Saudi Pharmaceutical Journal 32 (2024) 102089

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Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Apixaban and clopidogrel in a fixed-dose combination: Formulation and in vitro evaluation

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ARTICLE INFO

Keywords:
Fixed-dose combination tablets
Apixaban
Clopidogrel
Extended-release
Factorial design
In vitro dissolution

ABSTRACT

Fixed-dose combination (FDC) products represent a novel, safe, and cost-effective formulation. Combined use of anticoagulant and antiplatelet medications is common among comorbid cardiovascular patients. This study aimed to formulate FDC tablets for Apixaban and Clopidogrel, as prophylaxis and treatment of thrombo-embolic events. FDC tablets were developed by combining small tablets of Immediate-Release Clopidogrel 75 mg and Extend-Release Apixaban 5 mg through direct compression and wet granulation. Particularly, Apixaban tablets were developed using design expert software, and various types and concentrations of polymers were entered. For Clopidogrel tablets, various diluents were used to develop the formulation. Then, the dissolution profile for each formula was studied. Finally, the optimized formulations were encapsulated within hard gelatin capsules. Apixaban formulation followed zero-order with super case II transport mechanism as the dominant mechanism of drug release. The Apixaban drug release rate was affected by the type and concentration of the polymers in the formulation ($P < 0.05$). As the HPMC concentration was increased, Apixaban release was retarded. For, Clopidogrel, the formulated tablets with spray-dried lactose filler and sodium stearyl fumarate lubricant were found to be stable with good properties. In conclusion, the optimum formulation yielded Clopidogrel and extended-release Apixaban for 24 h with the desired in vitro drug dissolution.

1. Introduction

Cardiovascular disease (CVD) is a major cause of morbidity and mortality globally, consequently, the rising cost of health care. The yearly number of new cases increased and the total number of prevalent cases doubled between 1990–2019 (Brodmann et al., 2020). Antithrombotic agents are used in single, dual, or triple therapy based on patient diagnosis and risk factors as prophylaxis for cardiovascular events and complications. However, using multi-antithrombotic agents simultaneously increases the risk of bleeding, as the main side effect, and hospitalization. Many reviews were performed to evaluate these regimens and revealed that Clopidogrel (CLOP) is the preferable agent from the P2Y12 receptor antagonist due to its lower adverse medication effect of bleeding. Furthermore, in a clinical trial, dual therapy, including CLOP and Apixaban (APX), was superior to triple therapy with Aspirin or dual therapy with warfarin due to reduced medication-related reversed effects and less hospitalization (Andreou et al., 2018; Barnes, 2020; Bergmark et al., 2022; Lip, 2020; Lupercio et al., 2020; Virginia et al., 2021). These patients have a therapy regimen composed of multi-drug components that can be formulated as fixed-dose combination (FDC) therapy.

Apixaban (Eliquis®) is a direct oral anticoagulant agent, that highly selective active site inhibitor of factor Xa, administered as 5 mg twice daily and reduced to 2.5 mg in certain cases, and its biopharmaceutics classification system (BCS) is class III, as good soluble poor permeable substances (Bell et al., 2009; EMC, 2022; Lexicomp, 2022). The chemical structure of APX is shown in Fig. 1 A (Rxlist, 2022). The Food and Drug Administration (FDA) approved APX to be marketed on December 28, 2012, by Bristol-Myers Squibb/ Pfizer (FDA, 2012). After that, many studies were performed and patents were registered to develop APX in different dosage forms; immediate release (IR) tablets or capsules (Gambhire et al., 2017; Jia et al., 2017; Kumar Shaha and Mehdi Hasan, 2018), IR crushed tablet (Song et al., 2015), dispersible tablets (Vadaliwala et al., 2019), and sublingual film (Shah et al., 2022). In addition, other studies formulated APX for prolonged-release transdermal patches (Patel et al., 2021), transdermal nano-emulsion (Abdulbaqi and Rajab,

Abbreviations: APX, Apixaban; CLOP, Clopidogrel; FDC, fixed dose combination; DC, direct compression; WG, wet granulation; IR, immediate release; SR, sustained release; MDT, mean dissolution time.

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https://doi.org/10.1016/j.jsps.2024.102089
Received 24 February 2024; Accepted 24 April 2024
Available online 29 April 2024

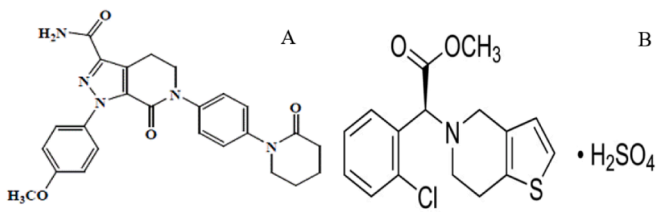

Fig. 1. Chemical structure of A) Apixaban B) Clopidogrel hydrogen sulfate.

2021), and a patent formulated it as extended-release (ER) tablets using only hydroxypropyl methylcellulose (HPMC) (马玉国, 2016). APX as ER tablets is characterized to cause lower gastrointestinal irritation, reduce the bleeding risk, stabilize the drug release, and decrease administration frequencies (马玉国, 2016). Furthermore, clinical studies were performed to assess the blood concentration after administration of an IR tablet of 10 mg once a day and revealed that more fluctuation in blood concentration was observed compared to a dose of 5 mg twice a day. So it is favored to be administered twice a day (Frost et al., 2013; Lassen et al., 2007).

Clopidogrel (Plavix®) is an anti-platelet agent, P2Y12 antagonist, administered as 75 mg once daily, and its BCS is class II, as poor soluble good permeable substances (FDA, 1997; Lexicomp, 2022). Fig. 1 B shows the chemical structure of Clopidogrel (CLOP) Hydrogen Sulfate salt (Merck, n.d.). The FDA approved Sanofi to market CLOP in 1997 (FDA, 1997). Many formulations were developed later in different dosage forms to enhance CLOP solubility and bioavailability. A literature search revealed formulating CLOP as IR tablets (Kavya et al., 2014; Kumar et al., 2013), gastro retentive system as floated tablets (Rao and Lakshmi, 2014), high-density tablets (Desai and Purohit, 2017) or floated osmotic capsules (Shah and Prajapati, 2019), liquisolid compact (Ali et al., 2021; Mohammed and Mohammed, 2018), micro-emulsion (Patel et al., 2010), nano-suspension (Jassim and Hussein, 2014), oral disintegrating tablets (Mahrous et al., 2016), or fast disintegrating films (Alladi Saritha, 2018), and as FDC system (Huang et al., 2011; Seo and Han, 2019).

A single-pill combination, known as FDC, is a system formulated to deliver two or more active pharmaceutical ingredients as one unit. These products are increasing and represent an important segment of the global pharmaceutical market (Kaushal, 2017). The number of FDC approvals significantly increased in the 2000 s and represents an important segment of the global pharmaceutical market (Hao et al., 2015; Kaushal, 2017). Formulation of FD multidrug therapy may give a synergistic and sustained therapeutic effect associated with positive therapeutic outcomes, greater efficacy, safety improvement, and fewer side effects compared to a single maximum dose. Better patient control was also achieved due to fewer frequent and missed doses (Bell, 2013; Kavanagh et al., 2018). Add that patient adherence increased due to less dosing burden and lower cost (Gambhire et al., 2017; Kim and Weon, 2021).

However, these benefits are combined with potential challenges associated with FDC formulations, including drug solubility, dissolution profile, dose, possible drug interaction, and individual drug characteristics. After FDC usage, other challenges may be faced; lack of inclusion in treatment protocol, less dose flexibility to fit individual medications, and the ability to determine the source of side effects (Kavanagh et al., 2018; Kim and Weon, 2021).

Multi-drug therapy can be formulated using different technologies, including monolithic, multilayer, multi-particulate, or 3D-printing systems, depending on the compatibilities between the active constituents and the dissolution profile for each (Kaushal, 2017).

APX and CLOP have different solubility and permeability, so it is better to formulate the FDC as a multi-particulate system.

The present study aimed to develop novel FDC tablets of multi-tablet system and evaluate their in vitro dissolution profile. The FDC consisted of one CLOP (75 mg) tablet and one or two APX (5 mg each) tablets, matrix type, encapsulated within translucent gelatin shell capsules (0 size).

2. Materials and methods

2.1. Materials and reagents

A pharmaceutical grade APX and CLOP hydrogen sulfate salt were received as gifts from Pharmacare PLC (Palestine). Tablet excipients including hydroxypropyl methylcellulose, 28–30 % methoxyl, 7–12 % hydroxypropyl, viscosity (2 % aq. soln., 20 °C) 7500–14000 mPa.s (HPMC) and hydroxypropyl cellulose (HPC) were purchased from Alfa Aesar (by Thermos Fisher Scientific, United Kingdom). Methocel, sodium laurilsulfate (SLS), spray-dried lactose, mannitol, microcrystalline cellulose 112 (MCC 112), colloidal silica, sodium stearyl fumarate (SSF), magnesium stearate (Mg. stearate), and anhydrous ethanol were donated from Pharmacare PLC (Palestine). For buffer preparation, sodium hydroxide pellets, sodium phosphate tribasic dodecahydrate, 98 %, and hydrochloric acid were analytical grades and purchased from Carlo Erba reagents, Acros organics, and Merck Serono respectively. HPLC grade solvents for analysis including; acetonitrile (ACN), trifluoroacetic acid (TFA), and triethylamine (TEA) were purchased from Merck (Merck Serono Amman, Jordan). Water was obtained by filtration using a cellulose nitrate filter (0.45) micron manufactured by Sartorius stedim biotech company (Jordan). Translucent hard gelatin shell capsules (0 size) were donated from Jerusalem Pharmaceuticals Co. Ltd. (Palestine).

2.2. Instruments

APX and CLOP tablets were formulated on a manual single-punch tablet compression machine. Pharma- test® (Germany) instruments were used to perform the friability, hardness, disintegration, and dissolution tests. Copley® tapped density tester, Ohaus® electronic balance, Ohaus® loss on drying (LOD) tester, pH/orp meter, manual sieves, digital caliper, Elma® bath sonicator, and DHG- 9023A dry oven were used during formulation and analysis. Compatibility study experiments were conducted using a Bruker Fourier transform infrared (FT-IR) vacuum spectrometer, equipped with a platinum ATR unit with single reflection diamond crystal (Bruker Optik GmbH, Rosenheim, Germany), and the obtained spectra were compared using OPUS viewer software. Quantitative analysis was performed using an Agilent HPLC 1200 series with an AS thermostat (Santa Clara, USA), and UV/ VIS detector. The used column was BDS Hypersil C_{18}, (4.6*150 mm), 5 µm, thermo-scientific part # 28105–154630. PerkinElmer double beam UV/ VIS spectrometer lambda 25, sonicator, and vacuum filter pump were also used during the analysis. For stability studies, samples were kept in a climate chamber (Binder, Tuttlingen, Germany).

2.3. Compatibility study

A list of expected excipients including polymers, fillers, binders, disintegrants, and lubricants was chosen. A binary mixture of active pharmaceutical ingredients (APIs) and each excipient was prepared in a 1:1 ratio and stored in a stability chamber at room temperature and 40 ± 2 °C/ 75 ± 2 % relative humidity (RH) for four weeks, while other samples were stored in a dry oven at 60 °C for two weeks. These different stress conditions were defined by the international council for harmonization of technical requirements for pharmaceuticals for human use (ICH) guidelines (ICH, 2003). The samples were tested for their physical appearance, and their compatibility was evaluated using FT-IR Spectroscopy. The FT-IR peak matching method spectrum was used for compassion. The samples were scanned in the wavelength range of 4000—400 cm^{-1}.

2.4. Tablets formulation

2.4.1. Preparation of Clopidogrel tablets

The proposed formulas of CLOP tablets were formulated using direct compression (DC) technology. The proposed formulas are listed in Table 1. All ingredients were weighed for 150 tablets per batch for each of the three proposed formulas. All ingredients, except glidant and lubricant, were blended manually for 5 min using a mortar and a pestle. The glidant was then added and blended manually in a polypropylene bag for 2 min, followed by the lubricant, which was added and blended manually in a polypropylene bag for 2 min. Finally, the blends were directly compressed with a manual single-punch tablet machine using 6 mm biconcave punches.

2.4.2. Preparation of Apixaban tablets

2.4.2.1. Formulation development. APX formulation development aimed to obtain stable sustained-release tablets that were released completely within 24 hrs. The experiment included various manufacturing technologies, including direct compression, wet granulation with water, or wet granulation with absolute ethanol. Dissolution experiments were performed on three tablets of each formula to discriminate between formulas and identify the minimum and maximum amounts of excipients required to produce tablets with the intended characteristics. Eleven formulas (ER1- ER11), listed in Table 2, were prepared until we clearly understood the excipients' behavior and the appropriate manufacturing process.

2.4.2.2. Factorial design. For the determined excipients, a full factorial design (2^4), D-optimal level, was employed using design expert software version 6.0.4.1 (STAT- EASE), and 4 factors were evaluated. One replicate was run, and then the experimental trials were performed at the sixteen resulting combinations. The concentrations of HPMC (X1), HPC (X2), Methocel E5 (X3), and SLS (X4) were selected as independent variables (Table 3). At the same time, the three dissolution parameters, mean dissolution time (MDT), times 25 and 90 % of the drug released ($T_{25\%}$ and $T_{90\%}$), were identified as responses. The data were subjected to 3D response surface methodology to determine the influence of the three polymers and SLS on the dependent variables.

All the formulations contained the same quantities of APX and Mg. stearate (5 mg and 0.025 mg, respectively), but varying quantities of polymers and SLS, and the total tablet weight was 75.75 mg (Table 4). Apixaban ER tablets were formulated by wet granulation technology. All excipients were weighed separately for 150 tablets per batch for each formula. First, excipients, except SLS and Mg stearate, were added in the geometric method. Next, polymers and APX were blended manually for 5 min in a mortar and a pestle, and absolute ethanol was sprayed to formulate granules. The formulated granules passed through mesh size #12, dried in a dry oven at 40 °C for 30 min, and then crossed into mesh size #16. Finally, SLS and lubricant were manually added and blended in a polypropylene bag for 2 min. The final blend was compressed with a manual single-punch tablet machine using 6 mm biconcave punches.

The final FDC consisted of one CLOP (75 mg) tablet and one or two APX (5 mg each) tablets encapsulated within translucent hard gelatin shell capsules (0 size).

Table 1
Clopidogrel formulated batches.

Ingredients	Function	Formulation code (mg/ tablet)		
		CLOP 1	CLOP 2	CLOP 3
Clopidogrel hydrogen sulfate	API	98	98	98
Lactose spray dried	Diluent	42.9	0	0
Mannitol	Diluent	0	42.9	0
MCC 112	Diluent	0	0	42.9
Klucel	Binder and disintegrant	7.5	7.5	7.5
Sodium stearyl fumarate	Lubricant	1.5	1.5	1.5
Colloidal silica	Glidant	0.15	0.15	0.15
Total		150.05	150.05	150.05

2.5. Evaluation of blends

Various parameters, such as angle of repose and bulk/ tapped density were determined to characterize the final blend flow and compressibility. In addition, Carr's Index (CI%) and Hausner's ratio (HR) were also calculated as per USP General Chapter ⟨616⟩ and < 1174> (United States Pharmacopeial, 2011; USPC, 2014).

2.6. Evaluation of FDC tablets

2.6.1. Physical parameters

Ten tablets were randomly selected for each of the 16 formulas of APX and CLOP batch to perform weight variation, thickness, and hardness test. The friability test of IR CLOP and ER APX formulated tablets were evaluated using the pharm-test friabilator. The disintegration times of the IR CLOP formulated tablets were evaluated using a pharm-test disintegrator that operated at 37 ± 2 °C. Six tablets of CLOP were placed into the six cells of the rack, one per cell, and then immersed in water. For all evaluations, the means and standard deviations were calculated.

2.6.2. Assay

Ten tablets of each formula were powdered in a mortar and a pestle. The weight of one tablet (75.75 mg and 150.05 mg of APX and CLOP, respectively) was dissolved in 100 ml ACN, leftover a night to ensure complete dissolution, 5 ml of solution was filtered in a 50 ml volumetric flask through a 0.45 μm syringe filter, and the volume was made up using purified water. The drug content assay was evaluated by chromatographic separation of the APIs on a BDS Hypersil C_{18}, (4.6*150 mm, 5 μm), with ACN and TFA in the ratio 48:52 (v/v) as a mobile phase, at a flow rate of 0.9 ml/ min., column temperature 45 °C, and injection volume of 5 μL. The calibration curve was developed for each API and the correlation coefficient R^2 was more than 0.999 (Al-Shami et al., 2024).

2.6.3. In vitro drug release study

2.6.3.1. In vitro dissolution studies. For CLOP, in vitro drug release studies were carried out in 750 ml of 0.1 N HCL for 2 hrs. For APX, in vitro, drug release studies were carried out in 900 ml of 0.05 M Sodium phosphate buffer with 0.05 % SLS for 24 hrs. A USP type II dissolution apparatus (paddle type) was used to test both at 75 rpm and 37 ± 0.5 °C. All dissolution studies were performed on three tablets of each formula.

The in vitro drug release study for the final FDC, two strengths, was carried out using the USP delayed release method A (United States Pharmacopeial Convention, 2011). The study was performed in 750 ml of 0.1 N HCL. Samples were withdrawn at (5, 10, 20, 30, 45, 60, and 120 min.). After 2 hrs., 250 ml of 0.2 M tribasic sodium phosphate equilibrate 37 ± 0.5 °C was added to the dissolution media, and the pH of the media was adjusted to (6.8 ± 0.05). The apparatus continued to run for 24 hrs. A sample of 5 ml was withdrawn from the dissolution media in a specified period (4, 8, 12, 16, 20, and 24 hrs.). All of the experiments were tested using a USP type II dissolution apparatus (paddle type) at 75 rpm and 37 ± 0.5 °C. All dissolution studies were performed on six tablets of each formula.

A sample of 5 ml was withdrawn from the dissolution media using an auto-sampler, and no volume correction was made. The samples were filtered using a 0.45 μm Clarify® syringe filter. The absorbance of the samples was measured using a spectrophotometric method at 210 nm using HPLC developed method(Al-Shami et al., 2024) and the %

Table 2
Formulation trials of Apixaban extended-release tablets:

Ingredient	ER 1	ER 2	ER 3	ER 4	ER 5	ER 6	ER 7	ER 8	ER 9	ER 10	ER 11
Apixaban	5	5	5	5	5	5	5	5	5	5	5
HPMC	35	35	35	20	25	50	0	0	20	20	20
HPMC E5	0	0	0	0	0	0	20	50	30	0	25
HPC	35	35	35	30	45	0	30	0	0	0	25
Avicel 101	0	0	0	20	0	20	20	20	20	50	0
SLS	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mg stearate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	75.75 mg										
Manufacturing process	D.C*	WG. DW**	WG. Et-OH***								

* D.C.: direct compression.
** WG. DW: wet granulation with distilled water.
*** WG. Et-OH: wet granulation with absolute ethanol.

Table 3
Experimental design plan generated by software:

Component	Code	Minimum	Maximum	Coded low	Coded High
A. HPMC	X 1	20	35	+0	+0.495868
B. Methocel E5	X 2	0.25	25	+0	+0.818182
C. HPC	X 3	20	35	+0	+0.495868
D. SLS	X 4	0	0.25	+0	+0.0082F6446

cumulative release (% CR) was plotted using calculated mean values of cumulative drug release versus time.

2.6.3.2. Kinetic analysis of dissolution data. The release data were fitted to five kinetic models including; zero-order, first-order, Higuchi, Hixon-Crowell, and Korsmeyer-Peppas to determine the drug release mechanism with the aid of DD Solver add-in software. The drug release mechanism was considered according to the coefficient of determination; R^2 values (Grassi and Grassi, 2014).

2.7. Swelling and erosion studies

The swelling nature of tablets was studied by water gain on 3 tablets. The swelling index study was performed using USP dissolution apparatus-II in the final dissolution media at 37 ± 0.5 °C, rotated at 75 rpm. At predetermined intervals for 24 hrs., the tablets were withdrawn using a predetermined weight mesh, and excess dissolution media was removed with absorbent tissue, and then weighed. The percentage swelling of the tablet was determined according to the following equation (Gerogiannis et al., 1993):

$$\%Wateruptake = 100(W_t - W_0)/W_0$$

where W_t is the mass in the swollen state at time t, and W_0 is the initial tablet weight.

The matrix erosion was determined on the same tablets, at the same time intervals. After weighing the hydrated tablets, they were dried in an oven at 60 °C for 24 hrs., and the remaining dried were weighed. The percentage erosion (% mass loss) was determined according to the following equation (Ravi et al., 2007):

$$\%Erosion = 100(W_0 - W_r)/W_0$$

where W_o was the initial tablet weight, W_r was the dry weight after time t.

The remaining percentage was determined using the following equation:

$$\%Driedremaining = 100 - \%erosion$$

2.8. Stability study

Forty capsules of the optimized batch were packed in a screw-capped amber glass bottle and kept for accelerated stability study at 40 °C/ 75 % R.H. in a climate chamber (BINDER, Tuttlingen, Germany) for 90 days. Accelerated stability study samples were analyzed at 0 and 90 days for physical appearance and drug content concerning the initial results of the same batch.

Table 4
Ingredients of Apixaban ER tablets based on factorial design (APX1- APX16):

Formula code	Ingredient (mg/ tab.)						
	Apixaban	**HPMC**	**Methocel E5**	**HPC**	**SLS**	**Mg stearate**	**Total**
APX 1	5	35	0.5	35	0	0.25	75.75
APX 2	5	27.5	7.87	35	0.12	0.25	75.74
APX 3	5	35	0.25	35	0.25	0.25	75.75
APX 4	5	20	15.5	35	0	0.25	75.75
APX 5	5	20	15.25	35	0.25	0.25	75.75
APX 6	5	20	25	25.37	0.12	0.25	75.74
APX 7	5	25.5	25	20	0	0.25	75.75
APX 8	5	27.05	16.15	27.05	0.25	0.25	75.75
APX 9	5	25.25	25	20	0.25	0.25	75.75
APX 10	5	35	15.37	20	0.12	0.25	75.74
APX 11	5	35	7.87	27.5	0.12	0.25	75.74
APX 12	5	30.12	20.12	20	0.25	0.25	75.74
APX 13	5	20	20.12	30.12	0.25	0.25	75.74
APX 14	5	31.04	12.05	27.29	0.12	0.25	75.75
APX 15	5	24.85	20.61	24.85	0.19	0.25	75.75
APX 16	5	25.5	25	20	0	0.25	75.75

2.9. Statistical analysis

To investigate the significance of the differences between the results from the studied formulations, a one-way analysis of variance (ANOVA) test was used. The significance level was set at ($\alpha < 0.05$). Design Expert software version 6.0.4.1 (STAT- EASE) was used for analysis.

3. Results

3.1. Compatibility study

The FT-IR spectra were recorded for pure API and powder mixtures of API and each excipient to assess any possible chemical interactions between API and the excipients (Dave et al., 2015). Table 5 reflects the FT-IR peak values (cm^{-1}) for each functional group. Fig. 2 demonstrates the FT-IR spectra of pure APX, CLOP, and their physical mixture upon preparation and after exposure to stress conditions, Figs. 3 and 4 show each API with the physical mixture with each excipient. No physical changes were observed, and the obtained spectra for pure APIs correlated well with that of the APIs mixture and with excipients and showed all the characteristic peaks with no major changes. Hence, no significant chemical interaction occurs in the solid state at zero time. In addition, no shifting was observed for peaks of each mixture at zero time and after exposure to stress conditions. This finding concludes that the APX and CLOP are compatible with each other, and each API was compatible with its formulation components.

3.2. Formulation development

Based on the excipients compatibility studies and revising the reference listed drug (RLD) product, different laboratory scale experiments were prepared and tested to obtain the appropriate dissolution profile. The experiment included various manufacturing technologies, including direct compression, wet granulation with water, or absolute ethanol.

3.2.1. Development of IR CLOP tablets

Three formulations of CLOP (CLOP 1- CLOP 3) were formulated using different diluents including mannitol, MCC 112, and spray dried lactose. Observation of the characteristics of the powder bed showed that the formula with mannitol (CLOP 2) tended to stick with punches during the compression process. Further, the formula with MCC 112 (CLOP 3) showed a longer disintegration time (within 10 min) than CLOP 1 and CLOP 2 disintegrated within 4 and 7 min, respectively. The dissolution test results revealed that CLOP 1 and 2 were released completely (% CR > 96 %) within the first 30 min of the test, while 76 % of CLOP 3 was released within the first 30 min. Compared to mannitol and lactose, the formula with spray dried lactose (CLOP 1) was chosen as an optimum formula to scale up.

3.2.1.1. Development of ER APX tablets. As preliminary (scanning) experiments, eleven APX formulas were investigated (ER 1 – ER 11), with each formula containing different quantities and types of polymers, as well as different manufacturing technologies. Table 2 shows the tested formulas' composition and their manufacturing process.

The pre-formulation results revealed that the wet granulation technology with absolute ethanol that was sprayed on the powder bed and mixed was the optimum method of formulation. Subsequently, the quantities of polymers that give formulas with extended release within 24 hrs., were determined to be within the range of 20 to 35 mg/ tablet for HPMC and HPC, and less than 25 mg/ tablet for Methocel E5. Furthermore, several Apixaban ER formulas (APX 1 – APX 16) were developed and evaluated utilizing these findings and a factorial approach. Table 4 shows the sixteen developed formulas using design expert software.

Table 5
FT-IR data of Apixaban and Clopidogrel hydrogen sulfate.

API	Functional groups	IR values (cm^{-1})
Apixaban	N–H stretch	3482
	N–H stretch	3308
	C–H stretch	2902
	C = O stretch	1679
Clopidogrel hydrogen sulfate	N–H stretch	2501
	C-S-C stretch	2345
	C = O stretch	1751
	C = C stretch	1438
	C-O stretch	1062, 1152, 1185

3.3. Evaluation of blend

Table 6 presents the findings of various evaluations of the blend characteristics investigation for formulation APX 1–16. The results of bulk densities for all formulations of APX were in the range of 0.19–0.35 g/ ml and 0.33 g/ ml for CLOP. The findings of tapped density for APX formulations were in the range of 0.24–0.40 g/ ml and 0.38 g/ ml for CLOP. The angle of repose values were between 29° – 41° for APX and 34° for CLOP. Carr's Index and Hausner's ratio were between 7.1–17.6 % and 1.0–1.2, respectively for APX, and 13.3 % and 1.15 for CLOP. These results revealed excellent to good flow for all the formulas of APX except formulas APX 8, which showed fair flow and APX 4 which showed passable flow. These results indicated that all blends have acceptable flow properties and compressibility, and all pre-compression parameters were within the acceptable ranges.

3.4. Evaluation of tablets

The evaluation of the resulting tablets of the sixteen APX formulations and CLOP formulation is presented in Table 7. Among APX and CLOP batches the results revealed that all batches showed a total weight loss of less than 1 % after the friability test, had a uniform tablet thickness, whereas the hardness of tablets was variate in the range of 4.13–5.7 kilopond (kp) for APX formulations. The hardness and thickness values showed sufficient mechanical resistance in all the patches. Furthermore, a disintegration test was performed on IR CLOP 1 tablets, and the disintegration time range was between 3–4 min, within the accepted limits. For the assay test, APX tablets assay were between 95–102 %, which indicates getting accuracy in dosing. All evaluation parameter values were within acceptable limits according to the USP 38-NF 33 and European Pharmacopoeia.

3.5. Experimental design and response surface analysis

A full factorial design 2^4 was selected as it helps in understanding the effects of polymers and SLS concentrations on response parameters. Based on the preliminary studies, the quantities of polymers were determined. Changes on the concentration of the three polymers (HPMC, HPC, Methocel E5) and SLS quantity were spontaneously applied, since a single response optimization is thought to yield misleading results (Anderson et al., 2017).

The in vitro dissolution parameters and APX release profiles of the sixteen formulations (APX 1–16) are presented in Fig. 5 and Table 8. APX release was affected by the polymer type and amount used in each formula. The cumulative percent released after 24 hrs. of each of the APX formulations was complete except for APX 10, that only 80 % of the APX were released. The results illustrated that there is a relationship between the concentration of each polymer and its dissolution profile. Formulation with HPMC concentrations between (39.6–46.2 %) APX 1, APX 3, APX 10, APX 11, APX 12, and APX 14 showed the lowest cumulative amount of APX release within 20 h. (91.5, 89.7, 68.4, 84.0, 75.8, and 87.2 %, respectively). When the HPMC concentrations were between 32.8 – 33.7 % (APX 2, APX 7, APX 8, APX 9, and APX 15), a

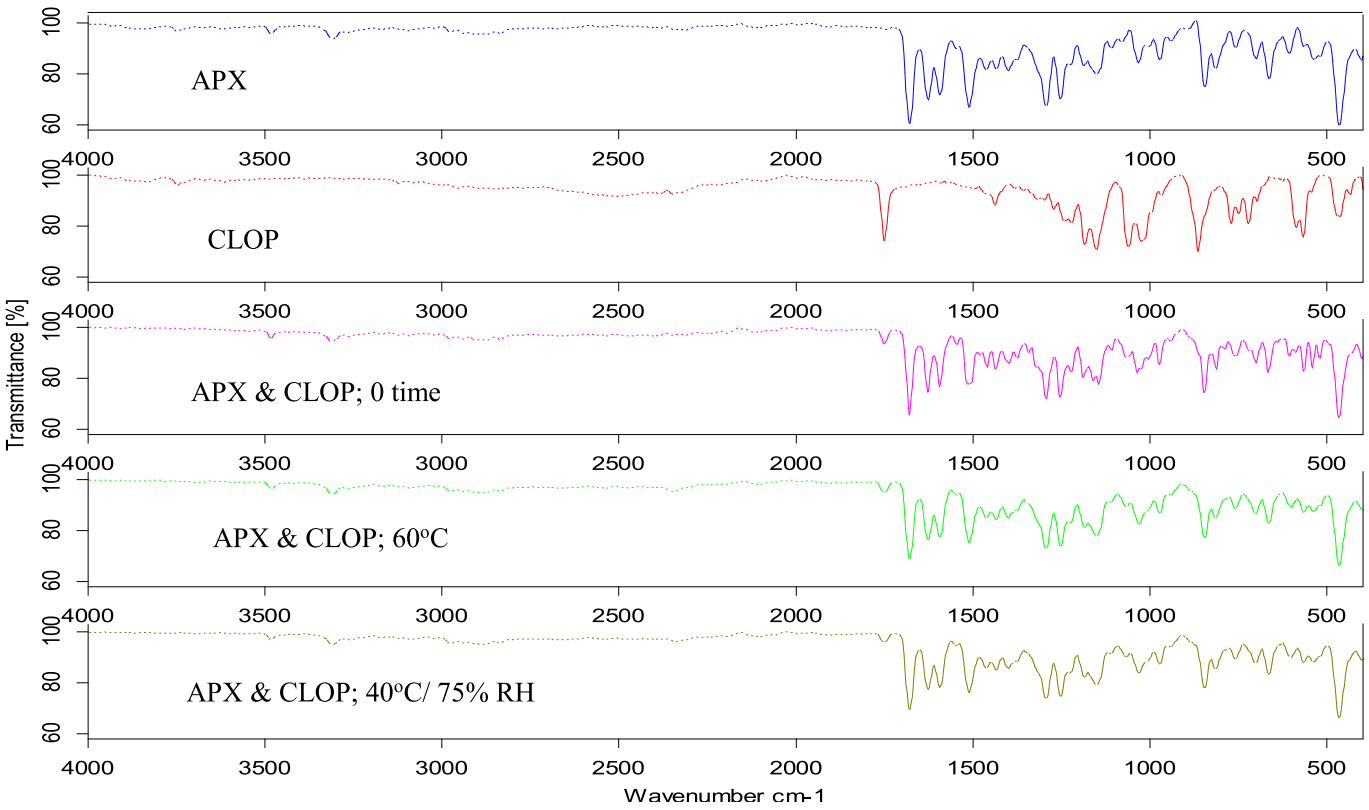

Fig. 2. FT-IR spectra of pure APIs and their physical mixture in different conditions.

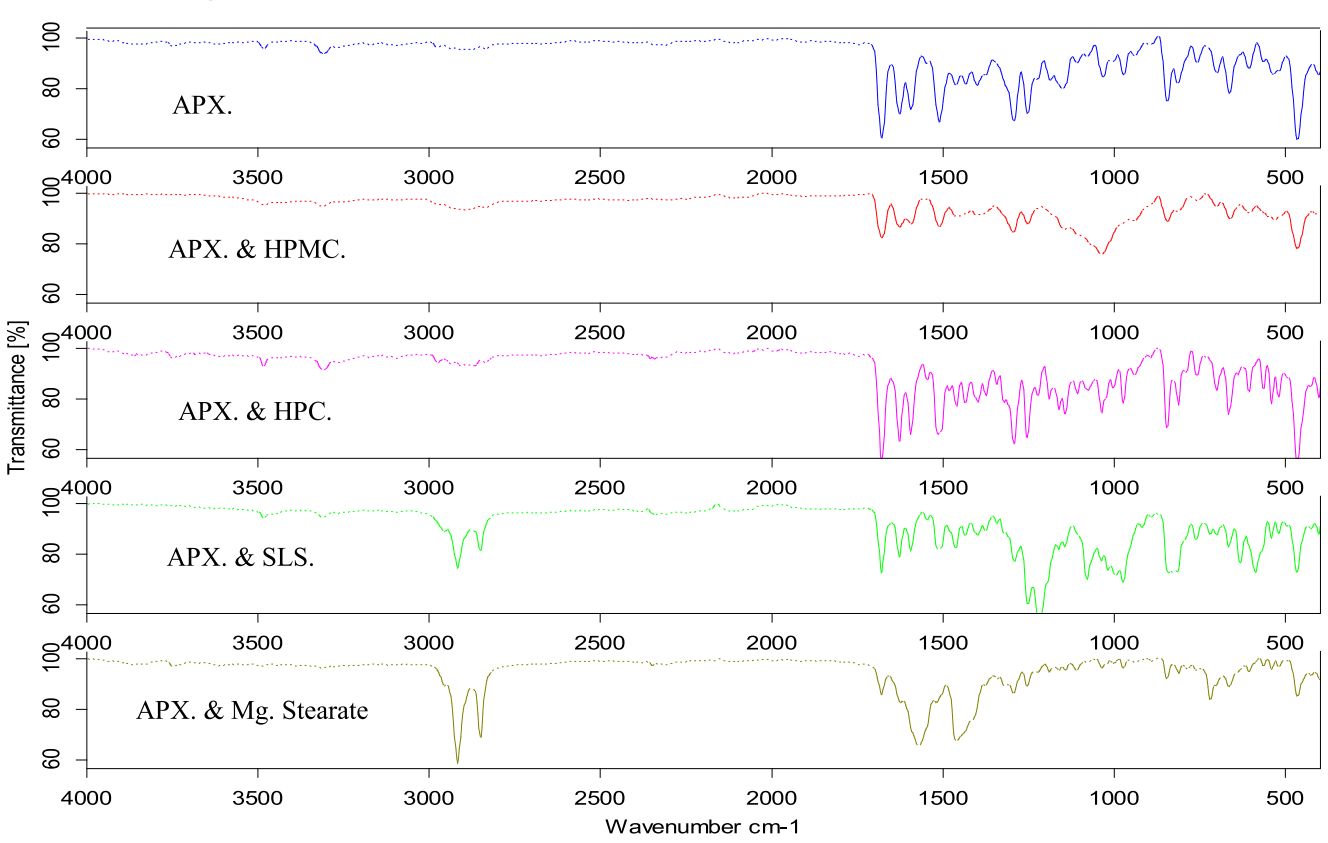

Fig. 3. FT-IR spectra of pure APX and a physical mixture of APX with each excipient. APX.: Apixaban, HPMC: Hydroxypropyl methylcellulose, HPC.: Hydroxypropyl cellulose, SLS.: Sodium lauryl sulfate, Mg. ST.: Magnesium Stearate.

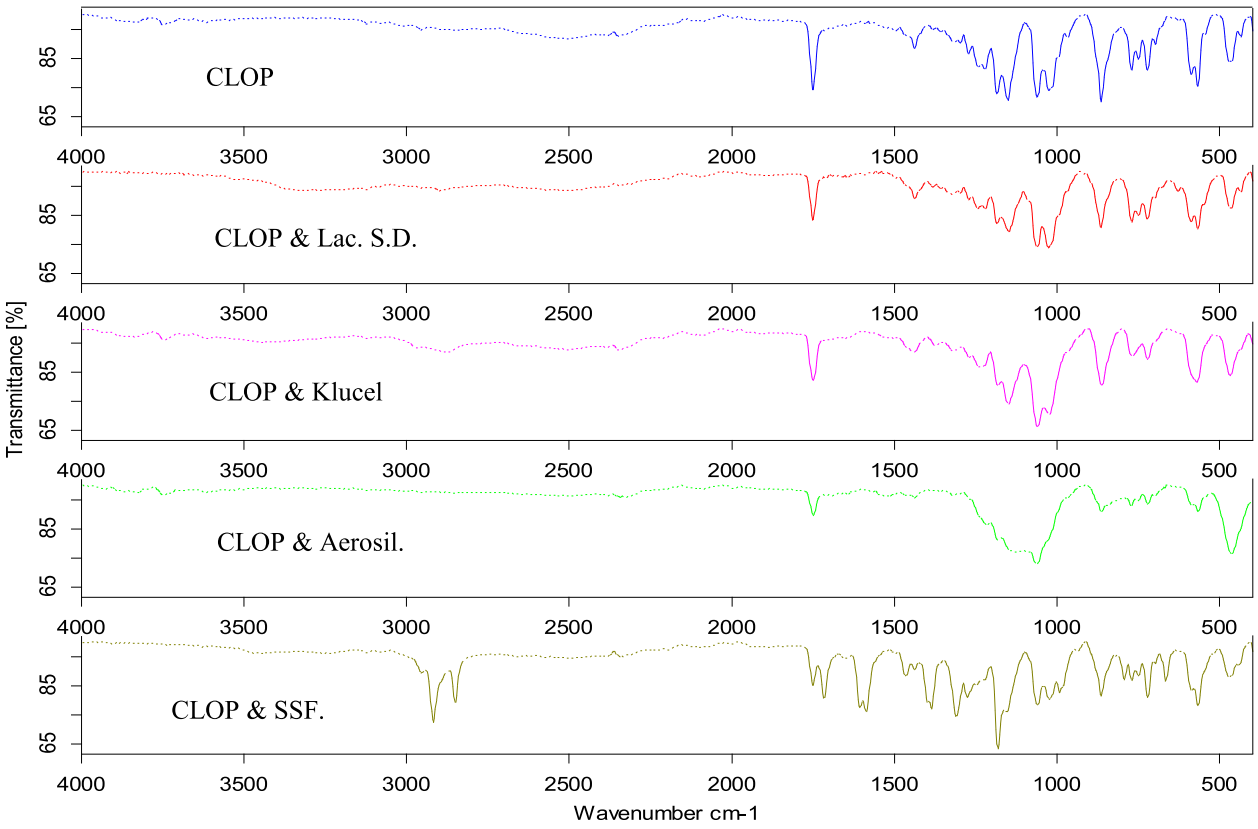

Fig. 4. FT-IR spectra of pure CLOP and a physical mixture of CLOP with each excipient. CLOP: Clopidogrel Hydrogen sulfate, Lac. S.D.: spray dried lactose, Aerosil: Colloidal silica, SSF.: Sodium stearyl fumarate.

Table 6
Evaluation of blend characteristics (n = 3).

Formulation code	Bulk Density	Tapped density	Angle of repose ⊖	Flow Property	Carr's index	Hausner's ratio	Scale of flowability
APX 1	0.31	0.333846	32	Good	7.142857	1.076923	Excellent
APX 2	0.250915	0.294825	35	Good	14.89362	1.175	Good
APX 3	0.246926	0.272122	35	Good	9.259259	1.102041	Excellent
APX 4	0.282214	0.348618	41	Passable	19.04762	1.235294	Fair
APX 5	0.278918	0.310614	35	Good	10.20408	1.113636	Excellent
APX 6	0.305957	0.343268	34	Good	10.86957	1.121951	Good
APX 7	0.357852	0.402583	32	Good	11.11111	1.125	Good
APX 8	0.297844	0.339316	39	Fair	12.22222	1.139241	Good
APX 9	0.302717	0.343827	31	Good	11.95652	1.135802	Good
APX 10	0.300867	0.347154	29	Excellent	13.33333	1.153846	Good
APX 11	0.2462	0.284077	32	Good	13.33333	1.153846	Good
APX 12	0.335605	0.390027	31	Good	13.95349	1.162162	Good
APX 13	0.310667	0.362444	35	Good	14.28571	1.166667	Good
APX 14	0.198118	0.240571	30	Excellent	17.64706	1.214286	Fair
APX 15	0.3032	0.36384	30	Excellent	16.66667	1.2	Fair
APX 16	0.331286	0.3865	31	Good	14.28571	1.166667	Good
CLOP 1	0.3267	0.3769	34	Good	13.3333	1.1538	Good

complete release was achieved after 20 hrs. of dissolution. While formulas with HPMC concentrations of 26.4 % showed complete release after 16 hrs., that obviously revealed a strong dependence of percent released (selected as a response) on the concentration of polymers. SLS is added to the formula as a wetting agent for the inherent hydrophobicity of APX (Jia et al., 2017). As the added quantity of API is 5 mg, the sink conditions were achieved and the SLS quantity showed no effect on the drug release.

Among all the developed formulations, APX 11 which contains HPMC: Methocel E5: HPC in the ratio 46.2: 10.4: 36.3 gave ER for 24 hr. was selected as the optimum formula.

For CLOP 1, complete drug release was achieved within 15 min, indicating that the used excipients did not retard drug release. The CLOP 1 dissolution profile is illustrated in Fig. 6.

The dissolution of the final encapsulated FDC after scale up is shown in Fig. 7. The same dissolution results were obtained, as a complete release of the APX 11 was achieved after 24 hrs, and within 15 min of CLOP 1, with slight rapid release in the percent of CDR of APX 10 mg, two tablets of APX 11, compared to 5 mg, one tablet of APX 11.

3.6. Mathematical model analysis

A mathematical model was constructed to quantify the effect of the variables on the response parameters within the experimental design boundaries. Table 9 summarizes the coefficients of model terms. For each response, ANOVA test model results were evaluated and revealed

Table 7
Evaluation of formulation batches of tablets.

Formula code	Weight variation	Thickness	Hardness	Friability (%loss)	Moisture content (%LOD)	Assay (%)
APX 1	76.09 ± 2.9	2.99 ± 0.07	5.69 ± 0.49	0.06	4.68	95.33
APX 2	75.5 ± 2.4	2.99 ± 0.07	5.3 ± 0.416	0.097	3.95	95.72
APX 3	75.8 ± 2.37	2.97 ± 0.07	5.03 ± 0.37	0.04	4.12	97.04
APX 4	75.18 ± 2.11	2.92 ± 0.06	4.75 ± 0.26	0.6	4.26	96.1
APX 5	75.04 ± 2.15	2.89 ± 0.06	4.82 ± 0.43	0.2	3.99	99.36
APX 6	75.81 ± 2.19	2.92 ± 0.07	4.76 ± 0.43	0.4	3.84	100.89
APX 7	75.31 ± 2.12	2.92 ± 0.07	4.22 ± 0.3	0.4	3.51	101
APX 8	74.47 ± 2.08	2.9 ± 0.05	4.13 ± 0.5	0.02	3.9	98.22
APX 9	75.38 ± 1.72	2.9 ± 0.08	4.92 ± 0.15	0.22	3.99	99.64
APX 10	74.49 ± 2.4	2.89 ± 0.08	4.77 ± 0.31	0.14	4.05	101.76
APX 11	75.48 ± 2.26	2.92 ± 0.06	5.38 ± 0.85	0.04	4.43	95.77
APX 12	76.49 ± 1.86	2.95 ± 0.07	4.48 ± 0.31	0.16	3.85	98.76
APX 13	76.52 ± 2.19	2.9 ± 0.08	4.89 ± 0.45	0.18	3.39	96.21
APX 14	75.69 ± 3.13	2.91 ± 0.09	5.29 ± 0.47	0.03	4.64	95.15
APX 15	75.51 ± 2.64	2.92 ± 0.07	4.42 ± 0.36	0.04	4.65	95.08
APX 16	76.86 ± 2.27	3.02 ± 0.04	4.65 ± 0.42	0.09	4.12	102.23
CLOP 1	148.9 ± 3.56	4.36 ± 0.08	6.63 ± 0.61	0.65	2.9	99.8

The upper limit is 83.3 mg for APX and 161.3 for CLOP.
The lower limit is 68.2 mg for APX and 138.8 for CLOP.

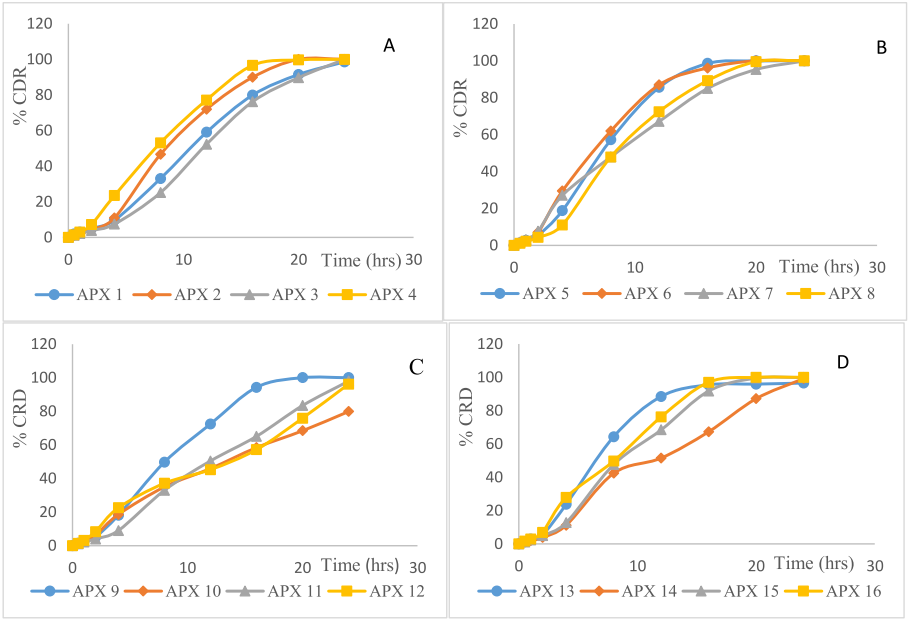

Fig. 5. In vitro dissolution of APX. formulations; (A) APX 1—4, (B) APX 5—8, (C) APX 9—12, (D) APX. 13–16.

Table 8
In vitro dissolution response parameters:

Formula Code	$T_{25\%}$ (h)	$T_{50\%}$ (h)	$T_{90\%}$ (h)	MDT
APX 1	5.618	11.237	20.227	10.901
APX 2	5.6	8.94	16	9.239
APX 3	6.785	12.293	20.498	12.047
APX 4	4.125	8.135	14.472	8.351
APX 5	3.849	7.699	13.858	7.956
APX 6	3.345	7.153	13.594	7.378
APX 7	3.373	8.945	18.730	9.629
APX 8	4.716	9.399	16.869	9.647
APX 9	4.201	8.851	16.557	8.765
APX 10	5.4	13.25	More than 24	10.756
APX 11	6.103	12.207	21.972	12.177
APX 12	5.951	12.519	23.521	12.177
APX 13	3.778	7.555	13.600	6.886
APX 14	5.886	11.772	21.190	11.567
APX 15	4.680	9.361	16.894	9.599
APX 16	4.050	8.099	14.579	8.318

that the sequential p-value was less than 0.05 for each response, and the lack of fit p-values were more than 0.05. The value of R^2 was greater than 0.7, the difference between the predicted R^2 and R^2 was less than 0.2, and the adequate precision values were greater than 8. These values represented a validated design with well fitted responses and insignificant model errors. All responses are shown to be fitted with a linear model.

Evaluating the effect of each component, a significant association was found between the concentration of polymers (HPMC, Methocel E5, and HPC) and the release time of APX. ($p < 0.001$, $p = 0.0003$, and $p = 0.0006$), respectively. An increase in the HPMC concentration was found to retard APX release while increasing Methocel E5 concentration enhances APX release at the beginning, then slowing the drug release. For HPC, increasing its concentration was shown to delay the release of APX at first, then enhancing the release rate later. SLS different quantities showed to have no significant effect on the release process ($p = 0.4843$). Table 10 and Fig. 8 represent the p-values for the 3 evaluated responses.

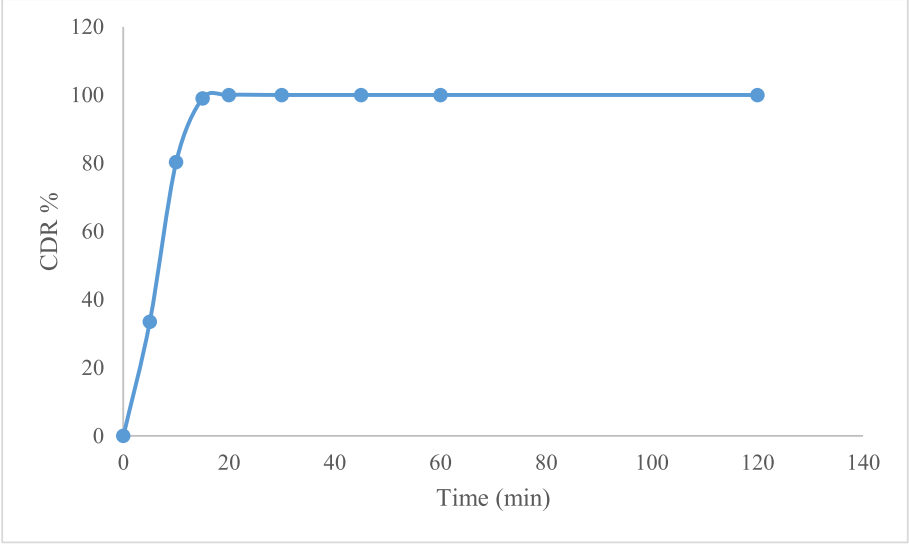

Fig. 6. In vitro dissolution of CLOP.

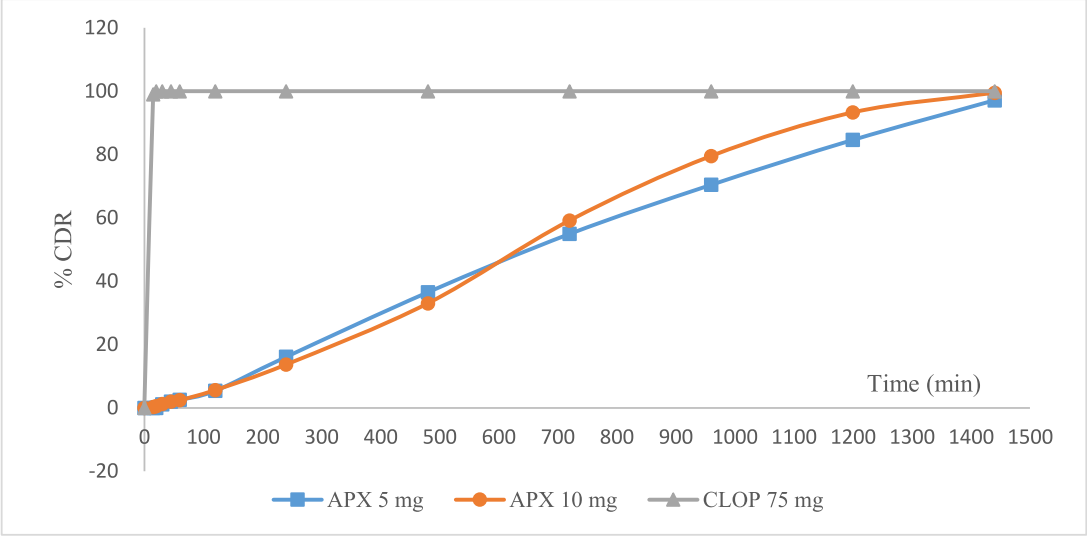

Fig. 7. In vitro drug release of the final dosage form of two doses of APX.5 mg and APX. 10 mg.(n = 6).

Table 9
ANOVA analysis for the selected different responses of APX formulas.

Response	Model	Sequential p-value	Lack of fit p-value	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	F- value
T25%	Linear	<0.0001	0.712	0.8697	0.8372	0.7412	15.0793	26.71
MDT	Linear	0.0001	0.7175	0.8165	0.7706	0.6501	10.8904	17.79
T90%	Linear	<0.0001	0.9236	0.8377	0.7972	0.6737	13.1650	20.65

Table 10
Estimated coefficients for responses.

Component	MDT		T25%		T90%	
	p-value	Co-efficient	p-value	Co-efficient	p-value	Co-efficient
HPMC	<0.0001	+0.305009	0.0001	+0.147837	<0.0001	+0.652761
Methocel E5	0.0003	+0.031030	<0.0001	−0.019282	0.0018	+0.08737
HPC	0.0006	+0.024017	0.0066	+0.027684	<0.0001	−0.058459
SLS	0.4843	+1.66680	0.0533	+2.43533	0.5933	+2.59188

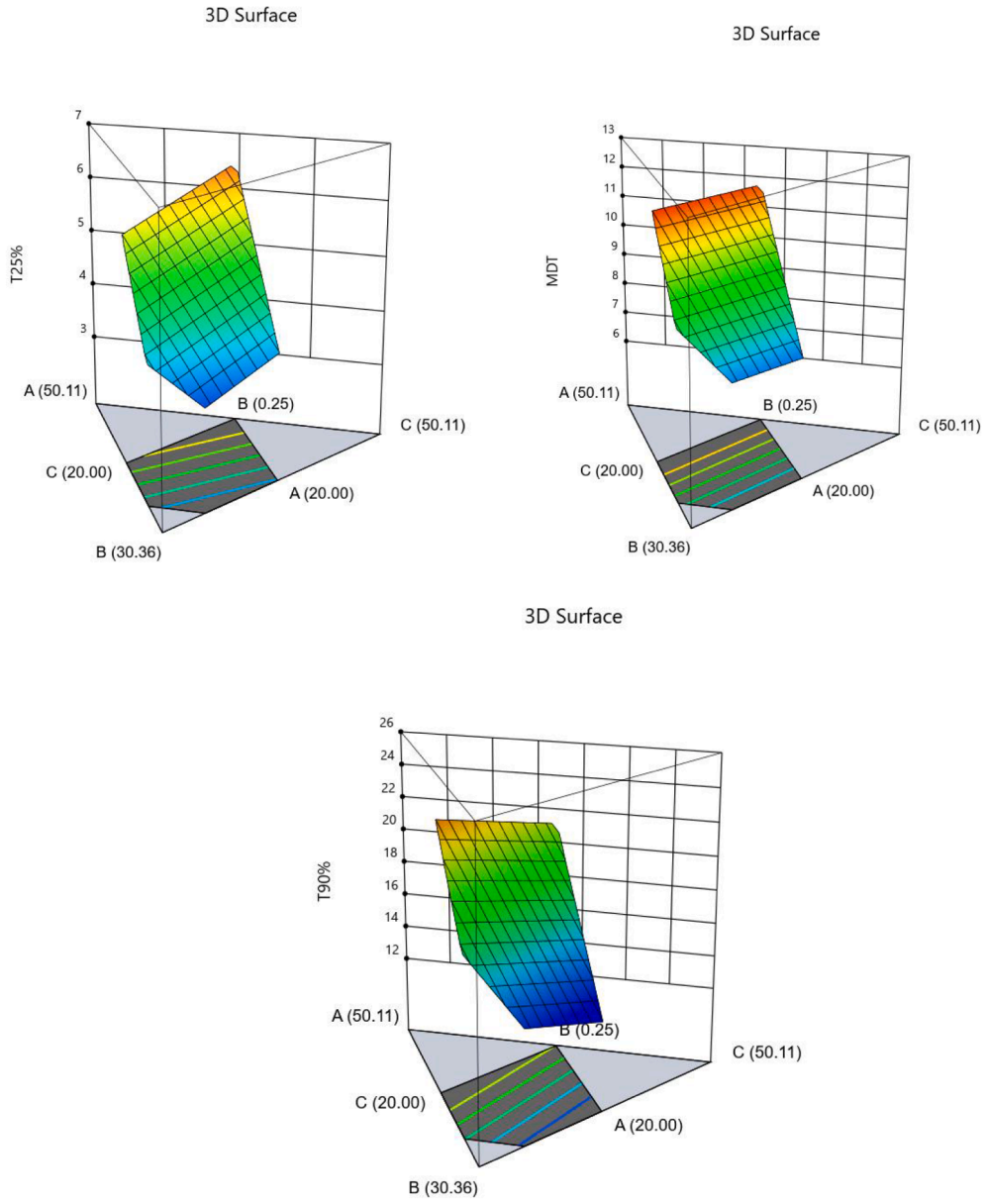

Fig. 8. 3D response –surface showing the influence of independent variables on responses.

3.7. Drug release kinetic study

The dissolution data of the sixteen formulas were fitted into different kinetic models and the regression coefficient (R^2) was close to 1 reflecting the most suitable model for selection (Singhvi and Singh, 2011). Table 11 shows the obtained data. Most of the formulated matrices fitted well into zero order, combined with Korsmeyer-Peppas model. Among formulas having "n" higher than 0.89, the release follows the super case II transport mechanism. While APX formulas 7 and 10 fitted the Hixson-Crowell model, and the "n" value was between $0.45 < n < 0.89$, which indicates the non-Fickian diffusion (anomalous diffusion) model.

3.8. Swelling and erosion studies

Fig. 9 showed the swelling and erosion plots. The plot revealed that the matrix tablets undergo both swelling and erosion spontaneously. The first two hours showed very rapid water uptake with no erosion and water uptake was the dominant process till twelve hours, followed by matrix erosion as the predominant process. After the first 0.5 hrs., an increase in weight was observed even after drying, which may be due to entrapped water within the matrix that cannot evaporate after drying. These results ascertain that the drug release was ruled out according to zero order, combined with the Korsmeyer-Peppas model.

3.9. Stability study

A short-term stability study results for FDC tablets are shown in Table 12. The study was carried out on the optimized formulation, a capsule containing (CLOP 1 and APX 11), for three months at 40 ± 2 °C/ 75 ± 5 % RH. Stability studies have shown no significant changes in the appearance and percent of APX drug content. For CLOP, a slight decrease in the active substance assay was found. So, it was considered that the formulation has good stability.

Table 11
In vitro release kinetics parameters:

Formula code	Zero order		First order		Higuchi Model		Hexon Crowell Model		Korsmeyer-Peppas Model		
	k_0	R^2	K_1	R^2	k_H	R^2	k_{HC}	R^2	k_{KP}	R^2	N
APX 1	4.450	0.9815	0.079	0.9309	17.615‘	0.8596	0.022	0.958	4.574	0.9792	0.991
APX 2	5.345	0.9778	0.094	0.9217	19.204	0.8413	0.027	0.9502	5.059	0.9748	1.02
APX 3	4.315	0.9749	0.072	0.8997	16.875	0.8185	0.021	0.931	2.997	0.9814	1.158
APX 4	6.202	0.9854	0.104	0.9419	19.889	0.8512	0.030	0.9918	5.891	0.9935	1.022
APX 5	6.499	0.9794	0.111	0.9173	20.743	0.8265	0.032	0.9468	5.684	0.977	1.054
APX 6	6.672	0.9736	0.122	0.9457	21.632	0.8655	0.035	0.9698	8.295	0.9725	0.915
APX 7	4.835	0.9701	0.099	0.9669	19.549	0.9235	0.027	0.9868	8.779	0.9860	0.794
APX 8	5.337	0.9763	0.094	0.9245	19.204	0.8438	0.027	0.9522	5.258	0.9763	1.005
APX 9	5.508	0.9814	0.101	0.9356	19.940	0.8660	0.029	0.9630	6.276	0.9796	0.952
APX 10	3.518	0.9864	0.056	0.9901	14.148	0.9173	0.016	0.9963	5.511	0.9944	0.845
APX 11	4.098	0.986	0.067	0.9276	16.121	0.8443	0.019	0.9591	3.241	0.9873	1.086
APX 12	3.892	0.9878	0.064	0.9533	15.554	0.8933	0.018	0.9690	4.755	0.9877	0.935
APX 13	6.624	0.9655	0.119	0.9313	21.372	0.8446	0.034	0.9572	7.577	0.9609	0.947
APX 14	4.254	0.9886	0.073	0.9407	16.855	0.8671	0.021	0.9635	4.353	0.9862	0.994
APX 15	5.341	0.9812	0.094	0.9264	19.223	0.8482	0.027	0.9543	5.203	0.9785	1.010
APX 16	6.173	0.9940	0.104	0.9436	19.841	0.8562	0.03	0.9674	5.990	0.9930	1.014

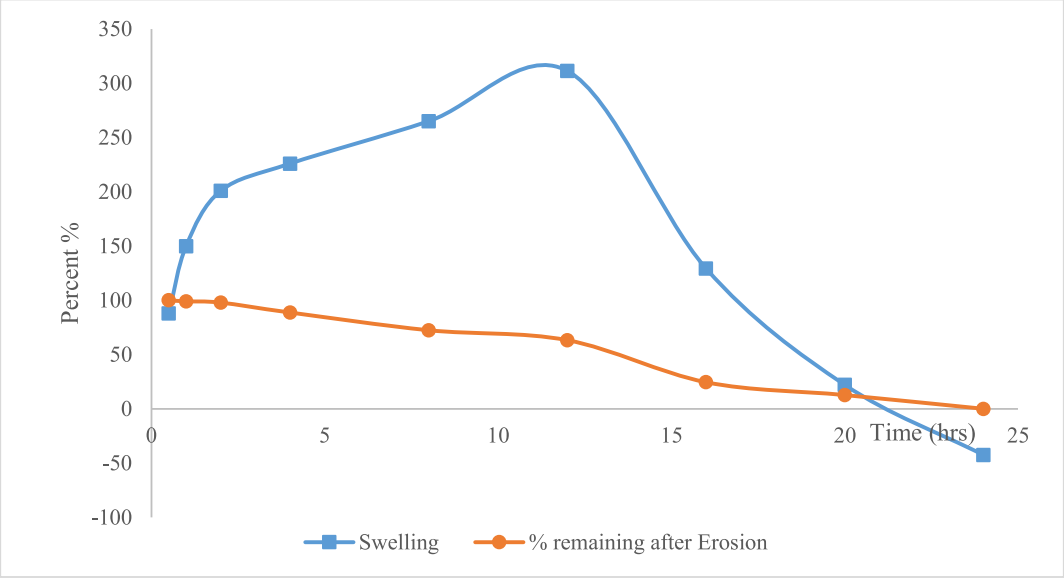

Fig. 9. Swelling and erosion study results.

Table 12
Stability studies of optimized batch.

Parameter		Appearance APX./ CLOP.	Drug content APX./ CLOP.
Initial	APX	Good appearance	95.77 %
	CLOP	Good appearance	99.80 %
After 3 months	APX	Good appearance	95.74 %
	CLOP	Good appearance	97.55 %

4. Discussion

Drugs excipients compatibility study was conducted to evaluate the compatibility of CLOP with APX and the compatibility of each API with pharmaceutical excipients expected to be used in the formulation process (WHO Expert Committee on Specifications for Pharmaceutical Preparations, 2012; World Health Organization, 2005). The most suitable excipients are chosen based on the literature and RLD product search to be suitable and fulfill the purpose of the study.

Since CLOP is formulated via direct compression, excipients with good flow properties are needed. The study results revealed using mannitol powder enhances the stickiness tendency as Sha et al. study (Sha et al., 2015). Using MCC founded to retard the disintegration and dissolution of the produced tablets due to the higher binding properties and hydrophobicity of that diluent (Chaerunisaa and Sriwidodo, 2019).

Regarding APX, it has low compressibility, low flowability (Gambhire et al., 2017), low dose, and is prepared with hygroscopic polymers; wet granulation technology was chosen. It aids in the preparation of a more stable formula that is less likely to cake or harden, has improved flowability and compression characteristics, decreased weight variation and loss of blended powder quality, enhanced drugs wettability, bioavailability as well as content uniformity (Augsburger and Hoag, 2010; Loyd and Allen, 2018).

Evaluating blend flow revealed accepted values, and that is related to the manufacturing process as the formulation was prepared in the wet granulation method (Augsburger and Hoag, 2010). Add that CLOP 1 formulation showed good flow even the bad flowability of CLOP using spray dried lactose. The spray dried process was known to yield spherical lactose particles with uniform size and flow easily (Vail and Cotts, n. d.), reflecting on the final blend's flowability.

In addition, this study revealed a strong dependence of % released (selected as a response) on the concentration of polymers that increasing the concentration of polymer increases the viscosity of the gel layer and retard the drug release (Salih, 2015; Samie et al., 2018). Increasing HPC

level enhances the drug release and indicates the predominance of swelling, which enhances the chance of diffusion over erosion (Dürig et al., 2011). While increasing the quantity of HPMC retarded the release due to the rapid formation of a strong, thick, and turbid gel layer, that resists water diffusion and surface erosion process; and that retard water uptake, drug diffusion, or release (Li et al., 2010; Mohamed et al., 2013). Comparing HPMC and Methocel E5, the polymer with a more hydrophobic methoxy group, HPMC, is less likely to form hydrogen bonding with water, within and between the polymer particles, so less hydration of the core occurs, and slower drug release is achieved compared to Methocel® (Chang et al., 2020; Li et al., 2010) and that explains the slight increase in the amount of methocel increased the percent of drug released.

Moreover, comparing the dissolution of the 2 doses of APX, a slight rapid release in the percent of CDR of APX 10 mg, two tablets of APX 11, compared to 5 mg, one tablet of APX 11. The total amount of SLS in the dissolution media of 10 mg dose tablets is doubled among the same volume of dissolution media compared to 5 mg. SLS is a surfactant that enhances the solubility of APX, which may be the cause behind the higher percentage of cumulative drug release for the dose of 10 mg compared to 5 mg (Sheskey et al., 2017).

Release kinetics results showed that most of the formulated matrices fitted well into zero order, combined with Korsmeyer-Peppas model. As APX has low water solubility and is formulated into a hydrophilic matrix, it is expected to follow zero order kinetics (Singhvi and Singh, 2011). These results indicate that both diffusion of the drug from the swelling gel layer and erosion of this layer are the drug release mechanisms. Reviewing the behavior of hydrophilic polymers such as HPMC and HPC with low water soluble API revealed that the hydrophilic polymers swell upon hydration, and then the drug dissolved and diffused out the system, then the matrix dissolved which explains the erosion process for drug release (Debotton and Dahan, 2017; Siepmann et al., 2002).

Analyzing the erosion study results, the first two hours showed very rapid water uptake with no erosions, which related to water diffusion into the system, relaxation of the polymer chain, and volume expansion upon exposure to biological fluid (Siepmann et al., 2002).

For CLOP, many studies performed to establish the compatibility between CLOP and different lubricants. Mg. stearate was found incompatible, and another alternative was found to have a less degradative effect such as stearic acid and SSF (Amer et al., 2016; Bharate et al., 2010; Sherman, 2005). This study found a slight decrease in the active substance assay in the formula due to SSF. This result was consistent with Sherman's study results, where around 1 % of CLOP degraded after applying stress conditions. He revealed that SSF is a more efficient lubricant than castor oil and PEG, and has a lower degradative effect than Mg. stearate, calcium stearate, zinc stearate, and stearic acid (Sherman, 2005). So, it was considered that the formulation has good stability, and a long-term stability study needed to be performed.

5. Conclusion

Oral dosage forms are the most popular route of administration. The FDC tablet offers safety and efficacy advantages by improving patient medication adherence, decreasing polypharmacy, and reducing medication costs. In addition, MPS for FDC products is a valuable tool for preparing medications with different dissolution profiles, pharmacokinetics, and pharmacodynamics. APX and CLOP are antithrombotic substances indicated for patients diagnosed with AF who had ACS and undergone percutaneous coronary intervention. The formulation of a novel FDC tablet of CLOP and APX will improve patient compliance and facilitate the dosing regimen.

The results revealed that CLOP 1 prepared using spray dried lactose as diluent gives a formula with good characteristics and a fast disintegrated effect, and sodium stearyl fumarate showed good compatibility with CLOP. For APX ER formula, the type and quantity of used polymers are shown to be important factors that can affect the drug release from the matrix. APX 11 which contains HPMC: Methocel E5: HPC in the ratios 46.2: 10.4: 36.3 gave ER for 24 hrs. was selected as the best formula. The drug release kinetics follows Korsmeyer-Peppas combined with zero order, and the mechanism was found to be super case II transport. The stability studies indicate that the selected formula was stable.

Further study may be implemented to evaluate the dissolution kinetics of FDC in simulated gastric and intestinal media mimicking fasting and fed conditions and to perform a bioequivalence study. A full stability study design following ICH guidelines is also recommended.

Funding.

This research was funded by the Scientific Research Committee, Faculty of Graduate Studies, Birzeit University, Palestine.

Ethical Consideration

Ethics approval was not sought for this study; experimental in-vitro research was conducted in the lab without involvement of any research subjects.

CRediT authorship contribution statement

Ni'meh Al-Shami: Writing – original draft, Writing – review & editing. **Hani Naseef:** Supervision. **Feras Kanaze:** Project administration, Supervision, Validation, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This paper is part of master's thesis in Industrial Pharmaceutical Technology at Birzeit University.

The authors are thankful to Assem Mubarak for analyzing compatibility study samples on FTIR. Thankful to Ramzi Moqadi for performing the HPLC analysis.

The authors are thankful to Pharmacare PLC (Palestine) for providing a gift sample of Apixaban, Clopidogrel Hydrogen Sulfate, and excipients.

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