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Preparation and *in vitro* evaluation of hot-melt extruded pectin-based pellets containing ketoprofen for colon targeting

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ABSTRACT

This work developed high drug-load pellets for colon targeting in minimal steps by coupling hot-melt extrusion (HME) with a die-surface cutting pelletizer, offering a potential continuous pellet manufacturing process. Ketoprofen (KTP) was selected as a model drug for this study due to its thermal stability and severe upper gastrointestinal side effects. Low and high methoxyl grade pectins were the enzyme-triggered release matrix, and hydroxypropyl methylcellulose (HME 4 M/HME 100LV) was used as a premature release-retarding agent. The powder X-ray diffraction technique and the differential scanning calorimetry results revealed that KTP exists in the solid-solution state within the polymeric matrix after the HME step. The scanning electron micrographs of the fabricated pellets showed a smooth surface without any cracks. The lead formulation showed the lowest premature drug release (~13%) with an extended KTP release profile over a 24 h period in the presence and absence of the release-triggering enzyme. The lead formulation was stable for 3 months at accelerated stability conditions (40 °C/75 ± 5% RH) concerning drug content, *in vitro* release, and thermal characteristics. In summary, coupling HME and pelletization processes could be a promising technology for developing colon-targeted drug delivery systems.

1. Introduction

Colonic diseases have become a critical public healthcare issue since colorectal cancer is the third most common cancer type worldwide, with approximately two million cases, were diagnosed in 2020. Colorectal cancer is the second most common cause of cancer death, leading to almost one million deaths yearly, according to the World Health Organization (Colorectal Cancer Awareness Month 2022 – IARC). Moreover, inflammatory bowel disease has become public health challenge in the past decade (Ng et al., 2017). >2 million and 1.5 million people in Europe and North America suffer from inflammatory bowel disease, respectively (Ng et al., 2017). Although the incidence of inflammatory bowel disease has become stable in western countries, the burden remains high, and the prevalence exceeds 0.3% (Ng et al., 2017). These colonic diseases necessitate effective local or systemic drug delivery for better therapeutic outcomes. Colon-targeted drug delivery systems have recently gained interest as potential local and systemic delivery carriers. The local treatment of colonic disorders with diminished systemic side effects is required.

Moreover, colon-targeted drug delivery systems can serve as promising oral delivery platforms for various actives susceptible to acidic and enzymatic degradation within the upper gastrointestinal tract (Lee et al., 2020). Colon-targeted drug delivery systems are intended to release the active pharmaceutical ingredient (API) in response to specific stimuli within the colonic environment with minimal premature release in the upper gastrointestinal tract (GIT). Therefore, it is necessary to explore the physiological properties of this organ and the environment surrounding colonic disease for the successful development and design of colon-targeted drug delivery systems. The microenvironment near the disease site is significantly different from normal colonic regions. For example, high levels of reactive oxygen species and inflammatory cytokines are associated with colonic diseases. Various approaches have been investigated to improve colon drug delivery, including pH-sensitive, enzyme-triggered, magnetically driven, and receptor-

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mediated (overexpression at the disease site) systems (Guo et al., 2018).

Pectins are complex non-toxic polysaccharides that have been widely used in the food and pharmaceutical industry for years and are referred to as a GRAS substance by the FDA (Khotimchenko, 2020). Pectins are classified into two categories based on the degree of esterification (DE) to high methoxyl pectins (HMP; DE > 50%) and low methoxyl pectin (LMP; DE < 50%) (Jantrawut et al., 2013). One of the unique properties of pectin is the ability to form gels (Sriamornsak, 2011). The main outstanding property makes this polysaccharide an important excipient in the pharmaceutical industry. The enzymes of the upper GIT do not digest pectins; however, the pectinolytic enzymes of colonic microflora degrade pectins completely. Therefore, pectins can maintain their gelling properties until reaching the colon and act as a successful matrix for an extended-release and enzyme-triggered systems for colon targeting. Moreover, decreasing blood cholesterol and promoting the removal of lipids and heavy metal ions from the body are additional health benefits for pectins (Khotimchenko, 2020; Wang et al., 2020).

However, the high water solubility of pectin makes the development of pectin-based formulations for colon targeting difficult (Chourasia and Jain, 2004). Pectin swells upon contact with the aqueous fluids of the gastrointestinal tract and causes the premature release of the entrapped API through diffusion before reaching the target. This problem could be overcome by choosing between pectin grades or including other polymeric excipients (Rubinstein et al., 1993). Therefore, a binary polymer matrix system with pectin and hydroxypropyl methylcellulose (HPMC) was developed for drug release profile tailoring to address this hurdle (Tung et al., 2016; Ugurlu et al., 2007).

Ketoprofen (KTP) is a nonsteroidal anti-inflammatory drug with analgesic and antipyretic properties, commonly used to treat rheumatoid arthritis symptoms and postoperative and trauma pain (Khotimchenko, 2020; Wang et al., 2020). The most common adverse effects of KTP are gastric ulceration and bleeding (Kuczyńska and Nieradko-Iwanicka, 2021; Pereira-Leite et al., 2017). This upper GIT toxicity made KTP an ideal candidate. In addition, KTP thermal stability makes it a good model drug to meet the study objective (Amidon et al., 1995).

The solvent-free hot-melt extrusion (HME) technology has gained much interest in the pharmaceutical field because of its continuous manufacturing ability. As a result, HME technology has been established for almost all routes of administration, including oral (Alshetaili et al., 2021), ocular (Khan et al., 2022), dermal, and parenteral formulations (Alzahrani et al., 2022a). Moreover, HME is a promising tool for producing high drug-load pellets in minimal steps by coupling HME with a die-surface cutting pelletizer, thus, offering a continuous pellet manufacturing process (Treffer et al., 2014; Vo et al., 2020).

The novel objective of the current study was to investigate pectin's suitability as an enzyme-triggered carrier matrix for colon drug delivery using HME technology. For this purpose, KTP was selected as a model drug, and HPMC was added to the bio-responsive polymeric matrix to prevent premature drug release in the upper gastrointestinal tract. This continuous manufacturing process could help develop extended-release systems for colon delivery. Moreover, the success of this approach could elucidate the possibility of protecting the drug from degradation within the upper gastrointestinal tract and protecting the upper gastrointestinal tract from ulceration and bleeding induced by many drugs, including KTP.

2. Materials & methods

2.1. Materials

KTP was purchased from PCCA (Houston, TX, USA) (Lot No. C171492). Genu® pectin type D slow set-Z (High methoxyl grade; Pectin HM) (Batch: GR10002240) and Genu® pectin type LM-104 AS-FS (Low methoxyl grade; Pectin LM) (Batch: SK30015244) were purchased from CPKelco Company (Atlanta, GA, USA). AFFINISOL™ HPMC HME 4 M (4 M) (Lot No. INR524352) and HME 100LV (100LV) (Lot No. INR524351) were kind gifts from Colorcon Inc. (West Point, PA, USA). Pectinex® Ultra SP-L (3300 PGNU/g activity) was purchased from Modernist Pantry LLC (Eliot, ME, USA). Transparent hard gelatin capsule shells, size 00 were purchased from Total Pharmacy Supply Company (Arlington, TX, USA). Acetonitrile and methanol solvents were of High-Performance Liquid Chromatography (HPLC) and purchased from ThermoFisher Scientific (Waltham, MA, USA).

2.2. HPLC analysis

KTP was quantified following an HPLC-UV method reported in the United States Pharmacopeia (USP) monograph of KTP extended-release capsules (USP-NF Ketoprofen Extended-Release Capsules). The analysis was carried out using a Waters HPLC system (Waters Corp., Milford, MA, USA) equipped with a UV/VIS detector. Chromatographic separation was achieved on a Phenomenex Luna® C18 column (25 cm × 4.6 mm, 5.0 µm). A detection λ_{max} of 254 nm was set during the analysis. The mobile phase (MP), consisting of acetonitrile, water, and glacial acetic acid (90:110:1), was pumped isocratically at a 1.2 mL/min flow rate through the instrument. The stock solution was prepared by dissolving KTP in the MP. The samples were analyzed with Waters Empower software chromatography data system. The HPLC method was linear over the KTP concentration range of 1.0–100 µg/mL with a 20 µL injection volume.

2.3. Preparation of physical mixtures

The composition of different physical mixtures (PMs) used for the HME process is shown in Table 1. The formulation excipients were blended in a porcelain mortar and pestle following the geometric dilution technique to ensure equal distribution within the PM for uniform drug content in the prepared pellets. First, all PMs were sieved (mesh# 45, US) to confirm the absence of aggregates within the mixture. Then, all PMs were tumble-mixed for 20 min in a V-shaped blender (Maxi-Blend™, GlobePharma, NJ, USA).

2.4. Blend uniformity

An accurately weighed amount (0.5 g) was collected from three different locations of each PM and dissolved in 100 mL of the MP. First, the KTP-methanol mixtures were vortexed (10 min) and subjected to 10

Table 1
Formulation composition of ketoprofen pellets.

Formulation	Ketoprofen (% *w*/w)	Pectin HM (% *w*/w)	Pectin LM (% *w*/w)	HPMC HME 4 M (% *w*/w)	HPMC HME 100LV (% *w*/w)
F0*	20	80	–	–	–
F00*	20	–	80	–	–
F1	20	–	–	80	–
F2	20	–	–	–	80
F3	20	16	–	64	–
F4	20	32	–	48	–
F5	20	48	–	32	–
F6	20	64	–	16	–
F7	20	–	16	64	–
F8	20	–	32	48	–
F9	20	–	48	32	–
F10	20	–	64	16	–
F11	20	16	–	–	64
F12	20	32	–	–	48
F13	20	48	–	–	32
F14	20	64	–	–	16
F15	20	–	16	–	64
F16	20	–	32	–	48
F17	20	–	48	–	32
F18	20	–	64	–	16

* Hot melt extrusion failed for this formulation.

min bath sonication. Then, the KTP-methanol mixtures were centrifuged (Fisherbrand™, Waltham, MA, USA) at 12,000 rpm for 10 min. The supernatant was collected with an Eppendorf® pipette and filtered through a Nylon membrane filter (0.45-μm). The filtrate was quantified for KTP with the HPLC method described above, following proper dilution with the extracting solvent.

2.5. Preparation of pellets

Pellets manufacturing was achieved using an 11 mm twin-screw corotating extruder (Process 11™, Thermo Fisher Scientific, TX, USA) with a standard Thermo Fisher Scientific screw design. The extruder was equipped with a volumetric feeder, conveyor belt, and a Varicut pelletizer (Thermo Fisher Scientific GmbH, Germany), as illustrated in Fig. 1. The PMs were successfully extruded at a screw speed of 50 rpm at 115 °C in all barrel zones, and a 5-set feed rate (1–2 g/min). The extruder barrel with die was heated to the required temperature and thermally equilibrated for 15 min before processing. The hot extrudate was stretched on the conveyor belt for each PM, and the first 5.0 g of HME extrudate was discarded. The extruded filaments were cooled and hardened on the conveyor belt before feeding to the pelletizer. The pellets were obtained during a steady state of extrusion, and the pelletizing speed was synchronized with the rate of extrudate formation. For each formulation, pellets (13 g) were collected and stored in tight-sealed plastic bags (20–25 °C) for further evaluation and characterization. Later, pellets (375 mg) were manually filled into hard gelatin capsule shells size 00.

2.6. Drug content

An accurately weighed amount (1.0 g) of the fabricated pellets (all formulations) was ground in a porcelain mortar into a fine powder. A sample equivalent to 50 mg of KTP (250 mg) was transferred to a volumetric flask (100 mL) containing the MP. The flask was sonicated for 10 min in Branson 2510 bath sonicator (Branson Ultrasonic Corp., Danbury, CT, USA). Samples (2 mL) were then centrifuged at 12,000 rpm for 10 min at 25 °C. The supernatant was collected with an Eppendorf® pipette and filtered through a Nylon membrane filter (0.45-μm). Then, the filtrate was diluted 10 times with the MP and analyzed for KTP content using the HPLC method described above.

2.7. Disintegration test

The disintegration testing was carried out using a DT2-IS disintegration tester (Dr. Schleuniger® Pharmatron) in 0.1 N HCl as the disintegration medium. The disintegration medium was thermally equilibrated (37 ± 2 °C) for 15 min before the test. Each tube (6 tubes) within the basket rack assembly contained one capsule, which was covered with a perforated plastic disc during the study. A standard motor raised and lowered the basket assembly at a distance of 5 to 6 cm with a 28 to 32 cycles/min basket oscillation frequency (USP).

2.8. In vitro dissolution testing

Three-stage dissolution testing for capsules was performed with three different media using a USP type-I basket apparatus (Hanson SR8-plus™; Hanson Research, Chatsworth, CA, USA) (Dumpa et al., 2018). The basket speed was set at 100 rpm, and a dissolution medium consisting of 0.1 N HCl (750 mL) was used. Dissolution media were thermally equilibrated at 37 ± 0.5 °C for 1 h before adding the dosage form. Next, the formulations were exposed to a 0.1 N HCl dissolution medium for 2 h. After 2 h in 0.1 N HCl, the pH was increased to 6.8 by adding 250 mL of sodium triphosphate buffer (0.2 M), and dissolution was carried out for another 3 h. In the last stage, the pH (7.4) of the dissolution medium was adjusted with sodium hydroxide (0.1 M) along with Pectinex® Ultra SP-L (3 mL), and dissolution was continued up to 24 h. Samples (2 mL) were collected at regular intervals and replaced with an equivalent volume of freshly prepared dissolution media. Samples were analyzed for KTP using the HPLC method described previously. It is worth mentioning that dissolution testing was repeated for the lead formulation in the absence of Pectinex® Ultra SP-L.

2.9. Differential scanning calorimetry (DSC)

DSC can assess the drug's thermal behavior, crystallinity, and miscibility with formulation excipients (Alzahrani et al., 2022a). DSC curves were recorded using a Discovery DSC 25 instrument (TA Instruments, Newcastle, DE, USA) equipped with a refrigerator cooling system (RCS90). The pellets were pulverized into a fine powder. Samples (5–10 mg) were loaded in hermetically sealed aluminum pans. The samples were then heated from 25 °C to 200 °C at a rate of 10 °C/min in an ultra-purified nitrogen environment at a 50 mL/min purge flow. The heat flow difference *vs.* temperature plots was obtained using Trios software (TA Instrument, DE, USA).

2.10. Powder X-ray diffraction (PXRD)

The crystallinity of KTP in the pure form and within the formulation was evaluated by PXRD analysis. The diffractograms were captured with a Rigaku X-ray system (D/MAX- 2500PC, Rigaku Corp., Tokyo, Japan) using Cu rays (λ = 1.54056 Å) at 40 kV and 40 mA over a 2–50° scanning range with a step width of 0.02°/s and a scan speed of 0.02 s (Narala et al., 2022).

2.11. Scanning electron microscopy (SEM)

The surface morphology of pure KTP, PM, and pellets of the lead formulation was studied using a JOEL JSM-7200FLV scanning electron microscope (JOEL, Peabody, MA, USA) with an accelerating voltage of 5.0 kV. Samples were placed and adhered to the SEM stubs using double-sided film adhesive tape. All samples were sputter-coated with platinum under an argon atmosphere using a fully automated Denton Desk V TSC Sputter Coater (Denton Vacuum, Moorestown, NJ, USA).

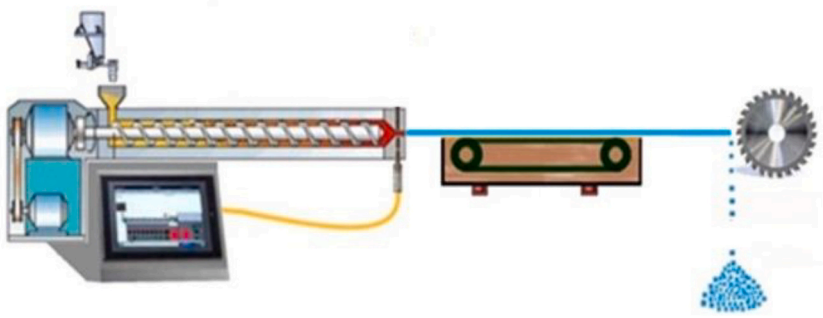

Fig. 1. Schematic representation of the hot melt extrusion process coupled with a pelletizer. The figure was adapted with permission from ref. (Vo et al., 2020).

2.12. Uniformity of dosage units of the lead formulation

Ten capsules were weighed individually, and gross weights were recorded. Then, capsules were emptied, the shells were individually weighed, and the net weight was calculated by subtracting the weight of the empty shell from the respective gross weight. KTP content of each capsule was quantified following the same procedures adopted during drug content testing. Finally, the drug content of each capsule was recorded based on the net weight, and the acceptance value was calculated.

2.13. Stability studies

The physicochemical stability of capsules was investigated over 3 months (last time point tested) of storage in glass scintillation vials at accelerated conditions (Climatic zone I; 40 ± 2 °C/75 ± 5% RH) (Nyavanandi et al., 2022). Samples were collected and evaluated at predetermined intervals for any change in drug content, *in vitro* release, and thermal characteristics.

2.14. Statistical analysis

Statistical comparisons of mean values of data were performed using one-way analysis of variance (ANOVA). The difference was deemed significant when the *p*-value was <0.05. SPSS 28 software (IBM®, Armonk, NY, USA) was used for this study.

3. Results and discussion

3.1. Blend uniformity

The content uniformity of the blend can adversely affect the performance of the final dosage form if the formulation excipients are not blended effectively. The FDA stated that the data should be within ±10% of the theoretical concentration to fulfill an acceptable level of mixing. Each formulation's mean drug content value was 90 to 110%, as shown in Fig. 2a, indicating that an excellent PM preparation technique was adopted before the HME process.

3.2. Hot-melt extrusion and pelletizing process

The HME process parameters like screw speed and feed rate were optimized for all the investigated formulations from the preliminary trials. Zone barrel temperature (115 °C) was optimized depending on the physical mixture's thermal data intending to minimize thermal degradation of the formulation excipients. A standard screw configuration with three mixing zones was used to extrude all the formulations. The extrusion temperature was still higher than the drug melting temperature (92 °C); thus, the HME process followed a miscible regime (Vo et al., 2020). When the extrusion temperature was ≤100 °C, the extruded filaments were less stretchable, resulting in difficulty in controlling the pellet size. This observation could be due to the presence of pectin that can affect the Tg of the HPMC polymer. The temperature of the feeding zone was kept lower than the remaining barrel zones through water recirculation from a chiller (15 °C) to prevent the agglomeration of the feed material in zone 1. To minimize variations, the torque and feed rate were controlled throughout the extrusion process. A 4 mm die was used to extrude all physical blends. The HME process produced uniform (~ 4 mm) circular strands. However, the HME process of physical blends of KTP and pectin alone was unsuccessful at 115 °C. When the processing temperature increased to 130 °C, the blend was converted to a dark brown to black color filament. This observation could be due to the degradation of pectin (Fraeye et al., 2007). Another reason for adding HPMC to pectin was to help process the pectin at low temperatures and improve the filament quality. It is worth mentioning that the drug load of the dark brown to black extrudates was comparable with the theoretical values and there was no new or abnormal peak in the HPLC chromatograms of the extrudate compared with that of the physical mixture, which confirmed the stability of the drug during the extrusion process.

3.3. Drug content for capsules

The drug content for all prepared capsules was found to be in the range of 93.9 ± 2.5 to 99.3 ± 2.1%, as shown in Fig. 2b. Generally, a ± 10% variation in drug content from the label claim (100%) is acceptable and accounted for the variability in the manufacturing and shelf-life stability. This limit is based on the observation that this variation in the drug content is less expected to have any observable adverse effect on the desired therapeutic outcome and toxicity profiles of the fabricated dosage form according to the USP.

3.4. Disintegration and In vitro release testing

The capsules of all 18 formulations showed rapid disintegration (~6.5 min). The *in vitro* drug dissolution testing was performed in three different pH conditions representative of the gastrointestinal tract to investigate the premature release phenomenon for all prepared capsules in this study. The *in vitro* drug dissolution profiles are graphically presented in Fig. 3. Additionally, the percentage of drugs released at the end

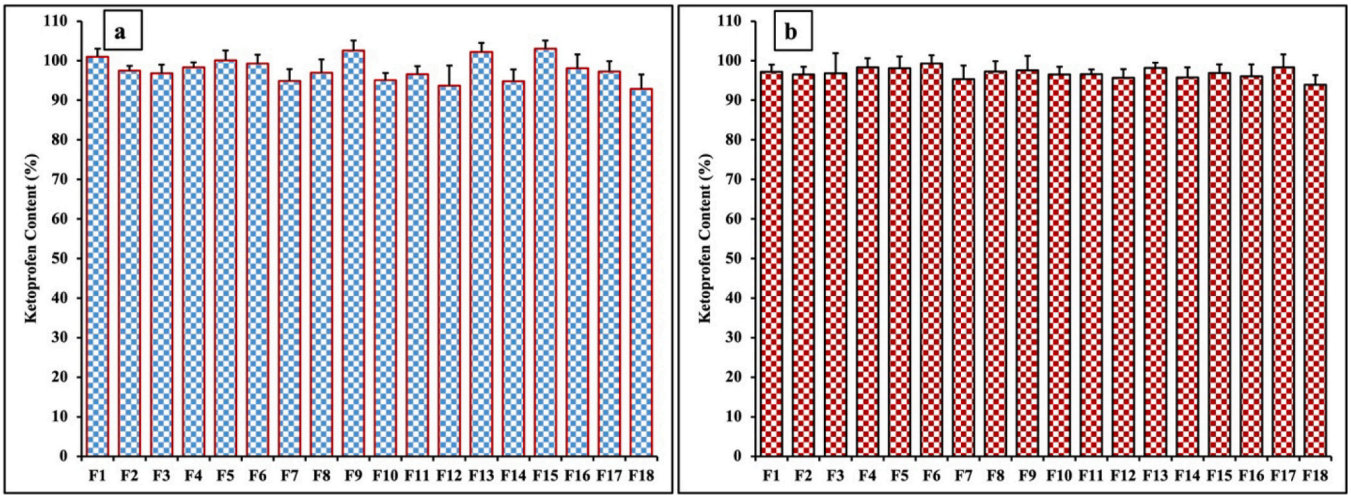

Fig. 2. Drug content (%) of all prepared a) physical mixtures before the HME process (Mean ± SD, $n = 3$) and b) ketoprofen-containing capsules (Mean ± SD, n = 3).

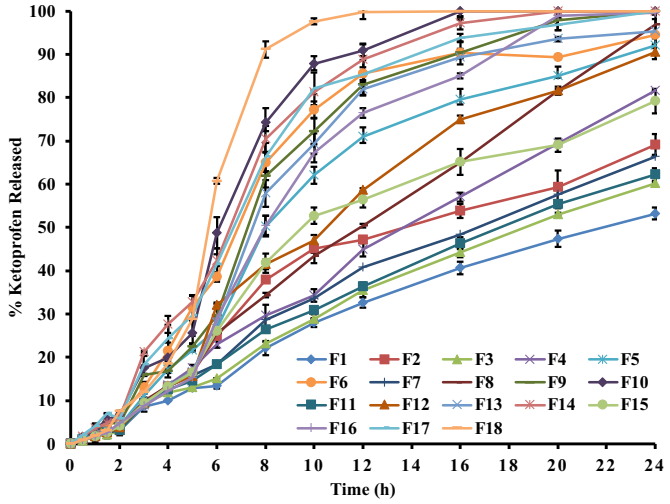

Fig. 3. Enzyme-triggered dissolution profiles of ketoprofen capsules (Mean ± SD, n = 3).

of each dissolution stage is represented in Table 2**.** A low % (~3–7.5) of KTP was released from all developed formulations at a pH of 1.2. Among all the formulations, F1 and F2 showed the lowest % drug release. When only HPMC was used with the drug in the formulation, the KTP release was slower than in other formulations depending on the polymer concentration. The increase in HPMC concentration of both grades significantly decreased KTP release. In the second stage, the F3 formulation demonstrated the lowest premature drug release (13.11%) among all formulations. This outcome could be due to the higher viscosity grade polymer HPMC HME 4 M, which inhibits the KTP diffusion out of the formulation. In addition, the high methoxyl pectin is less aqueous soluble and delays the KTP release.

Alternatively, F18 showed relatively the highest premature drug release (32.68%) due to the low viscosity grade polymer HPMC HME 100LV that allows easy drug diffusion outside the polymeric matrix (AFFINISOL™ - Nutrition & Biosciences). It is worth mentioning that the superior water solubility of the polymeric network containing the low-methoxyl pectin showed the highest premature release values within the first 5 h of the dissolution test. All these observations were consistent with many earlier published investigations (Hiremath and Saha, 2008). The dissolution study was continued for another 19 h by adding the degrading enzyme (Pectinex® Ultra SP-L) and adjusting the pH (7.4) of the dissolution media to mimic the colonic conditions. A relatively similar drug release profile was observed with the developed formulations. The increase in HPMC concentration of both grades significantly decreased KTP release in this dissolution stage. The F3 formulations showed the lowest drug release value (60.10%) at 24 h because it consists of a high methoxyl grade pectin and the highest concentration of the higher viscosity grade polymer HPMC HME 4 M.

Table 2
Percentage of ketoprofen release throughout the three-stage dissolution testing, performed with three different media using a USP type-I basket apparatus (Mean, $n = 3$).

Formulations	% Ketoprofen released			
	0.1 N HCl (pH 1.2) at 2 h	PB (pH 6.8) at 5 h	PB (pH 7.4) at 24 h with enzyme	PB (pH 7.4) at 24 h without enzyme
F1	3.07	12.83	53.23	Not applicable (NA)
F2	3.04	16.23	69.15	NA
F3	3.11	13.02	60.10	49.10
F4	3.62	17.41	81.57	NA
F5	4.16	21.54	92.12	NA
F6	5.90	31.57	94.42	NA
F7	3.25	15.82	66.31	NA
F8	3.80	16.44	96.83	NA
F9	4.57	22.46	100	NA
F10	6.74	25.59	100	NA
F11	3.13	14.65	62.28	NA
F12	3.72	15.55	90.54	NA
F13	4.29	16.62	95.27	NA
F14	6.02	28.75	100	NA
F15	4.10	16.39	79.20	NA
F16	4.75	15.27	99.64	NA
F17	5.85	29.59	100	NA
F18	7.49	32.68	100	NA

3.5. *Effect of pectin grade on drug release*

HPMC is a unique immediate and extended-release matrix forming polymer depending on the grade of the polymer. The dissolution study revealed that the 4 M grade (F1) provided a slower drug release rate than 100LV (F2). HPMC HME 100LV and 4 M molecular weights are ~179.3 and 552.8 kDa, respectively (Huang et al., 2016). This difference in the molecular weight between different polymer grades can affect the polymer viscosity after hydration. The polymer viscosity can affect polymer chain disentanglement. Therefore, there is a strong relationship between HPMC molecular weight and polymer chain disentanglement (Krese et al., 2016). Thus, high viscosity polymers induce greater chain entanglement than low viscosity polymers. Hence, it is difficult for longer polymer chains to dissolve because of the high energy needed to pull them out of the matrix. Consequently, polymers with higher viscosity form a thicker gel layer upon contact with the aqueous medium and reduce drug diffusion out of the polymeric matrix. The results were consistent with many published reports (Hiremath and Saha, 2008).

All the pectin-based formulations (F3 – F18) demonstrated higher drug release profiles over HPMC-based formulations (F1 and F2). This outcome could be due to the degradation of pectin by the enzyme (Pectinex® Ultra SP-L) in the dissolution media (Macleod et al., 1999), which allows the KTP to be released at a faster rate. Low methoxyl pectin matrices (F8 and F16) showed faster drug release in colonic pH than their respective high methoxyl pectin matrices. Furthermore, the KTP release from the pectin matrix-based pellets was altered by changing the pectin grade (Fig. 4) and concentration (Fig. 5a and b). These observations could be attributed to the difference in the swelling and erosion characteristics of the pectin matrix (Sriamornsak et al., 2007). Therefore, the pectin-containing matrix can form a gel layer upon contact with an aqueous medium, thus, undergoing swelling and erosion (Turkoglu and Ugurlu, 2002). The low methoxyl pectin matrix formulations release

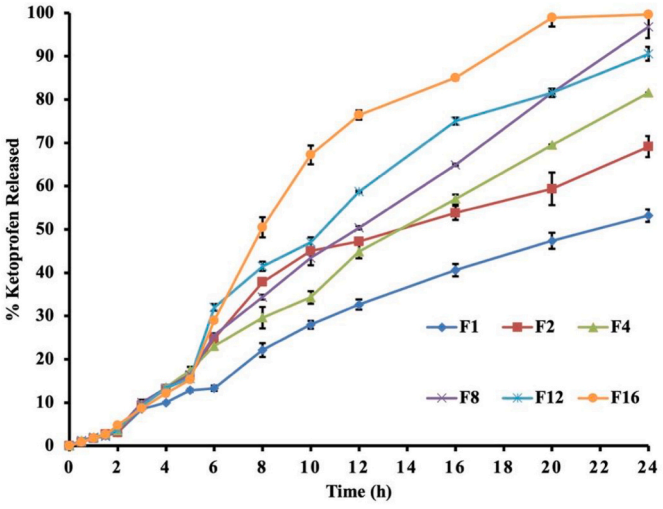

Fig. 4. Effect of pectin grade on ketoprofen release from hydroxypropyl methylcellulose-containing capsules (Mean ± SD, $n = 3$).

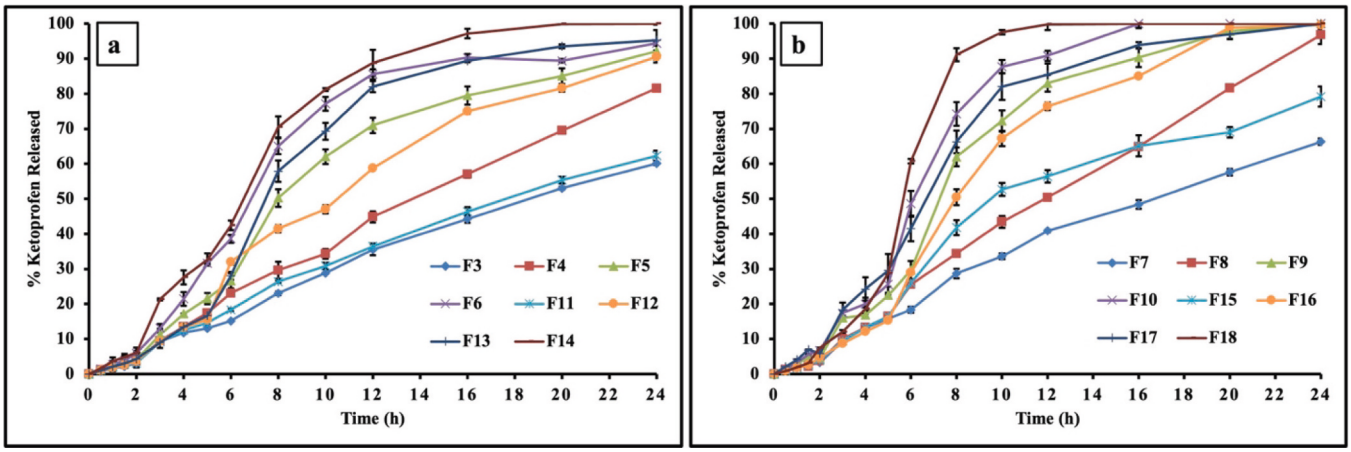

Fig. 5. Effect of pectin concentration on ketoprofen release from hydroxypropyl methylcellulose-containing capsules a) High methoxyl pectin (Mean ± SD, $n = 3$) and b) Low methoxyl pectin.

the drug faster than high methoxyl pectin matrices due to more ionizable carboxylic groups imparting high hydrophilicity and aqueous solubility to low methoxyl pectin. The lower the molecular weight, the weaker the gel. Thus, the swelling capacity of the matrix was low and provided a faster drug release.

3.6. Effect of pectin concentration on drug release

The dissolution data showed (Figs. 5a and b) that the KTP release was increased with increasing pectin concentration. It is worth mentioning that this observation was valid for both grades of this polymer. This outcome could be due to the increase in pectin concentration within the formulation, allowing more and easy degradation by the enzyme at the colonic conditions (pH 7.4), thus, allowing more drug release. Furthermore, higher pectin concentration resulted in more premature drug release at the end of the first and second dissolution stage (pH 1.2 and 6.8). Based on the dissolution testing observations, F3 showed the lowest premature release value– ~13% at 5 h– and released only ~60% of its KTP content over the 24 h release experiment because the formulation contained the highest concentration of HPMC HME 4 M polymer–release-retarding agent–that effectively controlled the drug diffusion outside the polymeric matrix. Thus, with the primary objective of extending drug release with the lowest possible premature release during colon targeting, F3 was selected as the lead formulation for serving the study objective and evaluated further.

Therefore, an *in vitro* release study was performed without adding the pectinolytic enzyme in dissolution media to investigate the effect of the degrading enzyme on the matrix. The dissolution data were illustrated in Fig. 6. Both profiles (presence and absence of the degrading enzyme) did not show a significant difference in the % release of KTP during the first 5 h. However, 60% of the drug was released in the presence of Pectinex® Ultra SP-L at the end of 24 h compared to only 49% released in the absence of the matrix-degrading enzyme within the dissolution media. These observations came in accordance with many earlier reported studies (Sriamornsak, 2011; Wong et al., 2011). Overall, a binary system of pectin and HPMC could help provide successful colon-targeted drug delivery systems.

3.7. Uniformity of the dosage units

The uniformity of dosage units can be evaluated based on content uniformity or weight variation tests based on the dosage or the drug load within the formulation. For example, for hard capsules containing less than <25% drug load, a content uniformity test was performed according to the USP "Uniformity of dosage units (905)" as presented in Table 3. As a result, the calculated acceptance value (6.7) was less than

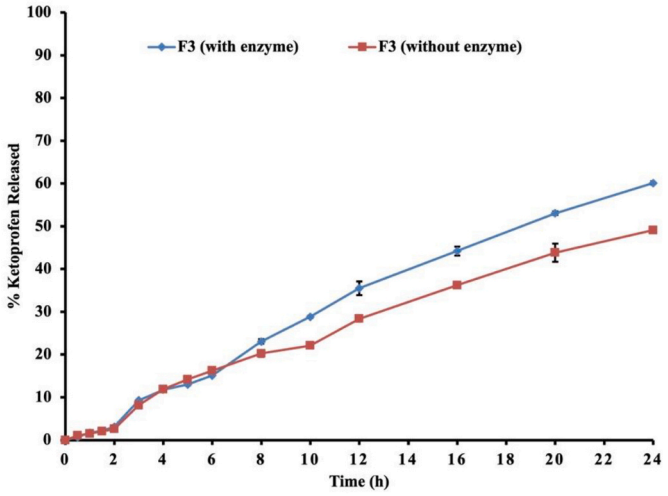

Fig. 6. Enzyme- and non-enzyme-triggered dissolution profiles of the lead ketoprofen capsules (Mean ± SD, n = 3).

L1 (15). Moreover, all drug content values of the ten tested dosage units were within the range of the lower side of L2 (73.9) and the higher side of L2 (123.1). Therefore, the capsules of the lead formulation met the USP acceptance criteria for content uniformity testing.

3.8. DSC analysis

DSC is a powerful thermoanalytical tool for evaluating and characterizing various thermal transitions of drugs and polymeric materials. The DSC curves of the KTP, both polymers, and lead pellet formulation are shown in Fig. 7. The DSC curves displayed a melting endothermic drug peak at approximately 94 °C. The KTP peak disappeared in the prepared pellets DSC curves, indicating the crystalline drug's conversion into an amorphous form and complete solubilization in the selected polymeric carriers (Alzahrani et al., 2022b).

3.9. PXRD characterization

The PXRD technique evaluated the thermal transitions of the drug before and after drug loading within the polymeric matrix. The PXRD patterns of the pure KTP and lead pellet formulation are shown in Fig. 8. The PXRD pattern of pure KTP showed two intense crystallinity peaks at 2θ angles of 18.38° and 22.84°, which is attributed to the crystalline

Table 3
Content uniformity test as per USP for the lead formulation capsules.

Capsule #	1	2	3	4	5	6	7	8	9	10
Assay (%)	97.4	96.3	98.1	94.9	96.7	101.3	98.5	93.8	95.1	97.9
n	10	**L1**	15.0 unless otherwise specified			**L2**	25.0 unless otherwise specified			
Average	97.0	**SD**	2.2	**M**	**98.5**	**Acceptability constant (k)**				**2.4**
Acceptance value		**6.7**	**Low side**	**73.9**	**High side**	**123.1**				
Low side	[1-(0.01)(L2)] M		**High side**	[1 + (0.01)(L2)] M		**Acceptance value**		98.5 – Average + k.SD		
Case	**M (case 1) to be applied when target assay is ≤ 101.5**									
Acceptance criteria	**The acceptance value should be ≤ L1**									
	The assay of each capsule should be between the calculated low and high side									

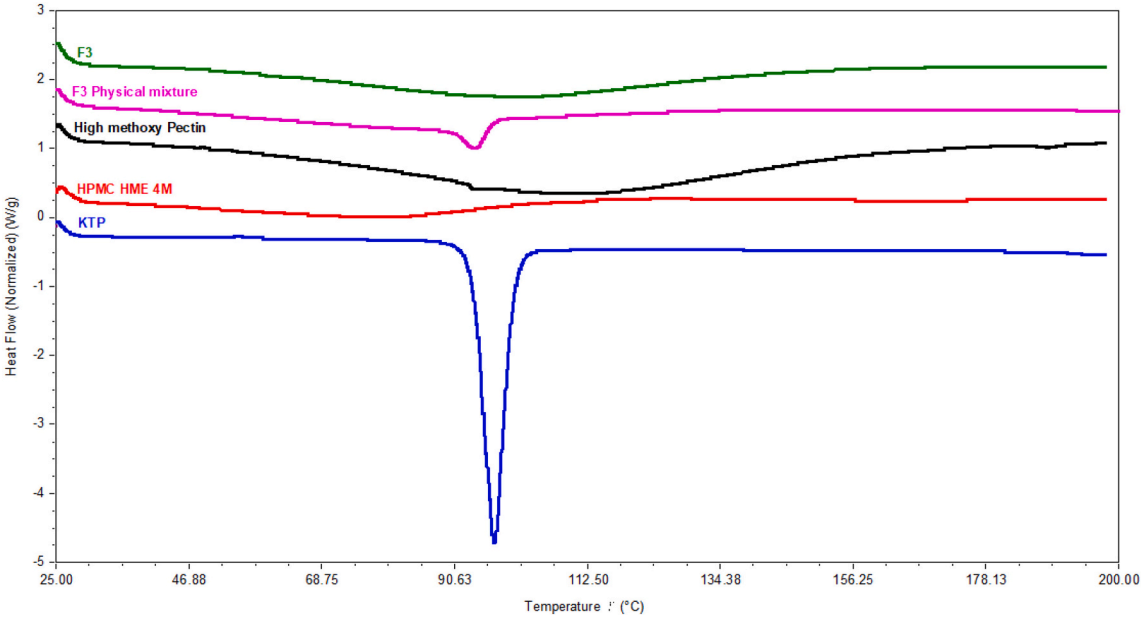

Fig. 7. DSC curves of ketoprofen, hydroxypropyl methylcellulose HME 4 M, High methoxyl grade pectin, F3 physical mixture, and pellets of the lead (F3) formulation.

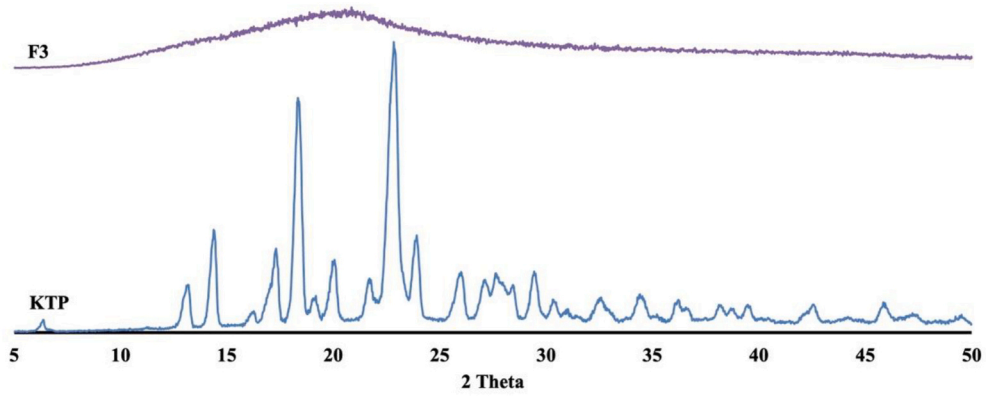

Fig. 8. Powder X-ray diffractograms of ketoprofen and the pellets of the lead formulation.

nature of the drug (Bhatia et al., 2021). The complete disappearance of the characteristic drug crystalline peaks from the PXRD pattern of the pellets suggests the conversion of crystalline KTP into an amorphous form. This thermal transition was attributed to both polymers and the HME process in which the high extrusion temperature and mixing shear led to the transformation to the amorphous state. These findings and the DSC data confirmed that the KTP exists in a solid-solution state within the pectin-HPMC polymeric matrix.

3.10. Morphological studies–SEM

SEM was used to analyze the surface morphology of the prepared pellets. SEM micrographs of pure KTP powder showed needle-like crystals forming crystalline masses (Fig. 9). Furthermore, the drug also preserved its crystallinity in the PM of the F3 formulation. The pellets of the lead showed a smooth surface without any cracks indicating molecular level mixing of the KTP and polymers during the extrusion process. Therefore, SEM micrographs supplemented the DSC and PXRD

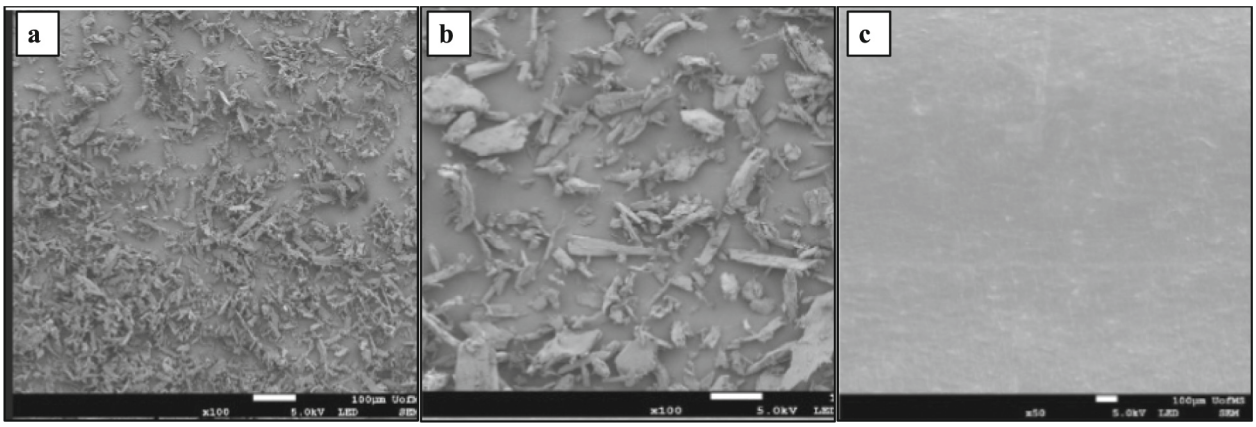

Fig. 9. Scanning electron micrographs of a) ketoprofen (100×), b) physical mixture (100×), and c) pellets of the lead formulation (50×).

result by displaying an amorphous drug form within the polymeric carrier.

3.11. Stability studies

The physicochemical stability was evaluated at scheduled intervals for any change in drug content, *in vitro* release behavior, and thermal characteristics. The effect of the accelerated storage conditions on the *in vitro* release and thermal behavior is shown in Fig. 10a and b. All tested lead capsule formulations did not show any significant ($p > 0.05$) change in KTP content–97.3 ± 2.5% (initial) and 96.1 ± 3.1% (3 months)–over the tested period. Furthermore, the initial and 3-month DSC curves showed no endothermic melting peaks for KTP, revealing no crystal form transformation during storage. Moreover, the initial and 3-month KTP release profiles were quite similar. These observations demonstrated that the pectin-HPMC matrix-based pellets are physiochemically stable under accelerated stability conditions through the tested period.

4. Conclusions

Pectin's suitability as an enzyme-triggered carrier matrix for colon drug delivery using HME technology was successfully investigated. Coupling HME technology with a die-surface cutting pelletizer was achieved to prepare amorphous solid dispersions of KTP for colon targeting. Pectin and HPMC matrix-based pellets containing various concentrations of both polymers were prepared and subjected to three-stage *in vitro* drug release testing. The amount and the grade of pectin significantly affected KTP release. The lead formulation did not show any significant change in drug content, *in vitro* release profiles, and thermal characteristics over 3 months (last time point tested) storage at accelerated conditions (40 °C/75 ± 5% RH). The fabricated capsules showed a short disintegration time and met the USP acceptance criteria for content uniformity testing. Thus, this innovative coupling approach could offer a continuous pellet manufacturing process with minimal steps for colon targeting.

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Author statement

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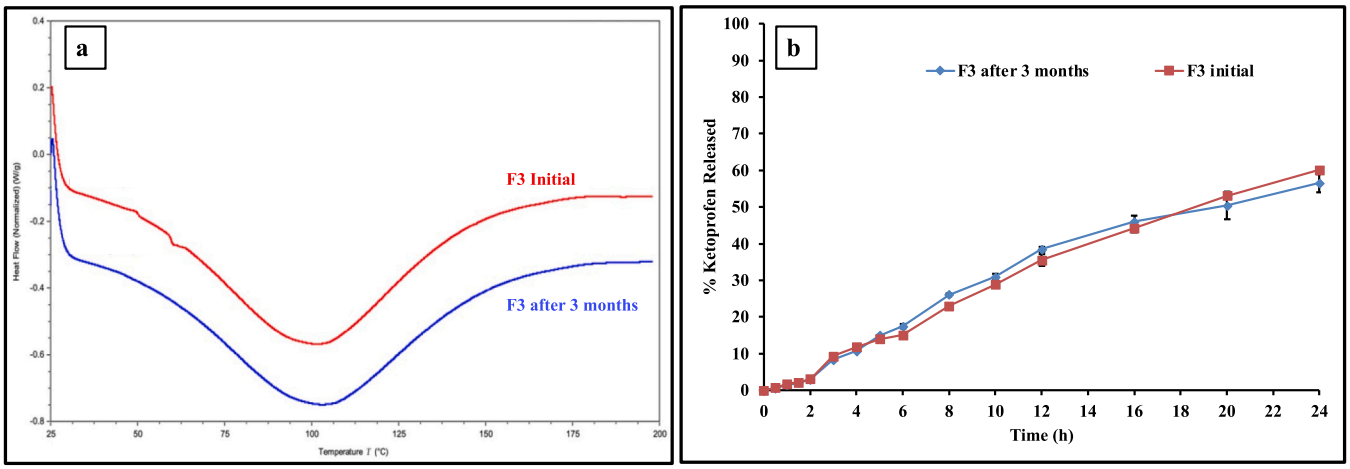

Fig. 10. The lead ketoprofen capsules over 3-month storage at accelerated conditions (40 ± 2 °C/75 ± 5% RH) a) DSC curvesand b) Enzyme-triggered *in vitro* dissolution profiles (Mean ± SD, $n = 3$).

Declaration of Competing Interest

The authors believe that there are no conflicts of interest to declare for the research article entitled "Preparation and *In vitro* Evaluation of Hot-Melt Extruded Pectin-Based Pellets Containing Ketoprofen for Colon Targeting".

Data availability

Data will be made available on request.

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