased conditions were checked on rifampicin dissolution profile using dissolution studies in Electrolab tablet dissolution tester USP XXIII, TDT-06P (Electrolab, Mumbai, India) in different biorelevant media based on the diseased states of Crohn's disease (concentrations of bile salts<2 mmol/l) and Cholestasis (concentrations of bile salts>10 mmol/l). The normal concentrations of bile salts in normal condition are 3 mmol/l. The agitation rate was set at 75 rpm corresponding to a normal hydrodynamic stress experienced by dosage form during peristalsis movement of GIT [14].

The release of the Rifampicin fast-dissolving formulation was checked in fasted simulated intestinal fluid (FaSSIF) that is in pH 6.5. Dissolution tests were performed using a six-stage dissolution apparatus with USP apparatus II (paddle) specifications at 37 °C±0.5 °C. The volume of the media was chosen to be 900 ml. During the entire dissolution studies, media sink conditions were maintained. According to published research, rifampicin readily solubilizes in the range of 1–3 mg/ml in near-neutral intestinal pH and media containing bile salts. Consequently, the saturation capacity in 900 ml dissolution medium exceeds 900 mg, and the tested dose of 100 mg (≈ 0.11 mg/ml) remains well below one-third of saturation solubility, confirming sink conditions [10, 20, 31]. This method made sure that drug solubility did not restrict dissolution under any of the examined circumstances. The dissolution parameters and methodology were kept constant in all the experiments except dissolution medium (varied as per diseased conditions). At the 5, 10, 20, 30-, 40-, 50-and 60-minute, 5 ml aliquots of the sample were withdrawn. The withdrawal sample was replaced with preheated fresh buffer. The samples were filtered with the help of 0.6 µm polycarbonate filters, diluted, and analyzed immediately by UV-Vis spectrophotometry at 260 nm for the samples of Crohn's disease and 263 nm in the case of Cholestasis. Percentage release was calculated with respect to the reference vessel based on labeled amount of rifampicin in the formulations. Evaluation of release profile was done by calculating dissolution efficiency (DE) at 45 min, which is defined as area under the dissolution curve to a certain time point, expressed as percentage of area of the rectangle described by 100% release at the same time [30].

The adsorption behaviour of rifampicin on 0.6µm polycarbonate membranes was not evaluated through a dedicated filter-recovery experiment. However, all dissolution samples were filtered using the same membrane type and pore size prior to analysis to ensure consistency across experimental conditions. Polycarbonate membrane filters are widely employed in dissolution tests because of their comparatively low indiscriminate drug-binding in comparison to cellulose-based membranes. Additionally, even the minor adsorption that possibly occurred would not substantially bias comparative dissolution profiling because all samples were handled with the same type of filter [33].

Sample processing and data analysis

The collected samples, at various time points, were analyzed to determine the amount of rifampicin released under these disease-specific conditions. The UV-Visible based method of estimation was used for quantification. The drug release profiles obtained under disease conditions to those under standard physiological conditions (e. g., fasting state) were compared to check the possible variation. Dissolution efficiency (DE) was calculated to quantitatively compare the drug release profiles, as it represents the area under the dissolution curve up to a certain time relative to the maximum possible release. This comparison was expected to help identify how the disease conditions affect drug release. The collected data was interpreted to draw conclusions about the impact of Crohn's disease and cholestasis on rifampicin release in which various factors such as altered pH, motility, drug solubility, and interactions with diseased tissues or components in the biorelevant media were considered for evaluation.

$$DE = \frac{\int_0^t y\,dt}{y_{100}.t} \times 100$$

Where DE is the dissolution efficiency, y is the percentage of drug released at time t, y_{100} is 100% drug release, and t is the total dissolution time.

Statistical analysis

Dissolution profile comparisons were conducted using the similarity factor (f_2), calculated according to regulatory guidelines, to quantitatively assess similarity between dissolution profiles. An f_2 value between 50 and 100 was considered indicative of similarity. All analyses were performed using replicate dissolution data, and results are expressed as mean±standard deviation.

RESULTS

This study presents a first-of-its-kind mechanistic evaluation of rifampicin dissolution at intestinal pH under simulated gastrointestinal conditions relevant to Crohn's disease and cholestasis. Various investigation were carried out prior to the release study and suitable method of estimation was evaluated. For this purpose, we prepared five types of standard curve to check the best fit conditions. First two standard curves (in acidic media) were prepared to check which condition is most suitable to for the drugs solubility in GI tract environment. The next method was developed to find its release and presence around normal intestinal pH (in phosphate buffer). The last two methods (in biorelevant media) were developed to check its actual release at intestinal pH buffer with the most appropriate components mimicking the actual GI tract environment exposed with Crohn's disease and cholestasis.

Method of estimation of rifampicin in different solvent

As far as standard curve of Rifampicin in 0.1 M HCl is concerned, it showed well linearity in the range of 3-30 µg/ml with the R^2value 0.9978. The equation of linearity was found to be y = 0.0281x-0.032. The method accuracy was found to be 99.92% and 0.1 µg/ml LOQ. Standard curve of Rifampicin in 0.1 M H_3PO_4 showed linearity in the range of 3-30 µg/ml with the R^2value 0.9983. The equation of linearity was found to be y = 0.0319x-0.0576. The method accuracy was found to be 99.97% and LOQ of 0.15 µg/ml (fig. 1).

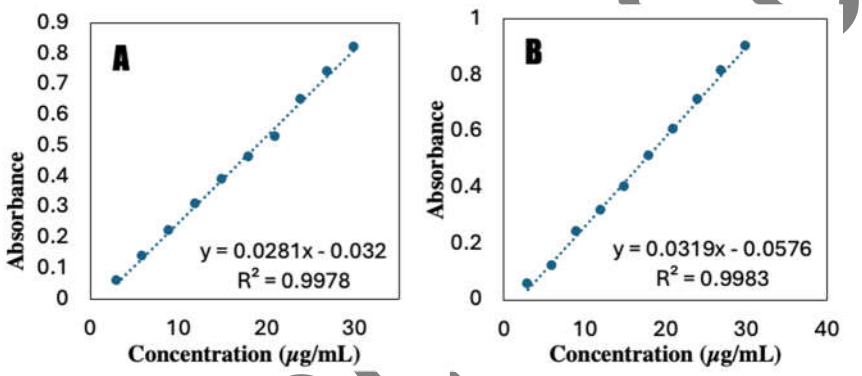

Fig. 1: Standard curve of Rifampicin in acidic and alkali condition. A) Curve in 0.1 M HCl absorbance's taken at 263 nm. B) Curve in 0.1 M H_3PO_4 absorbance's taken at 259 nm

The standard curve of Rifampicin in pH 6.5 phosphate buffer showed linearity in the range of 9-39 µg/ml with the R^2value 0.9942. The equation of linearity was found to be y = 0.0282x-0.1328. The method accuracy was found to be 99.81% and LOQ of 2.5 µg/ml (fig. 2).

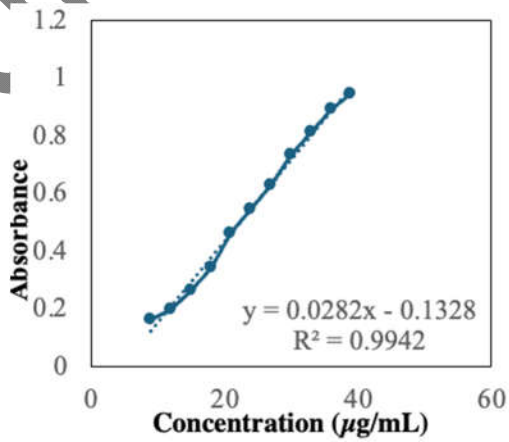

Fig. 2: Standard curve of rifampicin in pH 6.5 phosphate buffer at 260 nm

The standard curve of Rifampicin in in biorelevant media specific to Crohn's disease showed linearity in the range of 2-22 µg/ml with the R^2value 0.9905. The equation of linearity was found to be y = 0.0363x-0.0657. The method accuracy was found to be 99.95% and LOQ of 1.0 µg/ml. However, the standard curve of Rifampicin in biorelevant media specific to cholestasis showed linearity in the range of 2-22 µg/ml with the R^2 value 0.996. The equation of linearity was found to be y = 0.0358x+0.006. The method accuracy was found to be 99.99% and LOQ of 0.22 µg/ml (fig. 3).

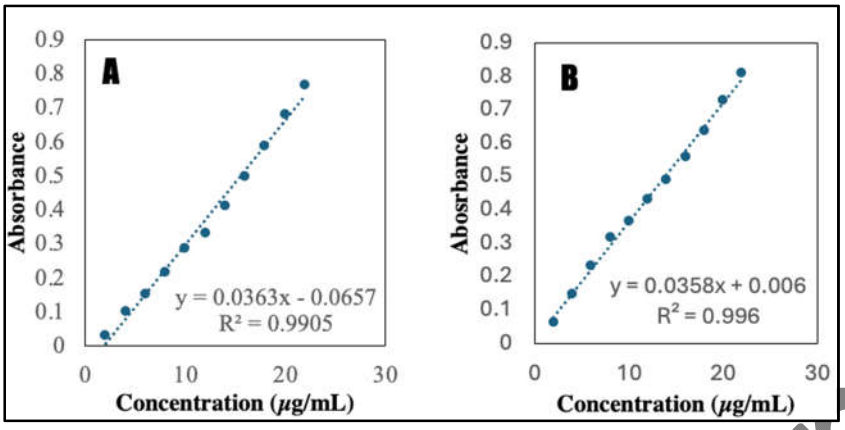

Fig. 3: Standard curve of Rifampicin in biorelevant media specific to Crohn's disease and cholestasis. A) Curve in biorelevant media specific to Crohn's disease and absorbances taken at 260 nm. B) Curve in biorelevant media specific to cholestasis and absorbances taken at 263 nm

Rifampicin release from fast-dissolving formulation in disease-specific biorelevant media

Dissolution of the fast-dissolving rifampicin formulation in disease-specific biorelevant media was carried out for 60 min. The results of the investigation are presented in fig. 4 represent the mean of six determinations±standard deviation. The maximum rifampicin concentration observed in each dissolution media remained well below the solubility threshold, indicating that sink conditions were maintained throughout the investigation. In FaSSIF-NA, rapid drug release was observed, with more than 45% of rifampicin released within the first 5 min, followed by approximately 63% release at 10 min and nearly 80% release at 20 min. The dissolution profile in FaSSIF-Cholestasis exhibited a broadly comparable release pattern to FaSSIF-NA, with minor variations observed between 5 and 30 min. Notably, at 20 min, FaSSIF-Cholestasis demonstrated approximately 13% higher drug release compared to FaSSIF-NA, indicating an influence of cholestasis-specific media components on rifampicin dissolution behaviour.

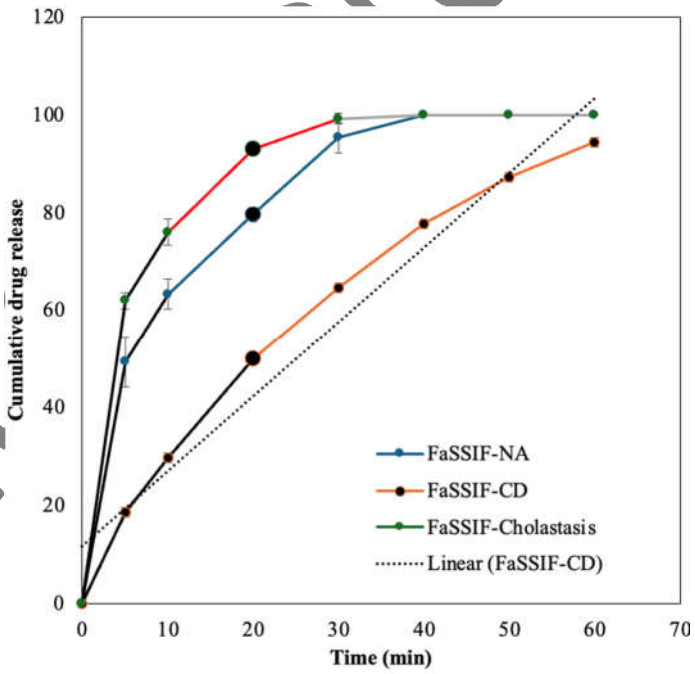

Fig. 4: Graphical presentation of comparative release profile of Rifampicin in biorelevant media specific to Crohn's disease and cholestasis at fasted condition along with normal biorelevant media at fasted condition. Data expressed as mean±SD (n = 3)

Accelerated drug release was observed in FaSSIF-Cholestasis, with nearly complete drug release achieved within 30 min compared to approximately 40 min in FaSSIF-NA; however, except for the 20 min time point, differences between the profiles were not pronounced.

In contrast, rifampicin dissolution in FaSSIF-CD showed a comparatively slower and distinctly altered release pattern relative to FaSSIF-NA and FaSSIF-Cholestasis. The formulation exhibited approximately 18% drug release at 5 min and 29% at 10 min, followed by a gradual increase in drug release over time. More than 90% drug release was achieved at 60 min, whereas similar extents of release were attained earlier in FaSSIF-NA and

FaSSIF-Cholestasis. Notable differences in drug release were observed across multiple time points between 10 and 50 min, indicating a sustained and delayed dissolution behavior under Crohn's disease–simulated conditions.

When rifampicin dissolution profiles were compared pairwise using the similarity factor (f_2), there was a clear difference between healthy and disease-simulated medium fig. 5. The non-similar dissolving behaviour across the studied conditions were indicated by all f_2 values being below the regulatory similarity criterion of 50. An f_2 value of 29.39 was obtained when PBS pH 6.5 was compared with FaSSIF-NA, however much lower f_2 values were found when PBS was compared with FaSSIF-CD (f_2 = 4.74), indicating significant changes in dissolution under Crohn's disease-simulated conditions. Comparing FaSSIF-NA and FaSSIF-CD also yielded a f_2 value of 2.68, indicating a significant difference in release profiles between diseased states and healthy conditions. In contrast to healthy controls, comparisons including cholestasis-simulated media also showed f_2 values below 50 (25.26–29.39), indicating altered dissolution behaviour. Collectively, the f_2 analysis quantitatively confirms that disease-associated media significantly impact rifampicin dissolution characteristics.

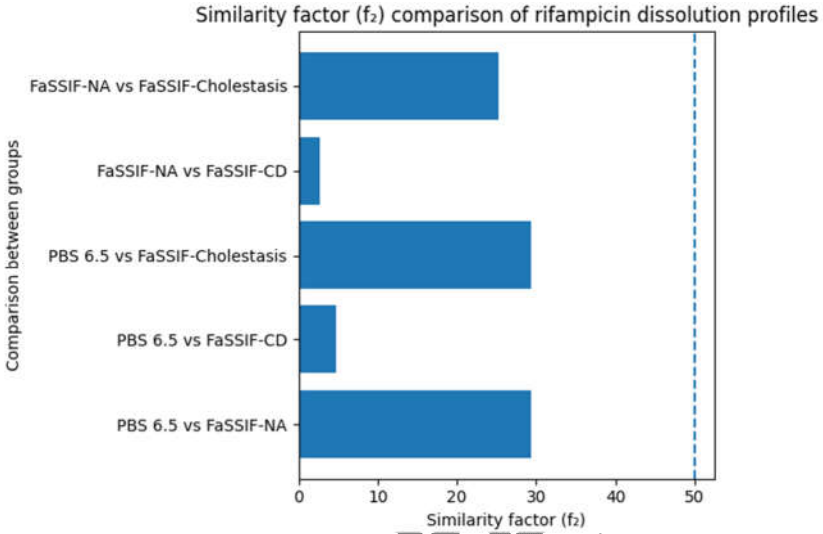

Fig. 5: Similarity factor (f_2)–based comparison of rifampicin dissolution profiles across healthy and disease-simulated media. The dashed vertical line represents the regulatory similarity threshold (f_2 = 50). All comparisons yielded f_2 values<50, indicating dissimilar dissolution behaviour

DISCUSSION

The current research explored the dissolution behaviour of rifampicin under gastrointestinal circumstances that mimic Crohn's disease and cholestasis, using disease-specific biorelevant media. The results demonstrated that rifampicin release was substantially diminished in FaSSIF-CD, whereas FaSSIF-cholestasis showed dissolution behaviour comparable to what was observed in healthy adult fasted-state conditions. These observations highlight the significant impact of pathological luminal shifts on drug solubilization and potential systemic drug exposure.

The findings based on model independent f_2 approach quantitatively support the influence of disease-associated luminal factors on rifampicin dissolution and underscore the importance of incorporating disease-specific biorelevant media during *in vitro* performance evaluation.

Rifampicin displays solubility that is dependent on pH and bile salts, with micellar solubilization playing a crucial role in its dissolution efficiency and intestinal absorption. Studies indicate that higher concentrations of bile salts and phospholipids substantially enhance the solubilization of lipophilic drugs through formation of mixed micelle. The concept that gastrointestinal physiological variations could serve as important determinants of pharmacokinetic outcomes is further supported by earlier research showing that bile salt disparities might explain variability in systemic drug exposure for poorly soluble substances [30-33]. The considerably decreased bile salt concentration (approximately 1.2 mmol) in the FaSSIF-CD medium may have contributed to the reduced drug release reported in the present research. This is in agreement with prior research indicating impaired bile recycling and solubilization capacity in inflammatory bowel disease [34-36]. The increased bile salt concentration in FaSSIF-cholestasis (10 mmol) enhanced micelle stability and drug solubilization capacity, which accounts for the minimal variance from the dissolution patterns observed in healthy FaSSIF-NA conditions.

The mucin concentration in FaSSIF-CD (2 mg/ml) was determined based on previous studies demonstrating that mucin structures significantly influence drug diffusion as well as create a hydrophilic viscoelastic wall surrounding drug particles, thereby limiting transport and dissolution rates [37-38]. The presence of inflammatory mediators, including TNF-α, IL-1β, and CRP, indicates the elevated inflammatory milieu characteristic of active Crohn's disease. Cytokines are recognized for their ability to compromise epithelial tight-junction integrity, modify mucus architecture, and change luminal physicochemical properties [39-40]. Collectively, these disease-associated factors may contribute to the reduced rate and delayed extent of rifampicin dissolution observed in FaSSIF-CD relative to healthy and cholestasis-simulated media. The observed dissolution behaviour demonstrates increased diffusional resistance within the disease-simulated medium; however, interpretation is limited to qualitative trends supported by model-independent dissolution profile comparison [41-42].

It is important to note that the FaSSIF-CD medium used in this study is an exploratory mechanistic dissolution model rather than a validated physiological surrogate. The observed dissolution differences should be interpreted as directional mechanistic indicators rather than quantitative *in vivo* predictors considering the individual contributions of mucin, cytokines, and microbial components were not isolated.

From a clinical perspective, the substantial change in dissolution profile under conditions of Crohn's disease may lead to decreased oral bioavailability, potentially requiring dose modifications, therapeutic drug monitoring, or enhanced formulation strategies for optimum therapeutic efficacy. Conversely, cholestasis conditions were not found to significantly influence dissolution, suggesting that modifications to dosage related to

dissolution may not be required. *In vivo* validation is necessary prior to the establishment of clinical recommendations, and physiologically based pharmacokinetic (PBPK) modeling may serve as a valuable approach to facilitate *in vitro–in vivo* correlation.

Limitations and future directions

The present research addresses biorelevant dissolution medium for Crohn's disease as an exploratory model. A disadvantage of the current work is the lack of experimental validation of cytokine stability, pH-dependent rheological changes, and possible microbial degradation of rifampicin. As a result, the results produced with this medium should be interpreted qualitatively, and comprehensive physicochemical and biological validation should be included in future research before translational use. Future research ought to include the characterization of medium rheology, including pH and viscosity, the persistence of cytokines as measured by ELISA, and the degradation behaviour analyzed through HPLC/lC-MS. Although organic solvent content was maintained below 1% v/v, experimental validation of solvent effects on bile salt aggregation and dissolution dynamics was not performed. Therefore, the cholestasis dissolution medium should also be regarded as a mechanistic tool rather than a fully physiologically representative system.

Furthermore, One disadvantage of the current work is that recovery tests were not used to quantitatively assess the potential adsorption of rifampicin onto the filter membrane. However, all samples received the identical filtration processes, thereby eliminated systematic bias when comparing dissolution profiles across media.

Since formal release model fitting was not done in this investigation, dissolution data are summarized using model-independent comparative profile analysis instead of kinetic model classification; such model-based approaches are described in formulation-focused release studies reported in the literature [40]. This study establishes that Crohn's disease conditions significantly impede rifampicin dissolution compared to cholestasis. The results generated from this study highlights the need for further mechanistic and *in vivo* pharmacokinetic investigations before establishing therapeutic dosing implications.

CONCLUSION

The present research demonstrates that Crohn's disease significantly affects the *in vitro* release profile of rifampicin, while cholestasis conditions have a minimal influence. Reduced drug release in Crohn's disease can originate from pathological alterations in the gastrointestinal tract, such as variations in pH and motility, changes in bile salt composition, the presence of inflammatory mediators, elevated mucin production, and disruption of microbiota. The results suggest that patients with Crohn's disease may have reduced oral bioavailability of rifampicin, highlighting the need for tailored therapeutic strategies, including dose optimization or modified-release formulations. Integrating disease-specific gastrointestinal parameters in formulation development and evaluation may improve the predictability of clinical outcomes. It is recommended to undertake additional research utilizing advanced dynamic dissolution systems and PBPK modeling to translate these *in vitro* findings into clinically relevant results. The results further highlight the necessity of incorporating gastrointestinal disease representative physiology into drug development process for enhanced therapeutic effectiveness and patient-centric treatment outcomes.

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AUTHORS CONTRIBUTIONS

AT: Conceptualization and study design; critical reviewing and editing of the manuscript; overall scientific supervision of the work.

TN: Continuous academic guidance, methodological oversight, and technical review throughout the study.

PS: Experimental work, data collection and analysis, media preparation, dissolution studies, fig. and table preparation, literature review, and primary manuscript drafting.

CONFLICT OF INTERESTS

Declared none

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