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Development of bicarbonate buffer flow-through cell dissolution test and its application in prediction of *in vivo* performance of colon targeting tablets

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ABSTRACT

The purpose of this study was to develop a bicarbonate buffer flow-through cell (FTC) dissolution test. Mesalazine colon targeting tablets of a generic development product (test formulation, TF; Mesalazine 400 mg tablet) and the original product (reference formulation, RF; Asacol® 400 mg tablet) were used as model formulations. A clinical bioequivalence (BE) study was conducted on 48 healthy male subjects under fasting conditions. The oral absorption time profiles were calculated by point-area deconvolution. The compendial paddle and FTC apparatus were used for dissolution tests. Bicarbonate or phosphate-citrate buffer solutions (McIlvaine buffer) were used as the dissolution media. A floating lid was used to maintain the pH value of the bicarbonate buffer solution in the vessel (paddle) or the reservoir (FTC). In the development of bicarbonate FTC method, the pH changes of bicarbonate buffer solution (pH 5.5–7.5; 5–50 mM bicarbonate) were evaluated. For the evaluation of colon targeting tablets, the dissolution profiles of TF and RF were measured at a pH of 7.5. The TF and RF formulations were exposed to 0.01 HCl (pH 2.0) for 2 h before pH 7.5. In the clinical BE study, drug dissolution started 4–8 h after oral administration and continued slowly more than 10 h. Both the area under the curve (AUC) and maximum plasma concentration (Cmax) of TF were approximately twice as high as those of RF. In the development of the bicarbonate FTC method, the pH change of the bicarbonate buffer solution was suppressed by the floating lid within $\Delta pH < 0.1$ over 10 h. In the dissolution test of McIlvaine buffer solutions, TF and RF showed faster disintegration and higher dissolution than those observed in the clinical BE study. When using the paddle apparatus the dissolution profiles of TF and RF in both buffer solutions were not consistent with those of the clinical result. In bicarbonate FTC, the disintegration time, dissolution rate, and dissolution inequivalence between TF and RF were consistent with the results of the clinical BE study. In conclusion, the bicarbonate FTC method was constructed for the first time in this study. This method is simple and practically useful for predicting *in vivo* performance of colon targeting tablets during drug development.

1. Introduction

In the development of generic drug products, it is necessary to demonstrate bioequivalence (BE) between test and reference formulations (Davit et al., 2013; Good ANDA Submission Practices Guidance for Industry, 2022; Kuribayashi et al., 2016). Therefore, in formulation development, it is important to use a dissolution test that can accurately predict *in vivo* performance. Compendial dissolution test apparatuses such as the paddle, rotating basket, flow-through cell (FTC), and reciprocating cylinder methods are widely used in formulation development. Phosphate and citrate buffer solutions are typically used as dissolution media. However, *in vivo*, the lumen of the small intestine and colon only in the fasted state are mainly (not exclusively) buffered with bicarbonate regardless of the gastric conditions (Amaral Silva et al., 2019; Boni et al., 2010; Litou et al., 2016). Phosphate and bicarbonate buffer solutions have been reported to exhibit different dissolution properties for different drugs (Krollik et al., 2022; Sakamoto and Sugano, 2021). Therefore, a dissolution test using a phosphate buffer solution may not necessarily be predictive of *in vivo* performance. This difference was especially pronounced in the dissolution profiles of enteric-coated formulations (Al-Gousous et al., 2019; Amaral Silva et al., 2021; Fadda et al., 2009; Liu et al., 2011; Shibata et al., 2016). The onset time of dissolution (that is, the dissolution of the enteric coating) has been reported to be slower in a bicarbonate buffer solution than in a

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phosphate-citrate buffer solution. Therefore, it is desirable to use bicarbonate buffer solution for dissolution testing.

Bicarbonate shows the following chemical equilibrium in aqueous media:

$$HCO_3^- + H^+ \rightleftarrows H_2CO_3 \rightleftarrows H_2O + CO_2 \quad (1)$$

When a bicarbonate buffer solution comes into contact with the atmosphere, the pH value rapidly increases as CO_2 volatilizes from the solution. Previously, to maintain the pH during the dissolution test, CO_2 was added to the solution through bubbling (Amaral Silva et al., 2019; Merchant et al., 2014). However, CO_2 bubbling requires special equipment and its operation is complicated. In addition, surfactants, such as bile acid and Tween 80, cannot be used because CO_2 bubbling causes foaming.

Recently, we developed a simple method to maintain the pH of a bicarbonate buffer solution using a floating lid in paddle dissolution tests (Sakamoto et al., 2021; Sakamoto and Sugano, 2021). The floating lid prevents CO_2 volatilization from the solution. Although this method is very simple, it can sufficiently maintain the pH value for the duration of dissolution testing ($\Delta pH < 0.1$ over 3.5 h and $\Delta pH < 0.7$ over 22 h). However, the use of bicarbonate buffer solution in the FTC method has not been reported. The FTC method is often used to evaluate controlled-release products because (i) a sink condition can be maintained by continuously supplying a dissolution medium to the dissolution cell, (ii) the dissolution medium can be easily switched during dissolution testing (buffer composition and pH), and (iii) the flow rate can be matched to that *in vivo*.

The purpose of this study was to develop a bicarbonate buffer FTC method. A generic development product (test formulation, TF) and the original product (reference formulation, RF) of mesalazine colon targeting tablets were used as model formulations. This clinical BE study was conducted under fasting conditions. The dissolution profiles were evaluated by the compendial paddle and FTC methods using bicarbonate and phosphate-citrate buffer solutions. The floating lid was used to avoid pH changes in bicarbonate buffer solutions in the vessel (paddle method) and reservoir (FTC method) (Fig. 1).

2. Materials and methods

2.1. Materials

Asacol® 400 mg tablets (RF) were purchased from Zeria Pharmaceutical Co., Ltd. (Tokyo, Japan). Mesalazine 400 mg tablets (TF) were manufactured by Nipro Corporation. Sodium bicarbonate ($NaHCO_3$) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Diluted McIlvaine buffer solution (pH 7.5), HCl, NaCl, and KOH were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Citrate monohydrate and tetrabutylammonium hydrogen sulfate were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

2.2. Methods

2.2.1. Clinical BE study

The BE study was an open-label, two-period, randomized crossover study. The inclusion criteria were as follows: healthy Japanese men, age between 20 and 35 years old, with a body mass index (BMI) ranged from 18.5 to 24.9 kg/m^2; written intention to voluntarily participate in this study after receiving a sufficient explanation of the purpose and content of this study. The main exclusion criteria were as follows: a history of major gastrointestinal surgery; aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and creatinine exceeding the upper limit of the standard values within the implementing medical institution; drug allergy or its history; alcoholism, drug dependence. The subjects ($n = 48$) were divided into three groups. In each group, the subjects ($n = 16$) received three, six, or twelve tablets with approximately 200 mL of water after fasting for a minimum of 10 h. There was a minimum of 10 days between doses in each treatment period. Blood samples were collected before dosing (0 h) and at 4, 8, 12, 13, 14, 15, 16, 18, 24, 36, 48, and 72 h after dosing in each period. Samples were frozen and stored at −90 to −70 °C until anaysis. The plasma concentrations of mesalazine were determined using LC-MS/MS. The analytical method was validated and limit of quantification (LOQ) was 2.00 ng/mL. The values of plasma concentration of four to five subjects were determined to be outliers using the Smirnov–Grubbs test (significance level $\alpha = 0.05$) (Grubbs, 1950). The subjects with outliers were excluded from further analysis. The study was conducted in accordance with the principles of good clinical practice and was approved by the institutional review board (IRB) of the hospital.

2.2.2. Deconvolution method

In this study, point-area deconvolution was used to calculate the *in vivo* drug absorption rate. Response, input, and weight functions were used in this method. The deconvolution integral was defined as follows:

$$R(t) = \int_0^t I(\theta) \cdot W(t-\theta) d\theta \quad (2)$$

where $R(t)$ is the response function, which is the plasma drug concentration at time t following the oral administration of reference or test tablets. $W(t)$ represents the weight function, which is the plasma drug concentration after *i.v.* administration. $I(t)$, the input function, represents *in vivo* drug absorption rate. $W(t)$ was calculated using Eq. (3), using the dose (D), volume of distribution (Vd), and elimination constant (k_{el}) (Bondesen et al., 1991).

$$X(t) = \frac{D}{Vd} \cdot e^{-k_{el}t} \quad (3)$$

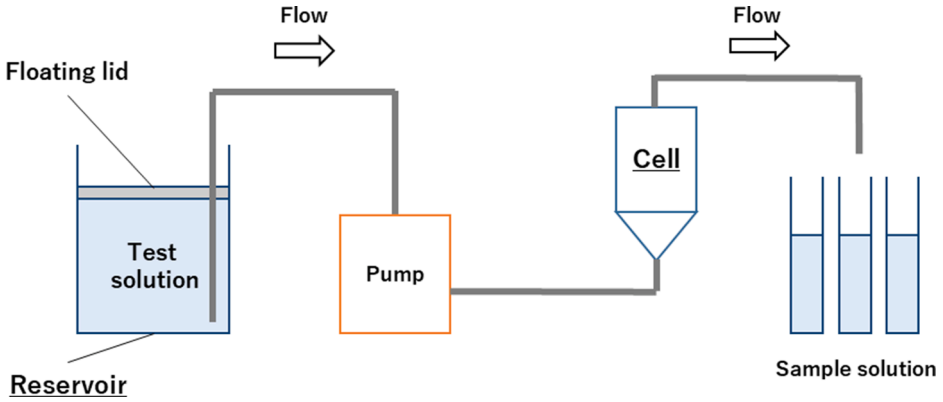

Fig. 1. Schematics of the FTC apparatus. The floating lid was used to maintain the pH value of bicarbonate buffer solutions.

The *in vivo* drug absorption rate *I(t)* was estimated using the *in vivo R (t)* data and *W(t)*. Point-area deconvolution was performed in Microsoft Excel (Vaughan and Dennis, 1978; Yan et al., 2015; Yeh et al., 2001).

2.2.3. Development of bicarbonate buffer FTC method

The bicarbonate buffer FTC method is illustrated in Fig. 1. To maintain the pH, a floating lid was used in the reservoir to prevent volatilization of CO_2 from the bicarbonate buffer solution. The floating lid was made of foamed styrol (5 mm thick). It was designed to cover more than 95% of the buffer solution surface. HCl solution was added to a sodium bicarbonate solution containing 140 mM NaCl to prepare bicarbonate buffer solutions (pH 5.5, 6.5, and 7.5; 5, 10, and 50 mM bicarbonate). The flow rate was set to 4 mL/min. The buffer solution was maintained at 37 °C. The pH values in the reservoirs and cells were measured over 10 h using a portable pH meter (D-220P; Horiba, Kyoto, Japan). For pH values measurement in the cells, the flow was stopped and the cells were removed from the instrument. The pH values were measured by inserting a pH meter into the buffer solution in the cells. The pH values in the reservoir were measured by temporarily removing the floating lid and inserting the pH meter into the buffer solution.

2.2.4. Dissolution test of TF and RF

2.2.4.1. Paddle method. A compendial paddle dissolution apparatus (NTR-6600AS; Toyama Sangyo Co., Ltd., Osaka, Japan) was used. One tablet was initially exposed to 30 mL of 0.01 M HCl containing 140 mM NaCl (pH2.0) at 37 °C for 2 h in a 50 mL polypropylene centrifuge tube (AGC Techno Glass Co., Ltd., Shizuoka, Japan). Each tablet was then moved and placed in a 900 mL vessel filled with 500 mL of a diluted McIlvaine buffer solution (pH 7.5) or a bicarbonate buffer solution containing 140 mM NaCl (pH 7.5) (37 °C, rotation rate: 50 rpm). The surface of the bicarbonate buffer solution was covered with a thick floating lid (5 mm thick). Dissolution samples were filtered with a PTFE filter (0.45 μm), and the drug concentration was measured using high-performance liquid chromatography (HPLC). The buffer capacity (β) of the diluted McIlvaine buffer solution was measured by dropwise addition of HCl (Yoshida et al., 2020). 1 M HCl aqueous solutions were added to 500 mL of a buffer solution while monitoring the pH value using a pH meter (D-220P; Horiba, Kyoto, Japan). The buffer capacity was calculated from the volume of HCl needed for one pH value change of the buffer solution. The buffer capacities of the bicarbonate buffer were calculated using the pKa values (Sakamoto and Sugano, 2021).

2.2.4.2. FTC method. A FTC dissolution apparatus (CE7 Smart; SOTAX AG, Aesch, Switzerland) and piston pumps (CP 7–35; SOTAX AG, Aesch, Switzerland) were used in open-loop mode. A ruby bead (5 mm in diameter) was placed at the bottom of a dissolution cell, which had an inner diameter of 22.6 mm. The conical space of the cell was filled with approximately 6 g of 1 mm glass beads. A glass filter with pore sizes of 0.5 μm (GC-50; Advantec, Tokyo, Japan) was placed at the outlet of the cell. One tablet was then placed on the glass bead layer. The dissolution tests were performed using 0.01 M HCl containing 140 mM NaCl (pH 2.0) initially for 2 h, followed by the diluted McIlvaine buffer solution (pH 7.5) or the bicarbonate buffer solution containing 140 mM NaCl (pH 7.5) for 10 h. The buffer solution was maintained at 37 °C. The flow rates were set at 2, 4, and 8 mL/min. A floating lid was placed on the surface of the bicarbonate buffer solution in the reservoir. The drug concentration in dissolution samples was measured using HPLC.

2.2.4.3. HPLC analysis. The concentration of mesalazine was determined through HPLC using a Shimadzu Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan). The analytical column was an Inertsil C8–3 column (5 μm, 4.6 mm × 150 mm) (GL Sciences Inc., Tokyo, Japan). The mobile phase consisted of acetonitrile: water with 0.25% citrate monohydrate adjusted to pH 6.0 using KOH (15: 85, v/v) containing 0.2% tetrabutylammonium hydrogen sulfate. The column temperature was maintained at 25 °C. The injection volume was 10μL, the flow rate was 1.2 mL/min, and the detection wavelength was 254 nm. The Limit of quantification (LOQ) for each buffer solution was 0.018, 0.003, 0.006 and 0.010 mg/mL (McIlvaine, 5, 10 and 20 mM bicarbonate buffer, respectively).

3. Results

3.1. Clinical BE study

The plasma concentration-time profiles after oral administration of TF and RF are shown in Fig. 2. In the clinical BE study, the area under the curve (AUC) and maximum plasma concentration (Cmax) values of TF were not equivalent to those of RF. The plasma concentrations of TF after oral administration were approximately twice as high as those of RF (Table 1).

The absorption rate was calculated by deconvolution of the Cp-time data (Fig. 3). Deconvolution results showed that the onset of absorption (disintegration time) was 4–8 h after oral administration for both formulations. Drug absorption was continued for over 10 h after the onset of absorption. Approximately 2.7% and 5% of RF and TF were absorbed in 20 h after administration, respectively (six tablets).

3.2. Development of the bicarbonate buffer FTC method

Fig. 4 shows the pH change in the reservoir and the dissolution cell at pH 5.5, 6.5, and 7.5, with bicarbonate buffer concentrations of 5, 10, and 50 mM. For all pH and bicarbonate concentrations, the pH change was less than 0.1 over 10 h.

3.3. Dissolution test of TF and RF

The buffer capacity (β) of the McIlvaine buffer solution was 20.9 ± 0.1 mM/ΔpH (n = 3, mean ± s.d.) . The β values of the bicarbonate buffer solution were 0.4, 0.7, and 1.5 mM/ΔpH (5, 10, and 20 mM bicarbonate, respectively). Fig. 5 shows the dissolution profiles of TF and RF measured using the paddle method with McIlvaine buffer solution. The disintegration time was < 1 h. Approximately 80% of the drug dissolved within 6 h. No significant difference was observed between TF and RF.

Fig. 6 shows the dissolution profiles of TF and RF measured using the paddle method with bicarbonate buffer solution. The disintegration times were 2–4 h. Approximately 50% of the drug dissolved within 10 h. TF dissolved slower than RF.

Fig. 7 shows the dissolution profiles of TF and RF measured using the FTC method with McIlvaine buffer solution. The disintegration times were within 1 h for TF and 2 h for RF. Approximately 100% of the drug dissolved within 6 h. TF dissolved faster than RF.

Fig. 8 shows the dissolution profiles of TF and RF measured using the FTC method with bicarbonate buffer solution. The disintegration time was approximately 4 h for TF and RF. Approximately 40% of the drug dissolved within 10 h. TF dissolved faster than RF.

The dissolution profiles were measured under various experimental conditions using bicarbonate buffer solutions in the FTC method (Fig. 9). The higher the concentration of bicarbonate buffer was, the faster the disintegration time. The disintegration time of TF was faster than that of RF at all concentrations.

The results of the dissolution tests at different flow rates are shown in Fig. 10. The higher the flow rate was, the earlier the onset time. The subsequent dissolution tended to be faster, depending on the flow rate.

4. Discussion

The RF (Asacol®) was a single-unit film-coated tablet using methacrylic acid-methacrylate copolymer (Eudragit® S) that dissolves above

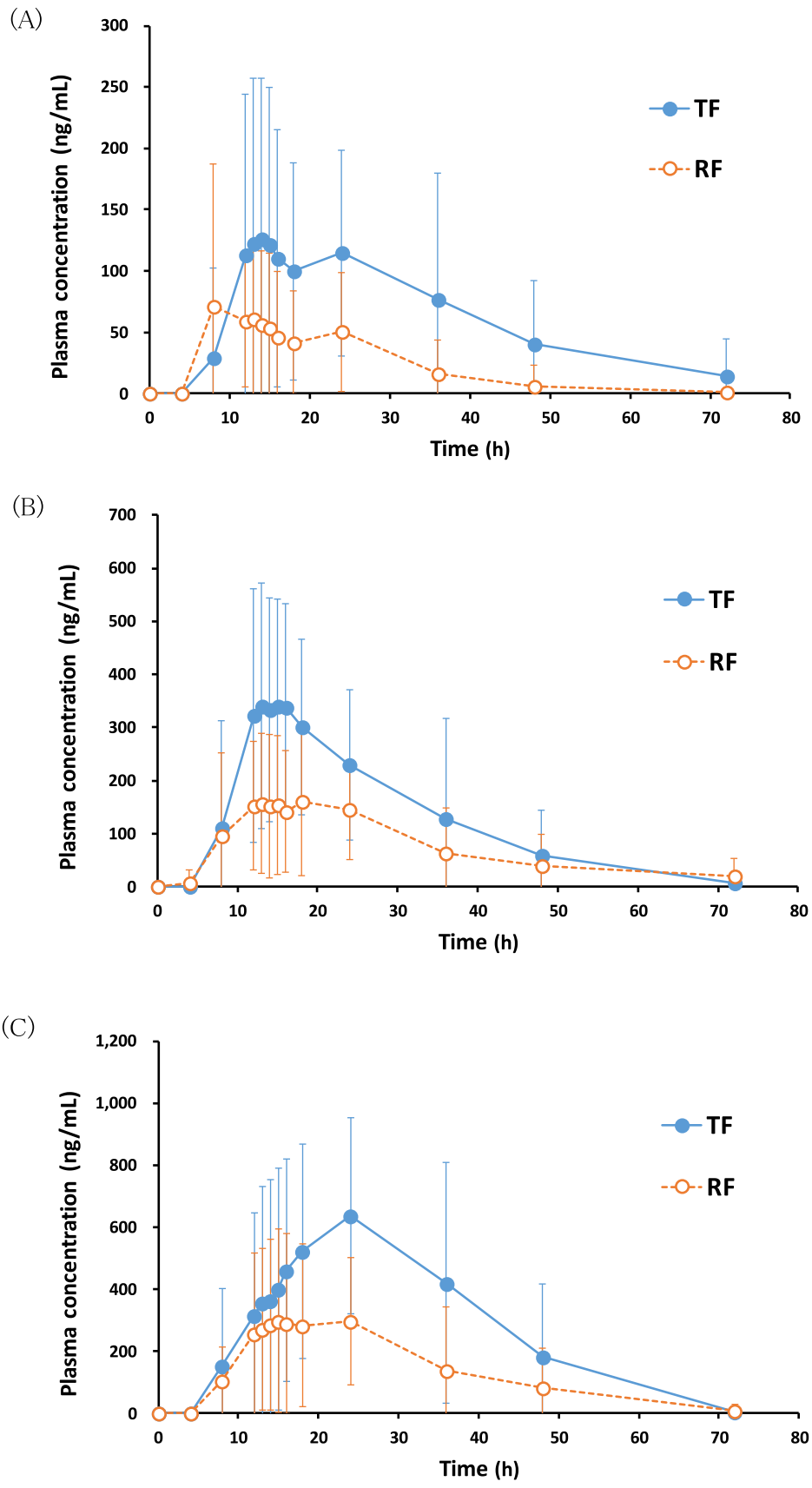

Fig. 2. Plasma concentration time profiles after oral administration of TF and RF formulations (n = 11–12, mean ± s.d.). (A) three tablets, (B) six tablets, and (C) twelve tablets.

Table 1
Pharmacokinetic Parameters obtained in clinical BE study.

Dose	AUC_t (ng·h/mL)			C_{max} (ng/mL)		
	TF[a]	RF[a]	TF/RF	TF[a]	RF[a]	TF/RF
3 tablets	4180 (2390)	1610 (1310)	2.60	221 (133)	108 (105)	2.05
6 tablets	8730 (6310)	5110 (4390)	1.71	407 (227)	211 (140)	1.93
12 tablets	19,400 (13,700)	9390 (8920)	2.07	717 (304)	367 (284)	1.95

AUC_t: area under the curve, C_{max}: maximum plasma concentration, TF/RF: mean ratio of test/reference formulation.
[a] Data shown as mean (s.d.).

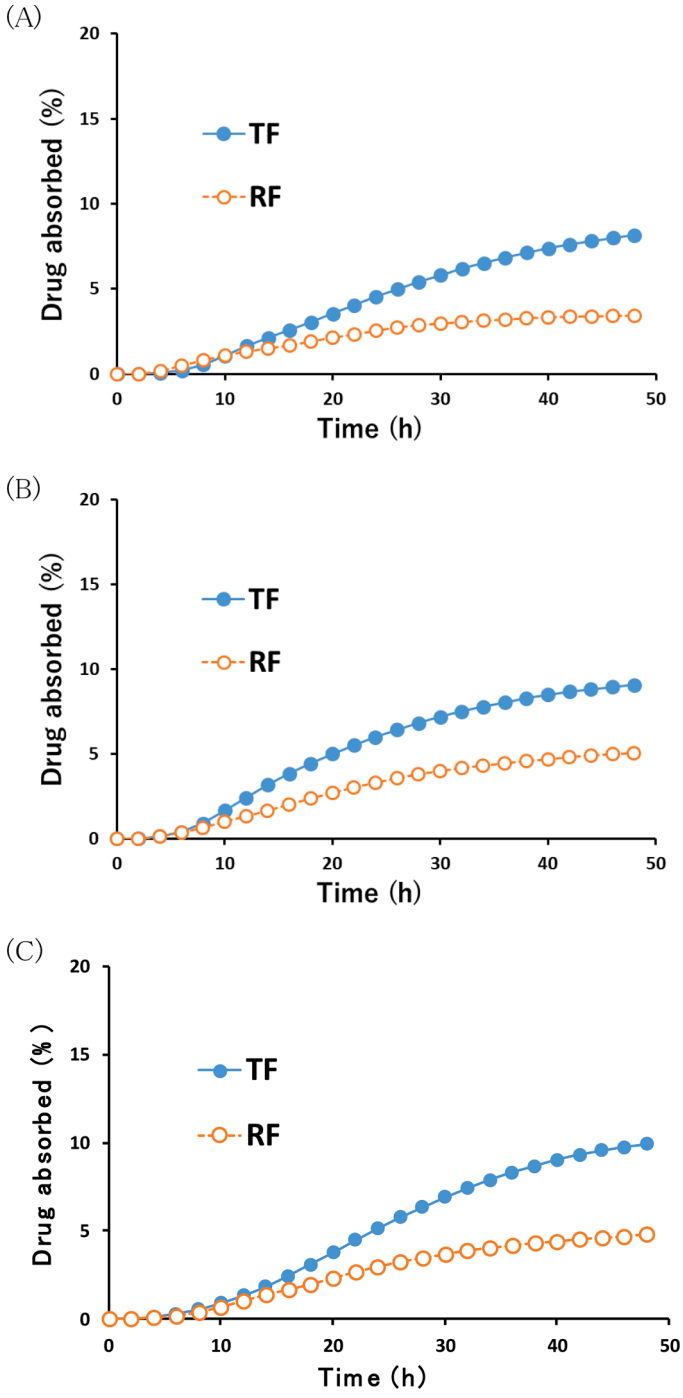

Fig. 3. Percentage of drug absorption. (A) three tablets, (B) six tablets, (C) and twelve tablets.

pH 7.0 (Ansari et al., 2019). It is designed to release mesalazine at the site of inflammation in the colon, where the pH value is 7.0–7.5 (Forbes et al., 2003). The developed product (TF) was also a single-unit film--coated tablet made of the same polymer.

In the clinical BE study, for both formulations, the release onset time was 4–8 h after administration; therefore, 1–5 h after reaching the colon (intestinal transit time is approximately 3 h) (Yuen, 2010). The drug was then slowly released for over 10 h. TF showed mesalazine plasma concentrations approximately twice as high as those of RF.

Exposure of an enteric drug product to acidic conditions can affect its subsequent dissolution at neutral pH (Kambayashi et al., 2013; Liu et al., 2011). Therefore, the TF and RF formulations were exposed to 0.01 HCl (pH 2.0) for 2 h before exposure to a pH of 7.5. In the paddle dissolution test using McIlvaine buffer solution (pH 7.5), the release onset time was less than 1 h for both formulations. No significant difference was observed between the release of RF and that of TF (Fig. 5). Therefore, the dissolution profiles under these conventional conditions did not correspond to the clinical data. In the paddle dissolution test using bicarbonate buffer solution (pH 7.5), the release onset time was 2–4 h, which is in agreement with the clinical data. However, RF dissolved faster than TF, which is in contrast to the clinical data (Fig. 6). Using McIlvaine buffer solution in the FTC method showed that TF dissolved faster than RF, which is in agreement with the clinical data (Fig. 7). However, the release onset time was less than 1 h. Using the bicarbonate buffer solution in the FTC method showed that the release onset time was approximately 4 h. TF dissolved faster than RF. These results are in agreement with the clinical data (Fig. 8). The condition of 10 mM bicarbonate and 4 mL/min flow rate were considered optimal setup from the dissolution onset time and discrimination between TF and RF. Overall, the bicarbonate buffer FTC method was suggested to be the most predictive of *in vivo* performance of mesalazine colon targeting tablets.

There may be several advantages of the bicarbonate buffer FTC method. The fluid flow caused by paddle agitation differs from that *in vivo*. The paddle method induces a stronger agitation force than the FTC method (Fotaki, 2011; Kukura et al., 2004; Morihara et al., 2002). In contrast, the FTC method creates a relatively slow flow that is similar to that *in vivo*. In addition, in the paddle method, coning of insoluble additives at the bottom of the vessel affects the dissolution rate (Higuchi et al., 2015; Mirza et al., 2005). The coning will occur even if the paddle rotation rate is reduced to mimic *in vivo* condition. Therefore, the FTC method has been used for controlled-release formulations (Andreas et al., 2017; Meruva et al., 2020; Özdemir et al., 2000; Skowyra et al., 2015).

It is well known that the onset of enteric formulations is slower in a bicarbonate buffer solution than in a citrate/phosphate buffer solution (Al-Gousous et al., 2019; Fadda et al., 2009; Liu et al., 2011; Shibata et al., 2016). Enteric polymers contain carboxylic acids as functional groups. Enteric polymers dissolve when carboxylic acid is ionized in the buffer solution. Bicarbonate buffer has a weaker buffering effect on the particle surface than phosphoric acid or citric acid buffers (Amaral Silva et al., 2019; Hens et al., 2017; Litou et al., 2020). It is important to use a bicarbonate buffer solution to evaluate enteric formulations (Amaral Silva et al., 2019).

This study demonstrates that it is possible to use a bicarbonate buffer

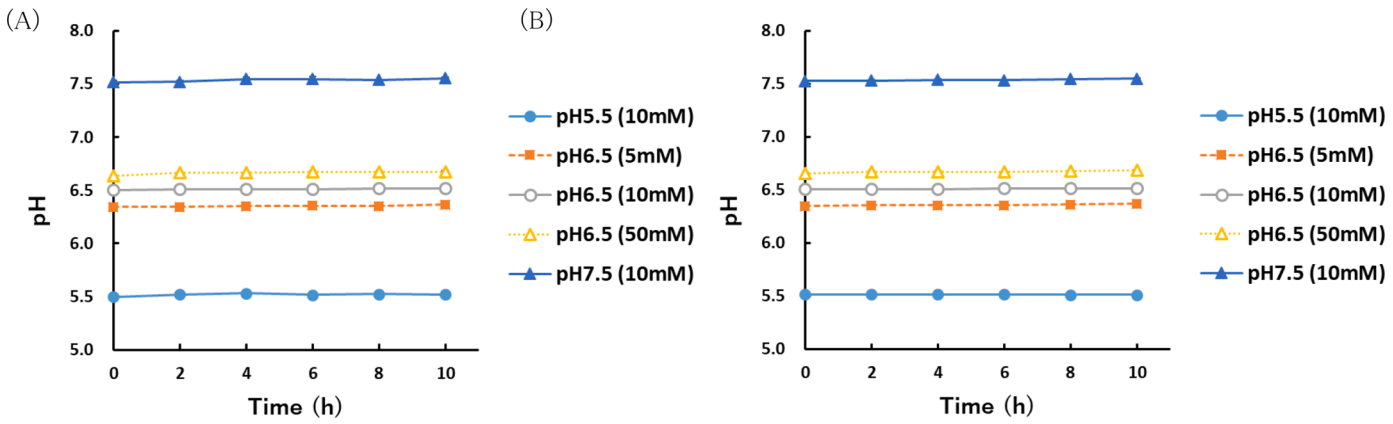

Fig. 4. pH in the reservoir (A) and the dissolution cell (B) (n = 3, mean ± s.d.).

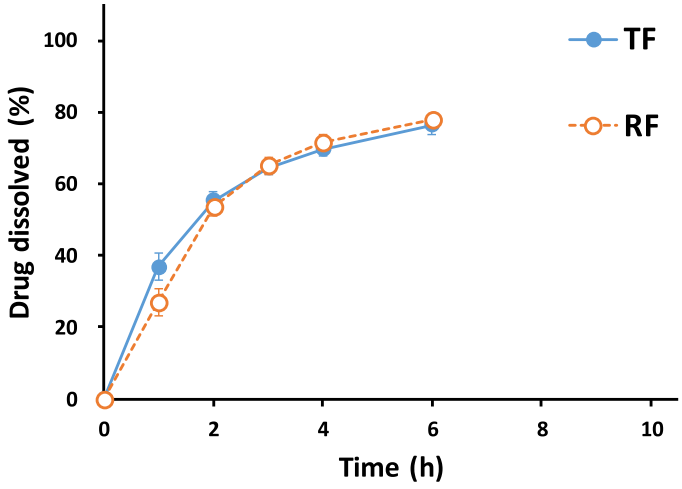

Fig. 5. Dissolution profiles of TF and RF formulations in the paddle dissolution test using the McIlvaine buffer solution (pH 7.5, β = 20.9 mM/ΔpH) (n = 3, mean ± s.d.).

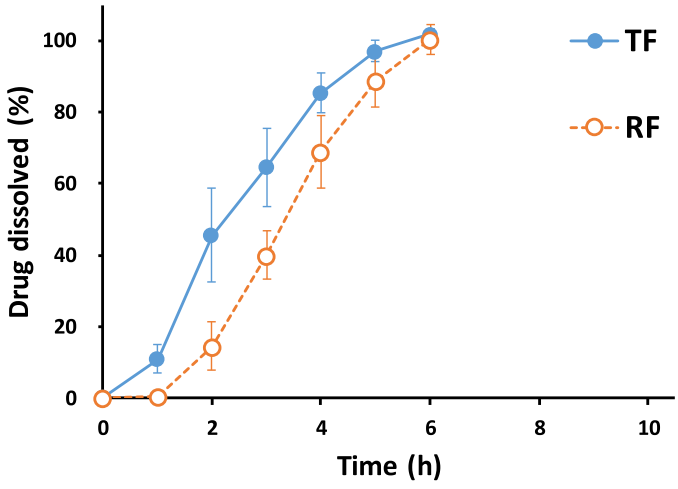

Fig. 7. Dissolution profiles of TF and RF formulation in the FTC dissolution test using the McIlvaine buffer solution (pH 7.5, β = 20.9 mM/ΔpH) (n = 3–4, mean ± s.d.). The X-axis is the time after the pH shift.

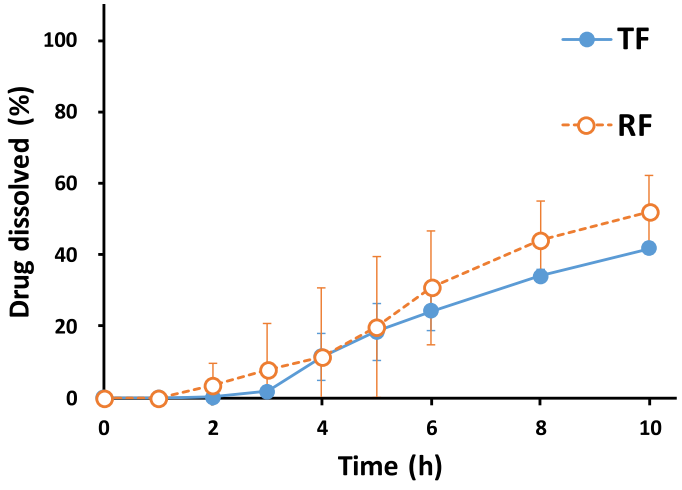

Fig. 6. Dissolution profiles of TF and RF formulation in the paddle dissolution test using the bicarbonate buffer solution (10 mM, pH 7.5, β = 0.7 mM/ΔpH) (n = 3, mean ± s.d.).

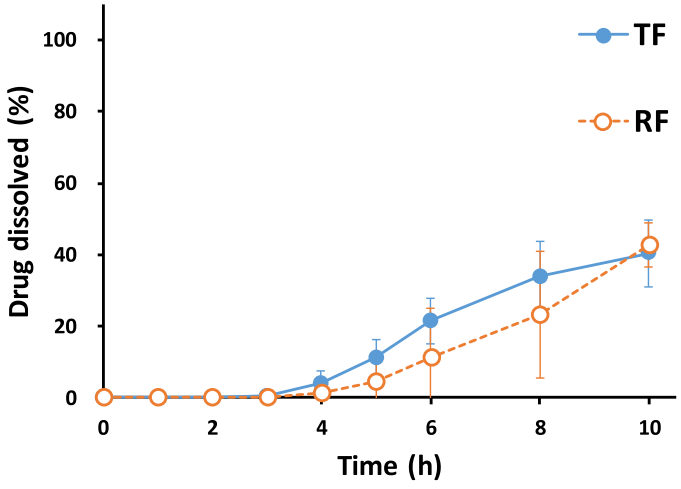

Fig. 8. Dissolution profiles of TF and RF formulation in the FTC dissolution test using the bicarbonate buffer solution (10 mM, pH 7.5, β = 0.7 mM/ΔpH) (n = 3–4, mean ± s.d.). The X-axis is the time after the pH shift.

solution in the FTC method. The floating lid method is simple to use and inexpensive. Therefore, the bicarbonate buffer FTC method is highly practical and versatile for use in drug development. The FTC method can be easily used for pH-shift dissolution tests that can reflect pH changes during gastrointestinal transit.

Some limitations of this study should be noted. In this study, the

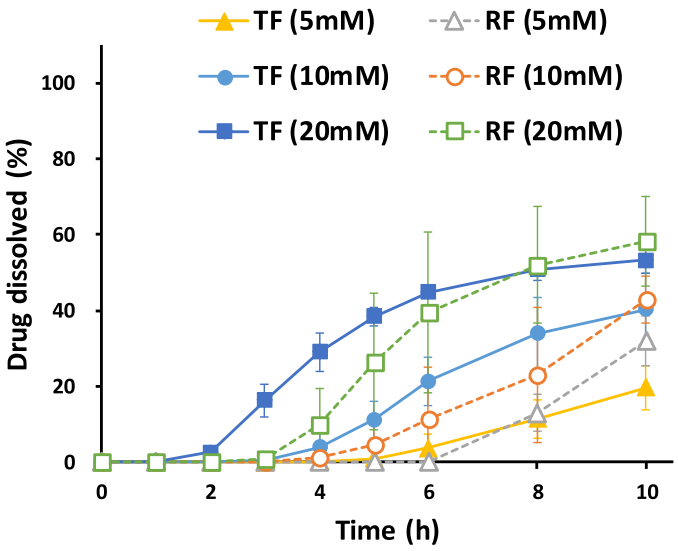

Fig. 9. Effect of bicarbonate buffer concentration on the dissolution profiles of TF and RF in the FTC method (pH 7.5; 5, 10, and 20 mM bicarbonate, $\beta = 0.4$, 0.7, and 1.5 mM/ΔpH, respectively) (n = 3–4, mean ± s.d.). The X-axis is the time after the pH shift.

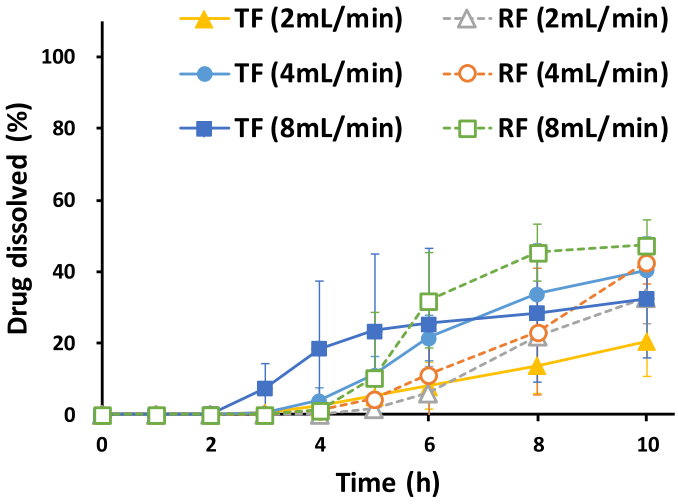

Fig. 10. Effect of flow rate on the dissolution profiles of TF and RF in the FTC method (flow rate: 2, 4, and 8 mL/min, pH 7.5, 10 mM bicarbonate, $\beta = 0.7$ mM/ΔpH) (n = 3–4, mean ± s.d.). The X-axis is the time after the pH shift.

numbers of tablets *in vivo* and *in vitro* tests were different (*in vivo*: 3–12 tablets, *in vitro*: one tablet). TF and RF showed similar differences in the clinical PK parameters independent of the number of tablets. In the clinical BE study, the dose strength was set to be the clinically effective dose (1200 mg (3 tablets) or above). The gastric emptying of a non-disintegrating tablet is affected by migrating motor complex (MMC) (Dooley et al., 1992; Tamás Katona et al., 2022). With single-tablet administration, *in vivo* PK data would vary greatly due to MMC. An administration of multiple tablets may have resulted in a more accurate assessment of TF and RF compared to single tablet administration. When multiple tablets are administered, they are thought to be scattered in the small intestine and/or the colon, rather than piled up in one place. On the other hand, in the dissolution tests, multiple tablets would be piled up at the bottom of the cells or vessels, making it difficult to discriminate differences in the disintegration time and the dissolution rate between TF and RF. According to the guidelines, dissolution tests are generally performed on a single tablet, even if multiple tablets are administered clinically. Therefore, in this study, the number of tablets for *in vitro* dissolution tests was set to be one in consideration of discriminating differences between TF and RF. The effective colonic permeability of mesalazine has been reported to be concentration-dependent. A physiologically-based biopharmaceutical modeling (PBBM) may be required for more accurate bioequivalence. For the use in PBBM, it may be desirable to match the number of tablets and the fluid volume in the *in vitro* and *in vivo* studies for a more accurate comparison.

5. Conclusion

In this study, we successfully developed a bicarbonate buffer FTC method. This system will be useful for predicting *in vivo* performance in drug development. Therefore, the results of this study will contribute to the improvement of prediction of *in vivo* performance using *in vitro* dissolution studies.

CRediT authorship contribution statement

Shotaro Ikuta: Funding acquisition, Data curation, Writing – original draft. **Hidetoshi Nakagawa:** Supervision. **Toshiya Kai:** Supervision. **Kiyohiko Sugano:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

None.

Data Availability

Data will be made available on request.

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