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Design and evaluation of oral formulation for apixaban

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ABSTRACT

Non-valvular atrial fibrillation (NVAF) is a common form of cardiac arrhythmia that affects 1–1.5% of adults and roughly 10% of elderly adults with dysphagia. Apixaban is an anticoagulant referred to as a factor Xa inhibitor, which has been shown to reduce the risk of stroke and systemic embolism in cases of NVAF. Our objective in the current study was to formulate an orally disintegrating film to facilitate the administration of apixaban to elderly patients who have difficulty swallowing. Researchers have used a wide variety of cellulose-based or non-cellulose-based polymers in a variety of combinations to achieve specific characteristics related to film formation, disintegration performance, drug content, *in vitro* drug release, and stability. One of the two formulations in this study was specify that bioequivalence criteria met with respect to Cmax of the reference drug (ELIQUIS®) in terms of pharmacokinetic profile. Further research will be required to assess the applicability of orodispersible films created using colloidal polymers of high and low molecular weights to other drugs with poor solubility in water.

1. Introduction

The aging of global populations is increasing the incidence of non-valvular atrial fibrillation (NVAF), the most common form of cardiac arrhythmia among adults [1,2], particularly among the elderly [3]. Extensive research has demonstrated that when used to treat patients with NVAF, anticoagulation therapy can reduce the risk of ischemic cerebral vascularization by 60% and mortality by 30% [4]. However, compliance with treatment regimens is a serious issue, particularly when dealing with conditions that require long-term therapy, such as NVAF [5]. Dysphagia further exacerbates the problem of compliance in taking oral anticoagulation formulations.

Apixaban is a widely prescribed oral immediate-release anticoagulation treatment used to treat patients with NVAF [6]. However, the fact that it must be swallowed intact can seriously undermine patient compliance. Research has shown that difficulties in

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swallowing can prompt patients to delay, skip, or even avoid medications altogether. Note that non-compliance is widely viewed as a form of medical error [7,8].

In an effort to promote compliance among patients with difficulty swallowing, researchers have developed orally disintegrating tablets [9,10], oral jelly formulations, and orally disintegrating films (ODF) [11]. Orally disintegrating tablets can be taken with little or no water and break down easily; however, the fact that these substances are insoluble means that they remain intact until after they have been swallowed. Jelly formulations are widely used to prevent choking, particularly among the elderly; however, the tablets are bulky and the formulations are prone to causing flatulence. Buccal films disintegrate in saliva without the need for water makes and ensure accurate dosing, and are easy to swallow for elderly and pediatric patients, while avoiding the gastric acid environment. Due to direct contact with the mucosal surface, they also offer site-specific and local effects, providing rapid disintegration and dissolution in the oral cavity. However, the main disadvantage of oral buccal films is that the relatively lower dose of active ingredients can only be accommodated within a limited surface area [12–15]. On the other hand, ODFs share the same advantages as buccal films, as they do not require water, ensure accurate dosing, and are easy to swallow for elderly and pediatric patients. The films provide rapid disintegration and dissolution in the oral cavity and can achieve at least 40% of the dose incorporated into the formulation. They should have tensile strength and non-sticky packaging material [16–18].

Preliminary experiments and clinical trials led us to develop a novel thin film that disintegrates under oral conditions, thereby making it easier for patients to swallow. The bioavailability of the proposed thin film is equivalent to that of the immediate-release tablet ELIQUIS®.

2. Materials and methods

2.1. Chemicals

Apixaban was obtained from Natco Pharma Ltd. (Telangana, India). The samples were more than 99.9% pure, as stated on a certificate of analysis provided by the manufacturer. Hypromellose 2910 K4M and E15 were purchased from Ashland Inc. (Covington, USA). Macrogol 6000 was purchased from NOF Corporation (Tokyo, JP). POLYOX™ Water-Soluble Resins (WSR) (Polyethylene oxide) were received as a gift from Colorcon Ltd (Wayne, USA). Glycerin was purchased from Merck (Darmstadt, Germany). Sucralose was purchased from JK Sucralose Inc. Maltose was provided by the manufacturer of SPI Pharm Inc (Michigan, USA). H_2O was purified using the Milli-Q system (Millipore Corp., Bedford, MA). Two formulations (A and B) were provided by TAHO Pharmaceuticals Ltd. ELIQUIS® 5 mg tablets were purchased from Pfizer and Bristol Myers Squibb [19].

2.2. Orodispersible films

2.2.1. Film preparation

The proposed film was prepared via solvent casting. The drug solution was prepared using a film-forming agent with a plasticizing agent, flavoring agent, and apixaban in a mixing vessel until homogenous. The drug solution was then cast on a release liner at a specific thickness and allowed to dry at 60–100 °C where the film has less than 10% water content. The resulting thin films were held at 25 °C for 24 h to reach the desired moisture saturation while maintaining their material properties. The dried film was then die-cut into squares (2×2 cm^2), each containing 5 mg of apixaban. The squares were then individually packaged in an aluminum foil pouch and stored at room temperature.

2.2.2. ODF: formulation and assessment

The effectiveness of polymer-based drug systems depends largely on the selection of excipient [20,21]. In the current study, multiple polymers (cellulose-based and/or non-cellulose-based) were used with a broad range of excipients, including plasticizers, disintegrants, and fillers. The formulations of two drug-loaded ODF were determined by reviewing the effects of excipient on pharmacokinetic properties of apixaban [16,17,20,22]. The excipients include film-forming polymers, plasticizers, sweeteners, colorants, fillers and flavors. Formulation A and B showed a nice film surface, good ductility, and rapid dissoluble properties. Based on these results, we used formulation A and B to measure further ODF technological properties in our study.

Currently the two prescriptions have been obtained through a series of tests within the above range. The two final formulations contained the active pharmaceutical ingredient, plasticizers, cellulose-based film polymers (HPMC K4M and HPMC 15 cps), and non-cellulose-based polymers (Polyox WSR (N80)). Formulation A consists of apixaban (10%), HPMC K4M (15%), HPMC 15 cps (20%), PEG 6000 (20%), maltose (25%), and glycerin (10%). Formulation B consists of apixaban (10%), HPMC K4M (10%), Polyox WSR (N80) (20%), PEG 6000 (20%), maltose (20%), starch 1500 (15%), and glycerin (5%).

2.3. Pharmaceutical properties of apixaban ODF

2.3.1. Thickness and characteristic evaluation

The thickness of the film (in the middle and at each of the four corners) was measured using a micrometer screw (Mitutoyo, Neuss, Germany). Note that the values were averaged from ten samples, and the experiments were performed in TAHO Pharmaceuticals Ltd.

2.3.2. Tensile properties

The mechanical properties of tensile strength (TS) were measured using a tensile testing machine (H1KS; Tinius olsen, England)

[23]. The film strips (sample size: 20 mm × 20 mm) were vertically fixed with two grips at the initial separation distance of 10 mm, and then pulled by a constant rate of 1.0 mm/s. The maximum fracture force (the force reached just before the film strips ruptured) was recorded. The measurements were repeated in triplicate using three film samples for each type of formulation. The TS (N), elastic modulus (MPa), break stress, and the strain at break (%) were computed during the tensile test, and this method followed ASTM D882-10: Tensile properties of thin plastic sheeting. The experiments were performed in TAHO Pharmaceuticals Ltd.

2.3.3. Evaluation of pH values

Determining the pH of OTFs is important for their solubility/dispersibility in the oral cavity, and rapid release of the active substance in the oral cavity. For this purpose, add 5 mL of artificial saliva (pH 6.8) was added to a 10 mL Centrifuge tube with the ODF sample containing active pharmaceutical ingredient and ensure swelling [24]. After swelling, measure the pH using a pH meter (S220, Mettler Toledo, Switzerland).

2.3.4. Disintegration testing

The term disintegration refers to a physical process involving the mechanical breakdown of a substance into constituent particles. The experiment setup included a basket rack, a 1000 mL low-form beaker containing 600 mL test medium, a fluid heating system with thermostatic control, and a system to immerse the disintegration device or remove the basket at a set rate. Note that the test fluid in the disintegration device was water (37 ± 2 °C). The experiment involved immersing samples in the liquid and then recording the disintegration of the film as a function of time. The experiments were performed in TAHO Pharmaceuticals Ltd.

2.3.5. Solubility evaluation and selection of suitable condition

Apixaban is an anhydrous compound exhibiting high thermodynamic stability, low permeability, and low solubility in water. Solubility testing was used the characterize the solubility of apixaban for use in devising a suitable dissolution method. Fig. 1 indicates the solubility of apixaban as a function of pH (pH 1.0–13.0). The highest overall solubility was observed at pH 4.0, while the lowest solubility was observed at pH 6.0. In accordance with standards established by the United States FDA (Division of Bioequivalence, Office of Generic Drugs), the analysis of dissolution was performed using 0.05 M Sodium Phosphate Buffer with 0.05% SLS at pH 6.8.

2.3.6. In vitro dissolution

The *in vitro* dissolution of the various platforms was assessed using the United States Pharmacopeia paddle-over-disk apparatus in tests lasting 1 h. Testing was performed in 900 mL of a phosphate buffer solution with 0.05% Sodium lauryl sulfate (SLS) (pH 6.8 at 37 °C) with the paddle speed operated at 75 rpm. The quantity of apixaban in the dissolution medium was measured via isocratic high-performance liquid chromatographic (HPLC) with UV detection at 275 nm. Samples (1.5 mL) were collected over a period at 5-, 10-, 20-, 30-, 45-, and 60-min after immersion to establish dissolution profiles. Samples were then analyzed to determine the drug content via HPLC with a UV–Vis detector at 275 nm (Agilent 708-DS, 10 μL loop; manual injector; UV–visible wavelength detector) with an XBridge C18 column (4.6 mm ID × 50 L mm, 3.5 μm particle diameter, 30 °C) using potassium phosphate monobasic buffer/acetonitrile (70/30, v/v) as the mobile phase delivered at a flow rate of 0.8 mL/min. The experiments were performed in TAHO Pharmaceuticals Ltd.

2.3.7. Quantitative chromatographic analysis

HPLC was developed for the identification of drug substances and analysis of contamination. This analysis involves isocratic and gradient reversed-phase separation using a pump (Waters e2695; Waters Corporation, Milford, MA, USA), fluorescence detector (W2475; Waters Corporation, Milford, MA, USA), and chromatographic Insert Sustain C18 column (4.6 mm × 250 mm, 5 μm; Waters Corporation, Milford, MA, USA) with UV detection at 275 nm. Testing was performed at 25 °C with the flow rate set to 0.8 mL/min.

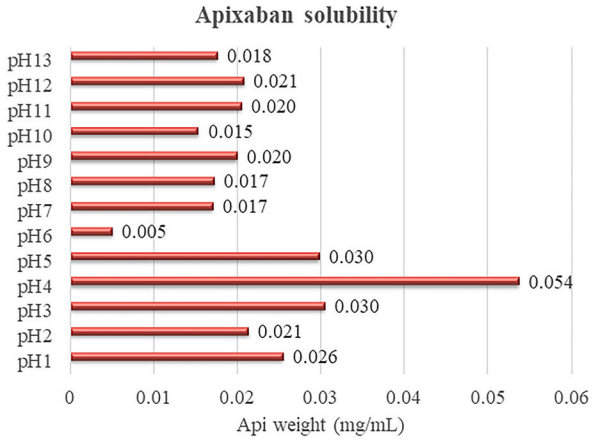

Fig. 1. Saturation solubility and relative pH buffer conditions of apixaban in dissolution media (TAHO Pharmaceuticals Ltd, Taiwan, R.O·C.).

Mobile phase A consisted of 10 mM potassium phosphate monobasic solution/ACN (80/20, v/v), whereas mobile phase B consisted of 10 mM potassium phosphate monobasic solution/ACN (20/80, v/v). The injection volume was 30 μL. The retention time of apixaban was 11.1 ± 10% min. Chromatograms were evaluated using Empower 2 Software (Waters Corporation, Milford, MA, USA).

2.3.8. Drug assay and impurity

Two Apixaban orodispersible films, each weighing 5 mg, were transferred to a 20 mL volumetric flask, and diluted with a diluent until reaching the mark. The diluent used is a mixture of acetonitrile and water in a ratio of 40:60 (v/v). Subsequently, 4 mL of the resulting solution was transferred to a 10 mL volumetric flask and further diluted with the same diluent until reaching the mark. The samples were thoroughly mixed and then filtered using a 0.45 μm nylon filter. The calculation formulas for assay of ODF and Impurities of ODF are as follows.

2.3.8.1. Assay of ODF

$$\text{Assay } (\%) = \frac{A_{SAM}}{A_{STD}} \times \frac{D_{SAM}}{D_{STD}} \times \frac{100}{W_{SAM}} \times W_{STD} \times P$$

2.3.8.2. Impurities of ODF

$$\text{Impurity } (\%) = \frac{A_{IMP}}{A_{STD}} \times \frac{D_{SAM}}{D_{STD}} \times \frac{100}{W_{SAM}} \times W_{STD} \times P \times \frac{1}{RRF}$$

AIMP = The peak area of impurity in the sample solution.
ASTD = The average peak area of Apixaban obtained from the Apixaban Standard Solution for Impurity injections.
DSAM = The dilution factor of the Sample Solution.
DSTD = The dilution factor of Apixaban Standard Solution for Impurity.
WSAM= Label claim (mg) of sample preparation.
WSTD= The weight (mg) of Standard Solution.
P = Purity Factor of Apixaban reference standard (as is).
RRF = Relative Response Factor of specified impurities.

The relative response factors (RRF) and relative retention times (RRT) for specific impurities, including Impurity-A[1], Apixaban, Impurity-B[2], Impurity-C[3], and Impurity-D[4], correspond to the following RRF and RRT values: Impurity A[1] (0.46, 0.89), Apixaban (1.0, 1.0), Impurity-B[2] (1.56, 0.72), Impurity-C[3] (1.73, 0.78), and Impurity-D[4] (2.08, 1.16). The numbers on the remark correspond to the following chemical formulas: [1] 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxo-1-piperidyl)phenyl]-4,5-dihydropyrazolo [3,4-*c*]pyridine-3-carboxylic acid (ABN-Acid). [2] *N*-formyl-1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxo-1-piperidyl)phenyl]-4,5-dihydropyrazolo [3,4-*c*] pyridine-3-carboxamide (*N*-formyl impurity). [3] Methyl 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxo-1-piperidyl) phenyl]-4,5-dihydropyrazolo [3,4-*c*]pyridine-3-carboxylate (methylester impurity). [4] Ethyl 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxo-1-piperidyl) phenyl]-4,5-dihydropyrazolo [3,4-*c*]pyridine-3-carboxylate (ABN-1).

2.3.9. Stability studies

Stability studies were conducted for formulation A and B films in accordance with the guidelines of International Conference on Harmonization (ICH). These studies included accelerated stability testing at 40 °C and 75% relative humidity, as well as storage stability testing under two additional conditions: room temperature (25 °C) and intermediate temperature (30 °C), both with 75% relative humidity. The stability tracking was performed at three time points: the beginning, one month, and six months. The parameters assessed during these stability tests included appearance, content, impurities, and moisture content. The experiments were performed in TAHO Pharmaceuticals Ltd.

2.4. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed using a model TA AQ2000/RCS 90 calibrated using indium. We recorded DSC thermograms of pure apixaban, polymers (HPMC 15 cps, HPMC K4M, Polyox N80), plasticizer (PEG 6000), blank film, and drug-loaded film. This involved weighing samples of roughly 3 mg into an aluminum pan, which was then sealed, before heating the samples from 40 to 300 °C at a ramp rate of 10 °C/min under an inert nitrogen atmosphere. Measurements to determine the melting and transition points were performed via software provided with the device, and the experiments were performed in TAHO Pharmaceuticals Ltd.

2.5. ATR-FTIR study

A Nicolet iS10 FTIR ATR spectrophotometer (Thermos Fisher Scientific) was used to obtain infrared transmission spectra of apixaban as well as 1:1 w/w physical mixtures of apixaban and excipients (HPMC K4M, HPMC 15 cps, and Polyox N80), film formulation A, blank film formulation A, film formulation B, and blank film formulation B. Scanning was performed over a wavelength range of 650–4000 cm^{-1}, and each sample was scanned 32 times to derive the final spectrum. The data were analyzed using OMNIC Spectroscopy software, version 9.5.9 (Thermos Fisher Scientific, U.S.), and the experiments were performed in TAHO Pharmaceuticals

Ltd.

2.6. In vivo studies in rats

2.6.1. The method of drug administration

The animal testing part involves administering the drug orally to the animals. The reference product (Eliquis Apixaban 5 mg) and the test product (ODF 5 mg) will be administered once a day by mouth, with a dose of 5 mg of Apixaban per kilogram of body weight. The drug concentration will be 5.0 mg/mL. Six-month-old rat was selected for testing and could be administered the drug while in a fasting state. According to rat weight, the drug could be converted to the appropriate dose before administration, the dosage was administered orally via gavage.

When evaluating the method of administration, since it was not possible to successfully put the orodispersible film into the mouth of the rat. Because the rat would keep spitting out the film during the process, the dosage was administered orally via gavage.

2.6.2. In vivo absorption

Animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and regulations related to Animal Care (National Institute of Health), and were approved by the Institutional Animal Care and Use Committee, Chang Gung University Taiwan (IACUC approval No. CGU110-059).

Experiments were performed on healthy 8-week-old male Wistar rats to compare proposed formulations A and B versus the reference drug (ELIQUIS®, Lot number ABB0859) in terms of *in vivo* absorption. The animals were fed ad labium. Apixaban was administered orally to six rats at a dosage of 5 mg/kg. The drug concentrations were 5.0 mg/mL in normal saline, and they were administered orally using gavage. At various time points after oral administration (0.25, 0.5, 1, 2, 4, 6, and 24 h) [25], blood samples were withdrawn into a blood collection tube containing EDTA. The samples underwent centrifugation at 1000 *g* at 4 °C for 15 min, after which the supernatant (plasma) was stored at −80 °C until assayed.

2.6.3. Sample preparation

Frozen rat plasma samples were left to reach room temperature. Individual samples (20 μL) were quenched using 200 μL ACN and then vortex-mixed for at least 15 s followed by centrifugation at 12,000 *g* at 4 °C for 25 min. Samples of the supernatant (100 μL) were then measured using LC-MS/MS.

2.7. Bioavailability

This study recruited 12 healthy subjects aged between 18 and 55 years with a body mass index (BMI) ranging from 18 to 30 kg/m2. The inclusion criteria involved assessing their overall health through a thorough evaluation of their medical history, physical examination, chest radiography, and serum laboratory analysis. Additionally, the assessor determined normal prothrombin and activated partial thromboplastin values during the screening process. Exclusion criteria were applied to individuals with a history or evidence of hepatic, hematologic, renal, or gastrointestinal abnormalities, as well as any acute or chronic comorbidities. Furthermore, participants were required to abstain from all forms of tobacco and nicotine. These precautions were implemented to ensure that any observed variations in independent variables were not influenced by underlying illnesses or medication use.

All subjects were instructed to fast (except for water) for at least 10 h prior to dosing and at least 4 h after dosing. On Day 1, all subjects were required to arrive at the clinic at least 10 h before the scheduled dosing time and remain confined for a minimum of 48 h after dosing. Each participant received a single 5 mg dose of either 1 × apixaban Oral Film [Formulation A or B] or 1 × ELIQUIS® (apixaban) Tablet [26,27].

Food does not have a clinically meaningful impact on the bioavailability of apixaban [28]. It is speculated that water administration should not affect the clinical performance of human bioavailability [29].

The oral film was placed on the top of the tongue, and subjects were instructed to close their mouth naturally and refrain from swallowing until the film completely dissolved. Once the film had fully dissolved, subjects were provided with 240 mL ± 2 mL of room temperature water to rinse and swallow, ensuring that any residue of the film was ingested. Additionally, the subjects who received the reference treatment (1 × ELIQUIS® (apixaban) Tablet) were instructed to swallow the tablet whole without chewing or biting, using 240 mL ± 2 mL of room temperature water.

The subjects were assigned to receive one of the two test formulations, or the reference drug based on a six-sequence randomization schedule. The sequence of administration, denoted as 'T1T2 R′ or 'RT1T2′ or 'T2RT1′ or 'T1RT2′ or 'T2T1 R′ or 'RT2T1′, was determined according to the randomization schedule, where R represents the reference product and T1 and T2 represent the test products. During each study period, blood samples were collected prior to dosing and at 48 h intervals after dosing. Subjects were required to remain confined at the clinic for at least 10 h prior to dosing and until the completion of blood sample collection at 48 h post-dosing. The doses were administered at intervals of at least 5 d. It is important to note that the half-life ($t_{1/2}$) of the apixaban oral film was determined to be 11.5 h. Therefore, a 5-d washout period was implemented between the initial platform and the administration of the alternate platform to minimize the potential for carry-over effects.

This study was performed in accordance with the current revision of the Declaration of Helsinki, Good Clinical Practice, and Good Laboratory Practice. The clinical investigators provided the eligible subjects with comprehensive information regarding the objectives and potential risks associated with the study. Written informed consent was obtained from all participants before their participation in any study-related activities. Experimental protocol was approved by the Ethics Review Board of Optimum Clinical Research Inc.

(approval no. 0062–21).

2.8. Statistics

The dissolution rate of the formulation, pharmacokinetic data in rats and humans were presented as mean ± SEM. Other data was presented as mean ± SD. Student's t-test was used for the statistical analysis, except for the pharmacokinetic study of apixaban, which employed Two-way ANOVA. $P < 0.05$ were considered to indicate statistically significant differences. Statistical analyses of the pharmacokinetic study of apixaban in humans were performed using the PROC MIXED SAS® program.

3. Results and discussion

Apixaban ODF was prepared via solvent casting with HPMC K4M as the main film former on a release liner. Propylene glycol was used as a plasticizer, and distilled water was used as a solvent. We assessed two formulations (A and B), which differed in terms of polymer type.

3.1. Pharmaceutical properties of apixaban ODF

3.1.1. Comparison of a and B formulations

Formulations A and B were compared in terms of appearance, size, and thickness. Overall, the observable differences were containing thickness, tensile strength, elastic modulus, break strain in the physical and mechanical characteristics of apixaban ODF containing formulation A and B (Table 1, and Table 2). Tables 1 and 2 present the pH and mechanical properties of the orodispersible films, including tensile strength, Young's modulus, and break strain. Formulation A exhibited higher mechanical properties compared to Formulation B in terms of tensile strength, Young's modulus, and break strain. However, the pH values of the orodispersible films were similar for both formulations.

3.1.2. Drug assay and uniformity of drug content

The drug content in the developed oral orodispersible films ranged between 98% and 102%. For prescription A and prescription B, the drug content was measured as 100.65 ± 1.20% and 101.11 ± 0.50%, respectively. In terms of content uniformity, the test values for formulation A and B were found to be 5.30 ± 0.12 mg and 5.10 ± 0.31 mg, respectively.

3.1.3. Disintegration test

The term disintegration refers to a specific physical process involving the mechanical breakdown of a substance into constituent particles. In order to clearly understand whether the orally dissolving film can be disintegrated in a short period of time, USP <701> is used to evaluate it. The main reason for this method is to understand the disintegration performance of the drug in oral preparations, and when disintegration does not imply complete solution of the unit or even of its active constituent.

The disintegration test is to observe the disintegration status of the test drug in a medium. The drug needs to be disintegrated before it can be further released. The release situation of the drug can be understood through its disintegration status. Therefore, it is hoped to simulate the disintegration time and appearance of the test drug in different mediums that appears to be drug release.

The rationale for using the US Pharmacopeia (USP) rapid disintegration test is that it is a widely accepted *in vitro* testing protocol for orally disintegrating formulations. The USP rapid disintegration test provides a standardized and reproducible method to evaluate the disintegration performance of orodispersible films and other orally disintegrating formulations in a short period of time. We believe that using this established method allows for a meaningful comparison and assessment of the disintegration properties of the developed ODFs.

These assessments were performed in four mediums, including artificial saliva (pH 6.5), PBS (pH 1.2), PBS (4.0), and water (Table 3). These tests were meant to simulate the disintegration of the ODF as it progresses through the human body, starting with the mouth (artificial saliva), followed by a gastric environment (PBS pH 1.2) and then the anterior segment of the small intestine (PBS pH 4.5), with water used as a control [30,31].

Our analysis revealed that Formulation B disintegrated more rapidly than did Formulation A, regardless of the buffer in which it was immersed. The disintegration of Formulation A was more rapid in PBS pH 4.5 than in the other buffers. This was not observed with formulation B. The fact that apixaban is pH-independent indicates that the pH-dependent variation in disintegration can be attributed to the constituents of the film polymers and other excipients.

Table 1
Properties of apixaban ODF containing formulation A and B.

Formulation	Appearance	Size (cm^2)	Thickness (mm)	Label claim (mg)
A	White and opaque	2 × 2	0.145 ± 0.007	5
B	White and opaque	2 × 2	0.202 ± 0.008	5

Physical evaluations were analyzed statistically using a Student's t-test. Probability values ($p = 0.0000000000015371$) were considered to indicate statistically significant differences.

Table 2
Physical and mechanical characteristics of apixaban ODF containing formulation A and B.

Formulation	Tensile strength (N/mm^2)		Elastic modulus (MPa)		Break strain (%)		pH	
	Ave	Std	Ave	Std	Ave	Std	Ave	Std
A	20.63	1.84	3.51	0.53	2.09	1.76	6.42	0.02
B	4.00	1.33	2.12	0.57	0.40	0.16	6.45	0.02

Method: ASTM D882-10: Tensile properties of thin plastic sheeting.
Physical evaluations were analyzed statistically using a Student's t-test. Probability values of $p < 0.05$ were considered to.
Indicate statistically significant differences.

Table 3
Disintegration time of ODF containing formulation A and B in different pH buffers.

Formulation/Medium	pH 1.2	pH 4.5	Artificial liquid (pH 6.5)	H_2O
Time (seconds)				
A	47 ± 5	38 ± 4	50 ± 6	53 ± 5
B	32 ± 7	29 ± 5	23 ± 3	24 ± 4

3.1.4. Water content

Water content plays a key role in the stability of thin films [32,33]. Researchers have demonstrated that under humid storage conditions, the absorption of moisture by co-amorphous systems can interfere with molecular interactions, lowering the Tg (glass transition temperature), increasing molecular mobility, as well as promoting amorphous-amorphous phase separation and recrystallization [34–36].

Active pharmaceutical ingredients and excipients differ in terms of hygroscopic properties. This can lead to unintended processing-induced phase transitions as well as solid-state phase transitions in the final dosage form [37]. As shown in Table 4, the water content of formulation A (6–7%) did not differ significantly from that of formulation B (4–6%), such that the long-term stability should be unaffected.

3.2. In vitro dissolution

In discussing the bioavailability of drugs, it is crucial to consider that the rapid disintegration of the film does not necessarily equate to rapid absorption *in vivo*. In other words, even complete dissolution cannot guarantee the complete release of the drug. Our objective was to determine how the difference in formulation affected drug release. At 10 min post-immersion, the dissolution rate of formulation B was 50% faster than that of formulation A (Fig. 2). Subsequent analysis was aimed to determine whether the difference in dissolution rate corresponded to a comparable difference in drug absorption in animals or humans.

3.3. DSC characterization

DSC was used to assess the compatibility of the active ingredient with the polymers used in the film. Fig. 3 presents DSC thermograms of apixaban, hypromellose, polyox PEO N80, PEG plasticizer, blank-formulation A film, formulation A film, blank-formulation B film, and formulation B film. The melting point of apixaban was 238 °C. PEG presented a significant endothermic peak at 63 °C with an exothermic peak at 151 °C. Polyox (a subbranch of PEG with a high molecular weight) presented the same

Table 4
Stability of the ODF by summarizing water content presence data for ODFs tested at long-term storage conditions and accelerated condition.

Formulation	Storage Condition	Test Interval	Appearance (film)	Water Content (%)
A	25 °C/60%RH	Initial	2 cm × 2 cm	7.44 ± 0.05
		1 month	2 cm × 2 cm	7.73 ± 0.63
		6 months	2 cm × 2 cm	6.29 ± 0.26
	30 °C/75%RH	6 months	2 cm × 2 cm	7.28 ± 0.38
	40 °C/75%RH	1 month	2 cm × 2 cm	7.94 ± 0.44
		6 months	2 cm × 2 cm	7.73 ± 0.33
B	25 °C/60%RH	Initial	2 cm × 2 cm	5.96 ± 0.13
		1 month	2 cm × 2 cm	5.26 ± 0.09
		6 months	2 cm × 2 cm	4.95 ± 0.95
	30 °C/75%RH	6 months	2 cm × 2 cm	6.05 ± 0.54
	40 °C/75%RH	1 month	2 cm × 2 cm	6.04 ± 0.24
		6 months	2 cm × 2 cm	5.13 ± 0.90

(TAHO Pharmaceuticals Ltd, Taiwan, R.O·C.).

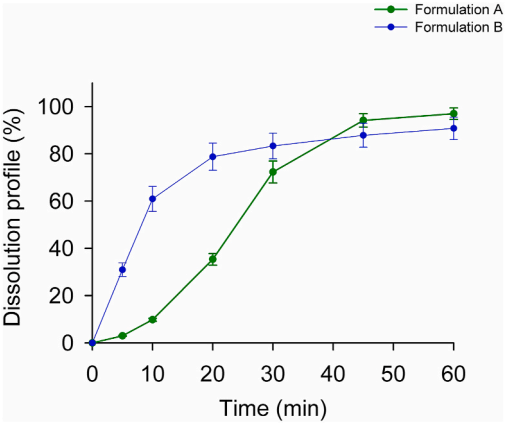

Fig. 2. Dissolution profiles of ODFs containing formulation A (A) and formulation B (B) stored at three conditions as initial state. Data illustrate stability of ODF by comparing dissolution profiles using 0.05 M Sodium Phosphate Buffer with 0.05% SLS, pH 6.8. Each data point is presented as mean ± SEM (n = 6) (TAHO Pharmaceuticals Ltd, Taiwan, R.O·C.).

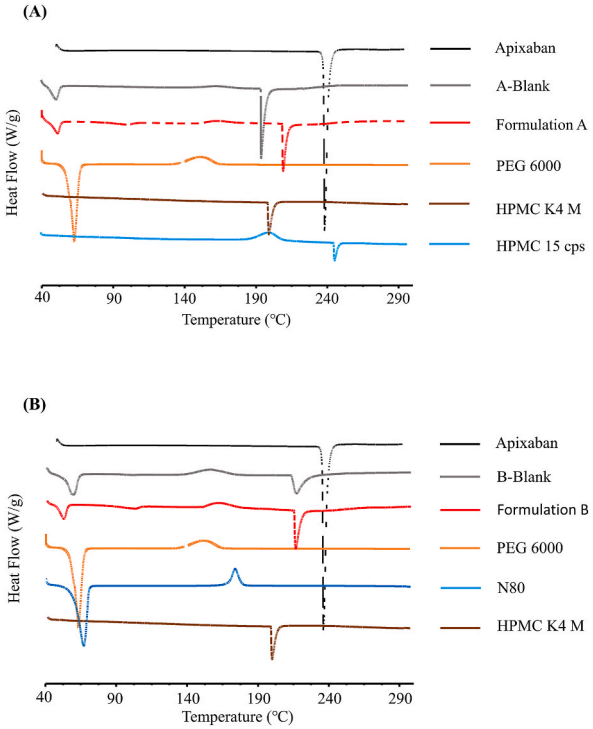

Fig. 3. Differential scanning calorimetry (DSC) thermogram generated for apixaban of each formulation and related potential excipients. (A) PEG 6000, HPMC 15 cps, HPMC K4M, A-blank, and formulation A. (B) PEG 6000, Polyox N80, HPMC K4M, B-blank, and formulation B. Blank-formulation is mean formulation without apixaban.

endothermic peak at 66 °C with an exothermic peak at 172 °C. HPMC 15 cps (a subtype of hypromellose with a lower molecular weight) presented an obvious exothermic peak at 200 °C with an endothermic peak at 246 °C. HPMC K4M (a polymer belonging to the hypromellose group with a high molecular weight) presented an endothermic peak at 199 °C. The film comprising a polymer and plasticizer presented the hypromellose structure of a polymer (with a high molecular weight) superimposed over the structure of the hypromellose (with a low molecular weight), resulting in an obvious endothermic peak.

The blank film retained the endothermic peak of PEG in the front section with an exothermic peak in the rear section. As shown in Fig. 3, the Tm of hypromellose (high molecular weight) decreased to 209 °C after loading the active pharmaceutical ingredient, while the peak at 238 °C disappeared entirely, indicating the dispersion of the active ingredient into the polymer matrix and resulting in an amorphous structure. As shown in Fig. 3B, polyox is essentially a high molecular weight PEG with notable plasticity, which preserved

the rear endothermic peak in roughly the same position, regardless of the presence or absence of the active pharmaceutical ingredient. Note that the temperature change associated with formulation and blank groups did not exceed 1°. The fact that the films based on formulation A and formulation B were prepared via solvent casting meant that the active ingredient coprecipitated with the polymer and PEG to form a solid dispersion.

3.4. ATR-FTIR study

Fig. 4A presents the spectra of apixaban, formulation A blank film, formulation A film, a physical mixture of apixaban with HPMC K4M (powder), and a physical mixture of apixaban with HPMC 15cps (powder). Fig. 4B presents the spectra of apixaban, formulation B blank film, formulation B film, a physical mixture of apixaban with HPMC K4M (powder), and a physical mixture of apixaban with Polyox N80 (powder). The primary functional groups of apixaban presented prominent peaks at 3483 cm^{-1} and 3311 cm^{-1} (corresponding to NH stretching), at 1360 cm^{-1} and 1595 cm^{-1} (corresponding to NH bending), and at 1682 cm^{-1} (corresponding to C═O stretching) [38]. The spectra of apixaban and formulation A blank film showed the absorption peaks characteristic of apixaban (at 3483 cm^{-1}, 3311 cm^{-1}, 1630 cm^{-1}, and 1595 cm^{-1}); however, the peak at 1682 cm^{-1} did not appear. The peaks at 3483 cm^{-1} and 3311 cm^{-1} did not shift to any obvious spectrum, due to the interaction between NH with OH from hypromellose [39]. Further

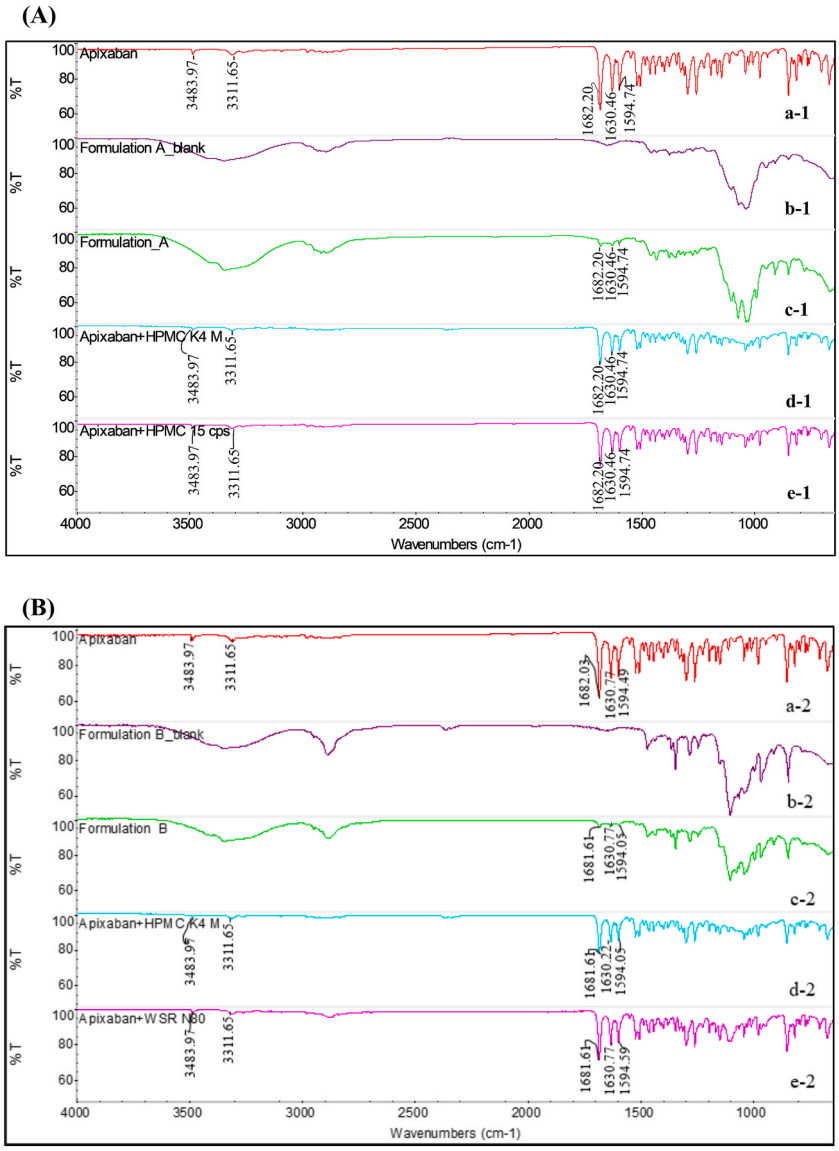

Fig. 4. (A) IR spectrum of apixaban (a-1), formulation A_blank (b-1), formulation A (c-1), mixture of apixaban and excipients as HPMC K4M (d-1), and HPMC 15cps (e−1). (B) IR spectrum of apixaban (a-2), formulation B_blank (b-2), formulation B (c-2), mixture of apixaban and excipients as HPMC K4M (d-2), and HPMC 15cps (e−2).

discussion about the spectrum of apixaban, formulation A film and HPMC 15 cps, after being loaded the ratio of mixture as one to one as apixaban and excipient like HPMC K4M or HPMC 15cps, the vibrations of NH and C═O of apixaban did not change. These results demonstrate that apixaban did not interact with the excipient in a solid state.

The spectra of formulation B presented the absorption peaks characteristic of apixaban (3483 cm^{-1}, 3311 cm^{-1}, 1630 cm^{-1}, and 1595 cm^{-1}); however, the peak at 1682 cm^{-1} did not appear. A similar result was obtained from analyzing the spectrum of formulation A with a blank film for formulation A, in which the peaks at 3483 cm^{-1} and 3311 cm^{-1} did not shift to any obvious spectrum, due to the interaction between water or alcohol and hypromellose or polyethylene oxide (see Fig. 4 (B)). This was shown to decrease the intensity of the NH group (3483 cm^{-1} and 3311 cm^{-1}) to the point where it all but disappeared. Finally, the evaluation of about the spectrum of formulation B blank film, HPMC K4M, and Polyox WSR N80, the vibrations of NH and C═O of apixaban did not change when adding the ratio of mixture as one to one as apixaban and excipient like HPMC K4M or Polyox WSR N80. These results demonstrate that apixaban was unaffected by the excipients in a solid form. Taken together, it appears that the interactions occurred only after a film formed between the polymers and apixaban.

3.5. Pharmacokinetic study

3.5.1. Pharmacokinetic study: rats

We assessed formulations A and B *in vivo* using the pharmacokinetic profiles of rats. Fig. 5 presents the time course showing changes in apixaban concentrations in rat plasma following the oral administration of the proposed film versus the oral administration of apixaban tablets. The T_{max} and AUC values of formulation A were higher than those of the reference drug (5 mg ELIQUIS® tablet), while C_{max} concentration and half-life were similar. The AUC, C_{max}, and T_{max} values of formulation B were lower than those of 5 mg ELIQUIS® tablets (see Table 5). These *in vivo* results show that the drug absorption of both film formulations was comparable to that of 5 mg ELIQUIS® tablets.

Previously, we determined that the dissolution of formulation B was faster than that of formulation A. Our *in vivo* results revealed that the drug absorption of formulation B exceeded that of formulation A, indicating a correspondence dissolution and pharmacokinetic absorption in rats. Note that in the current study, we observed a discrepancy between our T_{max} values and those in previous studies; therefore, we adopted the reference drug as the basis for evaluation. Specifically, the T_{max} values in the literature were 0.6 ± 0.3 h, while ours were 2.3 ± 1.4 h. This discrepancy can perhaps be attributed to differences in rat species and/or age. Note that this does not call into question our experiment results, as our primary objective was to quantify drug absorption in animals.

3.5.2. Pharmacokinetic study: humans

All the volunteers in this study tolerated the procedure and the drug very well. Fig. 6 presents the mean plasma concentration-time profiles following the oral administration of apixaban in three treatments. Table 6 lists the pharmacokinetics of apixaban.

Our primary objective in this study was to compare the drug absorption (i.e., bioavailability) of the proposed Orodispersible film versus that of the reference drug in the form of a conventional tablet. The pharmacokinetic profiles indicated that the bioavailability of formulations A and B were both comparable to those of the reference drug (5 mg ELIQUIS® tablets). In our study on human subjects, the mean $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, C_{max}, and T_{max} of both oral film formulations were similar to those of the tablets. In fact, peak plasma levels occurred at 2.7 ± 1.3 h after the administration of 5 mg ELIQUIS® tablets.

The absorption values of the reference drug were as follows: Cmax (199.3 ± 36.7 ng mL-1), AUC0–t (1793 ± 340.7 ng × h mL-1), and AUC0-∞ (1816 ± 347 ng × h mL-1). The absorption values of formulation A were as follows: Cmax (182.6 ± 51.3 ng mL-1), T max (3.1 ± 0.9 h), and AUC0–t (1714 ± 536 ng × h mL-1). The absorption values of formulation B were as follows: Cmax (206.8 ± 51.9 ng mL-1), T max (2.6 ± 0.8 h), and AUC0–t (1868 ± 373 ng × h mL-1) (see Table 5). Overall, apixaban absorption from the orodispersible films were very similar to that of the reference drug, as evidenced by the Cmax and Tmax values. As a widely marketed product, apixaban is thermodynamically stable in an anhydrous state. The solubility of apixaban in water (0.028 mg/mL at 24 °C) and the oral

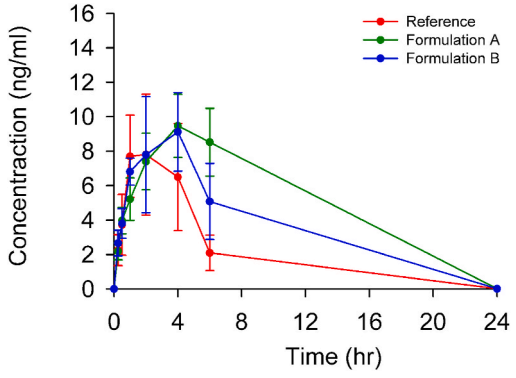

Fig. 5. Plasma concentration-time curves of apixaban after oral gavage in Sprague Dawley rats (n = 4) who received either test film or reference drug treatment. Blood samples for the pharmacokinetic analysis were drawn for up to 24 h. Data represent the mean ± SEM (n = 4).

Table 5
Comparative pharmacokinetics following oral gavage of test films and reference drug in Sprague Dawley rats.

Parameters	A (N = 4)		B (N = 4)		Reference (N = 4)	
	Mean	CV%	Mean	CV%	Mean	CV%
$AUC_{0\text{-}t}$ (ng/ml × hr)	121	42	90	72	53	86
$AUC_{0\text{-}inf}$ (ng/ml × hr)	121	42	90	72	54	86
C_{max} (ng/ml)	10.2	36	10.2	46	10.4	51
T_{max} (hr)	4.0	41	3.5	29	2.3	56
$t_{1/2}$ (hr)	2.0	7	2.1	11	2.6	27

T_{max} and C_{max} were determined from individual real values. Data were statistically evaluated by ANOVA test.

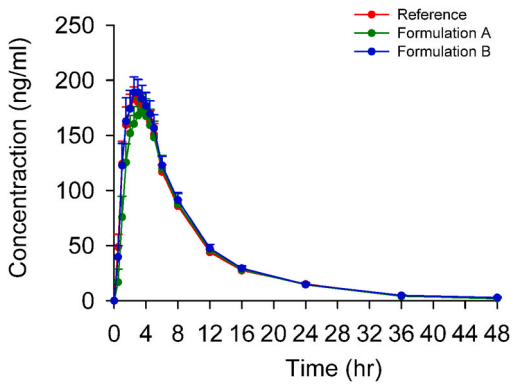

Fig. 6. Plasma concentration-time curves of apixaban after oral administration in healthy men (n = 11) who received either test film or reference drug treatment. Blood samples for the pharmacokinetic analysis were drawn for up to 48 h. Data represent the mean ± SEM (n = 11) (TAHO Pharmaceuticals Ltd, Taiwan, R.O·C.).

Table 6
Comparative pharmacokinetics following oral administration of test films and reference drug in human volunteers.

Parameters	A		B		Reference	
	Mean	CV%	Mean	CV%	Mean	CV%
$AUC_{0\text{-}t}$ (ng/ml × hr)	1714	31	1868	20	1793	19
$AUC_{0\text{-}inf}$ (ng/ml × hr)	1737	31	1907	19	1816	19
C_{max} (ng/ml)	182.6	28	206.8	25	199.3	18
T_{max} (hr)	3.1	29	2.6	31	2.7	47
$t_{1/2}$ (hr)	7.2	15	8.0	42	7.2	12

T_{max} and C_{max} were determined from individual real values. Data were statistically evaluated by ANOVA test.
(TAHO Pharmaceuticals Ltd, Taiwan, R.O·C.).

bioavailability (about 50% for a single 10 mg dose) are both relatively low; however, the drug absorption of apixaban in the current study was favorable. This can perhaps be explained by the polymer-based oral disintegrating film enhancing intestinal absorption.

3.6. Stability

3.6.1. Dissolution in the study of stability

Dissolution and stability properties of apixaban containing orodispersible films were previously discussed. Shah et al. explored different ratios of HPMC E5 and PEG 400 to optimize folding endurance, disintegration time, and drug release, and identified the best formulation with good stability after one month of observation. This demonstrates how the formulation structure evolved to reach the requirements of rapid drug release and stability, which this also applies to administering medication by placing a thin film on the tongue [18]. Furthermore, A. Joshi & A. Raval also used HPMC series E3, E5, E15, and PEG 400 for formulation studies to compare with commercial tablet in terms of dissolution [40]. This highlights how differences in formulation can be compared using related parameters to obtain the optimized formulation, which has better drug release ability than the reference product. These concepts demonstrate how the formulation is suitable for sublingual administration.

We sought to determine whether the films would meet the standards for commercial distribution by performing studies on long-term, intermediate, and accelerated stability. Samples were assessed after being stored for various durations up to six months under these conditions: 25 °C with RH of 60%, 30 °C with RH of 75%, and 40 °C with RH of 75%. We observed no impurities at the end

of any storage period without any added stabilizing agents (see Table 7). Note that an acceptable impurity level would be <0.2%, as defined by ICH Q1A, Q3B, and Q1F.

We also assessed the stability of formulations A and B by comparing the initial values with those obtained after accelerated degradation (40 °C/75% RH) for six months. No significant discrepancies were observed between values at the two time points or between values for the two formulations.

Furthermore, the physical appearance of the samples did not change considerably over the six-month accelerated degradation period.

4. Conclusion

At present, NVAF is the most common form of cardiac arrhythmia, which has been linked with an elevated risk of stroke and thrombosis. A number of drugs are available for the treatment of NVAF in the form of oral anticoagulant tablets, such as apixaban (ELIQUIS®). Patient compliance in taking oral anticoagulants is crucial to their efficacy and safety. ODFs provide a highly efficient approach to drug delivery, particularly for patients who find it difficult to swallow tablets. The apixaban film is created via solvent casting using various grades of hydroxypropyl methylcellulose and polyethylene glycol. The proposed films underwent thorough characterization in terms of film-forming capacity and visual appearance as well as consistency in thickness, weight, and drug content. The films were also assessed in terms of disintegration time, *in vitro* release, clinical applicability, and stability. In rat study, drug absorption of apixaban of two formulation was quite high. In further human study, Formulation B met the bioequivalence criteria with respect to Cmax of the reference drug (ELIQUIS®). Apixaban orodispersible films could potentially be used in the treatment of NVAF for patients with difficulty swallowing.

Ethics statement

Animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and regulations related to Animal Care (National Institute of Health), and were approved by the Institutional Animal Care and Use Committee, Chang Gung University Taiwan (IACUC approval No. CGU110-059). Bioavailability of human subjects was performed in accordance with the current revision of the Declaration of Helsinki, Good Clinical Practice, and Good Laboratory Practice. The clinical investigators provided the eligible subjects with comprehensive information regarding the objectives and potential risks associated with the study. Written informed consent was obtained from all participants before their participation in any study-related activities. Experiments were approved by the Ethics Review Board of Optimum Clinical Research Inc. (approval no. 0062–21).

Author contribution statement

Chien-Chiao Wang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yu-Li Chen: Analyzed and interpreted the data; Wrote the paper.

Ta-Chien Lu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Catherine Lee: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Xing-Rong Lin, Wen-Rung Hsieh, Ting-Yun Huang, Yu-Chia Chang, Hsin-Lan Huang, Yen-Fan Chan, Philip Mathew: Performed the experiments; Analyzed and interpreted the data.

Tsong-Long Hwang: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Table 7
Stability of the ODF by summarizing impurity presence data for the ODFs tested at long-term storage conditions and accelerated condition.

Formulation	Storage Condition	Test Interval	Appearance (film)	Assay	Impurities (%w/w)				
					RRT 0.46	RRT 0.74	RRT 1.59	RRT 1.75	RRT 2.11
A	25 °C/60%RH	Initial	2 cm × 2 cm	100.82	0.01	ND	ND	0.04	ND
		1 month	2 cm × 2 cm	104.21	0.01	ND	ND	0.03	ND
		6 months	2 cm × 2 cm	101.43	ND	ND	ND	0.03	ND
	30 °C/75%RH	6 months	2 cm × 2 cm	103.37	ND	ND	ND	0.03	ND
	40 °C/75%RH	1 month	2 cm × 2 cm	104.31	ND	ND	ND	0.04	ND
		6 months	2 cm × 2 cm	103.68	0.19	0.32	ND	0.04	ND
B	25 °C/60%RH	Initial	2 cm × 2 cm	102.03	0.01	ND	ND	ND	ND
		1 month	2 cm × 2 cm	105.14	0.01	ND	ND	ND	ND
		6 months	2 cm × 2 cm	102.06	ND	ND	ND	0.04	ND
	30 °C/75%RH	6 months	2 cm × 2 cm	105.00	ND	ND	ND	0.04	ND
	40 °C/75%RH	1 month	2 cm × 2 cm	107.04	ND	ND	ND	ND	ND
		6 months	2 cm × 2 cm	103.62	ND	0.04	ND	0.04	ND

Pass: expressed normal level, ND: not detected (TAHO Pharmaceuticals Ltd, Taiwan, R.O·C.).

Data availability statement

Data will be made available on request.

Declaration of competing interest

Ta-Chien Lu and Catherine Lee filed patent application for the discovery of pharmaceutical composition and use of Apixaban film products for the treatment and prevention of thrombosis and related disorders on 03 June 2022 (20220387415 A1). The authors declare that they have no competing financial interests or personal relationships that may appear to influence the work reported in this paper.

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References

[1] G. Erdem, M. Esme, B.B. Dogu, Systematic review and meta-analysis of randomized controlled trials on safety and effectiveness of oral anticoagulants for atrial fibrillation in older people, Ir, J. Med. Sci. 191 (6) (2022) 2517–2523, https://doi.org/10.1007/s11845-021-02891-x.
[2] Y. Miyasaka, M.E. Barnes, B.J. Gersh, S.S. Cha, K.R. Bailey, W.P. Abhayaratna, et al., Secular trends in incidence of atrial fibrillation in Olmsted County, Minnesota, 1980 to 2000, and implications on the projections for future prevalence, Circulation 114 (2006) 119–125, https://doi.org/10.1161/CIRCULATIONAHA.105.595140.
[3] Y. Zhan, J. Joza, M. Al Rawahi, R.S. Barbosa, M. Samuel, M. Bernier, et al., Assessment and management of the left atrial appendage thrombus in patients with nonvalvular atrial fibrillation, Can. J. Cardiol. 34 (2018) 252–261, https://doi.org/10.1016/j.cjca.2017.12.008.
[4] P. Merella, G. Lorenzoni, N. Marziliano, P. Berne, G. Viola, P. Pischedda, et al., Nonvalvular atrial fibrillation in high-hemorrhagic-risk patients: state of the art of percutaneous left atrial appendage occlusion, J. Cardiovasc. Med. 20 (2019) 1–9, https://doi.org/10.2459/JCM.0000000000000735.
[5] S.V. Garkina, T.V. Vavilova, D.S. Lebedev, E.N. Mikhaylov, Compliance and adherence to oral anticoagulation therapy in elderly patients with atrial fibrillation in the era of direct oral anticoagulants, J. Geriatr. Cardiol. 13 (2016) 807–810, https://doi.org/10.11909/j.issn.1671-5411.2016.09.010.
[6] C.B. Granger, J.H. Alexander, M.H. S, J.J.V. McMurray, R.D. Lopes, E.M. Hylek, et al., Apixaban versus warfarin in patients with Atrial fibrillation, N. Engl. J. Med. 365 (11) (2011) 981–992, https://doi.org/10.1056/NEJMoa1107039.
[7] P. Dodrill, M.M. Gosa, Pediatric dysphagia: physiology, assessment, and management, Ann. Nutr. Metab. 66 (S5) (2015) 24–31, https://doi.org/10.1159/000381372.
[8] D.J. Wright, D.G. Smithard, R. Griffith, Optimising medicines administration for patients with dysphagia in hospital: medical or nursing responsibility? Geriatrics 5 (2020) 9, https://doi.org/10.3390/geriatrics5010009.
[9] M.P. Ghourichay, S.H. Kiaie, A. Nokhodchi, Y. Javadzadeh, Formulation and quality control of orally disintegrating tablets (ODTs): recent advances and perspectives, BioMed Res. Int. 2021 (2021), 6618934, https://doi.org/10.1155/2021/6618934.
[10] N. Namiki, Clinical functionality required for orally disintegrating tablets selected as the next generation type, Yakugaku Zasshi 135 (2015) 237–243, https://doi.org/10.1248/yakushi.14-00228-3.
[11] A.F. Borges, C. Silva, J.F. Coelho, S. Simoes, Oral films: current status and future perspectives II - intellectual property, technologies and market needs, J. Contr. Release 206 (2015) 108–121, https://doi.org/10.1016/j.jconrel.2015.03.012.
[12] E.M. Hoffmann, A. Breitenbach, J. Breitkreutz, Advances in orodispersible films for drug delivery, Expet Opin. Drug Deliv. 8 (2011) 299–316, https://doi.org/10.1517/17425247.2011.553217.
[13] M.S. Pacheco, D. Barbieri, C.F. da Silva, M.A. de Moraes, A review on orally disintegrating films (ODFs) made from natural polymers such as pullulan, maltodextrin, starch, and others, Int. J. Biol. Macromol. 178 (2021) 504–513, https://doi.org/10.1016/j.ijbiomac.2021.02.180.
[14] S. Jacob, A.B. Nair, S.H.S. Boddu, B. Gorain, N. Sreeharsha, J. Shah, An updated overview of the emerging role of patch and film-based buccal delivery systems, Pharmaceutics 13 (8) (2021) 1206, https://doi.org/10.3390/pharmaceutics13081206.
[15] L. Shipp, F. Liu, L. Kerai-Varsani, T.C. Okwuosa, Buccal films: a review of therapeutic opportunities, formulations & relevant evaluation approaches, J. Contr. Release 352 (2022) 1071–1092, https://doi.org/10.1016/j.jconrel.2022.10.058.
[16] M. Irfan, S. Rabel, Q. Bukhtar, M.I. Qadir, F. Jabeen, A. Khan, Orally disintegrating films: a modern expansion in drug delivery system, Saudi Pharmaceut. J. 24 (5) (2016) 537–546, https://doi.org/10.1016/j.jsps.2015.02.024.
[17] A. Salawi, An insight into preparatory methods and characterization of orodispersible film-A review, Pharmaceuticals 15 (7) (2022) 844, https://doi.org/10.3390/ph15070844.
[18] R.P. Shah, A.K. Patel, V.M. Patel, Characterization and optimization of mouth dissolving film of an anticoagulant drug: apixaban, IJPRA 7 (3) (2022) 190–210, https://doi.org/10.35629/7781-0703190210.
[19] [Online available] Eliquis Tablets, 2021. https://packageinserts.bms.com/pi/pi_eliquis.pdf.
[20] R. Bala, P. Pawar, S. Khanna, S. Arora, Orally dissolving strips: a new approach to oral drug delivery system, Int. J. Pharm. Investig. 3 (2013) 67–76, https://doi.org/10.4103/2230-973X.114897.
[21] K. Olechno, A. Basa, K. Winnicka, Success depends on your backbone-about the use of polymers as essential materials forming orodispersible films, Materials 14 (17) (2021) 4872, https://doi.org/10.3390/ma14174872.
[22] J.R. Madan, S.V. Waghmare, R.B. Patil, R. Awasthi, K. Dua, Cocrystals of apixaban with improved solubility and permeability: formulation, physicochemical characterization, pharmacokinetic evaluation, and computational studies, Assay Drug Dev. Technol. 19 (2021) 124–138, https://doi.org/10.1089/adt.2020.1052.
[23] T. Potivas, T. Laokuldilok, Deacetylation of chitin and the properties of chitosan films with various deacetylation degrees, CM 13 (1) (2014) 559–567, https://doi.org/10.12982/cmujns.2014.0058.
[24] E. Özakar, R. Sevinç-Özakar, B. Yılmaz, Preparation, characterization, and evaluation of cytotoxicity of fast dissolving hydrogel based oral thin films containing pregabalin and methylcobalamin, Gels 9 (2) (2023) 147, https://doi.org/10.3390/gels9020147.
[25] K. He, J.M. Luettgen, D. Zhang, B. He, J.E. Grace Jr., B. Xin, et al., Grossman, preclinical pharmacokinetics and pharmacodynamics of apixaban, a potent and selective factor Xa inhibitor, Eur. J. Drug Metab. Pharmacokinet. 36 (2011) 129–139, https://doi.org/10.1007/s13318-011-0037-x.
[26] D. Zhang, K. He, N. Raghavan, L. Wang, J. Mitroka, B.D. Maxwell, et al., Comparative metabolism of 14C-labeled apixaban in mice, rats, rabbits, dogs, and humans, Drug Metab. Dispos. 37 (2009) 1738–1748, https://doi.org/10.1124/dmd.108.025981.
[27] J. Watson, G. Whiteside, C. Perry, Apixaban: first global approval, Drugs 71 (2011) 2079, https://doi.org/10.2165/11596820-000000000-00000. –2089.

[28] W. Byon, S. Garonzik, R.A. Boyd, C.E. Front, Apixaban: a clinical pharmacokinetic and pharmacodynamic review, Clin. Pharmacokinet. 58 (10) (2019) 1265–1279, https://doi.org/10.1007/s40262-019-00775-z.
[29] K. Mezue, C. Obiagwu, J. John, A. Sharma, F. Yang, J. Shani, Novel oral anticoagulants in atrial fibrillation: update on apixaban, Curr. Cardiol. Rev. 13 (1) (2017) 41–46, https://doi.org/10.2174/1573403x12666160720092024.
[30] M. Koziolek, M. Grimm, D. Becker, V. Iordanov, H. Zou, J. Shimizu, et al., Investigation of pH and temperature profiles in the GI tract of fasted human subjects using the intellicap® system, J. Pharmaceut. Sci. 104 (2015) 2855–2863, https://doi.org/10.1002/jps.24274.
[31] L. Ovesen, F. Bendtsen, U. Tage-Jensen, N.T. Pedersen, B.R. Gram, S.J. Rune, Intraluminal pH in the stomach, duodenum, and proximal jejunum in normal subjects and patients with exocrine pancreatic insufficiency, Gastroenterology 90 (1986) 958–962, https://doi.org/10.1016/0016-5085(86)90873-5.
[32] D.R. Heidemann, P.J. Jarosz, Preformulation studies involving moisture uptake in solid dosage forms, Pharm. Res. (N. Y.) 8 (1991) 292–297, https://doi.org/10.1023/a:1015877011807.
[33] G.K. Sakellariou, A. Vasilaki, J. Palomero, A. Kayani, L. Zibrik, A. McArdle, et al., Studies of mitochondrial and nonmitochondrial sources implicate nicotinamide adenine dinucleotide phosphate oxidase(s) in the increased skeletal muscle superoxide generation that occurs during contractile activity, Antioxidants Redox Signal. 18 (2013) 603–621, https://doi.org/10.1089/ars.2012.4623.
[34] K.T. Jensen, F.H. Larsen, K. Lobmann, T. Rades, H. Grohganz, Influence of variation in molar ratio on co-amorphous drug-amino acid systems, Eur. J. Pharm. Biopharm. 107 (2016) 32–39, https://doi.org/10.1016/j.ejpb.2016.06.020.
[35] J. Liu, T. Rades, H. Grohganz, The influence of moisture on the storage stability of co-amorphous systems, Int. J. Pharm. 605 (2021), 120802, https://doi.org/10.1016/j.ijpharm.2021.120802.
[36] A.C. Rumondor, L.S. Taylor, Effect of polymer hygroscopicity on the phase behavior of amorphous solid dispersions in the presence of moisture, Mol. Pharm. 7 (2010) 477–490, https://doi.org/10.1021/mp9002283.
[37] S. Airaksinen, M. Karjalainen, A. Shevchenko, S. Westermarck, E. Leppanen, J. Rantanen, et al., Role of water in the physical stability of solid dosage formulations, J. Pharmaceut. Sci. 94 (2005) 2147–2165, https://doi.org/10.1002/jps.20411.
[38] P.J. Larkin, M. Dabros, B. Sarsfield, E. Chan, J.T. Carriere, B.C. Smith, Polymorph characterization of active pharmaceutical ingredients (APIs) using low-frequency Raman spectroscopy, Appl. Spectrosc. 68 (2014) 758–776, https://doi.org/10.1366/13-07329.
[39] E. Leyk, M. Wesolowski, Interactions between paracetamol and hypromellose in the solid state, Front. Pharmacol. 10 (2019) 14, https://doi.org/10.3389/fphar.2019.00014.
[40] A. Joshi, A. Raval, Formulation and evaluation of sublingual films of apixaban, IJPBA 7 (2) (2019) 80–116. https://www.ijpba.in/index.php/ijpba/article/view/117.